EFFECT OF RD43 RICE (*Oryza sativa* L.) ON STARCH DIGESTIBILITY, PHYSICOCHEMICAL PROPERTIES, GLYCEMIC RESPONSES AND FOOD APPLICATIONS

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ผลกระทบของข้าว กข43 (*Oryza sativa* L.) ต่อความสามารถในการย่อยของแป้ง คุณสมบัติเชิงเคมีกายภาพ การเปลี่ยนแปลงระดับน้ำตาลในเลือดและการประยุกต์ใช้ในอาหาร

น.ส.พิมพ์อร สุขแล้ว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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พิมพ์อร สุขแล้ว : ผลกระทบของข้าว กข43 (*Oryza sativa* L.) ต่อความสามารถในการย่อยของแป้ง คุณสมบัติเชิงเคมีกายภาพ การเปลี่ยนแปลงระดับน้ำตาลในเลือดและการประยุกต์ใช้ในอาหาร. (EFFECT OF RD43 RICE (*Oryza sativa* L.) ON STARCH DIGESTIBILITY, PHYSICOCHEMICAL PROPERTIES, GLYCEMIC RESPONSES AND FOOD APPLICATIONS) อ.ที่ปรึกษาหลัก : ศ. ดร. สิริชัย อดิศักดิ์วัฒนา, อ.ที่ปรึกษาร่วม : ศ. ดร.ชิน-คุน หวัง

ข้าว กข43 (*Oryza sativa* L.) เป็นการผสมข้ามสายพันธุ์ระหว่างข้าวเจ้าหอมสุพรรณบุรีกับข้าวสุพรรณบุรี 1 ข้าว กข43 มีลักษณะปริมาณอะมิโลสต่ำ เนื้อสัมผัสนุ่มและมีกลิ่นหอม อย่างไรก็ตาม ยังไม่มีการศึกษาเกี่ยวกับความสามารถของข้าว กข43 ต่อผลกระทบด้านสุขภาพเมแทบอลิซึมและการประยุกต์ใช้ในอาหาร ในการศึกษาครั้งนี้พบว่าการบริโภคข้าว กข43 ที่ประกอบด้วยแป้งที่ไม่ถูกย่อย 14.10 กรัม เป็นระยะเวลา 12 สัปดาห์ ส่งผลให้ระดับน้ำตาลกลูโคส อินซูลิน น้ำตาลเฉลี่ยสะสม และตัวชี้วัดสำหรับการดื้อต่ออินซูลินในเลือดภายหลังอดอาหารลดลงอย่างมีนัยสำคัญในผู้ที่มีภาวะก่อนเบาหวาน นอกจากนั้น องค์ประกอบของร่างกาย ประกอบด้วย น้ำหนักตัว ดัชนีมวลกาย มวลไขมันในร่างกาย และเส้นรอบเอวลดลงอย่างมีนัยสำคัญหลังการบริโภคข้าว กข43 ในสัปดาห์ที่ 6 และ 12 เมื่อเปรียบเทียบกับข้าวที่บริโภคทั่วไปในไต้หวัน (ไท่เคน9) การบริโภคข้าว กข43 แสดงให้เห็นถึงระดับอินซูลินและตัวชี้วัดสำหรับการดื้อต่ออินซูลินที่ลดลงอย่างมีนัยสำคัญในสัปดาห์ที่ 12 ในคุณสมบัติทางเคมีกายภาพและเชิงหน้าที่พบว่า แป้งข้าว กข43 มีปริมาณอะมิโลส (ร้อยละ 19.04) สูงกว่าแป้งข้าวหอมมะลิ มีรูปร่างไม่แน่นอน มีหลายเหลี่ยม และแสดงโครงสร้างผลึกชนิดวีและมีความเป็นผลึกน้อย ส่งผลให้ค่าดัชนีการดูดซับน้ำ ค่าการพองตัว ค่าดัชนีการละลายน้ำ อุณหภูมิที่เกิดเจลาติไนเซชันและคุณสมบัติด้านความหนืดลดลงอย่างมีนัยสำคัญ ยิ่งไปกว่านั้น ยังมีความสามารถในการย่อยของแป้งต่ำกว่าแป้งข้าวหอมมะลิ เนื่องจากมีปริมาณแป้งที่ถูกย่อยได้อย่างรวดเร็วต่ำกว่าและมีแป้งที่ไม่ถูกย่อยในปริมาณสูงกว่า แป้งข้าว กข43 มีความสามารถในการรบกวนการรวมตัวของคอเลสเตอรอลเป็นไมเซลล์และจับกับกรดน้ำดี ในการประยุกต์ใช้ในอาหาร ขนมถ้วยฟูที่ทำจากแป้งข้าว กข43 มีความสามารถในการย่อยของแป้งต่ำกว่าขนมถ้วยฟูจากแป้งข้าวหอมมะลิ โดยไม่มีผลกระทบต่อคุณลักษณะทางประสาทสัมผัส นอกจากนี้ การทดแทนแป้งสาลีด้วยแป้งข้าว กข43 (ร้อยละ 10-40 โดยน้ำหนักต่อน้ำหนัก) ในบะหมี่ ลดความสามารถในการย่อยของแป้ง ดัชนีการย่อยและปริมาณแป้งที่ถูกย่อยได้อย่างรวดเร็วพร้อมทั้งเพิ่มปริมาณแป้งที่ไม่ถูกย่อย เมื่อเทียบกับบะหมี่ควบคุม บะหมี่ที่มีแป้งข้าว กข43 ร้อยละ 30 มีค่าดัชนีการดูดซับน้ำ ดัชนีการบวม ปริมาณของแข็งที่สูญเสียระหว่างการต้ม และค่าความสว่าง (_L*_) เพิ่มขึ้นเล็กน้อย นอกจากนี้มีค่าสีแดง (_a*_) ค่าสีเหลือง (_b*_) และค่าความแข็งลดลง ร่วมกับมีการยอมรับโดยรวมใกล้เคียงกัน การบริโภคบะหมี่ที่มีแป้งข้าว กข43 ร้อยละ 30 ส่งผลให้ระดับน้ำตาลในเลือดหลังรับประทานอาหารลดลง ร่วมกับระดับกลูคากอน ไลค์ เป็ปไทด์-1 และเป็ปไทด์ ไทโรซีน-ไทโรซีนที่เพิ่มขึ้นในอาสาสมัครชาย โดยสรุป ข้าว กข43 มีศักยภาพในการผลิตอาหารและการควบคุมระดับน้ำตาลในเลือด ความอยากอาหาร ตลอดจนองค์ประกอบของร่างกายในมนุษย์

สาขาวิชา อาหารและโภชนาการ ลายมือชื่อนิสิต ....
ปีการศึกษา 2563 ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
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KEYWORD: RD43 rice/ Physicochemical properties/ Food application/ Glycemic control/ Weight regulation

Phim On Suklaew : EFFECT OF RD43 RICE (*Oryza sativa* L.) ON STARCH DIGESTIBILITY, PHYSICOCHEMICAL PROPERTIES, GLYCEMIC RESPONSES AND FOOD APPLICATIONS. Advisor: Prof. SIRICHAI ADISAKWATTANA, Ph.D. Co-advisor: Prof. CHIN-KUN WANG, Ph.D.

RD43 rice (*Oryza sativa* L.) is a cross-breed between Khao Jow Hawm Suphan Buri and Supan Buri1. It is characterized as a low amylose content, soft texture, and mild fragrance. However, its potential of metabolic health effect and food application remains unknown. In the present study, daily consumption of RD43 rice containing 14.10 g of undigestible starch for 12 weeks showed a significant reduction in fasting plasma glucose, insulin, HbA1c, and HOMA-IR in prediabetic participants. Furthermore, a significant decrease in body composition including body weight, body mass index, total body fat, and waist circumference was observed after the consumption of RD43 rice at 6th and 12th week. In comparison with conventional rice in Taiwan (Taiken9), ingestion of RD43 rice showed a significantly reduction in fasting plasma insulin and HOMA-IR at 12th week. In physicochemical and functional properties found that RD43 rice flour had higher amylose content (19.04%) than Hom Mali rice flour. It presented an irregular polyhedral shape and manifested a V-type crystalline structure and less crystallinity, which lead to a significant reduction of the water absorption index (WAI), swelling power (SP), water solubility index (WSI), gelatinization temperature, and pasting properties. Moreover, it had lower starch digestibility than Hom Mali rice flour due to a lower content of rapidly digestible starch (RDS) and higher content of undigestible starch. RD43 rice flour also exhibited a greater ability to disrupt cholesterol micellization and bind bile acids. In food application, steamed muffin made from RD43 rice flour showed lower starch digestibility than Hom Mali steamed muffin with no alterations of sensory attributes. Moreover, the substitution of wheat flour with RD43 rice flour (10-40% w/w) into noodles decreased starch digestibility, hydrolysis index, and RDS content together with increasing undigestible starch content. Comparing to the control noodles, 30% RD43 rice flour noodles slightly elevated WAI, swelling index (SI), cooking loss, and lightness (*L**) as well as reduced redness (*a**), yellowness (*b**), and hardness with similar overall acceptability. In healthy men, consumption of 30% RD43 noodle caused a reduction in postprandial plasma glucose with increasing postprandial glucagon-like peptide-1 (GLP-1) and peptide tyrosine–tyrosine (PYY). In conclusion, RD43 rice has potential for food manufacturing and controlling glycemic, appetite as well as body composition in humans.

Field of Study:	Food and Nutrition	Student's Signature ....
Academic Year:	2020	Advisor's Signature ....
		Co-advisor's Signature ....

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INTRODUCTION

1.1 Background and linking of the study

Rice (*Oryza sativa* L.), one of the most important crops in the world, is primarily source of carbohydrate, protein, vitamins, and minerals (Bhattacharjee, Singhal, & Kulkarni, 2002). Rice is a staple food owing to intake nearly one-half of world's population (Kim, 2013). Particularly in Asia, 95% of global rice production is consumed which account for 40-80% of total calories of Asian diet (Bhattacharjee et al., 2002). Nevertheless, the consumption of rice is linked to health conditions because of their high glycemic index (GI). Intake of high GI food is rapidly increased postprandial glucose level, leading to greater insulin secretion (An, Bae, Han, Lee, & Lee, 2016; Arumugam et al., 2008; Hu, Pan, Malik, & Sun, 2012). After that, blood glucose level drops quickly and often leads to the hypoglycemia range, which is related to increase hunger and overconsumption of food (Campfield & Smith, 1990; Campfield, Smith, Rosenbaum, & Hirsch, 1996). Moreover, long term consumption of high GI foods significantly confers a substantially greater risk of obesity, type 2 diabetes (T2DM), and cardiovascular diseases (Augustin, Franceschi, Jenkins, Kendall, & La Vecchia, 2002; Ding & Malik, 2008).

In order to resolve these health issues, various rice cultivars have been established. For example, riceberry rice is a hybrid between two Thai rice cultivars which helps to control postprandial hyperglycemia (Thiranusornkij, Thamnarathip, Chandrachai, Kuakpetoon, & Adisakwattana, 2019; Adisakwattana et al., 2010; Nounmusig et al., 2018). Riceberry rice has been applied in many food products (e.g. bread, cake, biscuits, and noodles) (Klunklin & Savage, 2018a; Mau, Lee, Chen, & Lin, 2017; Sirichokworrakit, Phetkhut, & Khommoon, 2015; Thiranusornkij et al., 2019). Recently, Rice department, Thailand registered an *indica* rice named as RD43 (SPR99007-22-1-2-2-1 registered on 5 October 2010). It is cross-breed between Khao Jow Hawm Suphan Buri and Supan Buri1 cultivars characterized as low-amylose content rice (Rice Department of Thailand, 2018). Besides of soft texture and mild aromatic, RD43 rice lower glycemic index (GI) than other low amylose rice varieties such as RD15 and Hom Mali rice (Wasusun et al., 2017). Based on *in vitro* study, RD43 rice showed the lowest content of rapidly available glucose (RAG) when compared with other rice cultivars. Moreover, acute consumption of RD43 rice decreases postprandial blood glucose in T2DM patients when compared to low amylose rice (RD15 rice) (Wasusun et al., 2017). According to its special characteristics related to consumer acceptance and hypoglycemic effect, RD43 rice may be a promising beneficial for heath with possible practical for daily life. However, long-term effect of RD43 rice consumption and its beneficial for metabolic profiles and markers with possible practical in real-life remains unknown. Therefore, the first part of the present study is to investigate the effect of habitual RD43 rice consumption on metabolic profiles and markers in prediabetes participants. This finding would provide the alternative use of healthy white rice with high acceptance among consumers on prevention of diabetes (Hu, Pan, Malik, & Sun, 2012).

Aside from consumption as cooked granular form, rice has been used for traditional food in form of flour such as noodle, cake, and beverage (Kim, 2013). Due to its bland taste, high digestibility, hypoallergenic behavior, and low price, rice flour is recently used as an ingredient in specific products including infant foods, baking products, and processed foods (Klunklin & Savage, 2018b; Koh, Kasapis, Lim, & Foo, 2009; Steiger, Müller-Fischer, Cori, & Conde-Petit, 2014). However, various rice cultivars may display certain distinction in amylose content, color, crystallinity, gelatinization, and starch component, resulting in the quality of rice flour and its products (Cai et al., 2015; Kraithong, Lee, & Rawdkuen, 2018; Thiranusornkij et al., 2019). According to these important issues, the physicochemical and functional properties of RD43 rice flour must be addressed in order to utilize and develop new varieties of healthy food products, leading to be high value-added food ingredients with health-promoting properties (Falade & Christopher, 2015; Thiranusornkij et al., 2019; Yousif, Nhepera, & Johnson, 2012).

Moreover, rice flour is increasingly used for substitution of wheat flour by enhancing nutritional and functional properties of food products such as biscuit, cake, and especially noodle (Itthivadhanapong & Sangnark, 2016; Klunklin & Savage, 2018a; Wu et al., 2017). Noodle, a processing food, has been consumed since an ancient time in Asia, such as Japan, China, Indonesia, Malaysia, and Thailand (Karim & Sultan, 2014).

Nowadays, noodle consumption has been elevated resulting from being a quick and convenient food and the ease of cooking, palatability, transportation, and mechanizations (Li, Zhu, Guo, Brijs, & Zhou, 2014). In Southeast Asia, people mostly prefer to consume yellow alkaline noodle which regarding 48% of total flour consumption in that region (Karim & Sultan, 2014). However, a large cohort study found a correlation between noodle consumption and risk of T2DM such as hyperglycemia and insulin resistance in Asian people, relating to high GI value (Zuniga et al., 2014). Furthermore, it has been reported that consumption of wheat noodles exhibits a higher postprandial blood glucose than pasta and bread in healthy subjects (Matsushima, Yagi, Hamada, Ogura, & Yonei, 2014). This is the results of suppressing release of short-acting satiety hormones such as glucagon-like peptide 1 (GLP-1) and peptide tyrosine-tyrosine (PYY), and a consequent increase in excessive hunger and appetite as well as promoting food overconsumption (Arumugam et al., 2008; De Silva & Bloom, 2012). Today, the substitution of wheat flour with rice flour into noodles may be a new nutritional therapy for reducing starch digestibility, postprandial glucose response, and modulating short-acting satiety hormones and satiety. For instance, replacing wheat flour with brown rice flour in noodles significantly reduces the rate of starch digestion and absorption (Wu et al., 2017). Also, replacement of wheat flour with rice flour can enhance the noodle qualities such as reduce cooking time and elevate tensile strength and consumer acceptance (Ahmed, Qazi, & Jamal, 2015; Sirichokworrakit et al., 2015; Wu et al., 2017). According to these issues, use of RD43 rice flour as a functional ingredient in wheat noodles may be an alternative approach for enhancing the product qualities and providing benefits for health related to management of blood glucose and satiety. Therefore, it is hypothesized that noodles made from RD43 rice flour may suppress postprandial glycemic response and modulate short-acting satiety hormones related to satiety and appetite. To prove this hypothesis, RD43 rice noodle was developed in order to access its effect on postprandial glucose, short-acting satiety hormones, and appetite in healthy volunteers.

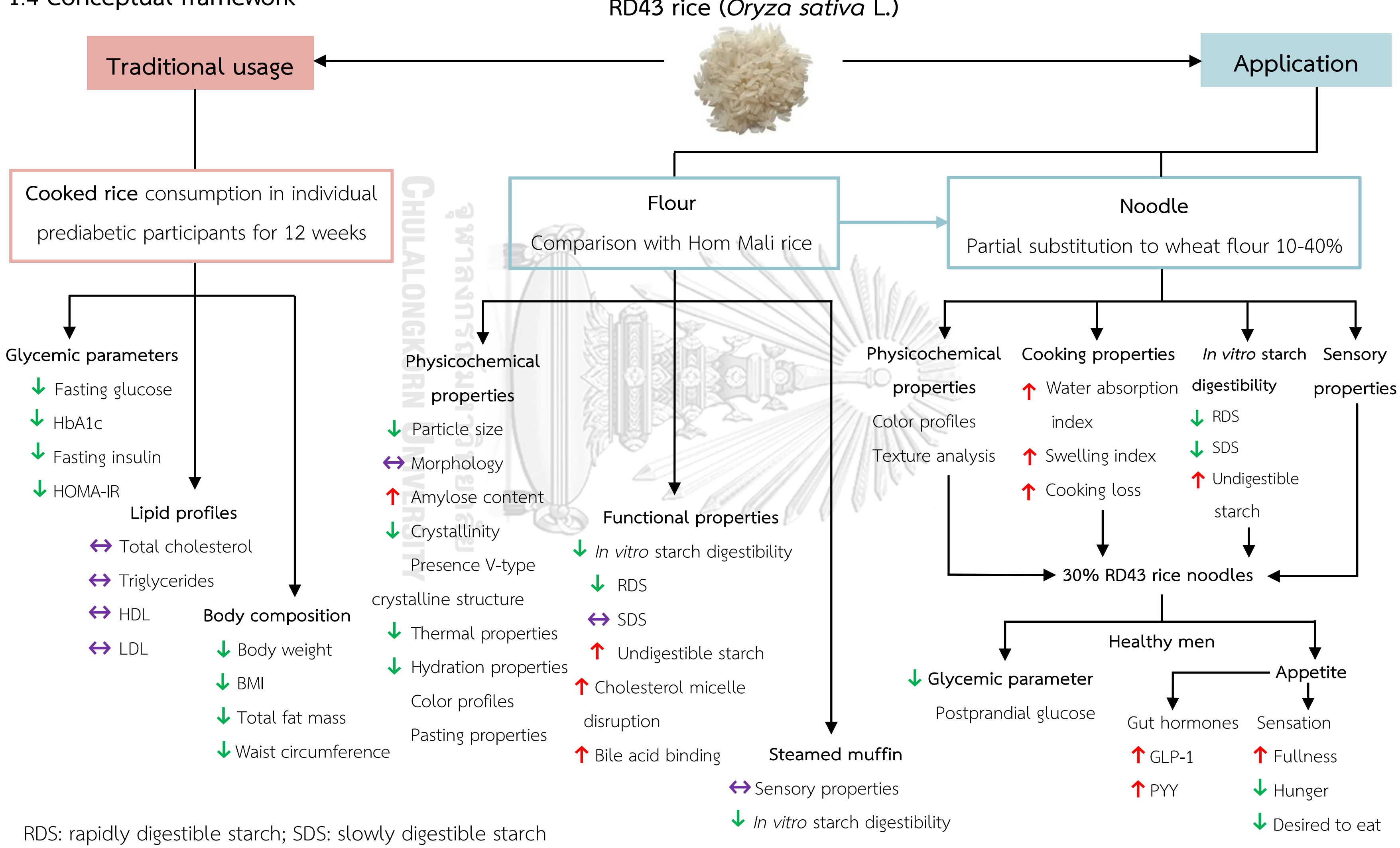
1.2 Objectives of the study

- To determine the effect of RD43 rice consumption on glycemic controlling, lipid profiles, and body composition in participants with prediabetes for 12 weeks.
- To investigate physicochemical, pasting, thermal, and functional properties of RD43 rice flour compared to Hom Mali rice flour.
- To evaluate the starch digestibility and sensory properties of application from RD43 rice flour in steamed muffin compared to Hom Mali rice flour.
- To assess the effect of wheat flour substitution with RD43 rice flour in noodles on *in vitro* starch digestibility, cooking characteristics, and physicochemical and sensory properties.
- To examine the effect of RD43 noodle consumption on glycemic response, short-acting satiety hormones (GLP-1 and PYY), and appetite in healthy men.

1.3 Hypotheses of the study

- Consumption of RD43 rice may improve glycemic parameters, lipid profiles, and body composition in participants with prediabetes for 12 weeks.
- In comparison with Hom Mali rice flour,
    - RD43 rice flour may have similar particle size, higher redness, yellowness, amylose content, thermal properties, and pasting properties, and lower lightness and hydration properties.
    - RD43 rice flour may have lower starch digestibility and higher ability to disrupt cholesterol micellization and bile acids binding.
- Steamed muffin made from RD43 rice flour may have lower starch digestibility and higher acceptance than steamed muffin made from Hom Mali rice flour.
- Substitution of RD43 rice flour to wheat flour may not alter physicochemical, functional, cooking, and sensory properties of noodles.
- Intake of RD43 noodle may decrease postprandial plasma glucose, increase short-acting satiety hormones (GLP-1 and PYY), and suppress appetite in healthy men.

1.4 Conceptual framework



RDS: rapidly digestible starch; SDS: slowly digestible starch

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CHAPTER II

IMPROVEMENT OF PARTIAL SUBSTITUTION FOR STAPLE FOOD BY RD43 RICE ON METABOLIC MARKERS OF PREDIABETIC SUBJECTS: A RANDOMIZED CLINICAL TRIAL

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2.3 Abstract

White rice is the most popular and accepted of the general population. Owing to its high glycemic index (GI) value, a positive correlation between white rice consumption and the risk of type 2 diabetes mellitus (T2DM). Consequently, strategic directions are to provide effective and healthier choices of rice for consumers. A low amylose *indica* rice namely RD43 had been reported low starch digestibility with high amounts of undigestible starch. Its potential and promising health effect in prediabetes remain unknown. In this study, the effect of partial substitution of RD43 rice for staple food on glycemic response, body composition and metabolic markers in prediabetes were investigated. A single blind, parallel, randomized controlled study was scheduled for 14 weeks. Thirty-four participants with prediabetes (aged from 32 to 68 years old) were randomly allocated to either ingesting RD43 rice group (GI=78) containing 14.10 g undigestible starch daily (substitute for 2 meals/day) or conventional rice group (GI=98) (2 meals/day) for 12 continuous weeks and 2 weeks of extra follow-up period (without tested rice). Biochemical parameters and body composition were assessed during baseline, 6th week, 12th week, and follow-up. In the 12th week, results clearly showed a significant decrease in fasting glucose, insulin, HbA1c, and HOMA-IR after partial substitute of RD43 rice. Ingestion of RD43 rice also greatly reduced body weight, body mass index (BMI), total fat mass, and waist circumference reduction at the 6th and 12th week, when compared with baseline. When compared with conventional rice group, fasting plasma insulin and HOMA-IR were significantly decreased in RD43 rice group at the 12th week. However, no significant difference in lipid profiles inter-and intra-group were detected. These findings suggested that RD43 rice could be a potential staple food to improve glycemic control and body composition for prediabetic people.

Key words: RD43 rice, glycemic index, glycemic control, HOMA-IR, body weight and fat

2.4 Introduction

Over a past few decades, the increased prevalence of type 2 diabetes mellitus (T2DM) is concerned as an epidemic health problem worldwide (Lovic et al., 2020). T2DM is a chronic metabolic disorder characterized by hyperglycemia, insulin resistance, and the impairment in insulin secretion. A sustained abnormally high level of plasma glucose is attributed to impair insulin action and sensitivities in the human liver and muscle, these biological effects referred to insulin resistance (Jellinger, 2007). A consequence of insulin resistance also promotes clinical presence of metabolic syndrome by changing the distribution of postprandial energy storage pattern such as increasing triglycerides and lowering high-density lipoproteins (HDL) level concomitant with escalating fat accumulation in ectopic tissue (Morigny, Houssier, Mouisel, & Langin, 2016; Petersen et al., 2007). Previous evidence supports that maintaining euglycemia is beneficial for preventing and delaying the progression of T2DM and its complications and improve overall quality of life (Stolar, 2010).

