BIOACTIVE *CLITORIA TERNATEA* L. FLOWER EXTRACT FOR THE GLYCEMIC CONTROL TO CARBOHYDRATE FOODS AND SUGARY BEVERAGES

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Food and Nutrition
Department of Nutrition and Dietetics
Faculty of Allied Health Sciences
Chulalongkorn University
Academic Year 2017

สารสกัดจากดอกอัญชันที่มีฤทธิ์ทางชีวภาพต่อการควบคุมน้ำตาลในอาหารประเภทคาร์โบไฮเดรตและเครื่องดื่มที่มีน้ำตาล

นางสาวจรูญศรี ชูศักดิ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาอาหารและโภชนาการ ภาควิชาโภชนาการและการกำหนดอาหาร
คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2560

Thesis Title	BIOACTIVE *CLITORIA TERNATEA* L. FLOWER EXTRACT FOR THE GLYCEMIC CONTROL TO CARBOHYDRATE FOODS AND SUGARY BEVERAGES
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จรูญศรี ชูศักดิ์ : สารสกัดจากดอกอัญชันที่มีฤทธิ์ทางชีวภาพต่อการควบคุมน้ำตาลในอาหารประเภทคาร์โบไฮเดรตและเครื่องดื่มที่มีน้ำตาล (BIOACTIVE *CLITORIA TERNATEA* L. FLOWER EXTRACT FOR THE GLYCEMIC CONTROL TO CARBOHYDRATE FOODS AND SUGARY BEVERAGES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.สิริชัย อดิศักดิ์วัฒนา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร.Christiani Jeyakumar Henry, หน้า.

ดอกอัญชันเป็นพืชชนิดหนึ่งที่อุดมไปด้วยสารแอนโทไซยานิน ปัจจุบันมีการใช้เป็นแหล่งของสารให้สีธรรมชาติในการผสมอาหาร เครื่องดื่มและขนมหวานทั่วโลก โดยรายงานก่อนหน้าระบุถึงความหลากหลายทางชีวภาพของดอกอัญชัน ประกอบด้วยฤทธิ์ในการต้านอนุมูลอิสระและต้านการเพิ่มขึ้นของระดับน้ำตาลในเลือด แต่อย่างไรก็ตาม การใช้ดอกอัญชันในอาหารและเครื่องดื่มมีความเข้มข้นที่หลากหลาย รวมทั้งยังไม่มีรายงานเกี่ยวกับผลของความเข้มข้นของดอกอัญชันต่อการย่อยของแป้งและการตอบสนองต่อน้ำตาลในเลือด ในการศึกษาครั้งนี้ จึงศึกษาผลของอาหารและเครื่องดื่มที่มีสารสกัดจากดอกอัญชันต่อการย่อยของแป้งในหลอดทดลอง การตอบสนองของน้ำตาลในเลือดและสารต้านอนุมูลอิสระหลังรับประทานอาหารในมนุษย์ รวมทั้งการยอมรับทางประสาทสัมผัส ผลการศึกษาพบว่าสารสกัดจากดอกอัญชัน (1 และ 2 กรัม) ช่วยยับยั้งระดับน้ำตาลในเลือดและอินซูลินหลังรับประทานอาหารอย่างมีนัยสำคัญทางสถิติ พร้อมทั้งเพิ่มระดับสารต้านอนุมูลอิสระในเลือดหลังรับประทานร่วมกับเครื่องดื่มที่มีน้ำตาลซูโครส อีกทั้งสารสกัดจากดอกอัญชันไม่ก่อให้เกิดภาวะระดับน้ำตาลในเลือดต่ำขณะอดอาหาร นอกจากนี้ร้อยละ 1 และ 2 (โดยน้ำหนักต่อปริมาตร) ของสารสกัดจากดอกอัญชัน ยับยั้งการทำงานของเอนไซม์แอลฟาอะไมเลส โดยใช้แป้งมัน แป้งมันสำปะหลัง แป้งข้าวเจ้า แป้งข้าวโพด แป้งข้าวสาลีและแป้งข้าวเหนียวเป็นสารตั้งต้น เพราะฉะนั้นร้อยละ 0.5 1 และ 2 (โดยน้ำหนักต่อปริมาตร) ของสารสกัดจากดอกอัญชัน แสดงให้เห็นถึงการช่วยลดลง ของการปลดปล่อยน้ำตาลกลูโคส ค่าดัชนีการย่อยของแป้งและค่าดัชนีน้ำตาลของแป้งที่ได้จากการคำนวณอย่างมีนัยสำคัญทางสถิติ การวิเคราะห์ทางสถิติแสดงให้เห็นถึงความสัมพันธ์ในเชิงบวกอย่างมีนัยสำคัญทางสถิติระหว่างร้อยละของสารสกัดจากดอกอัญชันและแป้งที่ไม่ถูกย่อยด้วยเอนไซม์ของแป้งข้าวสาลีและแป้งมันสำปะหลัง การเพิ่มสารสกัดจากดอกอัญชันร้อยละ 5 10 และ 20 (โดยน้ำหนักต่อน้ำหนัก) ลดอัตราการย่อยของแป้งในขนมปังที่ทำจากข้าวสาลีอย่างมีนัยสำคัญทางสถิติ ท้ายที่สุดการผสมของสารสกัดจากดอกอัญชันที่ร้อยละ 2.5 (โดยน้ำหนักต่อปริมาตร) ทำให้การย่อยของแป้งในข้าวหุงสุกลดลง พร้อมทั้งมีการยอมรับโดยรวมที่ดี ทั้งวิธีการหุงด้วยหม้อหุงข้าวและเตาอบไมโครเวฟ สิ่งที่น่าสนใจสารสกัดจากดอกอัญชันช่วยลดค่าดัชนีน้ำตาลของข้าวหุงสุกลงร้อยละ 13 การบริโภคข้าวหุงสุกที่มีสารสกัดจากดอกอัญชัน ทำให้ระดับน้ำตาลในเลือดหลังรับประทานอาหารลดลงในคนที่มีสุขภาพดี ดังนั้นสารสกัดจากดอกอัญชันอาจเป็นส่วนประกอบหนึ่งที่มีประโยชน์ในการลดการตอบสนองต่อระดับน้ำตาลในเลือดต่ออาหารและเครื่องดื่มที่มีคาร์โบไฮเดรตสูง

ภาควิชา	โภชนาการและการกำหนดอาหาร	ลายมือชื่อนิสิต	.....
สาขาวิชา	อาหารและโภชนาการ	ลายมือชื่อ อ.ที่ปรึกษาหลัก	.....
ปีการศึกษา	2560	ลายมือชื่อ อ.ที่ปรึกษาร่วม	.....

5777051837 : MAJOR FOOD AND NUTRITION
KEYWORDS: CLITORIA TERNATEA / GLYCEMIC CONTROL / FOODS AND BEVERAGES / CARBOHYDRATE / ANTHOCYANINS

CHAROONSRI CHUSAK: BIOACTIVE *CLITORIA TERNATEA* L. FLOWER EXTRACT FOR THE GLYCEMIC CONTROL TO CARBOHYDRATE FOODS AND SUGARY BEVERAGES. ADVISOR: ASSOC. PROF. SIRICHAI ADISAKWATTANA, Ph.D., CO-ADVISOR: PROF. CHRISTIANI JEYAKUMAR HENRY, Ph.D., pp.

Clitoria ternatea L. flower, an edible plant containing anthocyanins, has been currently used as the source of natural colorant in foods, beverages and desserts worldwide. The previous report demonstrated its biological properties including antioxidant and antihyperglycemic activity. However, the various concentrations of *Clitoria ternatea* were used in food and beverages but the effective concentration of *Clitoria ternatea* on the digestibility of starch and glycemic response remains unknown. In this study, the effects of foods and beverage containing *Clitoria ternatea* L. flower extract (CTE) on starch digestibility *in vitro*, postprandial glycemic response and antioxidant capacity in human as well as sensory acceptability were investigated. The results showed that CTE (1 and 2 g) significantly suppressed postprandial plasma glucose and insulin levels with enhanced antioxidant status after consumption of sucrose beverage. Moreover, CTE did not cause hypoglycemia in the fasting state. Likewise, the 1% and 2% (w/v) CTE inhibited the pancreatic a-amylase activity when using potato, cassava, rice, corn, wheat and glutinous rice flours as a substrate. Consequently, 0.5%, 1% and 2% (w/v) CTE showed a significant reduction in the glucose release, hydrolysis index (HI) and predicted glycemic index (pGI) of all flours. Statistical analysis demonstrated strong positive significant correlation between the percentage of CTE and the undigested starch of wheat and cassava flours. The addition of 5%, 10% and 20% (w/w) CTE significantly reduced the rate of starch digestion of wheat bread. Finally, the incorporation of 2.5% (w/v) CTE caused a reduction in starch digestibility of cooked rice with a good overall acceptability using an electric rice cooker and a microwave oven. Interestingly, CTE decreased the glycemic index (GI) of cooked rice by 13%. Consumption of cooked rice with CTE caused a reduction in postprandial blood glucose in healthy subjects Therefore, CTE may potentially be a useful ingredient to reduce glycemic response in high carbohydrate foods and beverages.

Department:	Nutrition and Dietetics	Student's Signature	.....
Field of Study:	Food and Nutrition	Advisor's Signature	.....
Academic Year:	2017	Co-Advisor's Signature	.....

ACKNOWLEDGEMENTS

First and foremost, I would like to express my special appreciation and thanks to my advisor, Associate Professor Sirichai Adisakwattana, Ph.D., for giving me the amazing opportunity to study Ph.D. and providing invaluable guidance through my university's life. He always supported me not only by assisting the academic and research careers but also by giving me the wonderful chances.

I would also like to thank my co-advisor, Professor Christiani Jeyakumar Henry, Ph.D., for his helpful and for giving me the precious experience to do research at Clinical Nutrition Research Centre (CNRC), Singapore Institute for Clinical Sciences (SICS), Agency for Science, Technology and Research (A*STAR), Singapore.

I am many thanks to my committees, Assistant Professor Suwimol Sapwarobol, DrPH., Assistant Professor Sathaporn Ngamukote, Ph.D., Kittana Mäkynen, Ph.D., as well as Assistant Professor Chatrapa Hudthagosol, DrPH., for their suggestion, motivation and kindness.

Many thanks go to my lab mates in Adisakwattana research group for sharing the knowledge with me, making a nice working atmosphere and friendship. I would also like to thank members in CNRC for their help and warm friendship while I was in Singapore.

Finally, I am extremely grateful to my mother, Pacharin Chusak, my father, Chanchai Chusak, and my brother, Kriangkrai Chusak for their love, understanding and always continuing support to complete my study and my life.

Special mention goes to all of my subjects to participate on this study, The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship, Overseas Research Experience Scholarship for Graduate Student, and the 90th Anniversary Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund), Graduate school and Faculty of Allied Health Sciences, Chulalongkorn University.

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CHAPTER I

INTRODUCTION

1.1 Background and significant of the study

Carbohydrates, the primary energy source, are accounted for 40-80% of total caloric intake in foods. However, an intake of high carbohydrate diet is regularly considered harmful with respect to energy imbalance because of their specific properties associated with postprandial metabolism (Aller, Abete, Astrup, Martinez, & Baak, 2011). The concept of GI is an instrument to classify carbohydrate foods as an index of the acute postprandial glycemic response of fixed amount of available carbohydrate from test food to the same amount of available carbohydrate from a standard food (Jenkins et al., 1981). The consumption of food having a high GI induce a rapid increase in plasma glucose and insulin secretion (Jenkins et al., 1981). In contrast, an intake of low GI foods causes the slower rate of digestion and absorption than high GI foods, resulting in attenuated postprandial glycemia and insulinemia. A number of evidences suggested that consumption of low GI diets leads to reduced risks of obesity, diabetes and cardiovascular diseases (Brand-Miller, McMillan-Price, Steinbeck, & Caterson, 2009).

The predicted glycemic index (pGI) is a common technique used to measure the rate of carbohydrate hydrolysis in foods (Goñi, Garcia-Alonso, & Saura-Calixto, 1997). It has been found that *in vitro* methods used to classify foods based on their digestion

characteristics are similar to the *in vivo* situation (Jenkins et al., 1981). There is a positive correlation between *in vitro* and *in vivo* glycemic response (Goñi et al., 1997). In the focus of nutritional aspect, carbohydrate foods with low pGI value (<55) as the rate of carbohydrate hydrolysis in food can be considered as beneficial foodstuff for human health in term of prevention and treatment of the metabolic syndromes, diabetes and cardiovascular diseases (Englyst, Veenstra, & Hudson, 1996; O'dea, Snow, & Nestel, 1981).

Several studies have shown that the reduced rate of carbohydrate absorption and digestion is a significant strategy to suppress postprandial hyperglycemia by inhibition of carbohydrate digestive enzymes such as pancreatic α-amylase and intestinal α-glucosidase (Boivin, Flourie, Rizza, Go, & DiMagno, 1988; Englyst & Englyst, 2005; McDougall et al., 2005). Interestingly, plant bioactive compounds such as polyphenols and anthocyanins have been shown to inhibit pancreatic α-amylase and α-glucosidase activity (Adisakwattana et al., 2010; McDougall et al., 2005). For example, phenolic-enriched black rice flour extracts delayed starch hydrolysis by inhibiting carbohydrate digestive enzymes (An, Bae, Han, Lee, & Lee, 2016). After consumption of berry puree with sucrose, the amplitude and the peak postprandial plasma glucose were significantly lower than the consumption of sucrose alone in healthy subjects (Torronen et al., 2010).

The replacing or mixing flours with other ingredients such as fruits and vegetables is one of alternative approaches to reduce pGI in carbohydrate foods (Dewettinck et al., 2008). For example, pomelo incorporated bread showed lower level of predicted glycemic index probably by inhibiting carbohydrate hydrolyzing enzyme activity which could be attributed to polyphenols (Reshmi, Sudha, & Shashirekha, 2017). It is becoming clear that plant-based ingredients containing polyphenols delay carbohydrate digestive enzymes and thereby reducing the absorption rate of glucose (Barrett et al., 2013; Gao et al., 2008; Jeng et al., 2015).

Furthermore, consumption of plant-based antioxidants improved plasma antioxidant capacity in human subjects (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005). It has been demonstrated that consumption of berries, apples, mixed grapes or kiwifruits was associated with increased postprandial plasma antioxidants including oxygen radical absorbance capacity (ORAC) and ferric-reducing ability of plasma (FRAP) (Godycki-Cwirko et al., 2010; Nälsén, Basu, Wolk, & Vessby, 2006; Prior et al., 2007). Therefore, plant-based antioxidant may be considered as a rich source of natural antioxidants for protecting postprandial oxidative stress.

Clitoria ternatea L. or Butterfly pea is a plant species belonging to the Fabaceae family. This plant is the most widely distributed in tropical zones such as Asia, the Caribbean, Central and South America. In traditional Ayurvedic medicine, *Clitoria ternatea* L. is a well-known plant used for treatment of stress and depression

(Mukherjee, Kumar, Kumar, & Heinrich, 2008). It has been many pharmacological activities reported for this plant, such as antidiabetic (Talpate, Bhosale, Zambare, & Somani, 2013), antipyretic (Parimaladevi, Boominathan, & Mandal, 2004), anti-inflammatory (Mukherjee et al., 2008) and antimicrobial activities (Kamilla, Mnsor, Ramanathan, & Sasidharan, 2009). Several phenolic compounds have been found in the flower of *Clitoria ternatea* such as rutin, kaempferol, delphinidin and related glycosides (Terahara et al., 1996). A previous study showed the ability of *Clitoria ternatea* extract (CTE) to inhibit carbohydrate digestive enzymes including intestinal maltase, sucrease and pancreatic α-amylase (Adisakwattana, Ruengsamran, Kampa, & Sompong, 2012). Pasukamonset *et al*. reported that addition of *Clitoria ternatea* in cooked pork patties reduced lipid peroxidation and rancidity (Pasukamonset, Kwon, & Adisakwattana, 2017). In Asian, natural anthocyanin pigments of *Clitoria ternatea* flower had been widely used in foods, beverages and desserts. This colorant flower is regularly mixed with rice, bread, cookies, flours and other traditional foods and desserts with a different ratios. Although the pancreatic α-amylase and α-glucosidase inhibitory activity of CTE is well-documented, the effective concentration of *Clitoria ternatea* on the digestibility of starch and glycemic response remains unknown. Therefore, the effects of foods and beverage containing *Clitoria ternatea* L. flower extract (CTE) on starch digestibility *in vitro*, postprandial glycemic and antioxidant capacity in human as well as sensory acceptability were investigated.