Carbohydrates are a dietary nutrient that extremely impacts on blood glucose concentration (Ludwig, 2002). It has been shown that type of carbohydrate components such as sugar, starch, and dietary fibers influences on the rate of digestion and absorption, resulting in difference in blood glucose response (Ludwig, 2002). According to this concept, glycemic index (GI) has been used for classifying the carbohydrate, as well as the application in dietary guidance (Ludwig, 2002). Several researches demonstrate that an ingestion of high GI diet elevates postprandial plasma glucose concentration than the low GI diet among healthy and T2DM subjects (Nounmusig et al., 2018; Silva, Kramer, Crispim, & Azevedo, 2015). Rice (*Oryza sativa* L.), is routinely consumed in many regions of the world, especially Asia. It is one of the most important and stable carbohydrate-rich food, constituting a substantial proportion of the calorie requirements of a regular diet (Mir, Shah, Bosco, Sunooj, & Farooq, 2020). Rice is commonly passed through a series of processing such as milling process for removing hulks to obtain brown rice before removing the outer bran layer to gain the white rice (Boers, Ten Hoorn, & Mela, 2015). Although the polishing process caused increasing the GI of white rice, white rice is a predominant type of rice that globally consumed (Hu, Pan, Malik, & Sun, 2012). Previous data reported that average rice consumption daily among Asian people is three to four serving or 474-632 g (Hu et al., 2012). Nevertheless, a meta-analysis and cohort study found an association between high consumption of white rice (>450 g/day) and increased risk of T2DM in populations (Bhavadharini et al., 2020; Hu et al., 2012). According to this issue, the demand for healthier varieties of rice has been growing rapidly in recent years.

Several research attempts advocate brown rice consumption owing to low GI and high nutritional value and dietary fiber content (Malik et al., 2019; Zhang et al., 2011). Interestingly, substituting brown rice for white rice demonstrated a significant reduction on HbA1c among participants with the metabolic syndrome (Malik et al., 2019). However, it has been reported that intake of brown rice is relatively low because of less consumer acceptability (Mir et al., 2020). Many studies have been conducted to investigate the utilization of healthy white rice containing resistant starch. A previous clinical evidence showed that consumption of white rice containing resistant starch for 4 weeks significantly decreased fasting plasma glucose and insulin, HOMA-IR, and oxidative stress in patients with prediabetes or newly diagnosed T2DM (Kwak et al., 2012). Recently, a Thai *indica* rice variety, namely RD43, was developed by cross-breeding between Supan Buri1 and Khao Jow Hawm Suphan Buri (Suklaew, Chusak, & Adisakwattana, 2020). It is a non-GMO rice and approved by the Rice Department of Thailand (SPR99007-22-1-2-2-1) (Suklaew et al., 2020). RD43 rice presents special characteristics related to consumer acceptance such as soft texture and mild fragrance (Rice Department of Thailand, 2018). *In vitro* study found that RD43 rice, a low amylose content, exhibits a slow rate of digestibility, representing lower value of rapidly digestible starch (RDS) and higher content of undigestible starch (Suklaew et al., 2020).

This rice demonstrated the ability to disrupt cholesterol micellization and bind bile acids (Suklaew et al., 2020). Moreover, consumption of RD43 rice decreases postprandial plasma glucose in T2DM patients when compared to other low amylose rice in mixed meal model (Wasusun et al., 2017). According to these interesting properties of RD43 rice, its potential and promising beneficial for metabolic markers with possible practical in real-life remain unknown. Therefore, we examined the effect of RD43 rice consumption for 12 weeks on metabolic markers and body composition in prediabetic participants compared with consumption of conventional white rice.

2.5 Methods

2.5.1 Rice sample

RD43 rice was purchased from Rice department, Ministry of Agriculture and Cooperatives, Thailand. Taiken9 rice, a conventional rice in Taiwan, was obtained from supermarket, Taiwan. Nutrients composition of both rice varieties were analysed according to The Association of Official Analytical Chemists standard methods (AOAC) (Sullivan & Carpente, 1993), which were shown in Table 1.

Table 1 Nutrients composition of rice/100 g dry weight

Nutrients composition[a]	Taiken9 rice	RD43 rice
Total energy (kcal)	347.00	353.33
Carbohydrate (g)	79.00	80.64
Protein (g)	6.50	5.96
Fat (g)	0.50	0.77
Total dietary fiber (g)	2.00	1.10

[a] Data were carried out by AOAC methods.

Cooked RD43 rice had lower amounts of rapidly digestible starch (RDS) and higher undigestible starch contents than cooked Taiken9 rice (Figure 1A), which presented lower starch digestibility (Figure 1B). All data were carried out using *in vitro* digestion method and quantity of total starch (Goñi, Garcia-Alonso, & Saura-Calixto, 1997; Suklaew et al., 2020). The glycemic index (GI) of cooked Taiken9 and RD43 rice were 97.99 and 78.12, respectively. GI was obtained from ten health subjects after consumption 50g of carbohydrate of cooked rice, 50 g of glucose solution was used as reference (FAO/WHO, 1998). According to the information, 80 g dry weight of Taiken9 rice and 82 g dry weight of RD43 rice were provided in sealed plastic bag for one meal consumption. Both rice packs were matched for total carbohydrates contents (64.50 g/pack). The undigestible starch contents in one pack were 4.25 g for Taiken9 rice and 7.05 g for RD43 rice.

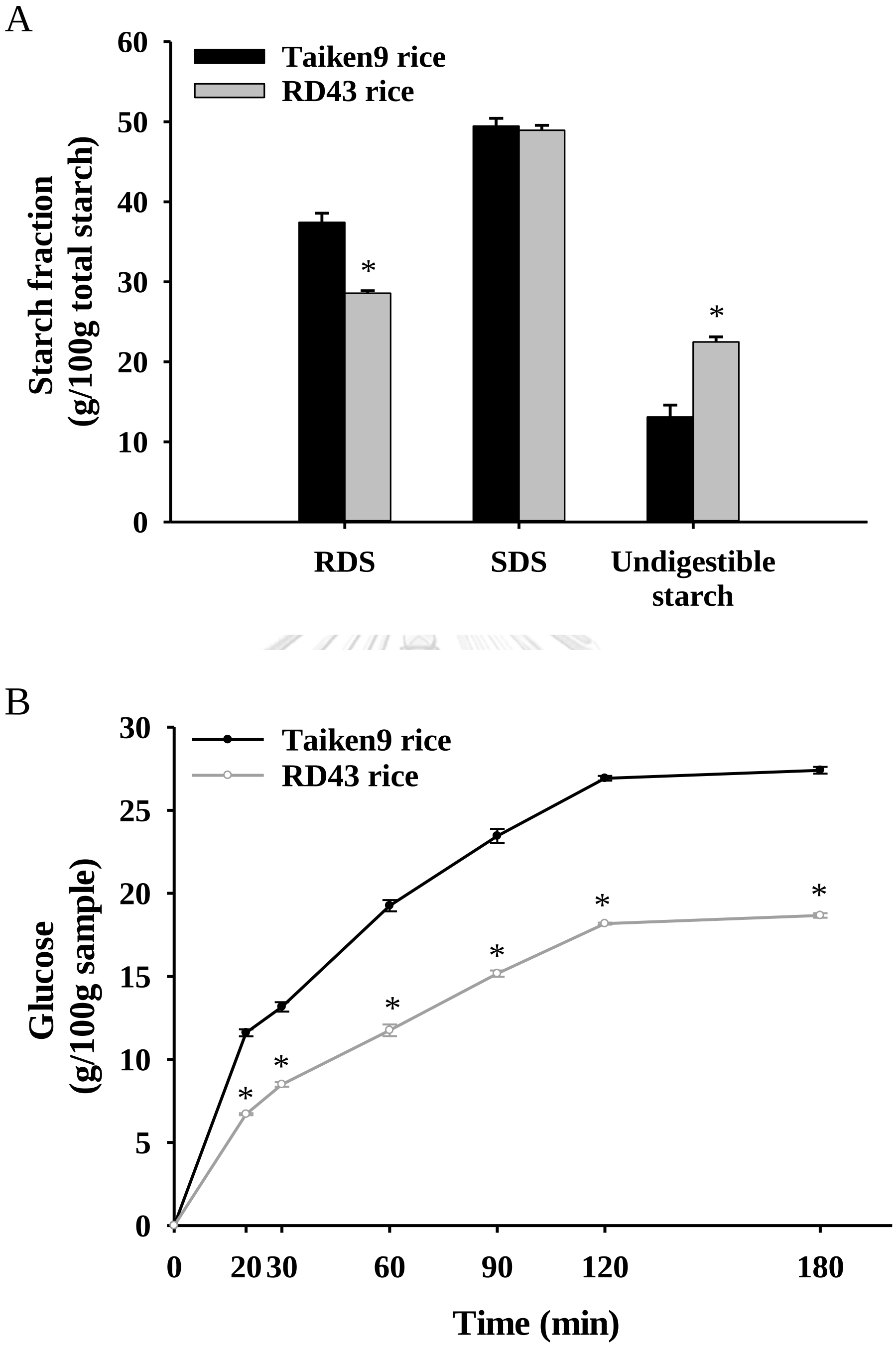

Figure 1 The *in vitro* starch digestion of cooked rice. Starch fractions (A) and the glucose release during *in vitro* starch digestion (B) from cooked Taiken9 and RD43 rice. Data are expressed as mean ± SEM, n = 3. * $p < 0.01$ compared to Taiken9 rice. RDS: rapidly digestible starch; SDS: slowly digestible starch.

2.5.2 Participants recruitment

The participants were recruited at Chung Shan Medical University, Taichung, Taiwan via advertisements in local website. Inclusion criteria were (1) aged 25-65 years; (2) fasting glucose 100-125 mg/dl or HbA1c 5.7%-6.4% according to prediabetes stage of American Diabetes Association guidelines (ADA, 2020); (3) stabilized drug administration of hypolipidemic and antihypertensive at the recent 3 months (if used). Excluded criteria were (1) weight loss or gain (≥10%) over the previous 6 months; (2) morbid obesity (body mass index (BMI) ≥ 40 kg/m^2 or BMI ≥ 35 kg/m^2 with co-morbidity); (3) diagnosis of vascular disease, cancer, thyroid disease, liver disease, renal disease, type 1 diabetes, chronic alcoholism, acute or chronic inflammatory diseases, and eating disorders; (4) pregnant or lactating; (5) taking minerals, vitamin, or antioxidant supplements in the past 30 days before study; (6) self-report of smoking and alcohol drinking. All participants gave written informed consent. The study protocol was approved by Institutional Review Board of the Chung Shan Medical University Hospital (CS1-20003) in accordance with the Declaration of Helsinki.

2.5.3 Study design

A single blind, parallel, randomized controlled study was conducted from May 2020 to August 2020 at Chung Shan Medical University, Taichung, Taiwan. This study was carried out in 3 periods: 2 weeks of run-in period, 12 weeks of intervention period, and 2 weeks of follow-up without rice consumption. Sample size of participants was calculated based on the significant difference of HOMA-IR from previous study (Kwak et al., 2012) with 0.05 of significant level (α), 80% power of test, and 30% dropout rate. Through a stratified randomization scheme, they were randomly allocated into Taiken9 rice (control, n = 25) and RD43 rice (treatment, n = 25) according to HbA1c and gender. All participants were asked for keeping a usual physical activity and dietary pattern without any antioxidant or herbal supplements consumption throughout the study. During intervention period, they were instructed to cook (ratio of RD43 rice:water was 1.4:1 and Taiken9 rice:water was 1:1) and consume rice for any 2 main meals/day (2 packs/day). Cooked rice was used and not allowed to cooling and reheating for prevention of retrogradation (Sonia, Witjaksono, & Ridwan, 2015). A consumption of 64.5 g carbohydrate from rice per meal (2 meals/day) was based on daily dietary guideline for Taiwanese and survey dietary intake of Taiwanese (2013-2016), considered as an acceptable amount for intake (Health Promotion Administration, 2018; Service, 2019). Participants received rice samples every 3 weeks and met the staff to monitor the rice consumption, dietary recall and routine measurements. Also, the cooking method and the measuring cup for water were prepared for participants. To ensure the consumption of rice, a photograph of remaining rice after consumption in each meal was estimated by researcher. Moreover, participants were asked to return all empty packets and bring the unconsumed rice packets back at the next visit (every 3 weeks). The adherence of rice consumption was ≥ 80% or ≥ 135 meals.

2.5.4 Parameter measurement

At baseline, 6th, 12th, and 14th week (follow-up), fasting blood samples were collected from forearm vein of participants by registered nurse and body composition was assessed. The primary outcomes of this research were fasting plasma glucose, insulin, glycosylated hemoglobin (HbA1c), and homeostasis model assessment of insulin resistance (HOMA-IR), while the secondary outcomes were body composition and lipid profiles.

2.5.4.1 Biochemical analysis

Blood samples were centrifuged at 3000 rpm for 15 min at 4°C and plasma was collected to Eppendorf tubes and stored at -80°C until analysis. Plasma glucose was analyzed by using the enzymatic colorimetric method from Human diagnostics (Wiesbaden, Germany). HbA1c was measured by ion-exchange HPLC (normal reference range 4.00-6.00%). Plasma insulin was analyzed by using ELISA kits from Sigma-Aldrich (St Louis, Missouri, United States). Insulin sensitivity was determined by homeostasis model assessment of insulin resistance (HOMA-IR) according the equation HOMA-IR = fasting glucose (mg/dl) × fasting insulin (μU/mL)/405 (Matthews et al., 1985). Total cholesterol, triglycerides, HDL, and LDL were measured using the commercial kits from Roche Diagnostics (Mannheim, Germany) with Shimadzu 7600 fully automated analyzer (Shimadzu Corp, Japan).

2.5.4.2 Body composition assessment

Body weight and total body fat were assessed by a non-invasive Omron Karada body scan instrument (Model HBF-375, Osaka, Japan) and participants wore light clothing and no shoes. Body mass index (BMI) was calculated from weight in kilograms divided by the square of height in meters. Waist circumference was determined by using measuring tape to record the nearest at the smallest horizontal circumference between the ribs and iliac crest.

2.5.5 Statistical analysis

Data were presented as mean ± SEM. Shapiro-Wilk test was used to test the normality of data such as fasting plasma glucose and insulin, HbA1c, HOMA-IR and body composition. Paired *t*-test was used to determine a difference intra-group. Independent *t*-test was used to assess a difference inter-group at each time point. *P*-values < 0.05 was considered as statistical significant.

A difference of rice starch fractions and starch digestibility was analyzed by independent *t*-test and a significant difference was set at less than 0.01. Statistical analysis was performed using IBM SPSS (version 22.0, SPSS Inc., United States).

2.6 Results

Based on the inclusion and exclusion criteria, only fifty eligible participants were enrolled in this study (Figure 2). During the intervention and follow-up periods, 8 participants in Taiken9 rice group (control) and 8 participants in RD43 rice group (treatment) dropped out due to poor compliance (9 participants) and personal reasons (7 participants). Consequently, data from 34 participants who completed the study were analyzed (Taiken9 group; *n* = 17 and RD43 group; *n* = 17). No adverse event relating to gastrointestinal symptoms including flatulence, diarrhea, and bloating from the consumption of rice containing undigestible starch was observed. The percentage of adherence rate was 92.55 ± 1.89, which considered high rate of rice consumption.

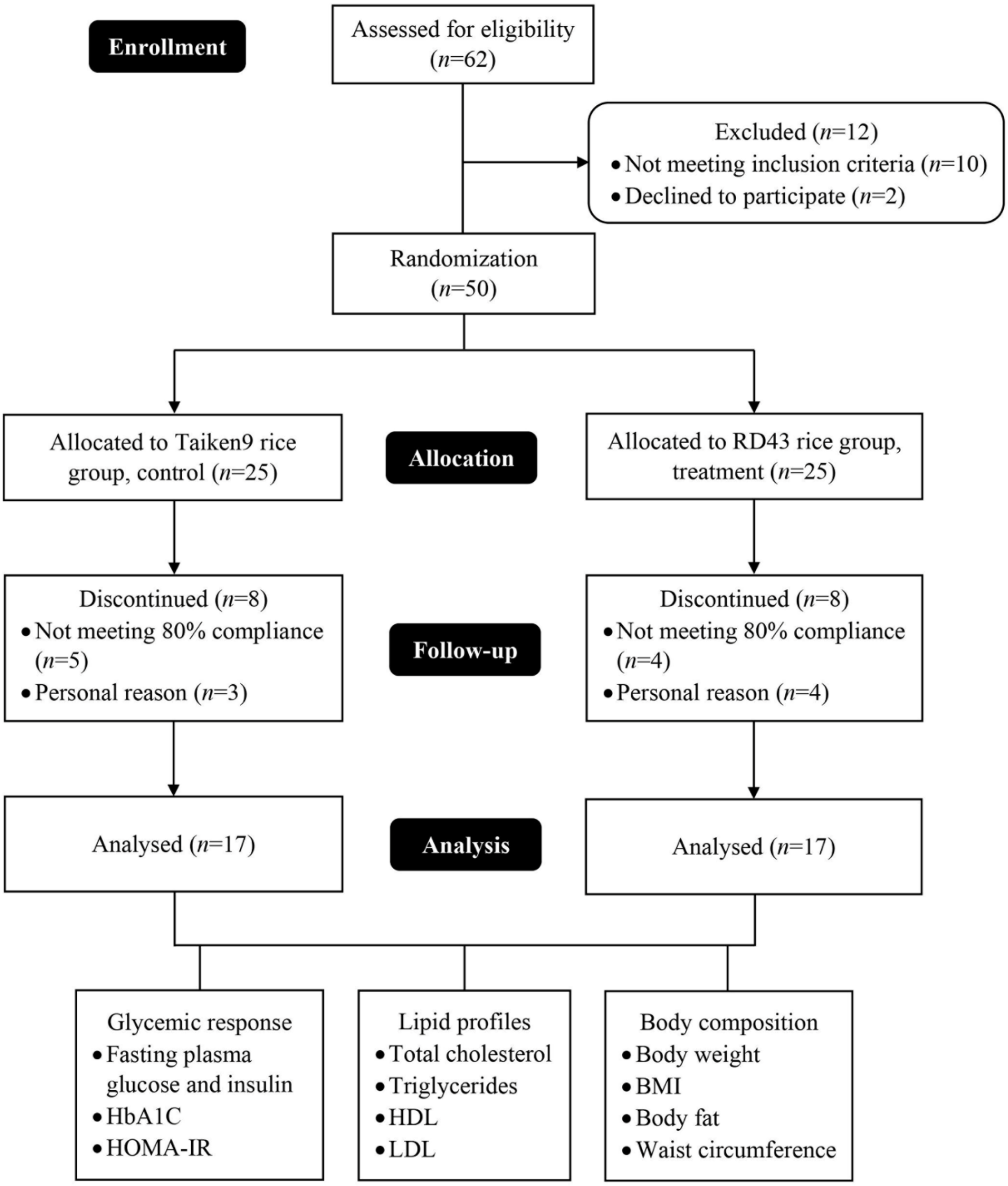

Figure 2 Study flow diagram of current study.

2.6.1 Baseline characteristics of participants

As demonstrated in Table 2, participants in each group consisted of 7 males (41%) and 10 females (59%). All participants were obese with prediabetes characteristic. At baseline, there were no significant differences in age, BMI, fasting plasma glucose, HbA1c, AST, ALT, creatinine, and systolic and diastolic blood pressure between Taiken9 and RD43 groups. In addition, consumption of both rice did not affect the function of kidney and liver such as creatinine, blood urea nitrogen (BUN), AST, and ALT (data not shown).

Table 2 Baseline characteristic

Parameters	Taiken9 rice (n = 17)	RD43 rice (n = 17)	P-value
Gender (male/female)	7/10	7/10	
Age (years)	53.59 ± 2.32	48.59 ± 2.28	0.218
BMI (kg/m^2)	26.26 ± 0.98	27.68 ± 0.94	0.375
Fasting blood glucose (mg/dl)	109.12 ± 0.04	112.41 ± 5.43	0.586
HbA1c (%)	5.85 ± 0.12	5.95 ± 0.23	0.413
AST (IU/L)	32.35 ± 3.43	34.52 ± 11.47	0.170
ALT (IU/L)	33.65 ± 4.03	33.91 ± 6.72	0.375
Creatinine (mg/dl)	0.93 ± 0.06	0.81 ± 0.04	0.205
Systolic blood pressure (mmHg)	126.82 ± 1.98	123.76 ± 4.16	0.375
Diastolic blood pressure (mmHg)	79.88 ± 2.19	75.65 ± 1.96	0.122

Data are expressed as mean ± SEM.

2.6.2 Changes in glycemic control

Consumption of RD43 rice for 12 weeks demonstrated a significant decrease in fasting plasma glucose (*p* = 0.005), HbA1c (*p* = 0.023), fasting plasma insulin (*p* = 0.018), and HOMA-IR (*p* = 0.014) when compared to baseline (Table 3). A slight raise in fasting plasma glucose, insulin, and HOMA-IR were observed at follow-up period (14th week) in RD43 rice group. Meanwhile, there was no significant difference in fasting plasma glucose, HbA1c, fasting plasma insulin, and HOMA-IR after consumption of Taiken9 rice when compared to baseline (Table 3). Interestingly, significant reduction in fasting plasma insulin (*p* = 0.031) and HOMA-IR (*p* = 0.040) was found at 12th week in RD43 when compared with Taiken9 (Table 3).

Table 3 Effects of consumption of Tiken9 rice or RD43 rice on biochemical parameters in prediabetic participants

Parameters	Initial	6th week	12th week	Follow-up
Fasting plasma glucose (mg/dl)				
Taiken9 rice	109.12 ± 2.11^{Aa}	111.94 ± 3.10^{Aa}	112.65 ± 3.15^{Aa}	112.59 ± 2.99^{Aa}
RD43 rice	112.41 ± 5.43^{Aa}	110.06 ± 5.34^{Aab}	105.47 ± 4.68^{Ab}	107.24 ± 7.71^{Ab}
HbA1c (%)				
Taiken9 rice	5.85 ± 0.12^{Aa}	5.84 ± 0.13^{Aa}	5.98 ± 0.11^{Aa}	5.89 ± 0.11^{Aa}
RD43 rice	5.95 ± 0.23^{Aa}	5.77 ± 0.18^{Ab}	5.81 ± 0.20^{Ab}	5.79 ± 0.19^{Ab}
Fasting plasma insulin (mIU/ml)				
Taiken9 rice	13.21 ± 1.71^{Aa}	12.24 ± 1.74^{Aa}	12.49 ± 2.11^{Aa}	17.62 ± 4.59^{Aa}
RD43 rice	16.13 ± 3.46^{Aa}	11.24 ± 1.24^{Aa}	7.82 ± 0.82^{Bb}	10.35 ± 2.11^{Ab}
HOMA-IR				
Taiken9 rice	3.55 ± 0.48^{Aa}	3.37 ± 0.49^{Aa}	3.61 ± 0.67^{Aa}	4.70 ± 1.09^{Aa}
RD43 rice	4.51 ± 1.02^{Aa}	3.10 ± 0.50^{Aa}	2.08 ± 0.26^{Bb}	3.31 ± 1.17^{Aab}
Total cholesterol (mg/dl)				
Taiken9 rice	188.35 ± 6.01^{Aa}	189.71 ± 10.38^{Aa}	199.00 ± 11.14^{Aa}	194.88 ± 10.78^{Aa}
RD43 rice	186.06 ± 8.69^{Aa}	176.71 ± 8.04^{Aa}	185.18 ± 9.73^{Aa}	185.00 ± 9.77^{Aa}
Triglycerides (mg/dl)				
Taiken9 rice	185.59 ± 41.76^{Aa}	164.35 ± 33.85^{Aa}	166.18 ± 32.14^{Aa}	171.24 ± 36.05^{Aa}
RD43 rice	127.18 ± 18.86^{Aa}	107.29 ± 14.91^{Aa}	110.94 ± 14.14^{Aa}	115.00 ± 13.94^{Aa}
HDL (mg/dl)				
Taiken9 rice	47.11 ± 2.23^{Aa}	45.36 ± 2.00^{Aa}	48.94 ± 2.91^{Aa}	45.95 ± 2.45^{Aa}
RD43 rice	49.16 ± 2.90^{Aa}	47.46 ± 2.92^{Aa}	50.02 ± 2.82^{Aa}	46.26 ± 3.97^{Aa}
LDL (mg/dl)				
Taiken9 rice	114.53 ± 6.56^{Aa}	120.66 ± 9.94^{Aa}	123.16 ± 11.21^{Aa}	118.77 ± 9.97^{Aa}
RD43 rice	108.85 ± 7.91^{Aa}	103.12 ± 6.42^{Aa}	109.38 ± 7.12^{Aa}	107.95 ± 8.46^{Aa}

Data are expressed as mean ± SEM. Difference capital letters in the same column of each parameter were considered significantly different as compared inter-group at each time point ($p < 0.05$). Difference lowercase letters in the same row of each parameter were considered significantly different as compared intra-group ($p < 0.05$).