1.2 The objectives of the study

- To determine the effects of the *Clitoria ternatea* L. flower extract (CTE) on postprandial glycemic response and antioxidant status in healthy subjects

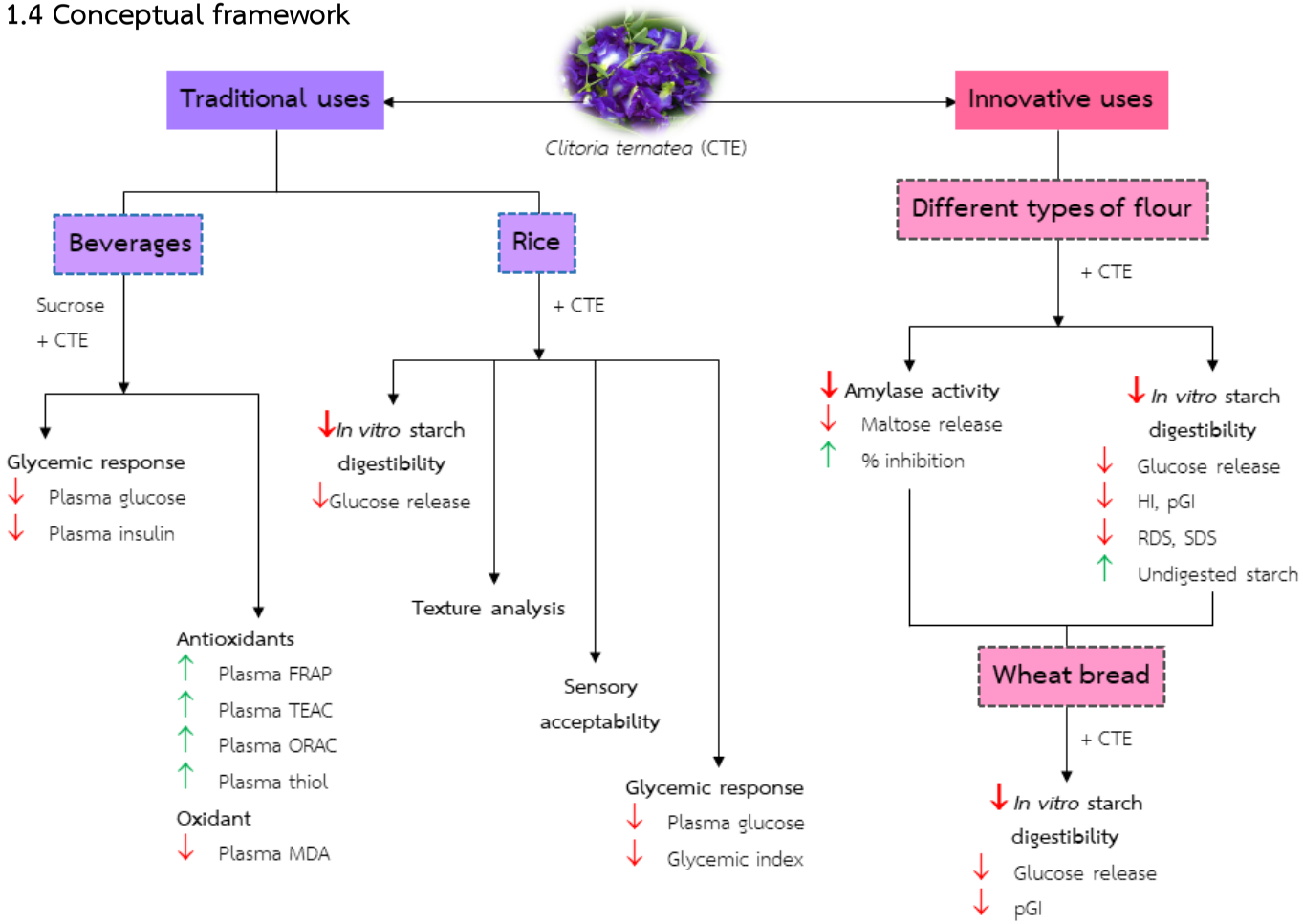
- To assess the effect of the *Clitoria ternatea* L. flower extract (CTE) on the inhibition of pancreatic α-amylase, *in vitro* starch hydrolysis and predicted glycemic index of different type of flours including potato, cassava, rice, corn, wheat and glutinous rice flour and to determine the application in a bakery product prepared from flour and CTE

- To evaluate the effectiveness of the *Clitoria ternatea* L. flower extract (CTE) incorporation into rice on *in vitro* and *in vivo* starch digestibility of cooked rice using domestic cooking methods and to determined textural properties and sensory evaluation of cooked rice with CTE

1.3 Hypotheses of the study

- *Clitoria ternatea* L. flower extract might improve in postprandial glycemic response and antioxidant status in healthy subjects
- The addition of *Clitoria ternatea* L. flower extract might reduce *in vitro* starch hydrolysis and predicted glycemic index of potato, cassava, rice, corn, wheat and glutinous rice flour and also improve the application in a bakery product prepared from flour and CTE.
- The incorporation of *Clitoria ternatea* L. flower extract into rice might decrease *in vitro* and *in vivo* starch digestibility of cooked rice using domestic cooking methods with acceptable sensory properties.

1.4 Conceptual framework



HI: Hydrolysis index; pGI: Predicted glycemic index; RDS: Rapidly digestible starch; SDS: Slowly digestible starch

CHAPTER II

REVIEW OF LITERATURE

2.1 Carbohydrate

Carbohydrate accounted for 40-80% of total caloric intake in various forms is common components of foods both naturally and as added ingredients, causing them the most important energy source. However, high carbohydrate intake and sugar consumption are regularly considered harmful with respect to energy imbalance because of their specific properties associated with postprandial metabolism, the balance between nutrient storage and oxidation and the effect on hunger and satiety (Aller et al., 2011).

2.1.1 Carbohydrate metabolism

Digestion of carbohydrate initiates in mouth by salivary α-amylase that hydrolyses α-1,4 linkage of starch and then convert to maltose (Dashty, 2013). The next enzyme is pancreatic α-amylase in small intestine that can digest approximately 60% of starches. Intestinal epithelial cell enzymes degrade 6-carbon carbohydrates. These specific enzymes such as maltase, sucrase and lactase degrade maltose, sucrose and lactose, respectively (Dashty, 2013; Englyst & Englyst, 2005). After these specific enzymes activity, monosaccharide units are produced and absorbed to blood stream via passive and active transport systems. After that, glucose is used in different

metabolic pathways for stability of blood sugar in the hypoglycemic state, energy supplier of the peripheral tissues, energy storage in the liver and skeleton muscle in the form of glycogen, energy storage in the adipose tissue following conversion to triglycerides and also stability of body temperature (Dashty, 2013). Moreover, glucose is up taken to tissue by activation of insulin, the blood glucose falls back to fasting levels. The amount of the rise and fall in plasma glucose and the duration over it occurs has been indicated as glycemic response.

2.1.2 Carbohydrate and oxidative stress

Macronutrient composition including carbohydrate, lipid and protein of the diet may differentially modify the postprandial pro-oxidative status (Gregersen, Samocha-Bonet, Heilbronn, & Campbell, 2012). In term of carbohydrate diet, high carbohydrate intake and sugar consumption are regularly considered harmful with respect to energy imbalance because of their specific properties associated with postprandial metabolism and the balance between nutrient storage and oxidation (Aller et al., 2011). Considering, hyperglycemia or high blood glucose is a major concern for oxidative stress in which imbalance between radicals and antioxidant defense. High concentration of glucose induce the formation of reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) and decrease in the capacity of antioxidants enzymes (Tousoulis et al., 2011) in several mechanisms (Figure 2.1). Moreover, excessive production and accumulation of free radicals in our body can

react with non-radicals, inducing DNA damage, lipid peroxidation and protein alteration (Shah, Iqbal, Karam, Salifu, & McFarlane, 2007).

Glycation, or direct binding of glucose to protein, can generate advanced glycation end products (AGEs) which can inhibit mitochondrial respiration and increase the formation of intracellular reactive oxygen species (ROS) and inflammatory cytokines which impair vascular function (Campos, 2012). Previous reports stated that hyperglycemia induced oxidative stress and cause hyperglycemic damage via four major pathways including polyol pathway, hexokinase pathway, protein kinase C pathway and advanced glycation end product (AGE) pathway which are indicated as an important pathway of microvascular and macrovascular complication as well as endothelial dysfunction (Choi, Benzie, Ma, Strain, & Hannigan, 2008; Rolo & Palmeira, 2006; Shah et al., 2007). In accordance with *in vitro* and *in vivo* reviews, acute effect of hyperglycemia caused free radicals production and induced endothelial dysfunction (Campos, 2012; Shah et al., 2007).

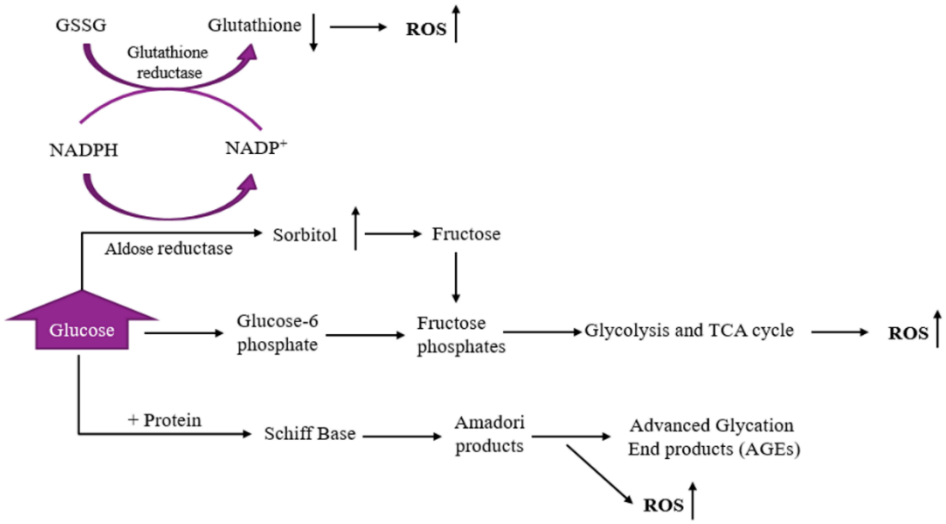


Figure 2.1 Hyperglycemia-induced oxidative stress (Campos, 2012)

In human study, high consumption of carbohydrate caused to increase postprandial plasma glucose and serum insulin and decrease postprandial plasma total antioxidative status and muscle Cu/Zn-superoxide dismutase in healthy individuals (Gregersen et al., 2012). Another study found that consumption a glucose solution and a carbohydrate-containing meal caused the reduction in postprandial plasma protein thiol groups, vitamin C, vitamin E, uric acid and total free radical trapping capacity in both healthy and diabetic volunteers (Ceriello et al., 1998). Therefore, hyperglycemia condition have stimulated free radicals and ROS production and inhibited antioxidant defense mechanism.

2.2 Glycemic index

The concept of a glycemic index (GI) is an instrument to provide a numeric classification of carbohydrate foods. The GI is showed as an index of the acute postprandial glycemic response of fixed amount of available carbohydrate from test food to the same amount of available carbohydrate from a standard food (glucose or with bread) consumed by the same subject (Jenkins et al., 1981). The GI has been supposed that the chemical composition of carbohydrate foods could predict the plasma glucose and insulin responses after consumption of carbohydrate diets. Carbohydrate containing foods are classified into low (<56), medium (56-69) and high (>69) GI categories as shown in Table 2.1 (Jenkins et al., 1981). As report in the observational studies, the results indicated that increasing dietary GI has been associated with higher prevalence of insulin resistance, metabolic syndrome, fatty liver and metabolic risk factors (Liu et al., 2001; McKeown et al., 2004).

Table 2.1 The average GI of common carbohydrate foods

High GI foods (>69)		**Medium GI foods (56-69)**		**Low GI foods (<56)**	
Glucose	(100)	Brown rice, boiled	(68)	Udon noodle	(55)
Rice milk	(86)	Pumpkin, boiled	(64)	Rice noodle	(53)
Cornflakes	(81)	Sweet potato, boiled	(63)	Banana, raw	(51)
Rice porridge	(78)	Wheat roti	(62)	Spaghetti	(49)
Potato, boiled	(78)	Honey	(61)	Orange, raw	(43)
White wheat bread	(75)	Pineapple, raw	(59)	Yoghurt	(41)
Whole wheat bread	(74)	Muesli	(57)	Carrot, boiled	(39)
White rice, boiled	(73)			Apple, raw	(36)
				Lentil	(32)
				Barley	(28)
				Chickpeas	(28)
				Kidney bean	(24)
				Soy bean	(16)

Modified from Atkinson, Foster-Powell, and Brand-Miller (2008)

2.2.1 Effect of glycemic index on glycemic response and health

Basically, the GI of diets depend on the rate at which the carbohydrate is digested in the gastrointestinal tract and the rate of glucose absorption into the blood circulation which influence the extent and duration of the rise in glucose concentration after a meal (Englyst, Englyst, Hudson, Cole, & Cummings, 1999; Jenkins et al., 1981). In addition, quickly digested and absorbed carbohydrate as high GI diets lead to a rapid increase in plasma glucose and insulin secretion that can promote glucose uptake to counteract the rise in plasma glucose concentrations (Jenkins et al., 1981). In contrast, intake of low GI foods causes the slower rate of digestion and absorption than high GI foods, resulting in decreased postprandial glycemia and insulinemia. A number of evidences from observational prospective cohort studies, randomized controlled trials and mechanistic experiments suggested that consumption of low GI diets lead to the prevention of obesity, diabetes and cardiovascular diseases (Brand-Miller et al., 2009). Figure 2.2 represented the effect of lowering postprandial plasma glucose by consuming low GI diets on the positive health outcomes for both healthy subjects and patients with insulin resistant. The selection of low GI foods has recently displayed advantages for both patients with metabolic syndrome and healthy persons in term of postprandial glucose and lipid metabolism (Rizkalla, Bellisle, & Slama, 2002). Reduction the GI or carbohydrate content of mixed meal reduced postprandial glycemia and insulinemia in overweight and obese adults which were indicated as an effective

method for controlling plasma glucose and insulin concentration (Liu et al., 2012). Comparing with low GI meal, high GI meal bring about the higher serum insulin, higher plasma epinephrine, lower plasma glucagon and lower serum fatty acids in obese teenagers (Ludwig et al., 1999). Also, energy intake after consumption of high GI meals was 53% and 81% greater than after medium and low GI meals, respectively. This results summarized that reduction in GI meals can improve a sequence of hormonal and metabolic changes that may reduce the food intake in obese.

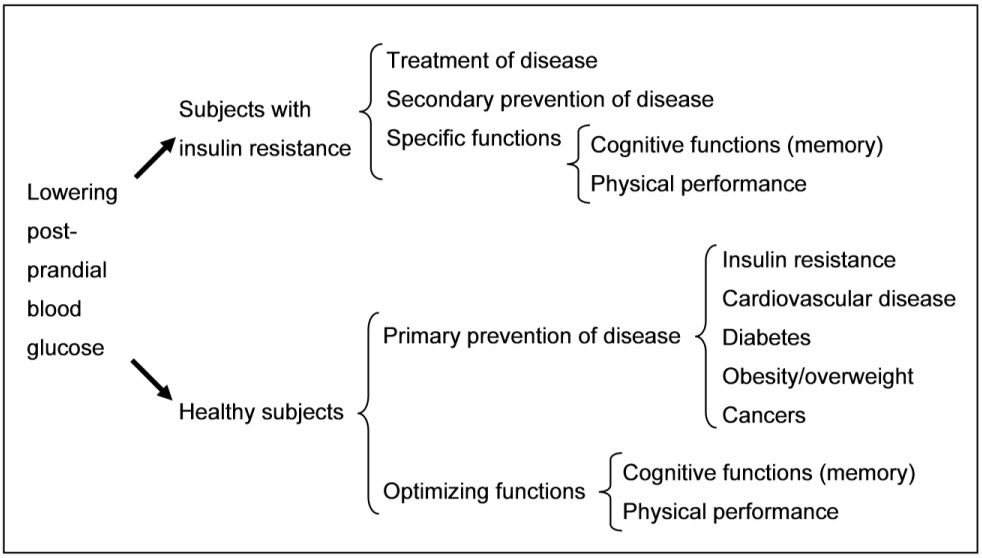


Figure 2.2 Potential health benefits of reduced postprandial plasma glucose (Lang, 2004)