2.6.3 Changes in lipid profiles

An alteration of lipid profiles after consumption of Taiken9 rice or RD43 rice is listed in Table 3. No significant difference in lipid profiles was found in Taiken9 and RD43 at 6th week, 12th week, and follow-up period. Also, there was no significant difference in lipid profiles when compared inter-group at each period.

2.6.4 Changes in body composition

The effect of dietary intervention on body composition was presented in Table 4. After comparison to the baseline, a considerable reduction in body weight (6th week, $p = 0.012$; 12th week, $p = 0.009$), BMI (6th week, $p = 0.011$; 12th week, $p = 0.005$), total fat mass (6th week, $p < 0.001$; 12th week, $p = 0.013$), and waist circumference (6th week, $p = 0.014$; 12th week, $p = 0.002$) were detected in RD43. In the follow-up period, a notable increase in body weight ($p = 0.045$) and BMI ($p = 0.024$) were observed in RD43 when compared with 12th week. There was no significant difference in body composition parameters after consumption of Taiken9 rice at 6th week, 12th week, and follow-up.

Table 4 Effects of consumption of Tiken9 rice or RD43 rice on body composition in prediabetic participants

Parameters	Initial	6th week	12th week	Follow-up
Body weight (kg)				
Taiken9 rice	69.32 ± 2.93^{Aa}	68.58 ± 3.10^{Aa}	68.42 ± 3.16^{Aa}	68.59 ± 3.00^{Aa}
RD43 rice	74.98 ± 3.91^{Aa}	73.30 ± 3.59^{Ab}	72.64 ± 3.47^{Ac}	73.02 ± 3.37^{Ab}
BMI (kg/m^2)				
Taiken9 rice	26.26 ± 0.98^{Aa}	26.31 ± 0.96^{Aa}	26.07 ± 1.00^{Aa}	26.12 ± 0.94^{Aa}
RD43 rice	27.68 ± 0.94^{Aa}	27.07 ± 0.84^{Ab}	26.84 ± 0.83^{Ac}	27.00 ± 0.81^{Ab}
Body fat (%)				
Taiken9 rice	32.31 ± 1.50^{Aa}	33.22 ± 1.40^{Aa}	32.14 ± 1.30^{Aa}	32.04 ± 1.48^{Aa}
RD43 rice	31.95 ± 1.22^{Aa}	30.59 ± 1.28^{Ab}	31.32 ± 1.20^{Ac}	30.95 ± 1.23^{Abc}
Waist circumference (cm)				
Taiken9 rice	88.47 ± 2.26^{Aa}	87.59 ± 2.33^{Aa}	87.09 ± 2.24^{Aa}	87.32 ± 2.54^{Aa}
RD43 rice	91.91 ± 2.83^{Aa}	88.88 ± 2.51^{Ab}	88.18 ± 2.51^{Ab}	89.44 ± 2.39^{Aab}

Data are expressed as mean ± SEM. Difference capital letters in the same column of each parameter were considered significantly different as compared inter-group at each time point ($p < 0.05$). Difference lowercase letters in the same row of each parameter were considered significantly different as compared intra-group ($p < 0.05$).

2.7 Discussion

Carbohydrate is a primary nutrient which provides the energy and influences metabolic parameters in the body. This is the first study to examine the effect of staple food consumption, namely, RD43 rice, on glycemic control, lipid profiles, and body composition in participants with prediabetes for 12 weeks and compared to conventional white rice (Taiken9). Consumption of conventional rice did not alter glycemic parameters. Our findings indicated that daily consumption of 309 g cooked RD43 rice for 12 weeks significantly improved the markers of glycemic control and body composition parameters in prediabetic participants. The significant reduction of fasting glucose by 6.94 mg/dl, HbA1c by 2.35%, fasting plasma insulin by 48.48%, and HOMA-IR by 46.12% among individual participants were found in RD43 rice consumption when compared to baseline. Interestingly, replacing conventional white rice with RD43 rice resulted in a significant reduction in fasting plasma insulin and HOMA-IR at 12th week. Our results are in agreement with the report of Malik *et al*. (2019), indicating that two meals/day substitution of high GI rice with low GI rice for three months significantly reduced HbA1c in overweight Indian participants. Our findings are consistent with Kwank *et al*. (2012), who reported that daily consumption of refined rice containing 6.51 g resistant starch for 4 weeks significantly decreased fasting plasma glucose and insulin concentration in subjects with prediabetes or newly diagnosed type 2 diabetes. The undigestible starch such as resistant starch content has been shown to influence the glycemic response (Halajzadeh et al., 2020). The presence of resistant starch could alter the process of carbohydrate digestion, providing a slow glucose release and absorption into blood circulation (Kwak et al., 2012; Malik et al., 2019; Wasusun et al., 2017). Moreover, the high amylose content of starch also decreases its digestibility (Panlasigui et al., 1991). Our present study indicates that RD43 rice presents higher content of undigestible starch when compared to conventional white rice. Furthermore, RD43 rice was shown to have higher amylose content as compared with jasmine rice (Suklaew et al., 2020). The current study suggests that the lower glycemic and insulinemic response elicited by RD43 rice may be attributed to high undigestible and amylose content.

Surprisingly, ingestion of RD43 rice significantly decreased body weight by 2.34 kg, total fat mass by 1.97%, and waist circumference by 3.73 cm among individual participants at 12^{th} week when compared to baseline. Also, a remarkable effect of RD43 rice consumption on body composition was observed at 6^{th} week. A potential mechanism could be explained by improving plasma glucose and insulin level after ingestion of RD43 rice, which probably enhanced other glucoregulatory hormones such as glucagon, amylin, GIP, and GLP-1, leading to prolong satiety and decrease food intake, causing a reduction in body composition (Aronoff, Berkowitz, Shreiner, & Want, 2004). Our results are in line with previous research conducted in overweight men, that a significant decrease in total fat mass together with improvement of insulin secretion was observed after consumption of lower GI diet for 5 weeks (Bouché et al., 2002). Total body fat indicates whole-body fat while waist circumference represents only central fat deposition (Nayak, Raghurama Nayak, Vidyasagar, & Kamath, 2018). The reduction in total body fat and waist circumference is important factor for lowering progression to diabetes whilst rising regression to normal glucose homeostasis (Nayak et al., 2018; Song et al., 2016). Previous research found a positive correlation between indices of overweight or obesity and fasting hyperglycemia in Taiwanese (Chien, Liou, & Chen, 2004).

In the present study, obesity was manifested in prediabetic participants based on classification of BMI in Asian population (Weir & Jan, 2019). The BMI has been used as the best available tool in obesity monitoring and predictor for T2DM progression (Hall & Cole, 2006; Song et al., 2016). Interestingly, our findings revealed that consumption of RD43 rice for 6 and 12 weeks significantly reduced BMI by 2.20% and 3.03%., respectively. The reduction of BMI in this study is a consequence of weight and total fat mass losses after RD43 rice ingestion. Moreover, our finding supported the study of Zhang *et al*. (2011) that presents a significant reduction in BMI among Chinese prediabetic and diabetic participants who consumed lower GI rice for 16 weeks. Although participants in this study were instructed to maintain physical activity and eating behaviour, several factors have been reported to influence a reduction in body composition, for example, educational level, income, emotion, and oral health (Kiesswetter, Keijser, Volkert, & Visser, 2020; Seppänen-Nuijten et al., 2009; Stookey, Adair, Stevens, & Popkin, 2001; Walther, Philipp, Lozza, & Ehlert, 2017). Thus, it could be suggested that the decreasing in body composition after consuming RD43 rice may be affected by these factors.

Type 2 diabetic and prediabetic patients commonly present abnormal lipid metabolism and dyslipidemia, especially hypertriglyceridemia (Nayak et al., 2018). The previous findings demonstrate the ability of RD43 rice on disrupting cholesterol micellization and binding bile acids which may be beneficial for reduction of cholesterol absorption (Suklaew et al., 2020). Nevertheless, the present study showed no significant differences in lipid profiles after 12 weeks of RD43 rice intervention. The reasons may be due to conducting in participants who had normal lipid profile level concomitant with low dose of resistant starch intake, which resulted in less altering lipid metabolism (Yuan et al., 2018). Besides, consumption of rice may present minor effect on serum lipids when comparing with fat and protein diet (Appel et al., 2005). However, this outcome is consistent with a previous study demonstrating no significant change in lipid profiles in prediabetic subjects after daily consumption of rice containing resistant starch for 4 weeks (Gower et al., 2016; Kwak et al., 2012).

The present study has some limitations such as a small sample size to pick up a statistically significant between group in secondary outcomes and high drop-out rate (32%), mostly due to inconvenient preparation of rice. There are no recorded dietary data to confirm the alteration in body composition. Moreover, some confounding factors such as lifestyle patterns and eating behavior may influence our results. Meanwhile, it reflects the free-living conditions which manifested real outcomes in normal living. Another strength is the observation of the impact of intervention diet after the intervention period, it helps to characterize the remaining effect and confirm the effect from intervention diet. Furthermore, this present study demonstrates the positive effect on glycemic controlling and body composition by consuming white rice containing high undigestible starch content which is the most popular and suitable for practical choice in a real-world setting.

2.8 Conclusion

Substitution of conventional white rice with RD43 rice for 12 weeks presents a potential effect for attenuation of glycemic parameters such as fasting glucose and insulin, HbA1c, and HOMA-IR in participants with prediabetes. It also reduces body weight, BMI, total fat mass, and waist circumference among those population. Since rice is one of important staple food, RD43 rice can be advantage for delaying and preventing the progression of T2DM. However, our findings are required to be confirm by further studies with larger sample sizes and difference population.

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CHAPTER III

PHYSICOCHEMICAL AND FUNCTIONAL CHARACTERISTICS OF RD43 RICE FLOUR AND ITS FOOD APPLICATION

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3.2 Article status

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3.3 Abstract

The increased use of a new rice cultivar is the result of increasing consumer demands for healthier choices. In this study, physicochemical, thermal, pasting and functional properties of flour from RD43 rice, a new rice variety, and its food application were investigated. RD43 rice flour demonstrated an irregular and polyhedral shape with a volume mean diameter of 103 ± 0.15 µm. In addition, the amylose content of RD43 rice and Hom Mali rice flour was 19.04% and 16.04%, respectively. The X-ray diffraction (XRD) and Fourier Transforms Infrared (FTIR) confirmed the presence of V-type crystalline and less crystallinity in RD43 rice flour, which resulted in a significant reduction of water absorption index (WAI), swelling power (SP), water solubility index (WSI), gelatinized temperature, and pasting properties. Comparing with Hom Mali rice flour, RD43 rice flour had greater ability to disrupt cholesterol micellization and bind bile acid. Furthermore, it had lower starch digestibility, with a lower percentage of rapidly digestible starch (RDS) and higher percentage of undigestible starch than Hom Mali rice flour. Moreover, steamed muffin based on RD43 rice flour had lower starch digestibility than Hom Mali steamed muffins. The sensory analysis showed no significant differences between Hom Mali and RD43 steamed muffins. The findings suggest that RD43 rice flour could be an alternative ingredient for lowering the glycemic index of food products.

Keywords: RD43 rice, physicochemical properties, starch digestibility, bile acid binding, cholesterol micellization, muffin

3.4 Introduction

Rice (*Oryzae sativa*, L.), one of the most important crops in the world, is a principal staple food in many regions of the world's human population, especially in Asia (Kaur, Ranawana, & Henry, 2016). Today, there are more than 4,000 identified rice varieties, which differ in their physicochemical and functional properties (Priya, Nelson, Ravichandran, & Antony, 2019). Apart from consumption as cooked granular form, rice flour is popularly used as a raw material for novel foods, because of its bland taste, high digestibility, hypoallergenic, and low price (Koh, Kasapis, Lim, & Foo, 2009). It has been shown that diversity in rice may include certain difference in granular size, amylose, lipid-complexed amylose, gelatinization, starch composition, affecting the quality of rice flour and its products (Cai et al., 2015; Kraithong, Lee, & Rawdkuen, 2018; Thiranusornkij, Thamnarathip, Chandrachai, Kuakpetoon, & Adisakwattana, 2019). Studies on physiochemical and functional properties of rice flour are necessary to indicate market value, consumer preference and food utilization (Falade & Christopher, 2015; Thiranusornkij et al., 2019; Yousif, Nhepera, & Johnson, 2012).

Hom Mali rice or jasmine rice, the most popular variety of any rice globally, has low apparent amylose content, which produces soft and sticky texture after cooking (Vanavichit et al., 2018). In particular, Hom Mali rice flour has been used in the formulation of flour-based products, such as bread and crackers (Jiamjariyatam, 2019; Thiranusornkij et al., 2019). Although Hom Mali rice provides good texture and mild floral aroma, it is considered as a food with high glycemic index (GI) (Thiranusornkij et al., 2019). Consumption of white rice contributes to a relatively large glycemic response associated with exacerbating impaired glucose tolerance. A meta-analysis revealed that over-consumption of white rice, categorized as high GI food, has been strongly associated with the development of non-communicable diseases such as type 2 diabetes (Hu, Pan, Malik, & Sun, 2012). Therefore, several efforts have been made to develop a new rice variety with improvement of physiochemical and functional properties, particularly in reduce the GI. For example, riceberry rice, a cross-bred strain between Hom Nin rice (known as a high-antioxidant rice) and Hom Mali 105 rice, demonstrated a lower starch digestibility and GI than Hom Mali rice because of the presence of anthocyanins and undigestible starch (Thiranusornkij et al., 2019). Recently, a new rice variety, RD43 rice (SPR99007-22-1-2-2-1 registered by the Rice Department, Thailand, 5 October 2010) was developed by a cross-breeding between Khao Jow Hawm Suphan Buri and Supan Buri1. The RD43 rice is non-GMO and approved by the Rice Department of Thailand (Wasusun et al., 2017). Most consumption of RD43 rice is as cooked rice served simultaneously with vegetables, poultry, and other dishes. However, there exists no available novel foods made from RD43 flour, due to limitations in its physicochemical and functional properties. Therefore, the objective of this research was to investigate physicochemical, pasting, thermal, functional properties, and *in vitro* starch digestibility of RD43 rice flour compared to Hom Mali rice flour. The application in steamed muffins made from RD43 rice flour was also evaluated.

3.5 Materials and methods

3.5.1 Materials

RD43 rice (*Oryza sativa* L.) and Hom Mali 105 rice (*Oryza sativa* L.) were obtained from the Rice Department, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. Pepsin from porcine gastric mucosa powder (250 U/mg), α-amylase Type VI-B from porcine pancreas (15.8 U/mg), pancreatin from porcine pancreas (4 × USP specifications), and pure amylose from potatoes, phosphatidylcholine, oleic acid, taurocholic acid, glycodeoxycholic acid, taurodeoxycholic acid, taurocholic acid and porcine cholesterol esterase (35 U/mg) were purchased from Sigma-Aldrich, Inc. (St. Louis, Missouri, United States). Amyloglucosidase (3,260 U/ml) was purchased from Megazyme International Ireland Ltd. (Bray, Ireland). Glucose oxidase kit (Glucose liquicolor) and Cholesterol test kits (Cholesterol liquicolor) were purchased from Human (Human diagnostics, Wiesbaden, Germany). Total bile acid kit was purchased from BIOBASE (Jinan, Shandong, China).

3.5.2 Flour preparation

RD43 and Hom Mali rice was grounded by using Pin Mill (Phoenix Equipment Corporation, United States) with a voltage of 50 Hz and a speed of 4800 rpm. Then, the ground rice was passed through 150 μm for removing large particles. The flour was stored in sealed plastic bags at room temperature until further analysis. To determine moisture content, each flour (5 g) was heated at 105°C for 10 min. Moisture content was measured using the infrared moisture determination balances (KETT FD-610, Japan). The moisture content of the RD43 and Hom Mali rice flours were 11.5% and 11.0%, respectively. Total dietary fiber was carried out using the AOAC method (AOAC, 2003). The total dietary fiber of RD43 and Hom Mali rice flour were 1.1% and 1.07%, respectively.

3.5.3 Scanning electron microscopy

Granular morphology of rice flour was observed using a scanning electron microscope (SEM) (JEOL, JSM-6400, Tokyo, Japan). The sample was mounted on an aluminium specimen holder using double-sided scotch tape and coated with gold at an accelerating voltage of 10 kV. The microstructure of the flour particles was scanned and photographed at various magnifications (100×, and 1500×).

3.5.4 Particle size distributions analysis

The particle size distributions (PSDs) of flour were analyzed using a Laser particle size analyzer (Mastersizer 3000, Malvern Instruments Ltd., Worcestershire, England) with dry dispersion. The PSD was calculated using the Mastersizer 3000 software and reported as volume mean diameter ($D_{4,3}$), average particle size (D_{50}), equivalent diameters at 10% and 90% cumulative volume (D_{10} and D_{90}). Three replicates were performed for each analysis.

3.5.5 Color

The color profile of flour was determined using a colorimeter (Color flex, Hunter Associates laboratory, Inc, United States). Before measurements, a standard black glass and white tile were used for instrument (45°/0° geometry, 10° observer) calibration.

The *L**, *a** and *b** parameters represent lightness, redness, and yellowness of flour. The measurement was performed in triplicates.

3.5.6 Determination of amylose content

The amylose content in rice flour was determined according to the previous study (Thiranusornkij et al., 2019). In brief, 0.1 g of rice flour was mixed with 1 ml of 95% ethanol and 9 ml of 1 N NaOH, and allowed to stand at room temperature for 10 min. The mixture was heated at 100°C in a water bath for 10 min and cooled at room temperature for 2 h. The mixture was made up to 100 ml with distilled water (DW) and vigorously vortex. A separating mixture (5 ml) was incubated with 2 ml of 1 N acetic acid, 50 ml of DW and 2 ml of iodine solution (2.0 g potassium iodide and 0.2 g iodine in 100 mL of aqueous solution) before adjusting final volume to 100 ml. The absorbance was recorded at 620 nm after standing for 20 min at room temperature. A calibration curve was derived using a set of pure amylose from potato.

3.5.7 X-ray diffraction analysis

X-ray diffraction patterns of rice flour were performed with X-Ray Diffractometer (Bruker AXS Model D8 Discover, Germany) equipped with a copper tube operating at 40kV, 45 mA, and the spectra scanned over a diffraction angle (2ϴ) range of 5-40° at a rate of 0.02° 2ϴ/second. The percentage of crystallinity was calculated using this equation (Cheetham & Tao, 1998):

$$\%\text{Relative crystallinity} = \frac{\textbf{Area above the smooth curve}}{\textbf{Total diffraction area above the baseline}} \times 100$$

3.5.8 Fourier transforms infrared (FT-IR) spectroscopy

The FT-IR of the rice flour was determined according to the previous study (Falade & Christopher, 2015). The rice flour samples (0.1 g, dry basis) were mixed with anhydrous KBr (0.1 g) and pressed for 20 min to obtain a transparent pellet. The pallet was transferred into the instrument and scanned in the absorption area of 450 to 4000 cm^{-1} by using a FT-IR spectrometer (Spectrum one, PerkinElmer Life and Analytical Sciences, Shelton, United States).

3.5.9 Water absorption index (WAI), swelling power (SP), and water solubility index (WSI)

Hydration properties of rice flour was determined following the previous method (De la hera, Gomez, & Rosell, 2013). The initial sample (W_i) (0.05 g) was added into 1 ml of DW, then the mixture was heated at 90°C for 10 min in the heat block. After that, the sample was cooled in ice water bath for 10 min and centrifuged at 3000g, 4°C for 10 min. The supernatant was separated and evaporated at 105°C until constant weight (W_s). The residue (W_r) was weighed. The results were calculated according the equation below:

$$\text{WAI (g/g sample)} = \frac{W_r}{W_i}$$

$$\text{SP (g/g sample)} = \frac{W_r}{W_i - W_s}$$

$$\text{WSI (g/100g sample)} = \frac{W_s}{W_i} \times 100$$

3.5.10 Thermal properties

Thermal properties of rice flour were determined using differential scanning calorimeter (DSC) (Netzsch DSC 204F1 Phoenix, Germany). An empty aluminium pan was used as the reference. The rice flour sample (3 mg, dry basis) was mixed with 10 μl of deionized distilled water and put into the sample pan. Then, the pan was sealed and allowed to stand at room temperature for 1 h before heating from 25°C to 100°C with a heating rate of 10°C/min. The DSC parameters, including onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH) were recorded.

3.5.11 Pasting properties

The pasting properties of rice flour samples were determined using a Rapid Visco Analyser (RVA 4500 Newport Scientific, Minnesota, United States). In brief, the sample (3 g) was suspended in 25 g DW. Then, the suspension was continuously heated from room temperature to 95°C at rate of 12°C/min. After that, the sample was held at 95°C for 2-3 min and finally cooled to 50°C at 12°C/min rate. Pasting temperature, peak time, peak viscosity, trough, breakdown, final viscosity, and set back were obtained from the pasting curves.

3.5.12 *In vitro* starch digestion of flour and steamed muffin

Starch digestibility of rice flour were performed according to a previous method with minor modifications (Yousif et al., 2012). In brief, 0.5 g of rice flour was boiled with 6 ml of 0.2 sodium acetate buffer, pH 6 for 15 min. Then, cooked rice flour was equilibrated at 37°C for 15 min in a shaker water bath (100 rpm/min). In the oral phase, the cooked rice flour or 0.5 g or steamed muffin were incubated with 1 ml of α-amylase (250U/ml in 0.2 M carbonate buffer, pH 7) for 15-20 s. After that, the sample was mixed with 5 ml of porcine pepsin solution (3200 U/ml in 0.02 N HCl, pH 2) and incubated for 1 h. Thereafter, gastric digesta was neutralized with 5 ml of 0.02 N NaOH and 25 ml of 0.2 M sodium acetate buffer, pH 6. Then, 5 ml of pancreatin (2 mg/ml) and amyloglucosidase (28 U/ml in 0.2 M sodium acetate buffer, pH 6) was added to the digesta at 37°C in a shaking water bath. The sample was aliquoted at 0, 20, 30, 60, 90,120, and 180 min and immediately heated at 105°C for 10 min. After centrifuged at 11000 rpm for 15 min, the glucose release was determined using the glucose oxidase kit. The results were expressed as mg glucose/100 g rice flour. The incremental area under the curve (iAUC) was calculated using the trapezoidal rule. The percentage of starch fraction in the sample was reported as rapidly digestible starch (RDS: 0-20 min digestion), slow digestible starch (SDS: 20-120 min digestion) and undigestible starch (120-180 min digestion) (Englyst, Kingman, & Cummings, 1992). Total starch was determined using a previous method and factor conversion from glucose to starch was 0.9 (Goñi, Garcia-Alonso, & Saura-Calixto, 1997).

3.5.13 Bile acid binding

The bile acid binding assay was determined according to the previous method (Mäkynen et al., 2013) The bile acid used in this experiment was glycodeoxycholic acid, taurodeoxycholic acid, and taurocholic acid. The rice flour (1 mg/ml) was incubated with each bile acid (2 mM) in 0.1 M phosphate buffered saline (PBS), pH 7, at 37°C for 90 min. The mixtures were filtered the through 0.2-mm filter for separating the bound from the free bile acid. The bile acid concentration was analyzed using bile acid analysis kit. The absorbance was measured at 540 nm. The results were reported as the percentage bile acid binding

3.5.14 Cholesterol micellization

The inhibition of cholesterol micellization formation was performed according to a previously method (Mäkynen et al., 2013). The mixture (2 mM cholesterol, 2.4 mM phosphatidylcholine and 1 mM oleic acid) was dissolved in methanol and dried under nitrogen before addition of 15 mM PBS containing 6.6 mM taurocholate salt, pH 7.4. The emulsion was sonicated for 45 min using a sonicator and incubated overnight at 37°C. The rice flour (10 mg/ml) and equivalent PBS using as control were added to the mixed micelle solution and incubated at 37°C for 2 h. The mixture was centrifuged at 16000 rpm for 20 min. The cholesterol in the mixture was determined using the total cholesterol test kit. The results were calculated as percentage inhibition.

3.5.15 Steamed muffin preparation

Rice flour and other ingredients as % on wet flour basis, including sugar (41%), yeast (0.6%), and water (94%) were mixed for 5 min and left for 90 min. Based on rice flour, baking powder (2.5%) was added and then mixed together for 2 min. After that, the muffin batter was poured into moulds and steamed them over boiling water for

15 minutes. Then, muffins were cooled at the room temperature and removed from the moulds.

3.5.16 Sensory evaluation of steamed muffin

The sensory evaluation of the steamed muffins was performed using untrained panelists to evaluate the acceptability for the two steamed muffins (Curtis, 2013). The 30 panelists, who had ever consumed steamed muffin, were recruited around Chulalongkorn University (23 females and 7 males, average age 39.09 ± 2.28 and 48.86 ± 3.74 years old, respectively). The steamed muffin was freshly prepared on the testing day. After cooling 10 min, all samples were served in a plastic box that presented three-digit numbers for identification. On the test day, the panelists were seated in individual sensory booths and asked to rinse the oral cavity with water before and between testing of samples. The analysis was performed in single replication (each subject evaluated each sample twice) as recommend by a previous report (Curtis, 2013). The sensory attributes were appearance, color, odor, taste, texture, hardness, and overall acceptance by using nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

3.5.17 Statistical Analysis

The results are reported as mean ± SEM. The mean comparison was carried out by independent *t*-test. The significance of difference was considered at $p < 0.01$. The analysis was performed by using an SPSS package (SPSS 21.0 for window, SPSS Inc, Chicago, Illinois).

3.6 Results and discussion

3.6.1 Morphology observation

The photograph and scanning electron micrographs (SEM) of RD43 and Hom Mali rice flour are demonstrated in Figure 3. The irregular and polyhedral shape were observed in both RD43 and Hom Mali rice flour. It has been shown that rice starches in tropical climates usually present polyhedral, round or irregular shapes (Wani et al., 2013). In addition, they presented the aggregation of starch granules into particle with the smooth cutting surface. The obtained results of this study are consistent with the previous study following the same method of flour preparation (Thiranusornkij et al., 2019). This milling process could produce the large aggregation of flour in the presence of protein and other substances on the surface of starch granule (Leewatchararongjaroen & Anuntagool, 2016).

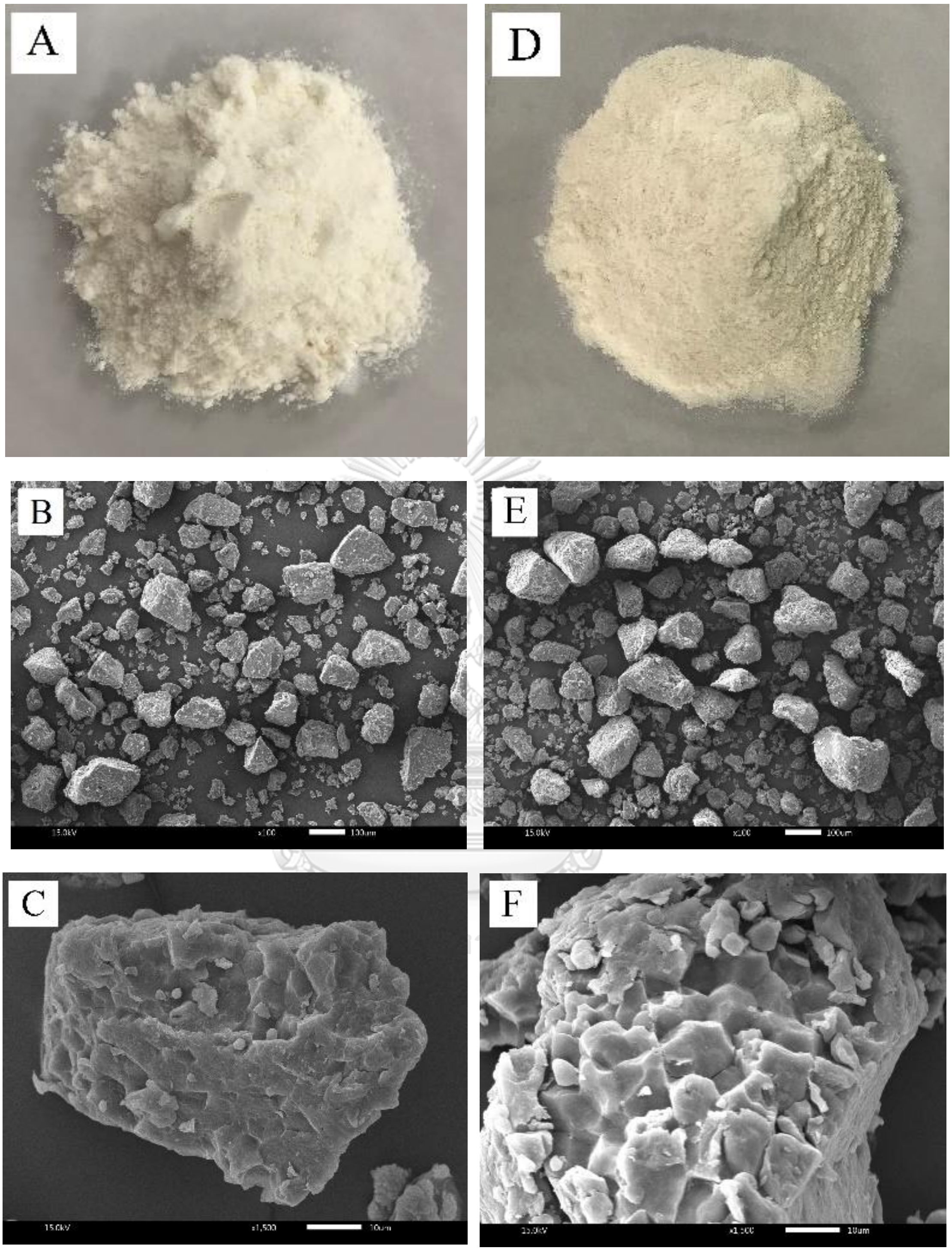


Figure 3 Photographs and morphology of rice flour. Photographs of Hom Mali (A) and RD43 rice flour (D). Scanning electron micrographs of Hom Mali flour, magnified 100x (B), and 1500x (C), and RD43 rice flour, magnified 100x (E), and 1500x (F).

3.6.2 Particle size distribution

The curve of particle size distribution of rice flour is presented in Figure 4. Hom Mali rice flour represented one peak ranging from 12.7-352.0 mm, while two peaks ranging from 4.03-9.86 μm and 11.2-400 μm were detected from RD43 rice flour. This result is consistent with a previous study showing two different particle diameters of riceberry rice obtained from the same milling equipment (Thiranusornkij et al., 2019). The first peak may be due to the damaged starch granule remnant (Leewatchararongjaroen & Anuntagool, 2016), whereas the second peak demonstrates the aggregation as indicating by SEM images. As shown in Table 5, the value of $D_{4,3}$, D_{10}, and D_{50} of RD43 rice flour was significantly lower than that of Hom Mali rice flour, suggesting that RD43 rice flour had smaller average particle size. The finer particle size of flour resulted from less grain strength (Keeratipibul, Luangsakul, & Lertsatchayarn, 2008). Previous study suggested that the small particle size of flour might produce fine and massive textures of finished products (Kraithong et al., 2018).

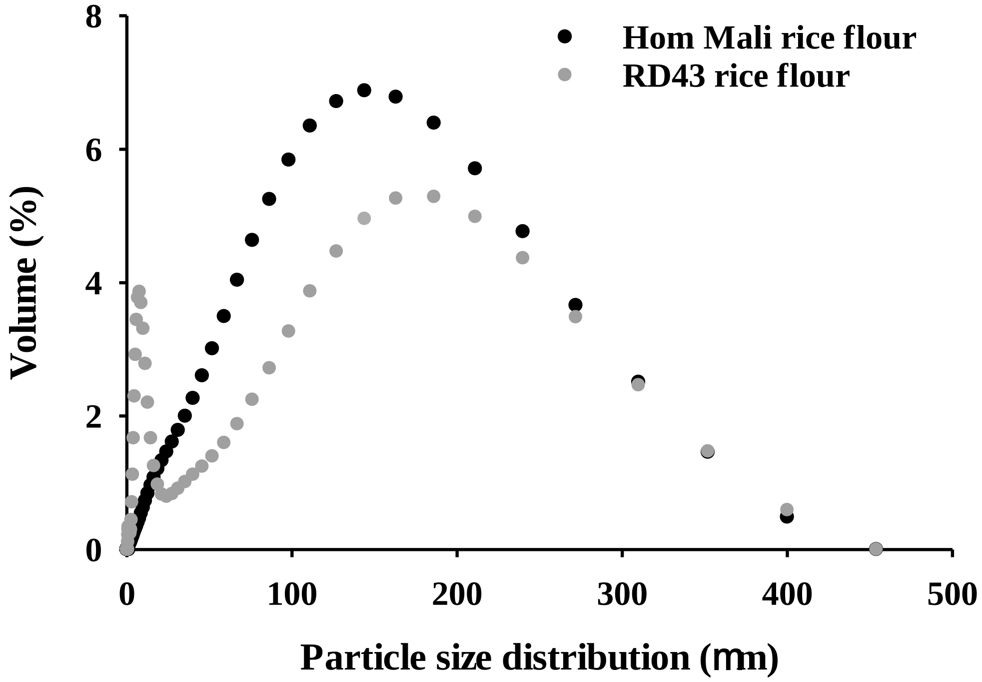


Figure 4 The particle size distribution by volume of rice flour.

3.6.3 Color of rice flour

As shown in Table 5, RD43 rice flour had significantly lower lightness (*L**) and higher redness (*a**) and yellowness (*b**) when compared to Hom Mali rice flour. The rice flour with a greater degree of yellowness and less intensity in lightness might result from different rice cultivars, storage time, and the exposure to high temperatures during milling process (Chrastil, 1990; Kraithong et al., 2018). In this study, the rice flour was prepared and kept in the same condition. This suggests that the difference in color values is most likely attributed to the rice cultivar.

3.6.4 The amylose content of rice flour

As demonstrated in Table 5, the amylose content of Hom Mali and RD43 rice flour was 16.05% ± 0.39% and 19.04% ± 0.30%, respectively, suggesting that they were classified as low amylose (12-20%) according to categorization of rice based on the amylose content (Juliano, 1992). Nevertheless, RD43 rice flour presented higher amylose content than Hom Mali rice flour. The variation of amylose content depends on cultivars and botanical source (Kraithong et al., 2018). The different content of amylose directly affects gelatinization, pasting behaviour, swelling power, crystallinity, and starch digestibility in relation to the content of resistant starch (RS) (Cai et al., 2015; Chung, Liu, Lee, & Wei, 2011). In food manufacturing, rice flour containing low amylose promotes an increase in moistness, chewiness, and softness texture (Falade & Christopher, 2015), whereas rice flour containing high amylose rice contributes to increase firmness and crispness in food product (Wang et al., 2016).

Table 5 The particle size distribution, color profile, and amylose content, as well as hydration, thermal and pasting properties of Hom Mali and RD43 rice flour

Physicochemical properties	Rice flour	
	Hom Mali	RD43
Particle size distribution (μm)		
$D_{4,3}$	130.00 ± 0.58	103.00 ± 0.15*
D_{10}	23.33 ± 0.13	5.41 ± 0.33*
D_{50}	115.67 ± 0.33	73.07 ± 0.17*
D_{90}	259.67 ± 1.73	257.00 ± 2.52
Color		
Lightness (L^*)	91.09 ± 0.06	89.4 ± 0.02*
Redness (a^*)	0.01 ± 0.02	0.16 ± 0.02*
Yellowness (b^*)	5.38 ± 0.07	6.29 ± 0.07*
Amylose content (%)^a	16.05 ± 0.39	19.04 ± 0.30*
Hydration properties^a		
WSI (g/g sample)	9.33 ± 0.13	7.33 ± 0.13*
WAI (g/g sample)	13.06 ± 0.13	12.41 ± 0.07*
SP (g/100g sample)	13.68 ± 0.11	12.35 ± 0.16*
Thermal properties		
T_o (°C)	65.63 ± 0.07	65.07 ± 0.26
T_p (°C)	71.20 ± 0.15	70.23 ± 0.22*
T_c (°C)	76.6 ± 0.10	75.20 ± 0.31*
ΔH (J/g)	7.85 ± 0.28	6.89 ± 0.31
Pasting properties		
Peak viscosity (RVU)	310.38 ± 3.71	223.50 ± 0.35*
Trough (RVU)	173.33 ± 0.94	156.17 ± 0.94*
Breakdown (RVU)	137.04 ± 2.77	67.33 ± 1.30*
Final viscosity (RVU)	302.71 ± 2.77	342.08 ± 3.30*
Setback (RVU)	129.38 ± 1.38	185.92 ± 2.36*
Peak time (min)	5.43 ± 0.05	5.70 ± 0.05*
Pasting temperature (°C)	84.05 ± 0.07	89.73 ± 0.04*
Total starch (%)	81.43 ± 0.58	80.72 ± 0.34

Data are expressed as mean ± SEM, $n = 3$. * $p < 0.01$ compared to Hom Mali rice flour. ^{a} Data are based on dry basis. L^*: lightness; a^* redness; b^*: yellowness; WSI: water insolubility index; WAI: water absorption index; SP: swelling power; T_o: onset temperature; T_p: peak temperature; T_c: conclusion temperature; ΔH: enthalpy gelatinization.

3.6.5 X-ray diffraction

As presented in Figure 5A and B, Hom Mali and RD43 rice flour presented diffraction peaks at 2θ of 15° and 23°, while they exhibited unresolved peaks at 17° and 18°. This peak pattern is the A-type crystalline structure containing densely packed forms of double helices in a monoclinic unit cell is more stable conformationally than other crystalline structures (Kraithong et al., 2018). Interestingly, the apparent diffraction peak at 20° was only observed in RD43 rice flour (Figure 5B). This peak indicates the V-type crystalline structure in relation to the existence of amylose-lipid complex in the rice flour (Ye et al., 2018). According to the calculation of the X-ray diffraction pattern, the degree of crystallinity in RD43 rice flour (24.55%) was significantly lower than observed in Hom Mali rice flour (26.80%). This result suggests that the lower degree of relative crystallinity in RD43 rice flour results from the higher presence of amorphous area (Colussi et al., 2014).

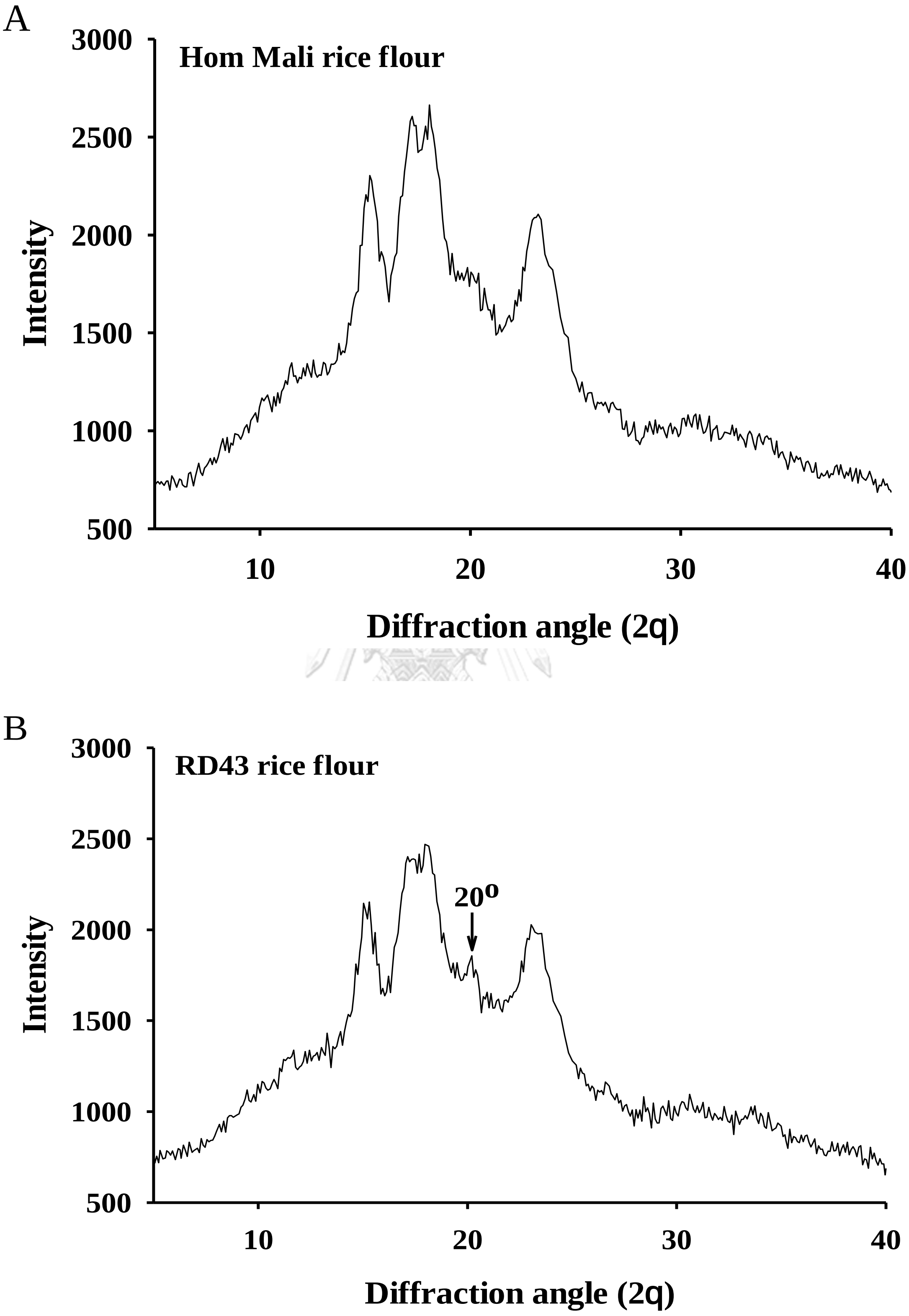
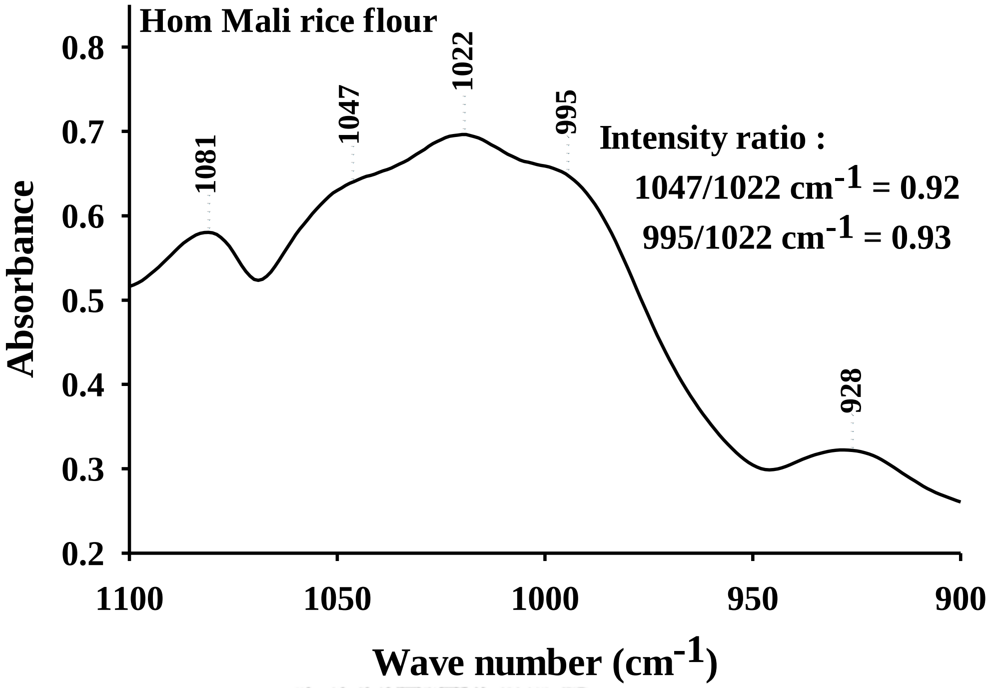


Figure 5 X-ray diffraction pattern of rice flour; Hom Mali (A) and RD43 rice flour (B).

3.6.6 FTIR Spectroscopy

The deconvoluted FT-IR spectra of Hom Mali rice and RD43 rice flour are illustrated in Figure 6A and B. The absorption bands in the region 1100-900 cm^{-1} represent the sensitive change in the starch structure. The observed peaks at 1081 and 1020 cm^{-1} indicate stretching of the anhydroglucose C–O ring, which related to polysaccharide molecules (Fang, Fowler, Tomkinson, & Hill, 2002). In addition, the peak at 928 cm^{-1} was assigned to polysaccharides with α-1,4 glycosidic linkage (Ratnaningsih, Harmayani, & Marsono, 2020). Several studies have reported that observed absorption bands at 1047 and 995 cm^{-1} are the crystalline of starch, whereas the band at 1022 cm^{-1} is characterized as an amorphous region of starch (Cai et al., 2015; Castillo, López, García, Barbosa, & Villar, 2019). In particular, the intensity ratio of 1047/1022 and 995/1022 cm^{-1} could refer the association between amount crystallinity and amorphous structure (Castillo et al., 2019; Ren, Ma, Xu, & Hu, 2020). Comparing with Hom Mali rice flour, RD43 rice flour expressed the lower intensity ratio of 995/1022 and 1047/1022 cm^{-1}, indicating the lower degree of double helices and ordered structure (Ren et al., 2020). The lower intensity ratio of 1047/1022 cm^{-1} especially is interpreted as a high amount of amylose with low amylopectin content (Cai et al., 2015). This result suggests that RD43 rice flour has lower degree of crystallinity than Hom Mali rice flour.

A



B

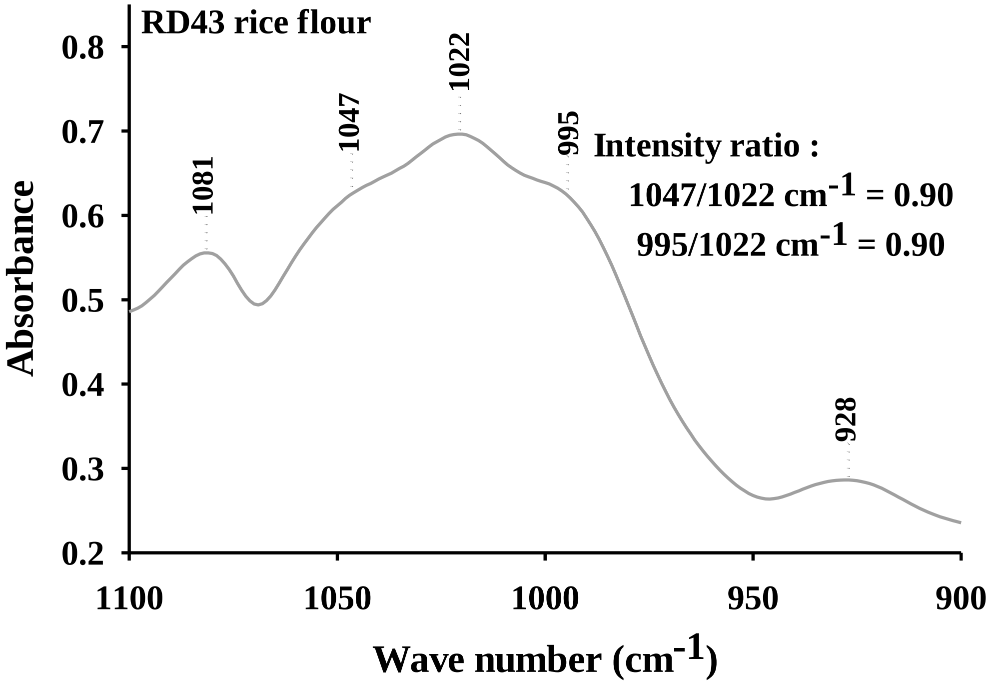


Figure 6 Fourier transform infrared (FTIR) spectra of rice flour; Hom Mali (A) and RD43 rice flour (B).

3.6.7 Hydration properties of rice flour

The WSI represents the ability of component to dissolve with water under an excess water condition. As demonstrated in Table 5, the lower WSI was obtained from RD43 rice flour, as compared to Hom Mali rice flour. The low WSI of RD43 rice flour may facilitate firm and dense internal structure of food products (Awuchi, Igwe, & Echeta, 2019). The WAI and SP represent the interactions between the water molecules and the starch chains in the crystalline and amorphous regions. In this study, RD43 rice flour was lower WAI and SP than Hom Mali rice flour (Table 5). Previously, the significant difference in WAI and SP was detected in various rice cultivars (Kraithong et al., 2018; Thiranusornkij et al., 2019). In addition, the WAI and SP demonstrated a negative correlation with the amylose content (Cai et al., 2015; Nakorn, Tongdang, & Sirivongpaisal, 2009). The lower SP and WAI of RD43 rice flour may be attributed to the higher amylose content.

3.6.8 Thermal properties of rice flour

As shown in Table 5, RD43 rice flour was significantly lower peak temperature (T_p) and conclusion temperature (T_c) than Hom Mali rice flour. There was no significant difference in onset gelatinization temperature (T_o) and gelatinization enthalpy (ΔH) between RD43 rice flour and Hom Mali rice flour. The thermal properties are normally affected by the chemical composition, the origin of starch, moisture, the presence of other biomaterials, processing and pretreatment conditions (Wijaya et al., 2019). Previous studies revealed positive correlation between T_p and T_c and crystallinity in rice flour (Kraithong et al., 2018). The greater degree of crystallinity causes an increase in transition temperature due to more resistance and stability of the granule structure to gelatinization (Alcázar-Alay & Meireles, 2015). Therefore, the lower transition temperature values of RD43 rice flour may be related to the presence of less degree of crystallinity.