2.2.2 Glycemic index in food application

As for glycemic index measurement, the *in vivo* measurement of GI requires the recruitment of human subjects under ethical committee approval, financial supports, time consuming, all these reasons led to widely accepted *in vitro* studies on starch digestibility as the predicted glycemic index (pGI) (Goñi et al., 1997; Woolnough, Monro, Brennan, & Bird, 2008). *In vitro* measurements may be regarded as reliable, simple and inexpensive tool that measure the rate of carbohydrate hydrolysis in food (Englyst et al., 1999). It has been found that *in vitro* methods used to classify foods based on their digestion characteristics are similar to the *in vivo* situation (Jenkins et al., 1981). There is a positive correlation between *in vitro* and *in vivo* glycemic response (Goñi et al., 1997). Moreover, differences of procedures during the *in vitro* digestion such as the duration of digestion and types of enzymes can represent more results which are rapidly available glucose, hydrolysis index and glycemic glucose equivalents (Englyst et al., 1999).

Carbohydrate foods with low predicted GI (pGI) value (<55) can be considered as beneficial foodstuff for human health in term of prevention and treatment of the metabolic syndromes, diabetes and cardiovascular diseases (Englyst et al., 1996; O'dea et al., 1981). In term of product application, The replacing or mixing flours with other ingredients such as fruits and vegetables is one of alternative approaches to reduce pGI in carbohydrate foods (Dewettinck et al., 2008). For example, pomelo incorporated

bread showed lower level of predicted glycemic index probably by inhibiting carbohydrate hydrolyzing enzyme activity which could be attributed to polyphenols (Reshmi et al., 2017). In addition, the substitution 94 wheat flour products with pulse ingredients (pea and lentil flour, pea protein and pea fiber) was observed a reduction in the GI value without changing palatability of the products (Fujiwara, Hall, & Jenkins, 2017).

2.3 Anthocyanins as natural pigment

Anthocyanins belong to flavonoid group of polyphenols which are the most important group of water soluble natural pigments (Castaneda-Ovando, de Lourdes Pacheco-Hernández, Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Anthocyanins consist of more than 500 compounds and their pigments are responsible for rich red and purples in many types of regularly consumed foods such as cereals, tubers, roots, pulses, flowers and fruits.

2.3.1 Structure and characteristics of anthocyanins

The structure of anthocyanins are composed of an anthocyanidin (aglycones) backbone with sugar and acyl conjugates (Tsuda, 2012). Anthocyanidins are included two aromatic benzene rings separated by an oxygenated heterocycle. Differences between several anthocyanidins found in the nature result form: (i) the number and the position of hydroxyl (OH) groups, (ii) the degree of methylation of these OH groups, (iii) the nature, number and location of sugars attached to the molecule and (iv) the

nature and number of aliphatic or aromatic acids attached to the sugar as shown in Figure 2.3 (Mazza, 2018).

Name	Substitution		Color	λ_{max} (nm) in HCl acidified MeOH
	R_1	R_2		
Cy	OH	H	magenta	535
Pn	OCH_3	H	magenta	532
Pg	H	H	red	520
Mv	OCH_3	OCH_3	purple	542
Dp	OH	OH	purple	546
Pt	OCH_3	OH	purple	543

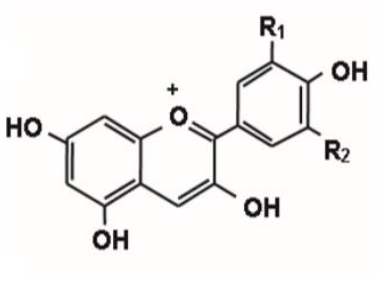


Figure 2.3 Basic structures of anthocyanins
(Kong, Chia, Goh, Chia, & Brouillard, 2003; Tsuda, 2012)

Based on difference in number of hydroxyl and methyl groups, there are six common types of anthocyanidins including pelargonidin, cyanidin (Cy), delphinidin (Del), peonidin, petunidin and malvidin as shown in Figure 2.4. The various colors of anthocyanins depend on aglycones with the B ring possessing more hydroxyl groups being more blue and more methyl groups being more red (Kong et al., 2003).

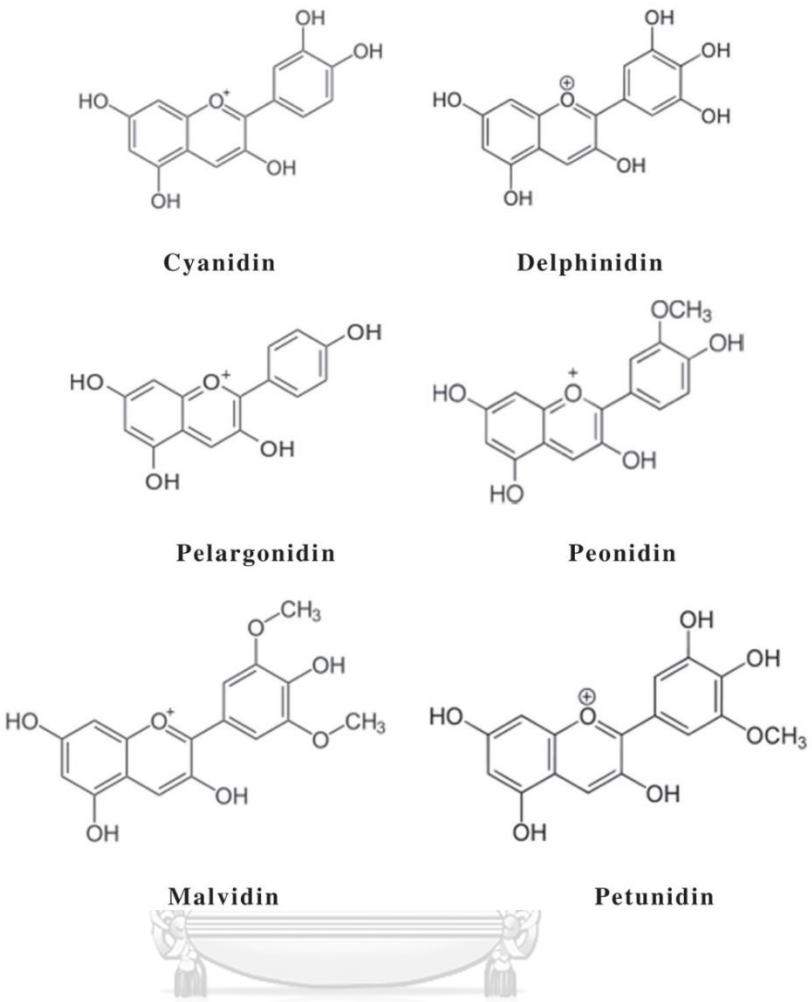


Figure 2.4 Common six anthocyanins in the nature
(Khoo, Azlan, Tang, & Lim, 2017)

2.3.2 Potential uses of anthocyanin in human diet

Anthocyanins are water-soluble plant pigments, several edible anthocyanin sources in nature involve in colored fruits, vegetables and other foods (Table 2.2). Interestingly, anthocyanins is one of the natural pigments responsible for red, blue and purple which are extracted from plants and their parts such as fruits, leaves, flowers, roots and grains (Khoo et al., 2017; Mazza, 2018). The plant pigments have been widely

used as natural food additives and colorants. Furthermore, pigments of anthocyanins also appear to be red or blue color due to acidic and basic condition, respectively. The use of natural pigments in food and beverages is considered as no or low toxicity and also essential for increasing consumer acceptability of these products (Khoo et al., 2017). Moreover, addition of natural anthocyanins pigments in food products have value-added properties due to the properties of anthocyanins including antioxidants, antihyperglycemic and antimicrobial as well as prevention and reduction of chronic diseases (Tsuda, 2012).

For the daily intake of anthocyanins, habitual diets are recognized as a major factor and the intake in human have been averaged to range between from several milligrams to hundreds of milligrams per day (Khoo et al., 2017). A report from the U.S. adults showed that the estimation of anthocyanins intake was approximately 12.5 mg/d (Wu et al., 2006). With regard to European countries, total intake of anthocyanins has estimated the range from 19.8 to 64.9 mg/d in men and 18.4 to 44.1 mg/d for women (Khoo et al., 2017). In addition, total anthocyanin content varies substantially across plant species or cultivars as shown Table 2.3. For instance, the most common anthocyanins in berries is high in cyanidins and its derivatives but low in malvidin, peonidin and petunidin (Khoo et al., 2017). Some anthocyanin beverages such as red wine generally contains glycosides of delphinidin, cyanidin, petunidin, peonidin and malvidin. Therefore, the major widespread anthocyanins in natural sources are cyanidin-3-glucoside.

Table 2.2 Edible anthocyanin sources in nature

Fruits	Vegetables	Spices	Other foods
Apples	Artichoke	Basil leaf	Cereals
Apricot	Black bean	Chili powder	Dates
Blackberry	Beets	Cinnamon	Figs
Blueberry	Broccoli	Curry powder	Ketchup
Cherries	Cauliflower	Mustard seed	Legumes
Cranberry	Corn	Paprika	Prunes
Elderberry	Eggplant	Pepper	Raisins
Gooseberry	Lettuces	Turmeric	Rice
Grapefruit	Peas		Tomato sauce
Grapes	Peppers		
Peaches	Potatoes		
Pears	Red onion		
Pineapples	Red cabbage		
Plum	Red kidney bean		
Raspberry	Red radishes		
Strawberry	Sweet potatoes		
Tangerines	Tomatoes		

Modified from Wu *et al*. (2006)

Table 2.3 Concentration of anthocyanins in edible sources

Edible sources	Total anthocyanins (mg/100 g of fresh weight or form consumed)
Apple	1.3-12.3
Blackberry	245.0
Blueberry	386.6-486.5
Cherry, sweet	122.0
Chokeberry	1,480.0
Cranberry	140.0
Black currant	476.0
Grape	26.7-120.1
Peach	4.8
Strawberry	21.2
Black bean	44.5
Eggplant	85.7
Red cabbage	322.0
Red onion	48.5

Modified from Wu *et al.* (2006)

2.3.3 Putative health-promoting effects of anthocyanins

In over the last 10 years, anthocyanin is one of the bioactive compounds as nutraceutical and traditional medicines. Anthocyanins rich plants have been applied to the traditional medicines in several countries in Asia. For traditional medicines, it is used for improvement of appetite, stimulant, diarrhea, liver diseases, urinary tract infection, choleretic agent and common cold (Khoo et al., 2017). Additionally, anthocyanins as a nutraceutical treatment have been reported in the various health benefits and the underlying molecular mechanism (Tsuda, 2012). With regard to *in vitro*, animal and human studies, the evidences stated that anthocyanins showed the relation in the prevention and suppression of obesity, diabetic, cancer, cardiovascular disease, visual function and brain function (Pojer, Mattivi, Johnson, & Stockley, 2013; Tsuda, 2012). Moreover, most abundant anthocyanins in natural sources showed the potential effect on antioxidant activity (Pojer et al., 2013). It is noted that anthocyanins showed the efficacy on the reduction in cellular lipid peroxidation and free radical oxidation, resulting in lowering the risk of several diseases (Khoo et al., 2017). According to Yang *et al*. (2017), a systematic review and meta-analysis of randomized controlled trial (RCTs) reported that the significant improvements in glycemic control and lipid support the benefits of anthocyanins in the prevention and management of cardiovascular diseases.

2.3.4 Antihyperglycemic activity of anthocyanins

Anthocyanins is one of the natural compounds that have the evidence in the inhibitory effect on carbohydrate digestive enzymes. Intestinal α-glucosidase and pancreatic α-amylase known as carbohydrate digestive enzymes are a main target of anthocyanins in order to inhibit or delay carbohydrate digestion (Belwal, Nabavi, Nabavi, & Habtemariam, 2017). The reduction in carbohydrate digestion leads to limit the availability of glucose released to the blood circulation. This mechanism is recognized as similar as some anti- diabetic drug such as acarbose. Various types of anthocyanins including cyanidin, peonidin, delphinidin and petunidin glycosides have reported the ability of carbohydrate digestive enzymes inhibition (Belwal et al., 2017; Khoo et al., 2017; Tsuda, 2012). The inhibitory effect on carbohydrate digestive enzymes helps to reduce and delay carbohydrate digestion, then it relates to delay glucose absorption into bloodstream. A number of studies revealed that anthocyanins rich fruits and vegetables such as cherries, grapes, roselle, strawberry and red cabbage have potential effects in the i*n vitro* intestinal α-glucosidase and pancreatic α-amylase inhibition (Belwal et al., 2017; Pérez-Ramírez, Castaño-Tostado, Ramírez-de León, Rocha-Guzmán, & Reynoso-Camacho, 2015). Also, the previous mentioned addition of anthocyanins from blue maize flour can affect starch digestibility by two possible mechanism by inhibition of digestive enzymes and by interaction with starch components restricting the amylolytic attack (Camelo-Méndez, Agama-Acevedo,

Sanchez-Rivera, & Bello-Pérez, 2016). Likewise, intake of high purify cyanidin-3-glucoside was exhibited to reduce the rise of blood glucose concentration and also improve insulin sensitivity in type 2 diabetic models as similar as intake of bilberry extract containing different types of anthocyanins (Takikawa, Inoue, Horio, & Tsuda, 2010; Tsuda, Horio, Uchida, Aoki, & Osawa, 2003). A study in type 2 diabetic mice demonstrated that bilberry anthocyanins improved hyperglycemia and insulin sensitivity via activation of AMP-kinase and also upregulated of GLUT-4 (Takikawa et al., 2010). The intake of anthocyanins also improved insulin resistance and regulated insulin sensitivity in mice after consumption of purple corn extract for 12 wk (Tsuda et al., 2003). Besides, anthocyanins namely pelargonidin and pelargonidin-3-galactoside induced a 1.4 times higher in insulin secretion in human with the normal glucose concentration (Christison & MacKenzie, 1993).

2.3.5 Antioxidant activity of anthocyanins

The anthocyanin pigments have been reported in antioxidant effects including both direct and indirect pathways (Pojer et al., 2013). In the direct pathways, anthocyanins have the ability to donate the hydrogen (electron) to free radicals and even reactive oxygen species which is indicated as free radical scavenging capacity of anthocyanins molecule (Fukumoto & Mazza, 2000). With regard to indirect pathway, anthocyanins can enhance endogenous antioxidant defense mechanisms (Pojer et al., 2013). So, antioxidants may be defined as being compounds that can help to delay, inhibit or

prevent the oxidation of protein, DNA and lipid molecules by scavenging free radicals and diminishing oxidative stress (Miguel, 2011). The presence of hydroxyl groups in B ring makes anthocyanins more powerful antioxidants. From the epidemiological study, consumption of anthocyanin rich fruits have shown to reduce the oxidative DNA damage and to increase reduced glutathione content markers of oxidative damage which was the reduction in oxidative (Weisel et al., 2006).

2.3.6 Evidences of anthocyanins from human studies

To illustrate the biological properties of anthocyanins, a number of human study have been investigated. Results suggest that diets rich in anthocyanins or anthocyanins rich foods are associated with the improvement of glycemic response and antioxidant properties. A study was conducted in healthy subjects found that anthocyanins in black currant and crowberry (300 ml) was able to improve postprandial plasma glucose and insulin response after consumption together with sucrose (15 g) (Törrönen, Kolehmainen, Sarkkinen, Mykkänen, & Niskanen, 2012). Similarly, a study in the acute effect of anthocyanin-rich blue berry supplementation (310 and 724 mg anthocyanins) suggested that blueberries significantly improved the postprandial glucose response and also reduced the peak of postprandial glucose concentrations compared to sugar-matched control in young adults (Bell, Lamport, Butler, & Williams, 2017). Besides, the treatment of wild-blueberry was associated with a significant elevation of serum antioxidant status indicated as ORAC and total antioxidant status in healthy subjects

(Kay & Holub, 2002). A study from Micallef *et al*. (2007) demonstrated that ingestion of 400 mL/day of red wine enriched with polyphenols significantly increased plasma thiol and decreased plasma MDA in young and old subjects, suggesting that polyphenols promoted the protection of both lipid and protein oxidation to avoid oxidative damage of the arterial walls and oxidative complication. Additionally, consumption of Chilean berries have been observed to decrease plasma MDA and protein carbonyl levels and increase in plasma antioxidant capacity in healthy male volunteers (Urquiaga et al., 2017). To date, a limited number of human studies have reported anthocyanins as for human health benefits.