3.6.9 Pasting properties of rice flour

The pasting curves of RD43 rice and Hom Mali rice flour are presented in Figure 7. When compared to Hom Mali rice flour, RD43 rice flour showed a lower value for peak, trough, and breakdown viscosity, whereas it exhibited higher value of peak time, pasting temperature, setback, and final viscosity (Table 5). These results suggest that RD43 rice flour had lower capacity to form a gel (low peak viscosity), which arises from a greater resistance to heating and shear stress (low breakdown) leading to highly stable form during cooking. RD43 rice flour also requires a higher temperature and longer time for the pasting process (high pasting temperature and peak time) than Hom Mali rice flour. In the cooling stage, RD43 rice flour had higher stability for the cooked paste (high final viscosity) and tendency toward retrogradation (high setback) than Hom Mali rice flour. The exhibition of larger final viscosity and setback value may be responsible for a huge tendency for retrogradation in food products during storage (Thiranusornkij et al., 2019), whereas the high-setback rice flour provides more food structure. This rice flour property is appropriately recommended for fried snacks, stick rice noodles, and pasta noodles (Van Hung, Maeda, Miskelly, Tsumori, & Morita, 2008).

The results from pasting properties indicate that RD43 rice flour may be more enhance the structure of food products than Hom Mali rice flour.

The distinct pasting behavior of rice flour is influenced by cultivars, resulting in variation of the undigestible starch and amylose content (Kraithong et al., 2018; Ye, Wang, Wang, Zhou, & Liu, 2016). Higher undigested starch content in rice flour may involve a compact linear structure that responsible for high setback and final viscosity (Yang et al., 2006). Moreover, the presence of amylose leads to decrease negative charges of the phosphate group in an amylopectin structure and binding of the hydrogen bond in water, which inhibits the swelling of starch granules (Wang et al., 2016). Ye *et al.* (2016) described that rice flour containing high amylose content contributes to increased pasting temperature, setback, and final viscosity, due to the flour's lower swelling ability and molecule rearrangement. Also, the amylose–lipid complex may influence pasting properties by increasing the hydrophobicity of starch granules, resulting in decrease in granule swelling and amylose leaching (Alcázar-Alay & Meireles, 2015). Consequently, this interaction causes a reduction of peak and breakdown viscosity and an increase in setback and final viscosity (Alcázar-Alay & Meireles, 2015; Van Hung et al., 2008). In addition, storage time, temperature during milling process, and environment are other factors on different pasting properties of the rice flour (Dang & Copeland, 2004; Huang & Lai, 2014; Saleh & Meullenet, 2015).

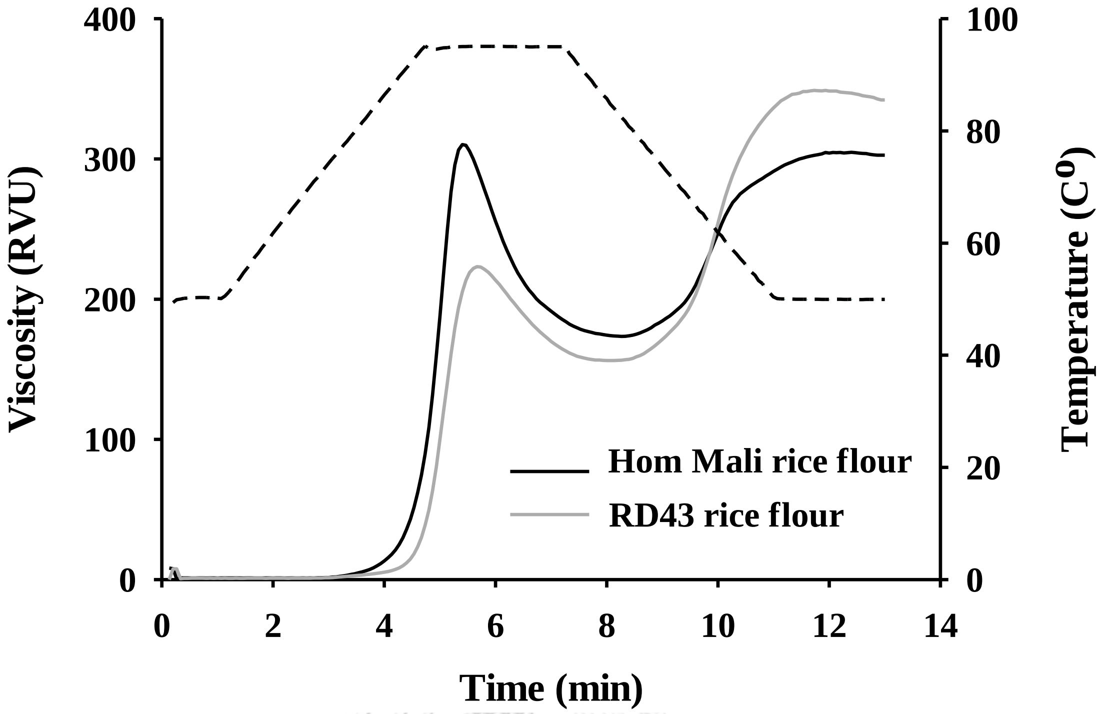


Figure 7 Pasting properties pattern of Hom Mali and RD43 rice flour.

3.6.10 *In vitro* starch digestion

The starch digestibility of RD43 and Hom Mali rice flour is demonstrated in Figure 8A. The release of glucose was observed from all samples over 20 min, and it gradually increased after 30 min of digestion. The results showed that RD43 rice flour exhibited lower glucose release and iAUC than Hom Mali rice flour throughout 180 min (Figure 8B). Moreover, RD43 rice flour presented a significantly lower percentage of RDS and higher percentage of undigestible starch than Hom Mali rice flour (Figure 8C). As shown in Figure 8D, both rice flours had a similar percentage of SDS, total starch (Table 5), and dietary fiber. It has been shown that the consumption of diets containing a low percentage of RDS and the high content of undigestible starch causes a reduction of postprandial blood glucose (Gourineni, Stewart, Skorge, & Sekula, 2017). The present findings can be explained by the presence of amylose and amylose–lipid complex content in RD43 rice flour (Ye et al., 2018). A rigid structure of amylose causes less accessibility to enzymatic action, such as α-amylase and amyloglucosidase (Chung et al., 2011), which provides a significant positive impact towards controlling postprandial blood glucose (Panlasigui & Thompson, 2006).

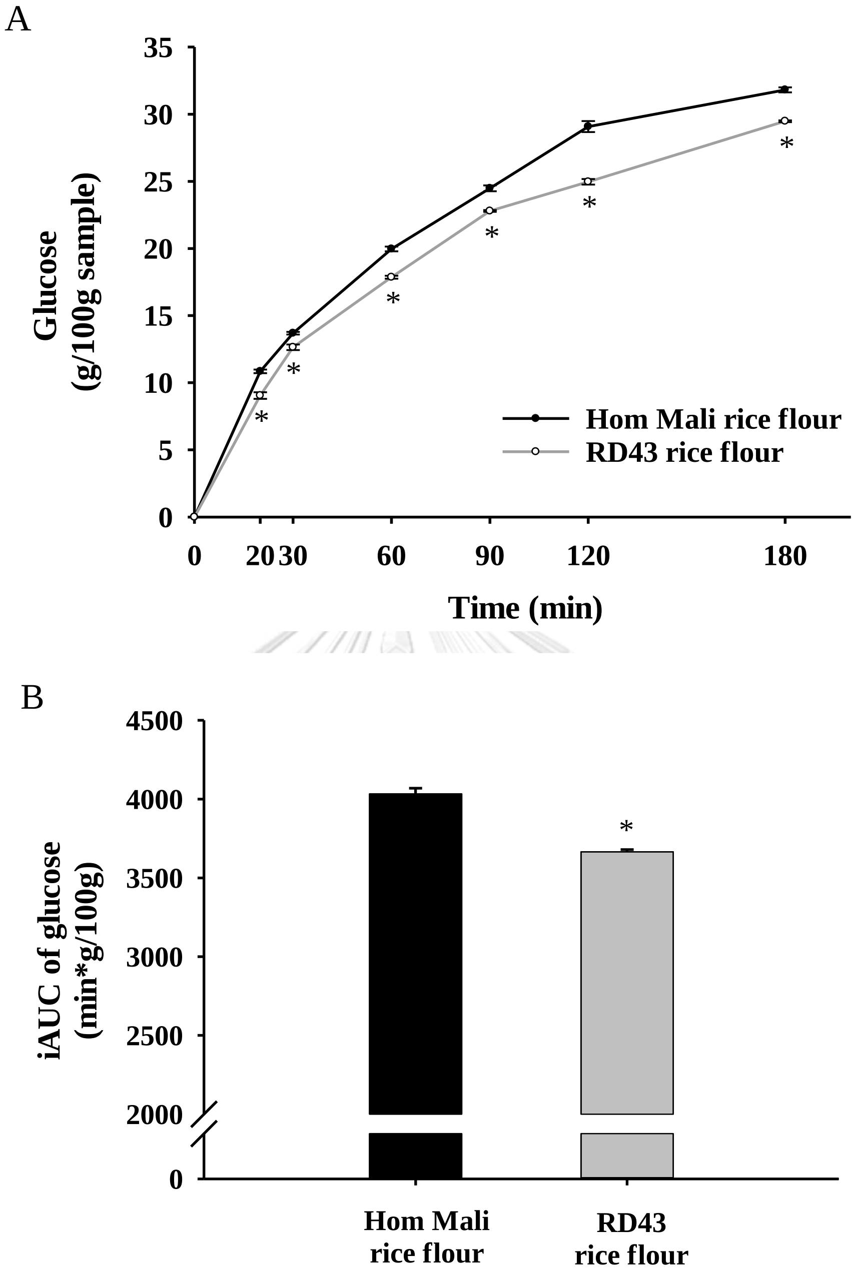


Figure 8 The *in vitro* starch digestion of rice flour. The glucose release during *in vitro* starch digestion from rice flour (A), incremental area under the curve (iAUC) of glucose (B), starch fraction (C), and total starch (D) of Hom Mali and RD43 rice flours. Data are expressed as mean ± SEM, *n* = 3. * $p < 0.01$ compared to Hom Mali rice flour. RDS: rapidly digestible starch; SDS: slowly digestible starch.

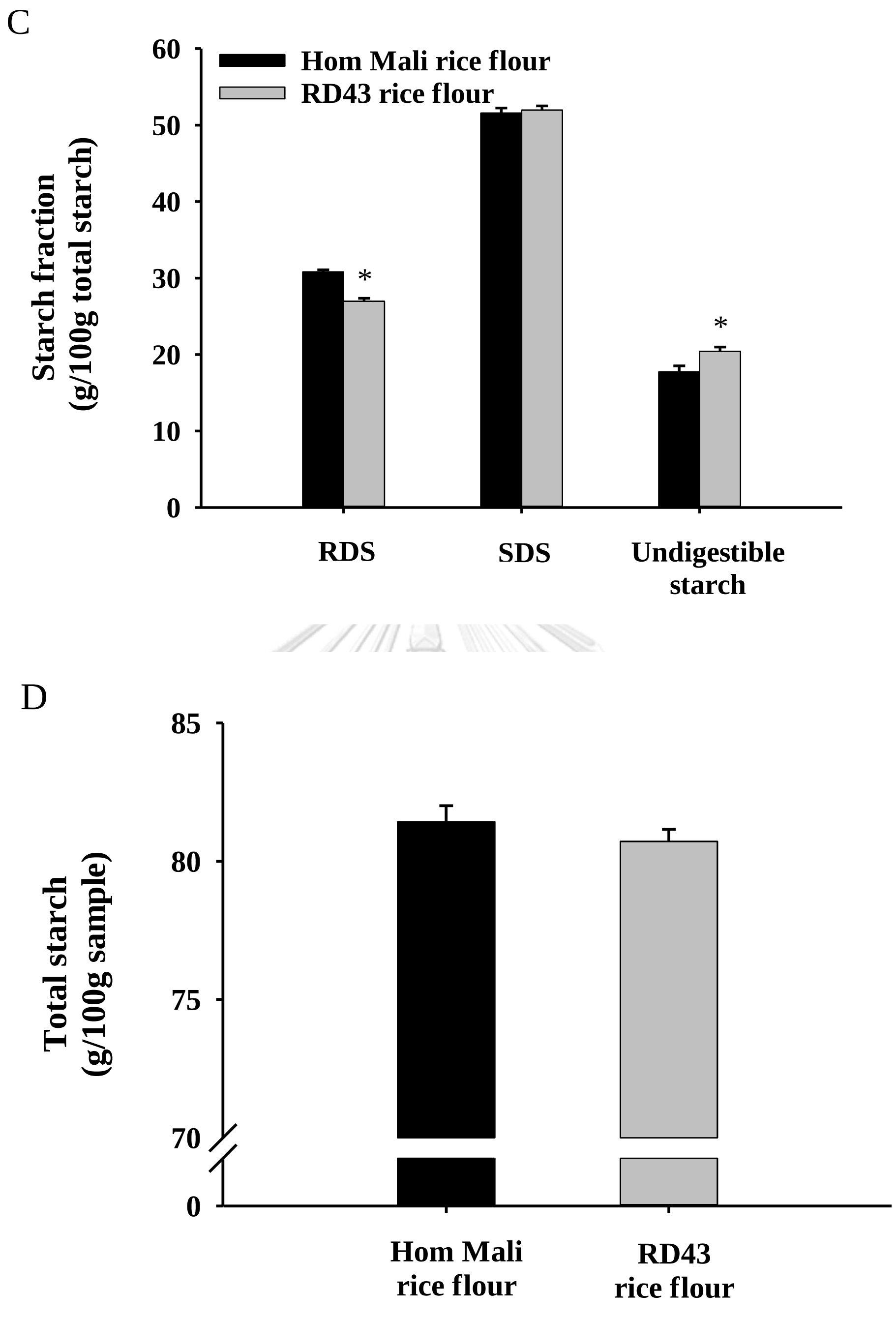


Figure 8. The *in vitro* starch digestion of rice flour. The glucose release during *in vitro* starch digestion from rice flour (A), incremental area under the curve (iAUC) of glucose (B), starch fraction (C), and total starch (D) of Hom Mali and RD43 rice flours. Data are expressed as mean ± SEM, n = 3. * $p < 0.01$ compared to Hom Mali rice flour. RDS: rapidly digestible starch; SDS: slowly digestible starch.

3.6.11 Bile acid binding and cholesterol micellization

The percentage of bile acid binding and the inhibition of cholesterol micellization of RD43 rice and Hom Mali rice flour are demonstrated in Table 6. The RD43 rice flour had greater ability to bind bile acids (taurocholic acid, taurodeoxycholic acid, and glycodeoxycholic acid). In addition, RD43 rice flour had greater ability to disrupt the formation of cholesterol micelles than Hom Mali rice flour. Due to the similar dietary fiber content, the inhibitory action of RD43 rice flour may be attributed to the existence of greater amylose content. Previous studies found that the helical structure of amylose could form complexes with bile salts and cholesterol (Takahama & Hirota, 2011; Villwock, Eliasson, Silverio, & BeMiller, 1999). It has been shown that the bile acid binding property and the disruption of cholesterol micellization is an attractive target for reducing cholesterol absorption into the small intestine (Mäkynen et al., 2013). It suggests that RD43 rice flour may help reduce the amount of cholesterol in blood circulation. Further research is warranted to resolve this issue in human studies.

Table 6 Effects of Hom Mali and RD43 rice flour on bile acid binding and the inhibition of cholesterol micellization.

Rice Flour	% Bile Acid Binding			% Cholesterol Micellization Inhibition
	Taurocholic acid	Taurodeoxycholic acid	Glycodeoxycholic acid	
Hom Mali	2.51 ± 0.23	NA	NA	9.22 ± 0.48
RD43	7.45 ± 0.36*	36.35 ± 0.19*	11.61 ± 0.12*	12.95 ± 0.52*

Data are expressed as mean ± SEM, $n = 3$. * $p < 0.01$ compared to Hom Mali rice flour. NA: no activity.

3.6.12 Starch digestibility and sensory evaluation of steamed muffins

The photograph of steamed muffins made from Hom Mali and RD43 rice flour are presented in Figure 9. Compared to the Hom Mali steamed muffin, the RD43 steamed muffin had significantly lower glucose release at 20, 90, and 120 min (Figure 10A). In addition, the iAUC of the RD43 steamed muffin was significantly lower than that of Hom Mali steamed muffin (Figure 10B). This indicates that the RD43 steamed muffin exhibited a greater ability to slow down starch digestibility than the Hom Mali steamed muffin. Our findings were consistent with previous studies that bread made from riceberry rice flour had lower glucose release than Hom Mali bread (Thiranusornkij et al.,2019). We suggest that RD43 flour may be a promising ingredient for reducing the glycemic index (GI) of food products, which helps to control postprandial blood glucose (Gourineni et al, 2017). The sensory evaluation of steamed muffin made from Hom Mali and RD43 rice is shown in Figure 11. Sensory parameters of the steamed muffins included appearance, color, odor, texture, hardness, taste, and overall acceptability. The findings present that sensory score of the steamed muffins made from RD43 and Hom Mali rice showed no significant difference in any evaluated attributes.

Figure 9 Photograph of rice flour steamed muffin (left: Hom Mali steamed muffin, right: RD43 steamed muffin).

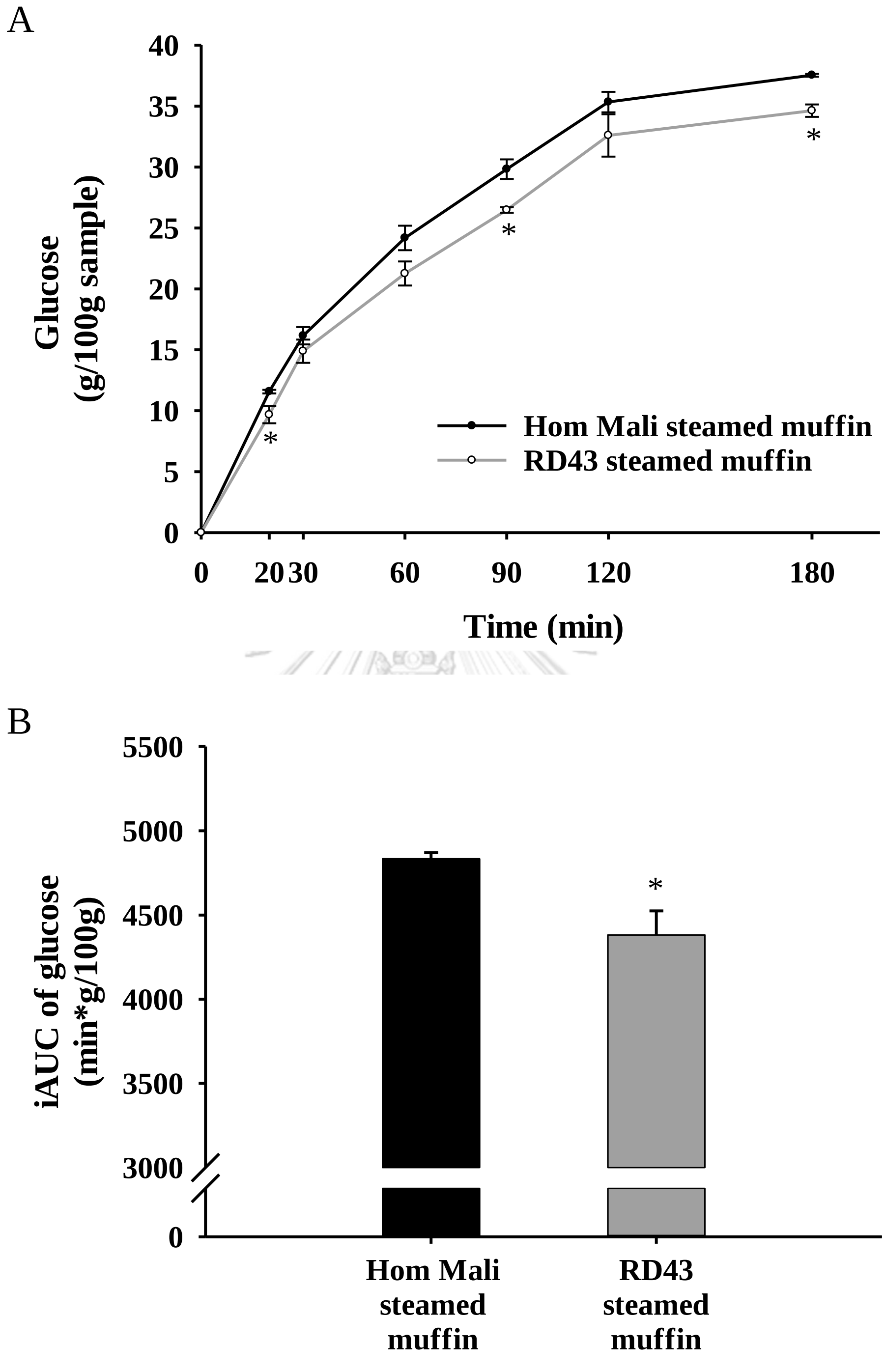


Figure 10 The *in vitro* starch digestion of rice flour muffins. The glucose release during *in vitro* starch digestion from rice flour muffins (A) and incremental area under the curve (iAUC) of glucose (B). Data are expressed as mean ± SEM, n = 3. * p < 0.01 compared to Hom Mali steamed muffin.

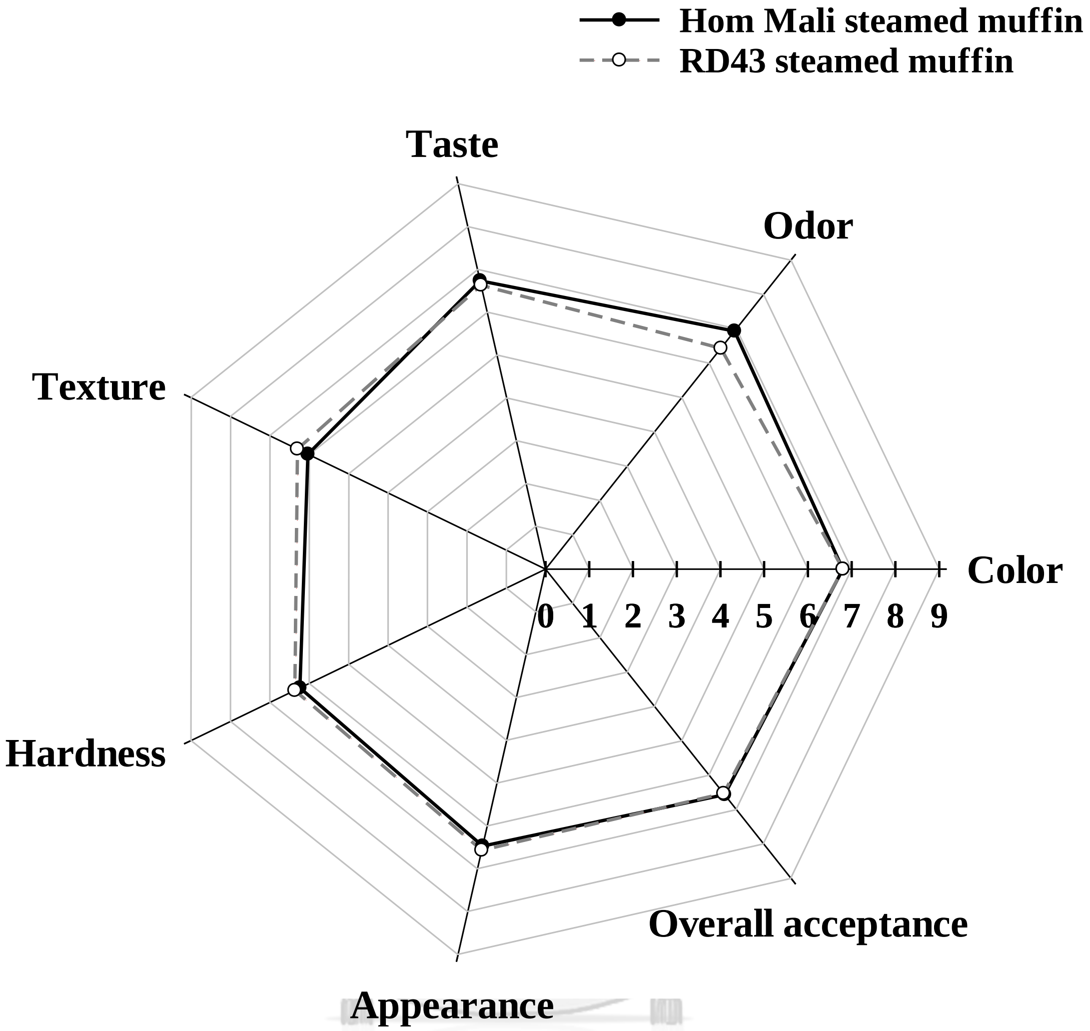


Figure 11 Sensory analysis of Hom Mali and RD43 rice steamed muffin. Data are expressed as the mean.