2.4 *Clitoria ternatea* L.

A large and increasing number of population in the world use medicinal plants and traditional herbs for health purpose. A previous study reported their therapeutic potential, biological properties and safety for their use (Al-Snafi, 2016). Interestingly, bioactive compounds containing in medicinal and traditional plants pointed out the wide range of pharmacological potentials including cardiovascular disease, antimicrobial, antioxidant, anticancer, anti-inflammatory, antioxidant and hypolipidemic effects (Al-Snafi, 2016; Khoo et al., 2017; Mukherjee et al., 2008). Based on the region, Asia and South-East Asia are known as the areas that have several traditional and medicinal plants in the use of health purpose. Several anthocyanins-rich plants in Asia and Southeast Asia are used as a traditional plant as well as a natural

food coloring. *Clitoria ternatea* L. or butterfly pea flower is an example of a plant flower that contains anthocyanin pigments and be popularly used in Asia, especially Southeast Asia (Mukherjee et al., 2008).

2.4.1 Origin and distribution

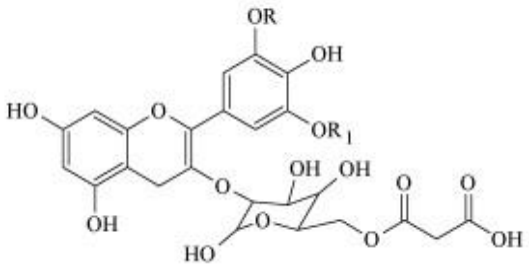
Clitoria ternatea L. is commonly known as un-chan, butterfly pea or blue pea which belongs to Fabaceae family in Plantae kingdom (Al-Snafi, 2016). It originated from the tropical equatorial Asia and then was distributed broadly in South and Central America, East and West Indies, African and Australia (Zingare, Zingare, Dubey, & Ansari, 2013). *Clitoria ternatea* is up to 2-3 m in height, growing wild and even in the garden, bearing dominant blue or white colors resembling a conch-shell Figure 2.5. However, the blue color is most commonly used. This plant is a well-known as Ayurvedic medicine and also as a medicinal plants in many parts of the world (Mukherjee et al., 2008). In addition, the flower of *Clitoria ternatea* has been applied in food and beverage in order to a nutraceutical products. The roots, seeds and other parts of the plant have been investigated as medical value and are used for slightly different purpose (Mukherjee et al., 2008).

Figure 2.5 *Clitoria ternatea* L.

2.4.2 Phytochemical substances

The analysis of major phytochemical compounds in *Clitoria ternatea* is the pentacyclic triter peniods such as taraxerol and taraxerone, ternatins, alkaloids, flavonoids, saponins, tannins and anthocyanins (Mukherjee et al., 2008). Focusing on the flowers, the blue flower pigment is traditionally applied as natural food coloring due to the high stability. A previous study reported that the aqueous extract of *Clitoria ternatea* flower had total phenolic compounds, flavonoid and anthocyanin in 53 ± 0.34 mg gallic acid equivalents/ g dried extract, 11.2 ± 0.33 mg catechin equivalents/ g dried extract and 1.46 ± 0.04 mg cyanidin-3-glucoside equivalents/g dried extract, respectively (Chayaratanasin, Barbieri, Suanpairintr, & Adisakwattana, 2015). In the blue petals of *Clitoria ternatea*, a predominant anthocyanins are ternatins including ternatins A1, A2, B1, B2, D1 and D2 which are recognized as the form of malonylated delphinidin 3,3',5'-triglucosides as shown in Figure 2.6 (Mukherjee et al., 2008). Furthermore, 3-O- (2"-O-alpharhamnosyl- 6"-O-malonyl)-beta-glucoside, 3-O- (6"-O-

alpha-rhamnosyl-6"-O-malonyl)-betaglucoside and 3-O-(2",6"-di-O-alpharhamnosyl)-beta-glucoside of kaemferol, quercetin and myricetin were isolated from the petals (Al-Snafi, 2016).



Delphinidin 3 - malonyl glucoside (G is D-glucose and C is p-coumaric acid)

Ternatin	R	R^1
Ternatin A1 (10)	GCGCG	GCGCG
Ternatin A2 (11)	GCGCG	GCG
Ternatin A3 (12)	GCG	GCG
Ternatin B1 (13)	GCGCG	GCGC
Ternatin B2 (14)	GCGC	GCG
Ternatin B3 (15)	GCGCG	GC
Ternatin B4 (16)	GCG	GC
Ternatin C1 (17)	GCGC	G
Ternatin C2 (18)	GCGCG	G
Ternatin C3 (19)	GC	G
Ternatin C4 (20)	GCG	G
Ternatin C5 (21)	G	G
Ternatin D1 (22)	GCGC	GCGC
Ternatin D2 (23)	GCGC	GC
Ternatin D3 (24)	GC	GC

Figure 2.6 Structure of ternatins

(Mukherjee et al., 2008)

2.4.3 Bioavailability of anthocyanins

Bioavailability is defined as the proportion of the nutrient or bioactive ingredient that is digested, absorbed and metabolized through human gastro-intestinal tract. However, little is known about the bioavailability of plant bioactive compounds in human. *In vitro* simulated gastrointestinal digestion is another way to represent the bioavailability of the plant compounds. For *Clitoria ternatea* L. flower extract, polyphenol contents loss after simulated gastric phase and intestinal phase were 12.5 % and 50%, respectively (Pasukamonset, Kwon, & Adisakwattana, 2016). The extract had 15.12 ± 0.14 % of inhibitory effect on pancreatic α-amylase *in vitro* after simulated gastrointestinal digestion. Moreover, simulated gastrointestinal digestion of *Clitoria ternatea* L. flower extract had shown antioxidant activities including ABTS and FRAP assay.

2.4.4 Antihyperglycemic and antioxidant activity of *Ciliria ternatea* L.

Clitoria ternatea is recognized as a traditional herbal medicine for hypoglycemic activity. *In vitro* study showed the effect of *Clitoria ternatea* extract on the inhibitory activity of carbohydrate digestive enzymes (Adisakwattana et al., 2012). The results indicated that IC_{50} values of *Clitoria ternatea* extract against intestinal maltase, intestinal sucrase and pancreatic α-amylase were 3.15 ± 0.19, 4.41 ± 0.15 and 4.05 ± 0.32 mg/ml, respectively. The inhibition of these carbohydrate digestion leads to reduce and delay digestion and absorption of carbohydrate. According to a study from

Daisy and Rajathi (2009), the aqueous extracts of *Clitoria ternatea* leaves and flowers (400 mg/kg body weight) exhibited a significant reduction in serum glucose, glycosylated hemoglobin and the activity of gluconeogenic enzyme and glucose-6-phosphatase but a significant increase in serum insulin, liver and skeleton muscle glycogen in rats with alloxan-induced diabetes mellitus.

In term of antioxidant activity, anthocyanins which is bioactive compound in *Clitoria ternatea* have the potential to be an alternative source of natural antioxidant (Patel, Kumar, Laloo, & Hemalatha, 2012). It is supported by Chayaratanasin *et al*. (2015), they showed the ability of *Clitoria ternatea* flower extract on protein glycation, advanced glycation end products (AGEs) and *in vitro* antioxidant properties. The results indicated that *Clitoria ternatea* flower extract was able to inhibit AGES formation which factor can impair diabetic complication. The extract markedly reduced the concentration of fructosamine and the protein oxidation by decreasing protein carbonyl content and preventing free thiol depletion. Moreover, *Clitoria ternatea* flower extract also had *in vitro* antioxidant activity, indicating as 1,1-diphenyl 2-picrylhydrazyl (DPPH) scavenging activity, trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant power (FRAP), superoxide radical scavenging activity (SRSA), and ferrous ion chelating power (FICP). A study in streptozotocin-induced diabetic rats found that ethanol extract of *Clitoria ternatea* at dose 200-400 mg/kg had markedly effect on hyperglycemia and oxidative stress in diabetic rats (Talpate et al., 2013).