3.7 Conclusion

RD43 rice flour presented a higher amylose content with a smaller particle size than Hom Mali rice flour. The results from X-ray diffraction indicated the presence of amylose–lipid complexes in RD43 rice flour. This structure influences hydration, thermal properties, and pasting properties of RD43 rice flour. Furthermore, RD43 rice flour had a greater ability to decrease starch digestibility, bind bile acid, and disrupt the formation of cholesterol micellization than Hom Mali rice flour. Steamed muffin made from RD43 flour had lower starch digestibility without affecting the sensory acceptability. Based on our findings, RD43 rice flour may be used as an alternative ingredient for lowering the glycemic index of food products.

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CHAPTER IV

RD43 RICE FLOUR: EFFECT ON STARCH DIGESTIBILITY AND QUALITIES OF NOODLES, GLYCEMIC RESPONSE, SHORT-ACTING SATIETY HORMONE AND APPETITE CONTROL IN HUMANS

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4.2 Article status

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4.3 Abstract

The aim of this study was to develop wheat noodles substituted with 10-40% RD43 rice flour. Starch digestibility and physicochemical and sensory properties of RD43 rice noodles and its effect on glycemic response, gut hormones, and appetite sensation in humans were also determined. The results demonstrated that substitution of wheat flour with 10-40% RD43 rice flour reduced starch digestibility, hydrolysis index, and rapidly digestible starch (RDS) while increasing undigestible starch in noodles. Noodles prepared with 30% RD43 rice flour slightly increased water absorption (WA), and swelling index (SI) without altering cooking loss. When comparing with the control, 30% RD43 rice showed higher lightness (*L**) and lower redness (*a**), yellowness (*b**) and hardness with similar overall acceptability. In human study, ingestion of 30% RD43 rice noodle significantly lowered post-prandial plasma glucose at 15-90 min. Interestingly, the postprandial concentration of glucagon-like peptide-1 (GLP-1) and peptide tyrosine–tyrosine (PYY) also significantly increased at 30 min after intake of 30% RD43 rice noodle. A significantly lower desire to eat and higher fullness were detected after RD43 rice noodle consumption until 120 min. This suggests that RD43 rice flour could be a potential ingredient in noodles for controlling glycemic response, short-acting satiety hormone, and appetite sensation.

4.4 Introduction

Consumption of carbohydrates has been increased rapidly during recent decades, especially among developing countries in the continents of Asia and Africa. In particular, the increased consumption of refined carbohydrates has been implicated as the risk factor of non-communicable diseases (NCDs) such as type 2 diabetes and obesity (Liu, 2002). Furthermore, it has been reported that a high carbohydrate diet attenuates the release of short-acting satiety hormones such as glucagon-like peptide 1 (GLP-1) and peptide tyrosine-tyrosine (PYY), leading to excessive hunger, an appetite increases and promoting food overconsumption (Arumugam et al., 2008; De Silva & Bloom, 2012). Previous reports indicate that Asian people consume a diet with 60% of total energy from carbohydrates (Cui & Dibley, 2012). Noodles are the main source of carbohydrate staples consumed as refined grains in Southeast Asia, China, and Japan (Karim & Sultan, 2014). Usually, noodles can be made from wheat and rice and various raw materials such as mung bean, sweet potato, and tapioca. In particular, wheat noodles display unique yellow color, smooth and non-sticky together with high protein content (Ahmed, Qazi, & Jamal, 2015; Hou, 2001). An intake of noodles has been increased due to being a quick and convenient food and the ease of cooking, palatability, transportation, and mechanizations (Karim & Sultan, 2014). However, recently published data suggest that excessive consumption of noodles has received a special attention, owing to its association with elevation of insulin resistance and hyperglycemia in Asian population (Zuniga et al., 2014). This is because noodles contain higher amounts of refined carbohydrates classified as high glycemic index (GI) and glycemic load (GL) foods. (Suk, Kim, Kim, Lim, & Choue, 2016). Reducing the glycemic impact of noodles is challenging strategies for the food industry and consumers.

Over the years, various attempts have been made to determine the effect of wheat flour substitution with rice flour containing high undigestible starch in carbohydrate diet (Sirichokworrakit, Phetkhut, & Khommoon, 2015; Wu et al., 2017). For example, partial replacement of wheat flour by brown rice flour in noodles resulted in a significant effect on the digestion rates and absorption of the starch (Wu et al., 2017). Furthermore, the substitution of wheat flour with rice flour influences on noodle quality and characteristics relative to a decrease in cooking time and an increase in tensile strength and consumer acceptability (Ahmed et al., 2015; Sirichokworrakit et al., 2015). RD43, a non-glutinous rice variety, is cross-bred between Khao Jow Hawm Suphan Buri rice and Supan Buri1 rice. A previous study indicates that RD43 rice flour has mild fragrance with low apparent amylose content (Rice Department of Thailand, 2018). This flour comprises a larger proportion of undigestible starch with less amount of rapidly digestible starch (RDS), causing a slower starch hydrolysis and digestibility (Suklaew, Chusak, & Adisakwattana, 2020). Interestingly, RD43 rice flour presents the ability to bind bile acids and disrupt cholesterol micellization (Suklaew et al., 2020). Besides, RD43 rice flour exhibits specific characteristics such as V-type crystalline structure, low water solubility index, and huge-setback value. These characteristics of RD43 flour have been suggested as an ingredient in high structural product such as pasta and noodles (Suklaew et al., 2020). Considering the well documented physicochemical properties and biological action of RD43 rice flour, replacement of wheat flour by RD43 rice flour may be potential approach towards the development of high quality and healthy noodles. Therefore, the objective of this study was to investigate the effect of wheat flour substitution with RD43 rice flour in noodles on *in vitro* starch digestibility, cooking characteristics, physicochemical, and sensory properties. The influences of RD43 rice noodle consumption on glycemic response, short-acting satiety hormones, and appetite in healthy participants were also determined.

4.5 Materials and methods

4.5.1 Materials

RD43 rice (*Oryza sativa* L.) was purchased from the Rice Department, Bangkok, Thailand. Pepsin from porcine gastric mucosa powder P-7000, α-amylase Type VI-B from porcine pancreas A-3176 and pancreatin from porcine pancreases P-1750 were obtained from Sigma-Aldrich (St. Louis, Missouri, United States). Human GLP-1, and PYY ELISA kits were obtained from Biobase Meihua Trading Co., Ltd. (Jinana, China). Glucose oxidase kit and (Glucose liquicolor) was purchased from Human diagnostics (Wiesbaden, Germany)

4.5.2 Preparation of RD43 rice flour

The RD43 rice flour was prepared following a previous method (Suklaew et al., 2020). RD43 rice grain was grounded at 4800 rpm of Pin Mill (Phoenix Equipment Corporation, United States) speed at voltage of 50 Hz. The sample was sieved through 100-mesh screen and kept in sealed plastic bag at temperature of 25°C until used.

4.5.3 Noodle preparation

The RD43 rice noodle was prepared by partial substitution of wheat flour with 10%, 20%, 30%, and 40% of RD43 rice flour, as shown in Table 7. Noodle formula was performed according to a previous study with a minor modification (Sirichokworrakit et al., 2015). All ingredients were mixed and kneaded for 15 min to make dough at 25°C. After leaving the dough for 30 min, it was pressed and cut to a width and length of 2.5 and 18-20 cm, respectively, by using the Pasta Machine (Changzhou Shule Kitchen Utensils Co. Ltd., Jiangsu, China). Then, fresh noodles were analyzed within 24 h.

Table 7 Formula of wheat and RD43 rice noodles

Ingredients	Formula				
	Control	10% RD43 rice	20% RD43 rice	30% RD43 rice	40% RD43 rice
Wheat flour (g)	100	90	80	70	60
RD43 rice flour (g)	-	10	20	30	40
Whole egg (g)	37	37	37	37	37
Water (g)	6	6	6	6	6
1% w/w $NaHCO_3$ (g)	7	7	7	7	7
Salt (g)	1	1	1	1	1

4.5.4 Cooking properties

The cooking properties including water absorption (WA), swelling index (SI), and percentage of cooking loss were determined according to the American Association for Clinical Chemistry standard methods (AACC) (AACC, 2000). First, raw noodles (10 g) were added to 100 ml boiling distilled water (DW) for 90 s until the hard and white core of noodles disappeared. Thereafter, the cooked noodle was added to 25°C DW for 5 min to prevent overcooking, then drained and weighed. A beaker containing rinse water and cooking water was dried at temperature of 105°C until constant weight. The cooked noodle was kept at temperature of 40°C until constant weight as well. The cooking quality parameters were calculated as follow:

$$\text{Cooking loss (\%)} = \frac{\textbf{Weight of dried residue in rinse water and cooking water}}{\textbf{Weight of raw noodles}} \times 100$$

$$\text{WA (\%)} = \frac{\textbf{Weigh of cooked noodles} - \textbf{weight of raw noodles}}{\textbf{Weight of raw noodles}} \times 100$$

$$\text{SI} = \frac{\textbf{Weight of cooked noodles – weight of dried cooked noodles}}{\textbf{Weight of dried cooked noodles}}$$

4.5.5 Color profiles

The measurement of noodle color profiles was done according a previous study (Kim, Kim, Bae, Chang, & Moon, 2017). Noodle sheet was prepared by cutting noodle sheets to 5×5 cm and cooked for 90 s (1:10 (w/v) noodles:water ratio). The color profile of cooked noodles was determined using Colorimeter (Hunter Associates Laboratory, Inc., Virginia, United States). The values of, *a** (redness), *b** (yellowness), and *L** (lightness) were resulted after calibration of instrument (45°/0° geometry, 10° observer) with standard black glass and white tile.

4.5.6 Texture profiles

Texture profiles (TPA) were determined as depicted in a previous study with minor modification (Tan, Phatthanawiboon, & Mat Easa, 2016). Noodle strands (5 cm in length) were cooked for 90 s (1:10 (w/v) noodle:water ratio). TPA was performed using a TA.XT-Plus Texture analyser (Technologies Corp. and Stable Micro Systems Ltd., Massachusetts, United States) with 35 mm diameter cylindrical probe. Five strands of cooked noodles were compressed at speed of 2.0 mm/s for pre-test and 0.8 mm/s for test and post-test with 75% depth from original noodle thickness. Texture profile parameters such as hardness, adhesiveness, cohesiveness, and springiness were resulted from the force (N) - distance (mm) curve.

4.5.7 Starch digestibility

The *in vitro* starch digestion was conducted following a previously published method with minor modifications (Yousif, Nhepera, & Johnson, 2012). In brief, cooked noodles (500 mg) were incubated with 1 ml of α-amylase (250 U/ml) in 0.2 M carbonate buffer at pH 7 for 20 s. Then, the solution was mixed with 5 ml of pepsin solution (3200 U/ml) in 0.02 N HCl at pH 2 and temperature of 37°C for 1 h in a water bath shaker at 100 rpm. After that, 25 ml of 0.2 M sodium acetate buffer at pH 6 and 5 ml of 0.02 N NaOH were added to the digesta for neutralization. Finally, 5 ml of the solution containing amyloglucosidase (28 U/ml) and porcine pancreatin (2 mg/ml) in 0.2 M sodium acetate buffer at pH 6 were added to the digesta. The aliquot was collected at 0, 20, 30, 60, 90,120, and 180 min. The reaction was terminated by heating at 105°C for 10 min. Then, centrifugation was performed at 11000 rpm (15 min at 25°C) before determination of glucose concentration in digesta by using the glucose oxidase kit. The results were reported as mg glucose/100 g cooked noodles. The incremental area under the curve (iAUC) of glucose released from 0 to 180 min was calculated using the trapezoidal rule. The starch fractions including rapidly digestible starch (RDS; 0-20 min digestion), slow digestible starch (SDS; 20-120 min digestion) and undigestible starch (120-180 min digestion) were calculated were calculated from the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose), and total starch by using the previous equation (Englyst, Kingman, & Cummings, 1992);

$$\%\text{RDS} = \frac{\text{G20}-\text{FG}}{\text{Total starch}} \times 0.9 \times 100$$

$$\%\text{SDS} = \frac{\text{G120}-\text{G20}}{\text{Total starch}} \times 0.9 \times 100$$

$$\%\text{Undigestible starch} = \frac{\text{Total starch}-\text{RDS}-\text{SDS}}{\text{Total starch}} \times 100$$

The quantity of total starch was obtained from measurement of glucose derived from hydrolysed sample by using a conversion factor of glucose to starch (0.9) (Goñi, Garcia-Alonso, & Saura-Calixto, 1997). Hydrolysis index (HI) was calculated according the formula GI = 39.71 + 0.549 HI (Goñi et al., 1997).

4.5.8 Sensory evaluation

All noodle formulas were freshly prepared for sensory evaluation according to a previous method (Ritthiruangdej, Parnbankled, Donchedee, & Wongsagonsup, 2011). Before the evaluation, cooked noodle samples were coated with 1% (w/w) vegetable oil for strands stick prevention. After that, 10 g cooked samples were kept in tightly covered plastic storage containers before testing and not more than 30 min (Ritthiruangdej et al., 2011). Sample containers were identified by different 3-digit code before testing and random providing to each panellist. Fifty untrained panellists (39 females aged 23.54 ± 0.77 years old, and 11 males aged 24.55 ± 0.85 years old) rinsed their mouth with water before and between evaluation of samples at individual sensory booths under artificial daylight condition. The cooked noodle was assessed for appearance, texture, hardness, springiness, color, flavor, and overall acceptability by using 9-point hedonic scale. The scale of value ranges from “extremely unpleasant” to “extremely pleasant” corresponding the lowest and highest scores to “1” and “9”.

4.5.9 Human study

4.5.9.1 Participants

Sixteen male participants were enrolled to participate in this study. Inclusion criteria were the following: (1) in the age of 20-40 years old males (this study was only conducted in male because its appetite is less influenced by sex hormones than female) (Hirschberg, 2012); (2) BMI range 18.5-22.9 kg/m^2; (3) fasting blood glucose < 100 mg/dl; (4) fasting serum cholesterol < 200 mg/dl; (5) creatinine ranged 0.7-1.4

mg/dl; (6) blood pressure < 140/90 mmHg. Exclusion criteria were: (1) presence of vascular disease, renal disease, liver disease, thyroid disease, diabetes, gastrointestinal diseases, eating disorders, and celiac disease; (2) use of dietary supplements or medication interfere with intestinal absorption or glucose homeostasis; (3) self-report of smoking and alcohol drinking.

4.5.9.2 Ethical approval

The study was approved by the office of Ethics Review Committee for Research Involving Human Research Subjects, Human Science Group, Chulalongkorn University (COA No. 162/2020). The study began in January 2020 and completed in February 2020 at Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University. Participants were written inform consent before enrolled the study and all information of participants were kept confidential. The study was enrolled in Thai Clinical Trials Registry (study ID: TCTR20210212002).

4.5.9.3 Study design

This study was a randomized crossover design with one-week wash-out period (Figure 12). The participants were randomized by using online random number generator in two groups of intervention which were wheat noodles (control) and RD43 rice noodles (treatment). Before and during the study, the participants were asked for keeping a normal activity and eating pattern without dietary or antioxidant or herbal supplements consumption. The primary outcome was glycemic responses. The secondary outcome was the appetite hormones including glucagon-like peptide 1

(GLP-1) and peptide tyrosine-tyrosine (PYY) and appetite sensation after test meal consumption including fullness, hunger, and desired to eat.

On the test day, the fasting participants arrived at Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University. At baseline, a catheter was inserted into forearm vein by registered nurse for blood sample collection. Also, the appetite sensation such as fullness, hunger, and desired to eat were determined by 100-mm a visual analog scale (VAS). Then, they were asked to consumption the test meal within 10 min. After that, blood samples were collected and VAS evaluation were performed at 15, 30, 60, 90, and 120 min. During the test session, drinking-water was limited (<300 ml) and they were not allowed to eat or drink any food or beverage.

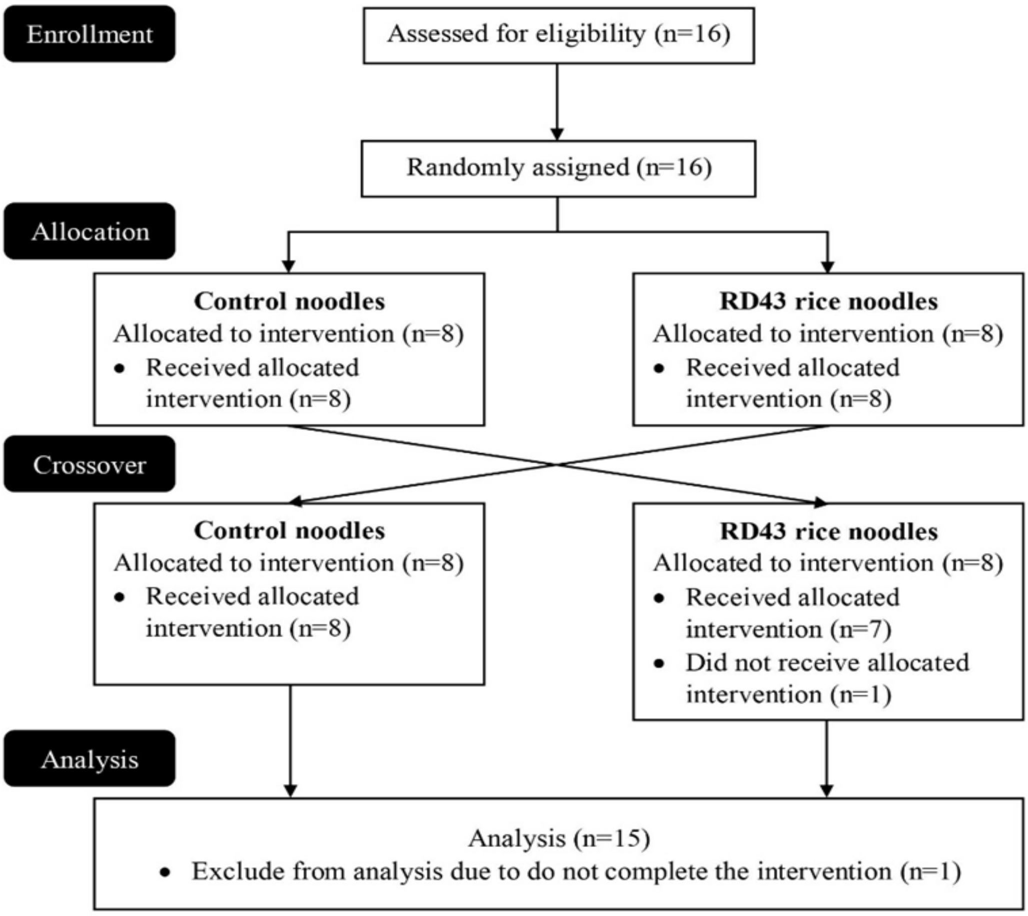

Figure 12 CONSORT diagram of selection of study subjects.

4.5.9.4 Test meal

In comparison with the control noodle, the test noodle made from 30% RD43 rice flour was chosen for this study. In test meal, cooked noodle was 120 g of raw noodles with 200 ml of clear soup. All test meals were given in the isoglycemic proportion of 61.3 g of carbohydrate. Proximate analysis of noodle samples was performed using The Association of Official Analytical Chemists standard methods

(AOAC) (Sullivan & Carpente, 1993). The nutritional composition of the noodle samples is reported in Table 8.

Table 8 Nutrient composition/ serving of the test meals

	Wheat noodle	RD43 rice noodle
Amount of raw noodle (g)	120	120
Carbohydrate (g)	61.28	61.28
Protein (g)	13.02	12.11
Fat (g)	3.98	3.90
Moisture (g)	39.78	43.78
Ash (g)	1.93	1.25
Total calorie (kcal)	333.07	328.68

4.5.9.5 Biochemical analysis

Blood samples were immediately centrifuged at 3000 rpm for 15 min at 4°C. After centrifugation, plasma was collected in Eppendorf tubes and kept at -80°C until analysis. Plasma glucose was analysed by using the enzymatic colorimetric method. Plasma GLP-1, and PYY were measured with enzyme-linked immunosorbent assay (ELISA kits). All parameters were determined in triplicate.

4.5.10 Statistical analysis

Data were reported as mean ± SEM. Starch digestibility, cooking properties, color and texture profiles, and sensory properties were evaluated using one-way ANOVA. Duncan's multiple-range test were used to compare the difference between individual means.

The incremental of plasma glucose, GLP-1, PYY and appetite sensation was calculated by subtracting values at 0 min from all subsequent values over 120 min. Postprandial incremental area under the curve (iAUC) was calculated using the Trapezoidal rule. Blood glucose, GLP-1, PYY, and appetite sensation were tested the normality by using Shapiro-Wilk test. Repeated measure ANOVA was used to determine the interaction variable of times, treatment, and times × treatment. Paired *t*-test was used to compare the difference between groups. *P*-values < 0.05 was considered as statistically significant.

4.6 Results and discussion

4.6.1 The cooking, color, and texture profiles of noodles

Water absorption is an important parameter for determining of cooking quality of noodles, indicating water uptake capacity of noodles structure during heating (Sirichokworrakit et al., 2015), whereas swelling index (SI) represents the swelling ability of starch and protein during cooking process (Kim et al., 2017). Significant increase in WA and SI values were observed in 10-40% RD43 rice noodles as compared with the noodle control (Table 9). Our results are consistent with a previous report showing an increase in WA and SI values of wheat noodles after incorporation with cereal flour (Kaur, Sharma, Nagi, & Dar, 2012). This might be due to decreasing continuous gluten network formation, leading to facilitate water absorption and increase swelling of starch granules (Chung, Cho, & Lim, 2012). Cooking loss is an indicator of noodle quality to resist the disintegration during the hot-water cooking process (Fu, 2008). No significant difference was observed in the cooking loss of noodles replaced with 10-30% RD43 rice flour which was comparable to that of control noodle (Table 9). Meanwhile, this value markedly increased in 40% RD43 rice noodle. This may be due to weaken of gluten network by replacement of non-gluten rice flour in noodles (Rayas-Duarte, Mock, & Satterlee, 1996). The good quality of noodles is prescribed as low cooking loss and moderate-high water absorption ability (Bruneel, Pareyt, Brijs, & Delcour, 2010). Our findings suggest that 10-30% RD43 rice flour can be a suitable proportion for the replacement of wheat flour without affecting the structural integrity of noodles during the cooking process.

The photographs of noodles with different proportion of RD43 rice flour replacement are illustrated in Figure 13. Remarkedly, the higher *L** value and the lower *b** and *a** value were perceived for RD43 rice noodles, suggesting that incorporation of RD43 rice flour made the noodle brighter with a slightly lower yellowness and redness (Table 9). The findings are agreed with the results of Suwannaporn *et al.* (2014) who reported that the replacement of wheat flour by rice flour decreased yellowness and redness with a concomitant increase in lightness of noodles. There are many factors affecting the quality of Asian noodles such as raw materials and food processing. In this study, the RD43 rice noodle presents a natural yellow because of raw material after milling process (Suklaew et al., 2020). In general, the basic requirements for good characteristic Asian noodles should have attractive bright yellow color and less discoloration within 48 h of preparation (Karim & Sultan, 2014). The discoloration in noodles could be resulted from a natural reaction after addition of alkaline salts (Karim & Sultan, 2014). Therefore, the substitution of wheat flour with RD43 rice flour produced a desirable color following noodle quality requirements (Hou, Kruk, & Center, 1998).

Figure 13 Photograph of cooked noodles; Control (A), 10% RD43 rice (B), 20% RD43 rice (C), 30% RD43 rice (D) and 40% RD43 rice (E).

Table 9 Cooking properties and color profiles of wheat flour and RD43 rice flour noodles

Sample	Cooking properties			Color profiles		
	%WA	SI (g water/g dried sample)	%Cooking loss	*L**	*a**	*b**
Control	142.39 ± 8.86^{a}	2.27 ± 0.09^{a}	3.58 ± 0.26^{a}	29.00 ± 0.68^{a}	2.65 ± 0.00^{a}	17.19 ± 0.23^{a}
10% RD43 rice	179.17 ± 6.97^{b}	2.67 ± 0.07^{b}	3.68 ± 0.05^{a}	29.79 ± 0.45^{ab}	2.45 ± 0.06^{b}	17.88 ± 0.14^{a}
20% RD43 rice	181.51 ± 0.48^{b}	2.76 ± 0.01^{b}	3.76 ± 0.16^{a}	31.28 ± 0.85^{bc}	1.81 ± 0.09^{c}	17.50 ± 0.28^{a}
30% RD43 rice	196.63 ± 4.69^{b}	2.89 ± 0.09^{b}	4.29 ± 0.12^{a}	31.92 ± 0.14^{c}	1.16 ± 0.02^{d}	15.28 ± 0.40^{b}
40% RD43 rice	214.72 ± 3.07^{c}	3.16 ± 0.04^{c}	6.28 ± 0.38^{b}	32.66 ± 0.34^{c}	0.77 ± 0.03^{e}	14.79 ± 0.41^{b}

Data are expressed as mean ± SEM (n = 3). Different letters in the same column were considered statistically significant differences as compared inter-formula (p<0.05). WA: water absorption; SI: swelling index; *L**: lightness; *a**: redness; *b**: yellowness.

The comparisons of wheat and RD43 rice noodles on texture characteristics are shown in Table 10. Texture properties of noodles are the most critical characteristic for determining the eating quality and consumer acceptance (Hou, 2001). Increasing the level of RD43 rice flour from 10% to 40% led to a decrease in hardness and adhesiveness of noodles. Furthermore, the springiness and cohesiveness values of 40% RD43 rice noodles were significantly lower than those of the control. These findings may be related to reducing formation of compact gluten network after addition of RD43 rice flour containing high fraction of undigestible starch, especially resistant starch (Pourmohammadi, Abedi, Amiri, Daneshgar, & Torri, 2019; Sozer, Dalgıç, & Kaya, 2007). Adhesiveness refers stickiness of cooked noodles, associated with starch content and gelatinization (Kolarič, Minarovičová, Lauková, Karovičová, & Kohajdová, 2020). Fu (2008) suggest that desirable Asian noodles should have low stickiness. Ritthiruangdej *et al*. (2011), also found a decrease in adhesiveness of noodles after addition of non-wheat flour. According to the findings, the substitution of wheat flour with RD43 rice flour markedly produces softer and less stickiness, whereas a slight reduction of springiness of noodles was presented.

Table 10 Texture profiles of cooked noodles by the replacement of wheat flour with RD43 rice flour

Sample	Hardness (N)	Springiness (mm)	Adhesiveness (g· sec)	Cohesiveness
Control	101.60 ± 1.06^{a}	0.79 ± 0.00^{a}	-3.09 ± 0.19^{a}	0.64 ± 0.01^{a}
10% RD43 rice	94.41 ± 0.66^{b}	0.83 ± 0.01^{a}	-2.55 ± 0.14^{b}	0.65 ± 0.01^{a}
20% RD43 rice	87.32 ± 0.72^{c}	0.79 ± 0.02^{a}	-2.53 ± 0.13^{b}	0.64 ± 0.00^{ab}
30% RD43 rice	85.06 ± 2.46^{c}	0.74 ± 0.01^{b}	-2.44 ± 0.06^{b}	0.63 ± 0.00a^{b}
40% RD43 rice	83.97 ± 0.96^{c}	0.72 ± 0.01^{b}	-2.51 ± 0.13^{b}	0.61 ± 0.01^{b}

Data are mean ± SEM (n = 3). Different letters in the same column were considered statistically significant differences as compared inter-formula (p<0.05).

4.6.2 Starch digestibility of noodles

The results of glucose released over 180 min in control and RD43 rice noodles are demonstrated in Figure 14A. The rate of glucose release from all noodle samples sharply increased up to 20 min and then gradually increased until 180 min. The replacement of RD43 rice flour (10%) did not show significantly different in glucose release when compared to the control. The reducing glucose release in RD43 rice noodles (20-40%) was significantly observed at all periods from 90 to 180 min. When increased proportion of RD43 rice flour in noodles, the amount of glucose release decreased. As the level of RD43 rice flour replacement increased, the incremental area under curve (iAUC), namely the decreasing glucose release (Figure 14B). The percentage of reductions in iAUC for 10-40% RD43 rice flour replacement in noodles were from

5.01 to 32.23%. The results showed that the total starch content of all noodles ranged from 20.84 to 24.56 g/100g sample. The relative fractions of RDS, SDS, and undigestible starch in noodles are demonstrated in Figure 14C. The replacement of wheat flour with 20-40% RD43 rice flour significantly decreased the RDS and SDS fraction as well as hydrolysis index of noodles (Figure 14D). This effect was accompanied by the increased undigestible starch fraction, to achieve values of the order of 28.24 to 56.84%. These findings suggest that the incorporation of RD43 rice flour (20-40%) in the formulation of noodles significantly retarded the digestibility of starch, leading to increase undigestible starch fraction of noodles. This effect may be influenced by the presence of V-type crystalline structure in RD43 rice flour (Giuberti, Gallo, Cerioli, Fortunati, & Masoero, 2015; Suklaew et al., 2020). This structure is densely packed to hinder an accessibility of carbohydrate hydrolysing enzymes (namely α-amylase, α-glucosidase). In addition, starch composition such as amylose-amylopectin ratio also influences starch digestibility (Ang et al., 2020). According to our previous study, the Fourier Transforms Infrared (FTIR) outcome of RD43 rice flour presents low intensity ratio of 1047/1022 cm^{-1} which indicating a high amount of amylose with a low amylopectin content (Suklaew et al., 2020). High ratio of amylose-amylopectin elevates the resistance of starch digestion, leading to reduction in starch digestibility (Ang et al., 2020). Therefore, lowering starch digestibility of noodles after addition of RD43 rice flour may attribute to these factors.

RDS (rapidly digestible starch) is defined as the starch fraction digested to release glucose in the first 20 min, whereas slowly digestible starch (SDS) represents the digestion in the range of time 20-120 min (Englyst et al., 1992). The increased proportion of RDS is detrimental to human health which causes a rapid surge in blood glucose level, resulting in postprandial hyperglycemia (Gourineni, Stewart, Skorge, & Sekula, 2017). Previous evidence revealed that long-term hyperglycemia alters cellular metabolism, leading to development of insulin resistance (Jellinger, 2007). Undigestible starch refers the proportion of starch which resists digestion and absorption in the small intestine after 120 min (Englyst et al., 1992). Studies on undigestible starch has received great attention because of its functional properties related to control fasting glucose, triglyceride, and cholesterol levels (Mudgil & Barak, 2013). Interestingly, it has been reported that starchy foods with a lower content of RDS and a higher undigestible starch content are preferred for management of postprandial blood glucose level owing to slow down starch digestibility and glucose release (Gourineni et al., 2017; Suklaew et al., 2020). Our findings suggest that the substitution of wheat flour with RD43 rice flour has a positive effect on decreasing RDS and increasing undigestible starch fractions of noodles which can be the potential food to reduce postprandial blood glucose responses.

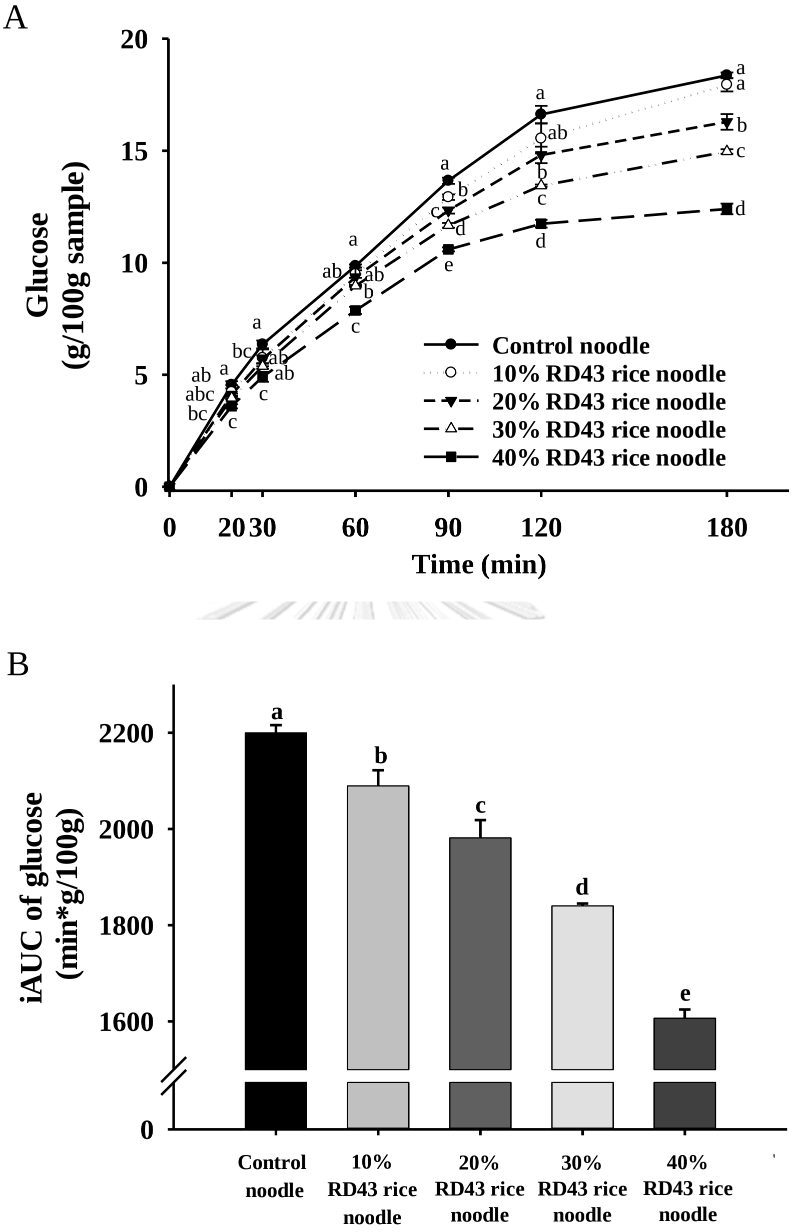

Figure 14 The *in vitro* starch digestion of cooked noodles. The glucose release during *in vitro* starch digestion (A), incremental area under the curve (iAUC) of glucose (B), starch fractions (C), and hydrolysis index (HI) (D) of cooked noodles. Data are expressed as mean ± SEM, $n = 3$. Different letters were considered statistically significant differences compared inter-formula ($p < 0.05$). RDS: rapidly digestible starch; SDS: slowly digestible starch.

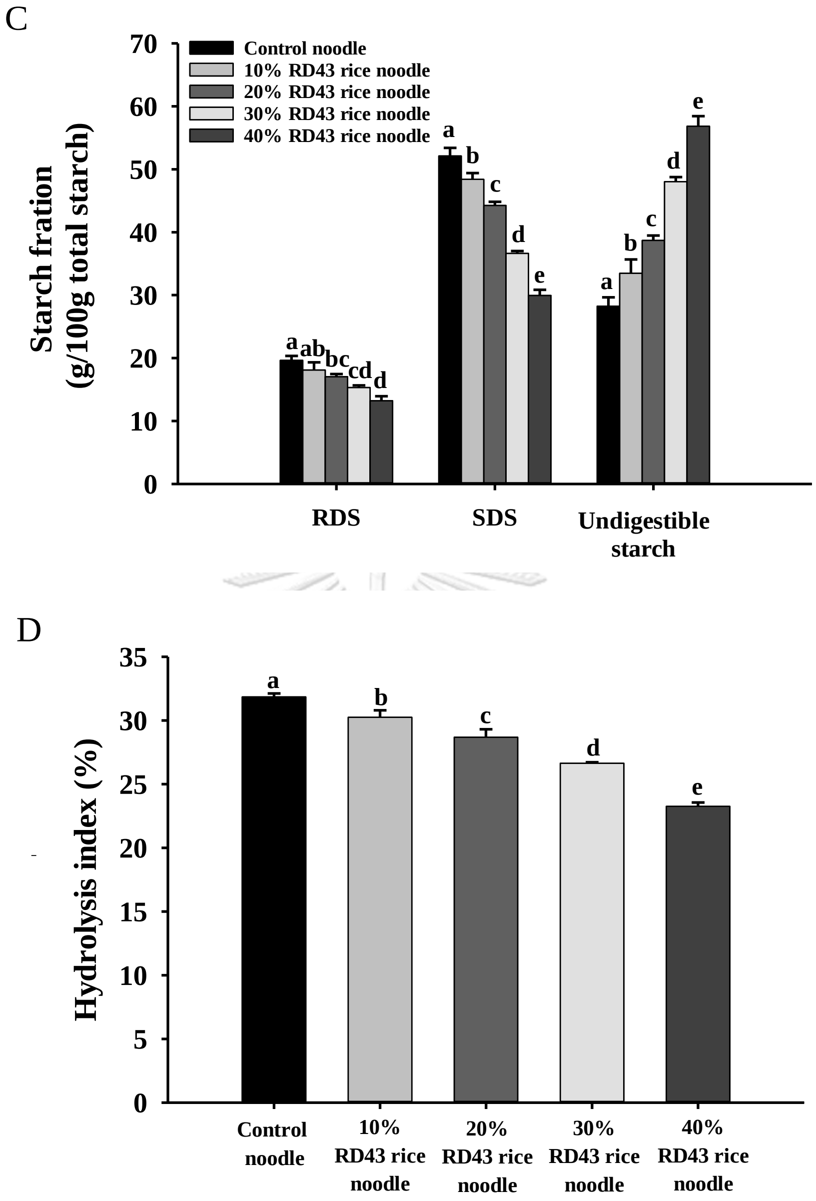

Figure 14. The *in vitro* starch digestion of cooked noodles. The glucose release during *in vitro* starch digestion (A), incremental area under the curve (iAUC) of glucose (B), starch fractions (C) and hydrolysis index (HI) (D) of cooked noodles. Data are expressed as mean ± SEM, $n = 3$. Different letters were considered statistically significant differences compared inter-formula ($p < 0.05$). RDS: rapidly digestible starch; SDS: slowly digestible starch.

4.6.3 Sensory evaluation

Sensory analysis of noodles made by partial replacement of RD43 rice flour is demonstrated in Table 11. The obtained results showed that 10-30% RD43 rice noodles did not alter all sensory attributes when compared to control. However, 40% RD43 rice noodle markedly decreased the score of sensory attributes including hardness, springiness, appearance and overall acceptance. According to the results from *in vitro* starch digestibility and physiochemical properties, the substitution of wheat flour with 30% RD43 rice flour remains a significant decrease in starch digestibility and hydrolysis index as well as an increase in undigestible starch content without affecting cooking loss and sensory evaluation scores. Although 30% RD43 rice flour substitution leads to a slight reduction in hardness and springiness but it causes a significant improvement of lightness and redness of noodles. These parameters are key determinants for consumer preference related to an acceptance and consumer selection (Ahmed et al, 2015; Hou et al., 1998; Karim & Sultan, 2014).

According to these results, 30% RD43 rice noodle were selected as the proper formulation for subsequent experiments in aspects of postprandial glycemic response and short-acting satiety hormones in the subjects.

Table 11 Sensory properties of cooked noodles by the replacement of wheat flour with RD43 rice flour

Sample	Color	Flavor	Texture	Hardness	Springiness	Appearance	Overall acceptance
Control	5.88 ± 0.23^{a}	5.08 ± 0.28^{a}	6.62 ± 0.20^{a}	6.40 ± 0.20^{a}	6.48 ± 0.24^{a}	6.28 ± 0.21^{ab}	6.50 ± 0.18^{ab}
10% RD43 rice	6.28 ± 0.21^{ab}	5.12 ± 0.28^{a}	6.72 ± 0.21^{a}	6.32 ± 0.21^{a}	6.50 ± 0.21^{a}	6.78 ± 0.19^{a}	6.92 ± 0.17^{a}
20% RD43 rice	6.56 ± 0.20^{b}	5.30 ± 0.27^{a}	6.68 ± 0.16^{a}	6.58 ± 0.20^{a}	6.24 ± 0.21^{ab}	6.70 ± 0.24^{a}	6.72 ± 0.17^{ab}
30% RD43 rice	6.38 ± 0.19^{ab}	5.06 ± 0.24^{a}	6.16 ± 0.24^{a}	6.04 ± 0.26^{ab}	5.72 ± 0.27^{bc}	6.38 ± 0.20^{ab}	6.24 ± 0.21^{bc}
40% RD43 rice	6.22 ± 0.10^{ab}	4.84 ± 2.70^{a}	5.52 ± 0.31^{b}	5.52 ± 0.30^{b}	5.24 ± 0.28^{c}	5.98 ± 0.24^{b}	5.80 ± 0.26^{c}

Data are expressed as mean ± SEM (n = 50). Different letters in the same column were considered statistically significant differences compared inter-formula ($p<0.05$).

4.6.4 Human study

4.6.4.1 Participants

Sixteen participants were enrolled at the beginning but only fifteen completed the study due to personal reasons. The data of recruitment and enrollment is demonstrated in Figure 12. The baseline characteristics of the participants were reported in Table 12. Participants were in aged range from 20 to 30 years old, weight from 58.30 to 77.30 kg, and BMI from 18.39 to 22.38 kg/m^2. Their fasting glucose concentrations ranged from 81 to 95 mg/dl, total cholesterol ranged from 144 to 195 mg/dl, and creatinine ranged from 0.79 to 1.21

Table 12 Baseline characteristics of participants

Characteristics	Mean ± SEM
Age (years)	24.73 ± 1.03
Weight (kg)	66.41 ± 1.52
BMI (kg/m^2)	22.06 ± 0.30
Fasting blood glucose (mg/dl)	86.60 ± 1.28
Total cholesterol (mg/dl)	188.33 ± 7.37
Creatinine (mg/dl)	0.95 ± 0.03
Systolic blood pressure (mmHg)	114.4 ± 2.6
Diastolic blood pressure (mmHg)	74.6 ± 2.2

Data are expressed as mean ± SEM, *n* = 15.

4.6.4.2 Postprandial plasma glucose response

The incremental postprandial plasma glucose response after noodles consumption is presented in Figure 15A. The postprandial plasma glucose was reaching a peak at 30 min after consumption of noodles. Moreover, consumption of RD43 rice noodles caused a decrease in incremental postprandial plasma glucose at 15, 30, 60, and 90 min when compared to the control. As demonstrated in Figure 15B, iAUC for glucose was 43.50% lower after RD43 rice noodle ingestion compared to the control. The undigestible starch normally contains resistant starch which resists carbohydrate hydrolyzing enzymes, namely α-amylase and α-glucosidase, leading to postpone the starch digestion, thus resulting in lowering postprandial plasma glucose (Mudgil & Barak, 2013). Recent study by Nilsson *et al*. (2008) reported that the replacement of resistant starch in starchy food beneficially influences on decreasing blood glucose in healthy volunteers. Previous clinical trials conducted in healthy adults reported that effective dose of undigestible starch, e.g., resistant starch that can achieve the attenuation of postprandial glucose were 38.20 and 72.60 g/100 g total starch (Nilsson et al., 2008; Nilsson, Östman, Granfeldt, & Björck, 2008). The 30% of RD43 rice noodle contained a sufficient amount of undigestible starch (48.05 g/100 total starch) that can explain the effect on reducing postprandial glucose. In meantime, the undigestible starch content of control noodle was reported to be 28.24 g/100 g total starch. It has been reported that intake of undigestible starch, i.e., resistant starch, has potential for glycemic and weight regulation in diabetic and obese people (Mudgil & Barak, 2013; Robertson, 2012).

Therefore, it could be suggested that RD43 rice noodle may be potentially used for management of glycemic control.

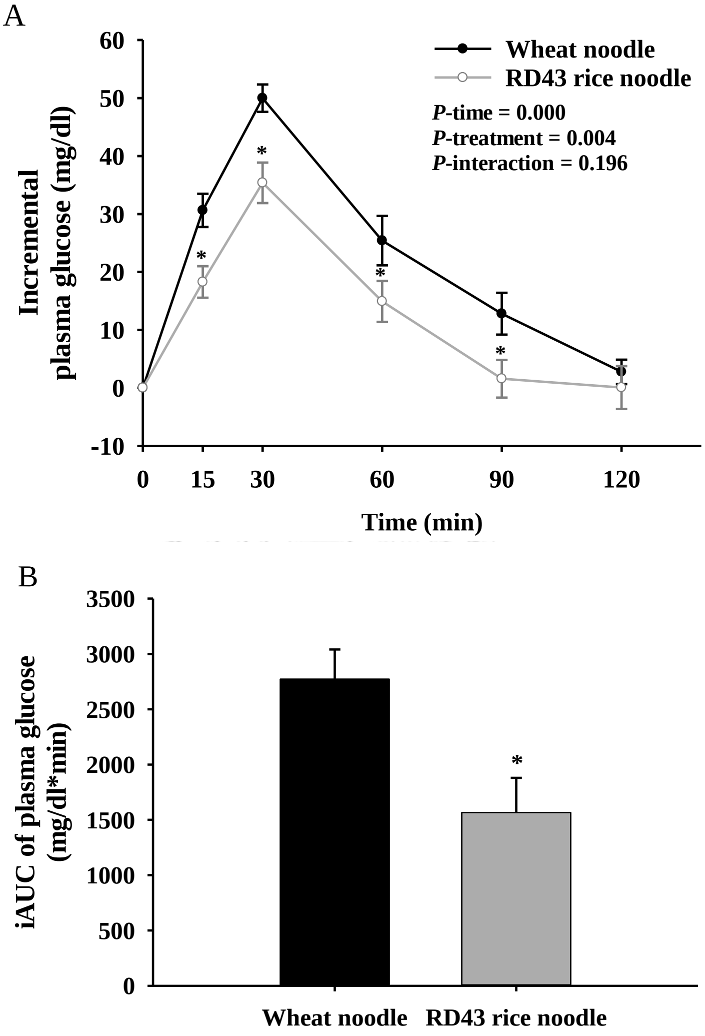

Figure 15 Incremental change from baseline of postprandial plasma glucose (A) and the incremental area under the curve (iAUC) of postprandial plasma glucose (B) in healthy males after consuming either wheat or 30% RD43 rice noodles. Fasting plasma glucose concentration was 78.71 ± 1.55 mg/dl and 82.40 ± 1.44 mg/dl for participants who consumed wheat noodle and RD43 rice noodle, respectively. Data are expressed as mean ± SEM, *n* = 15. * $p < 0.05$ compared to the wheat noodles.

4.6.4.3 Satiety hormones and appetite sensation

After food consumption, the nutrient content could stimulate the release of short-acting satiety hormones from gastrointestinal tract such as GLP-1 and PYY (De Silva & Bloom, 2012). Both noodles caused the stimulatory effect on the release of GLP-1 at 15 min. The postprandial plasma GLP-1 concentration was higher at 60 min after the consumption of RD43 rice noodle than the control (Figure 16A). There was 68.83% increase in plasma GLP-1 iAUC after the RD43 rice noodle compared to the control (Figure 16B). At the time point 15, 30, 60, and 120 min, there was statistically difference with the RD43 rice noodle ingestion leading to the larger increase in PYY secretion compared to the control noodle consumption (Figure 17A). Similarly, iAUC of RD43 rice noodle (59.40%) was remarkedly higher than the control noodle (Figure 17B). Our results were consistent with Stefoska-Needham *et al*. (2016) who found that consumption of undigestible starch (37.50 g/100 g total starch) enriched in starchy foods stimulates the release of GLP-1 and PYY in healthy volunteers. GLP-1 and PYY are synthesized and released from L-cell of distal small and large intestine in the response to a nutrient load (De Silva & Bloom, 2012). Releasing these hormones induce satiety sensation and suppress food intake through various mechanisms such as delaying gastric emptying rate and reducing gastrointestinal motility and signal in the gut-brain axis (De Silva & Bloom, 2012). As previously referred, undigestible starch such as dietary fiber and resistant starch was able to increase the emptying time (Rosén, Östman, & Björck, 2011; Stefoska-Needham et al., 2016). This caused the prolonged presence of nutrients in gastrointestinal tract to interact with gut receptors for stimulating the release of GLP-1 and PYY through its ability to hold water and thus altering chyme viscosity and delaying gastric (Mudgil & Barak, 2013).

The subjective rating scores of fullness, hunger, and desired to eat after noodles consumption are demonstrated in Figure 18A, C, and E. As compared to baseline, both noodles significantly increased fullness scores and decreased hunger and desired to eat scores. The fullness sensation significantly increased at 60, 90, and 120 min following RD43 rice noodles ingestion. However, significant lower hunger and desire to eat were observed at 30, 60, 90, and 120 min after the consumption of RD43 rice noodle in comparison to the control noodle. The iAUCs of fullness, hunger, and desired to eat indicated that consumption of RD43 rice noodles led to decrease hunger in and desire to eat and increased fullness (Figure 18B, D, and F). In general, appetite sensation is regulated by orexigenic and anorexigenic hormones (De Silva & Bloom, 2012). The increase of PYY level after consumption of starchy diet containing undigestible starch (54.79 g/100g total starch) had a correlation with increasing fullness rating scores in lean subjects (Gentile et al., 2015). We suggest that the RD43 rice noodle is able to modulate appetite sensation through an elevation of postprandial plasma GLP-1 and PYY level.

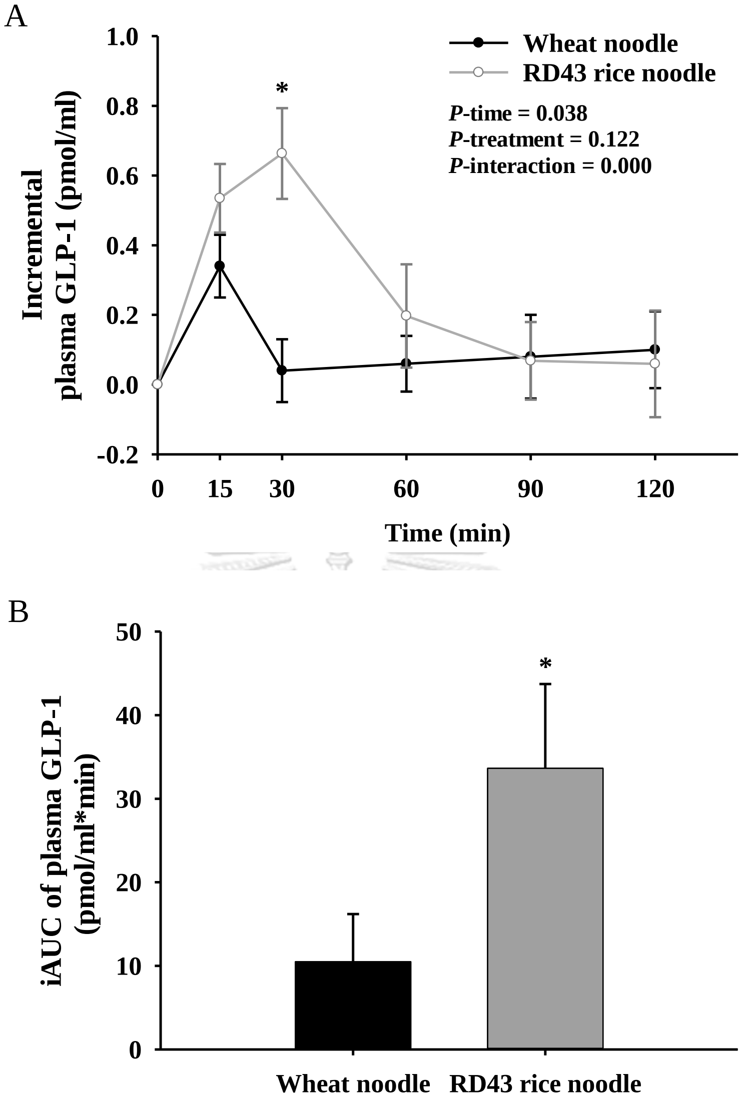

Figure 16 Incremental change from baseline of postprandial plasma GLP-1 (A) and the incremental area under the curve (iAUC) of postprandial plasma GLP-1 (B) in healthy males after consuming either wheat or 30% RD43 rice noodles. Fasting plasma GLP-1 concentration was 5.40 ± 0.17 mg/dl and 5.59 ± 0.45 mg/dl for participants who consumed wheat noodle and 30% RD43 rice noodle, respectively. Data are expressed as mean ± SEM, n = 15. * $p < 0.05$ compared to the wheat noodles.

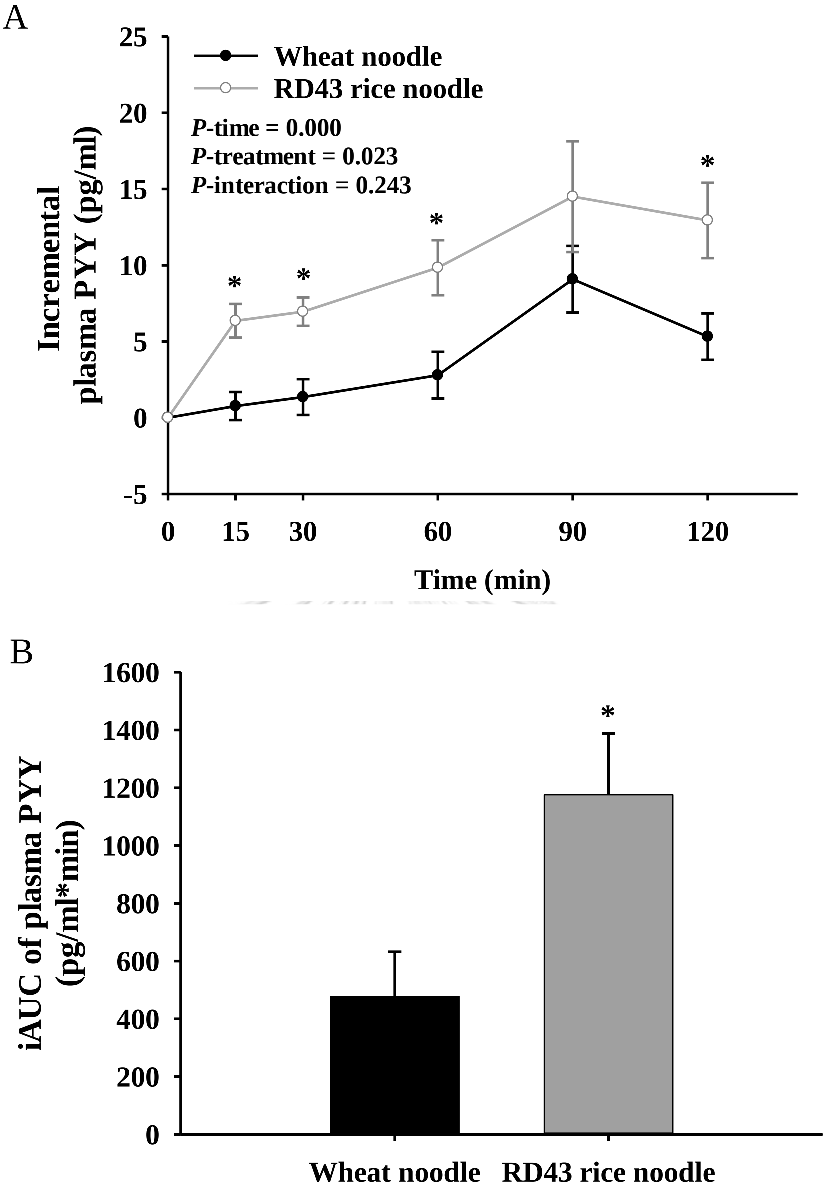

Figure 17 Incremental change from baseline of postprandial plasma PYY (A) and the incremental area under the curve (iAUC) of postprandial plasma PYY (B) in healthy males after consuming either wheat or 30% RD43 rice noodles. Fasting plasma PYY concentration was 72.90 ± 1.64 mg/dl and 73.62 ± 2.13 mg/dl for participants who consumed wheat noodle and 30% RD43 rice noodle, respectively. Data are expressed as mean ± SEM, *n* = 15. * $p < 0.05$ compared to the wheat noodles.

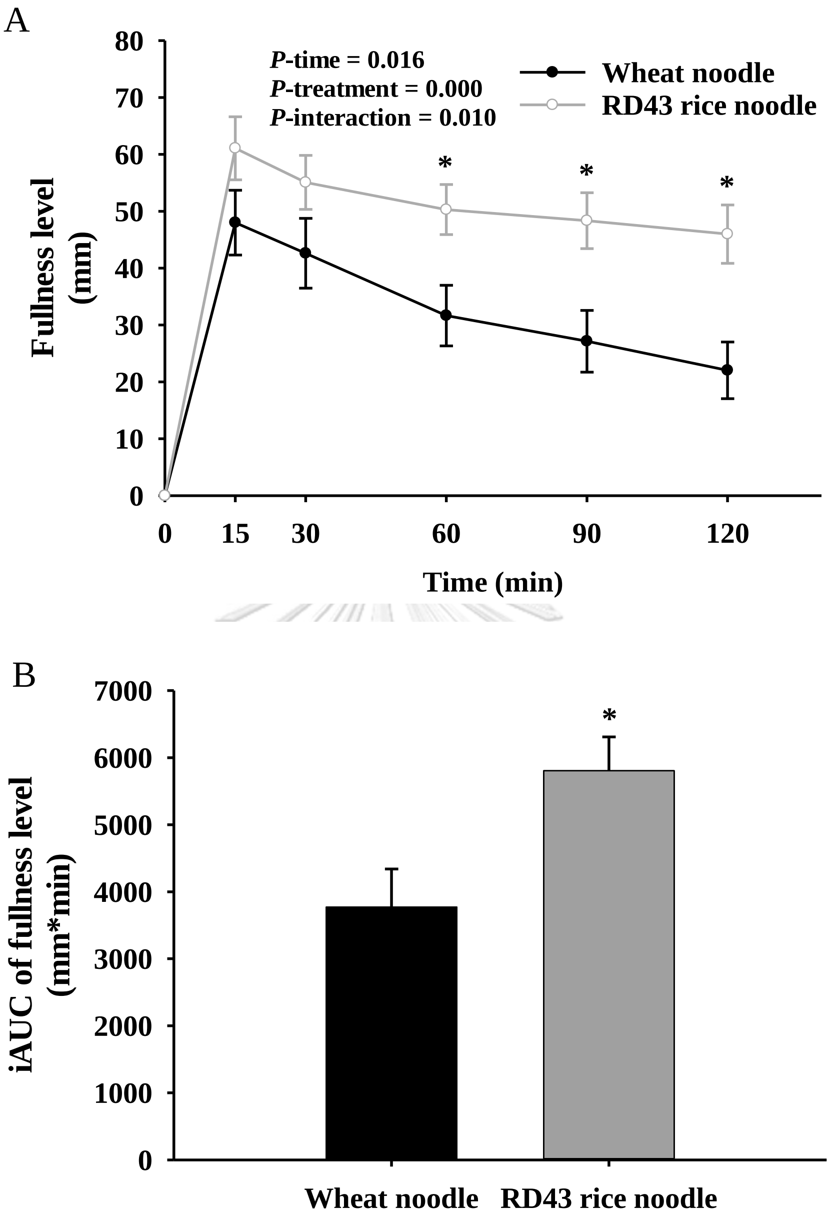

Figure 18 Changes in appetite sensation including fullness (A), hunger (C), and desired to eat (E). The incremental area under the curve (iAUC) of fullness (B), hunger (D), and desired to eat (F) in healthy males after consuming either the wheat or RD43 rice noodles. Data are expressed as mean ± SEM, *n* = 15. * $p < 0.05$ compared to the wheat noodles.

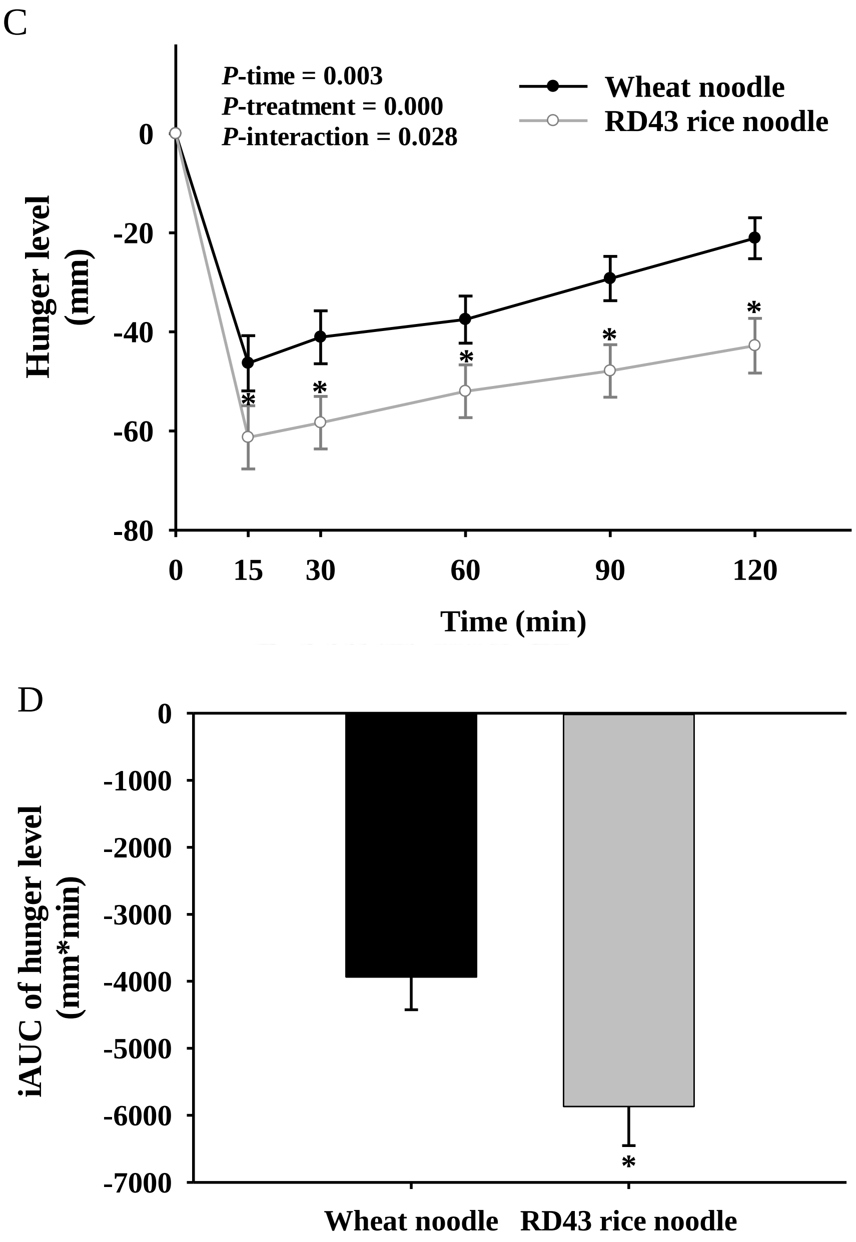

Figure 18. Changes in appetite sensation including fullness (A), hunger (C), and desired to eat (E). The incremental area under the curve (iAUC) of fullness (B), hunger (D), and desired to eat (F) in healthy males after consuming either the wheat or RD43 rice noodles. Data are expressed as mean ± SEM, *n* = 15. * *p* < 0.05 compared to the wheat noodles.

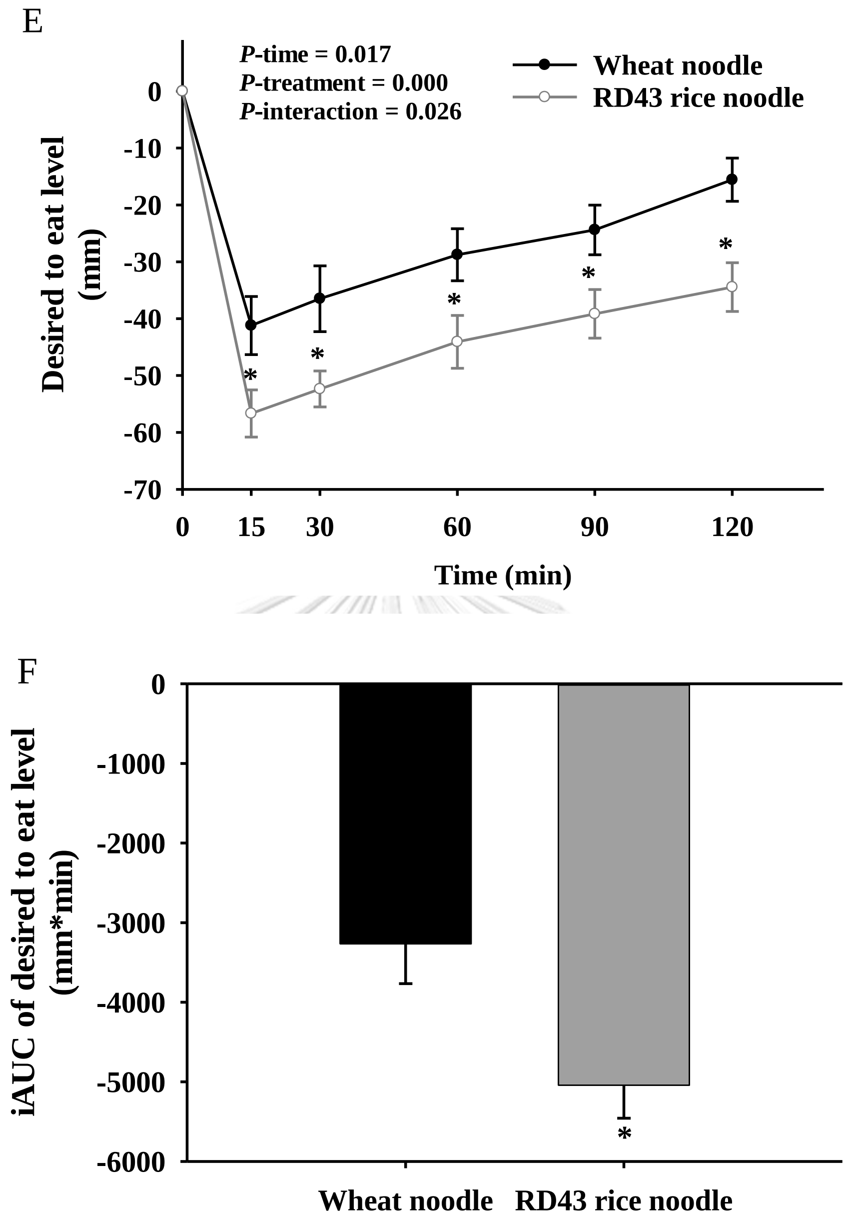

Figure 18. Changes in appetite sensation including fullness (A), hunger (C), and desired to eat (E). The incremental area under the curve (iAUC) of fullness (B), hunger (D), and desired to eat (F) in healthy males after consuming either the wheat or RD43 rice noodles. Data are expressed as mean ± SEM, n = 15. * $p < 0.05$ compared to the wheat noodles.

4.7 Conclusion

The substitution of RD43 rice flour (30%) in wheat noodles reduces RDS and increases undigestible starch with a slight decrease in WA and SI. Moreover, it does not alter cooking loss with improvement of the lightness and redness at sensory acceptable level. Consumption of RD43 rice noodles reduces postprandial plasma glucose and appetite sensation by increasing the level of GLP-1 and PYY. From our discovery, RD43 rice flour can be used as an alternative raw material for increasing the quality of noodles and controlling glycemic response and appetite sensations.

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CHAPTER V

CONCLUSION

RD43, an *indica* Thai rice cultivars, was developed by cross-breeding between Khao Jow Hawm Suphan Buri and Supan Buri1. It has special characteristics related to consumer acceptance such as soft texture and mild fragrance and biological properties such as lowering glycemic response. Replacement of conventional white rice with RD43 rice for 12 weeks resulted in attenuation of fasting glucose and insulin, HbA1c and HOMA-IR as well as body composition, such as total fat mass, waist circumference, body weight, and BMI in individual overweight participants with prediabetes. However, consumption of RD43 rice did not affect lipid profiles level in those participants. When compared to conventional white rice, intake of RD43 rice for 12 weeks markedly reduces fasting plasma insulin and HOMA-IR.

Regarding to the benefits for health, RD43 rice demonstrated potential to apply in food products. The investigation of physicochemical properties of RD43 rice flour showed a higher amylose content with a smaller particle size than Hom Mali rice flour. It displayed lower lightness value concomitant with higher values of redness and yellowness than Hom Mali rice flour. RD43 rice flour presented 24.55% of crystallinity with V-type crystalline structure. This structure influenced hydration, thermal properties, and pasting properties. of RD43 rice flour. Moreover, RD43 rice flour had lower starch digestibility and greater ability to bind bile acid and disrupt the formation of cholesterol micellization than Hom Mali rice flour. The application of RD43 rice flour in steamed muffin led to lower starch digestibility without affecting the sensory acceptability.

Owing to the maintaining structural characteristics of RD43 rice flour such as V-type crystalline structure, low water solubility index, and huge-setback value, indicating an appropriate application in noodles. The substitution of 30% RD43 rice flour in wheat noodles decreased rapidly digestible starch content and increased undigestible starch content with slightly reduced in water absorption and swelling index. Moreover, it did not alter cooking loss with improvement of the values of lightness, redness, and adhesiveness at sensory acceptable level. Furthermore, consumption of RD43 rice noodles attenuated postprandial plasma glucose and increased plasma GLP-1 and PYY concentration, resulting in appetite suppression in healthy men.

In summary, RD43 rice may be potentially used as a staple food as well as an ingredient in order to maintain the structure of product together with reduction in glycemic response, appetite, and body composition in humans.

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APPENDIX

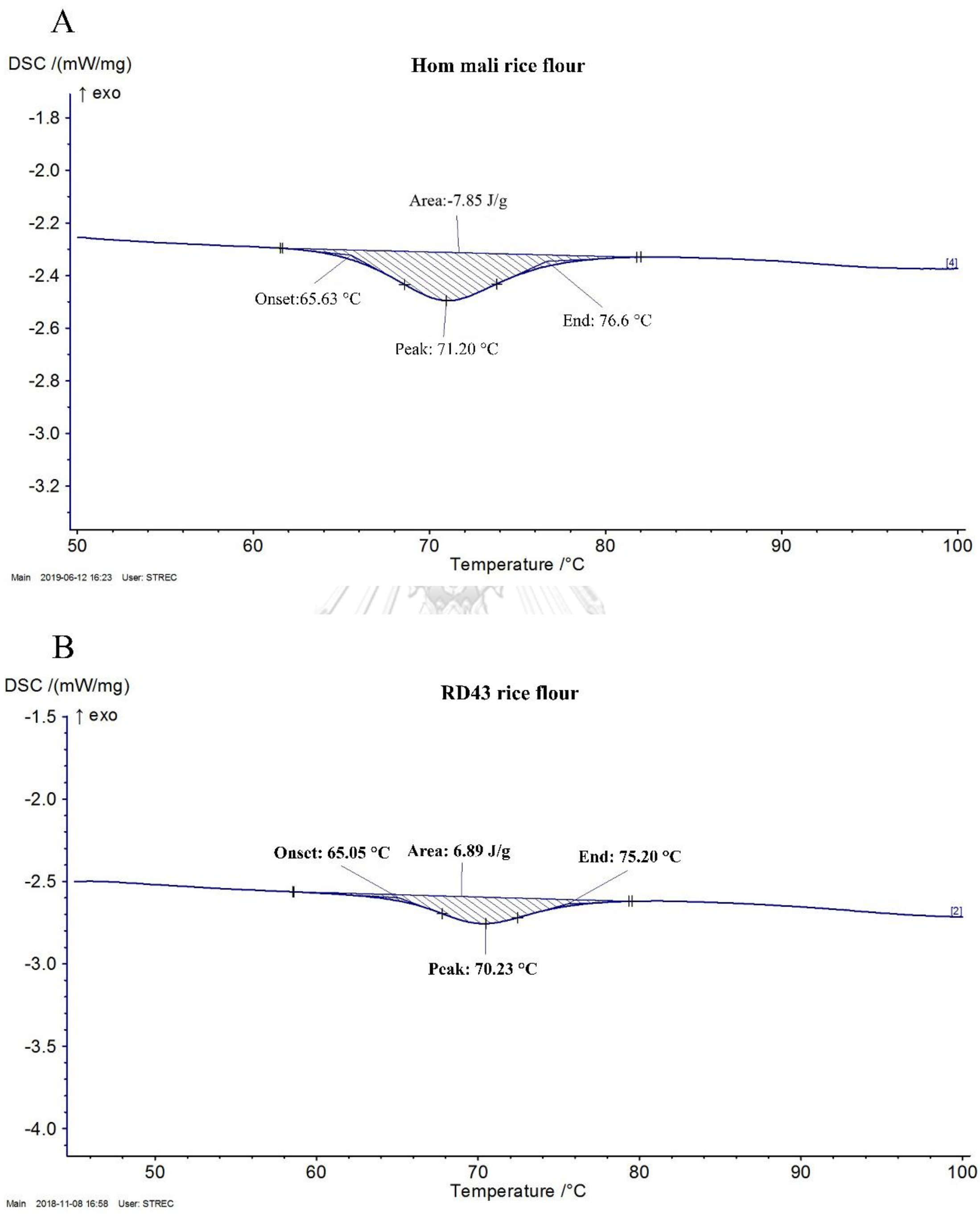

Figure 1. Differential scanning calorimetry (DSC) curves of Hom Mali and (A) RD43 (B) rice flour.

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