2.4.5 Toxicity and safety of *Clitoria ternatea* L.

Clitoria ternatea is widely used as medicinal properties, edible plants and natural food coloring since the ancient times. It is considered safe. Previous study demonstrated that acute oral toxicity was up to 3000 mg/kg in mice without mortality (Manalisha & Chandra, 2011). Moreover, no death or any other disorders up to 72 h after single dose 1000 mg/kg in rat (Taur & Patil, 2010). Similarly, the aqueous extract of *Clitoria ternatea* had a negative results and no mutagenic activities in both bacterial and mammalian cells by *Bacillus subtilis* rec assay, *Samonella typhimurium* Ames' test and micronucleus test (Punjanon & Arpornsuwan, 2009).

2.5 Starch fraction based on nutritional properties

Dietary carbohydrates are digested and absorbed at different rates and to different extents in the human small intestine, depending on source of starch, composition and processing conditions (Englyst, Englyst, Hudson, Cole, & Cummings, 1999). The fraction can be classified as following

2.5.1 Classification of starch fraction

- **Rapidly digestible starch (RDS)**

RDS is a fraction of starch granule that cause a rapid increase in blood glucose concentration after consumption of carbohydrates. Based on *in vitro*, this fraction is defined as the amount of starch digested within 20 min of a standard digestion reaction mixture (Englyst, Kingman, & Cummings, 1992). The rate of starch conversion to glucose

production is similarly kinetics in the human gastrointestinal tracts; although RDS is indicated by *in vitro* digestion (Dona, Pages, Gilbert, & Kuchel, 2010).

- **Slowly digestible starch (SDS)**

SDS is indicated as the fraction of slowly digested starch after RDS but completely in human small intestine within 120 min. The potential health benefits of SDS *in vivo* compose of stable glucose metabolism, diabetes management, mental performance and also satiety (Englyst et al., 1992).

- **Undigested starch**

Undigestible starch in the small intestine within 120 min is defined as resistant starch (RS). A number of studies showed the health effects of undigested starch on the controlling of glycemic and insulinemic response, improving lipid profile, increasing micronutrient absorption as well as synergistic interactions with other dietary components such as dietary fiber (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010).

2.5.2 Study and application of starch fraction

For nutritional purposes, starch in food is generally classified into three categories including rapidly digestible starch (RDS), slowly digestible starch (SDS), and undigested starch (Englyst, Liu, & Englyst, 2007). The various amount of starch fraction in different types of carbohydrates and their components depends on their physical and chemical characteristics (Cone & Wolters, 1990). Importantly, SDS is found to be the main

contributing factor to the GI (Meynier, Goux, Atkinson, Brack, & Vinoy, 2015). A report from Pinhero *et al*. (2016) revealed that estimated GI was positively correlated with RDS and negatively correlated with undigested starch. Thus, *in vitro* starch digestibility measured the term of content of RDS, SDS, and undigested starch could be used to predict glycemic response and help to make choices of food intake (Anderson et al., 2010). It has been indicated that an intake of diet containing high RDS level could induce a rapid hyperglycemic response and a subsequent glucose-induced insulin secretion from pancreatic β-cells (Jenkins et al., 1981). Furthermore, consumption of a meal containing RDS level resulted in higher peak of plasma glucose and insulin concentration in young healthy women subjects (Zhang, Venkatachalam, & Hamaker, 2006). Another study suggested the inverse relationship between the content of RDS and SDS fractions in selected Indian traditional foods (Sharavathy, Urooj, & Puttaraj, 2001). In contrast, undigested starch (RS) in human diets provides functional properties and applications for delaying postprandial glucose (Slavin, 2013). In contrast, undigested starch in human diets provides functional properties and applications for delaying postprandial glucose (Slavin, 2013) and improving postprandial insulin (Ells, Seal, Kettlitz, Bal, & Mathers, 2005).

2.6 The influencing factors to starch digestibility

2.6.1 Antinutritional factors

Antinutritional factors as structurally different compounds are widely referred to inhibit some specific enzyme activities or reduce digestion and absorption of nutrients (Kumar, Sinha, Makkar, & Becker, 2010). Plant bioactive compounds are one of the α-amylase inhibitor that have been reported in a previous study. In plant sources, many bioactive compounds such as anthocyanins, luteolin, tannins and flavonoids can against the activity of α-amylase to starch hydrolysis. From a clinical study, bioactive compounds from white beans significantly decreased the peak of postprandial glucose in healthy and type 2 diabetic subjects (Boivin et al., 1988). Similarly, plant compounds like luteolin and anthocyanins from strawberry extract as well as green tea polyphenols have been reported the inhibitory effect on α-amylase or reduce postprandial hyperglycemia (McDougall et al., 2005). In another study, polyphenols, (-)-epigallocatechin-3-gallate, green tea has been shown to inhibit corn starch hydrolysis, this result also supported the lowering postprandial blood glucose levels after consumption corn starch combined with green tea extract (Forester, Gu, & Lambert, 2012).

.6.2 Cooking methods

Before consumption, the starch products have mostly been processed and the common process uses the heating in the presence of water including boiling, steaming,

stir-frying and baking. The method of cooking and processing of carbohydrate food can affect to starch digestibility, glycemic index and glycemic response. In general, the starch in carbohydrate foods exhibits as large granules including amylose and amylopectin macromolecules which are available for hydrolysis. The native starch granules are attacked slowly by enzymes; however, the starch digestibility has been shown to increase by 35-40% during cooking (Singh, Dartois, & Kaur, 2010).

During cooking process, heating and moisture can affect to the starch granules and starch digestibility through gelatinization. Gelatinization is the disruption of hydrogen bonds in the starch granule in order to allow the H bonding sites to engage more water, exhibited irreversible changes in properties such as granular swelling of starch granules which can cause high starch digestibility by human carbohydrate digestive enzymes (Singh et al., 2010). There are three factors including the water content, temperature and duration of cooking that affect the gelatinization. Previously, Chung et al. reported the positive correlation between starch gelatinization and cooking temperature or duration when water content was fixed (Chung, Lim, & Lim, 2006). Moreover, cooling at the storage period cause the starch granule to become a gel which is called retrogradation of starch, depending on the amount of moisture, the amylose to amylopectin ratio as well as time and temperature of storage (Singh et al., 2010). Realignment of starch molecule in retrogradation can cause the starch complexes that are indicated as insoluble and not hydrolyze in the small intestine. So,

gelatinization increases starch digestibility but retrogradation decreases starch digestibility.

2.7 Lack of study in *Clitoria ternatea* L. extract

As mention, *Clitoria ternatea* has several purposes which are traditional medicinal plant and even natural food colorants. Various studies demonstrated the biological and pharmacological properties of *Clitoria ternatea* as presented in cell culture, *in vitro* and animal models (Adisakwattana et al., 2010; Al-Snafi, 2016; Chayaratanasin et al., 2015; P. Pasukamonset et al., 2016). However, *Clitoria ternatea* flower extract (CTE) are well-documented, clinical studies addressing the impact of CTE on glycemic response and antioxidant capacity remain unknown. In addition to biological activities, the flower of *Clitoria ternatea* has been used as a colorant in various foods, beverages and desserts in Asia. This colorant flower is regularly mixed with rice, bread, cookies, flours and other traditional foods and desserts with a variety of ratio. However, the effective ratio of CTE on reduced degree of hydrolysis index, predicted glycemic index of starch and even glycemic response remains unknown. Furthermore, addition of CTE in the carbohydrate foods and beverage and their *in vitro* glycemic index, sensory acceptability and glycemic response were determined in order to present more information.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

• 2,2'-Azino-bis-(3-ethylbenzothiazoline -6-sulphonic acid) (ABTS)	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• 2,2′-Azobis(2-methylpropionamidine) Dihydrochloride (AAPH)	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ)	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• 2,6-di-tert-butyl-4-methylphenol (BHT)	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• 3,5-dinitrosalicylic acid	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)	Calbiochem (Darmstadt, Germany)
• 6-hydroxyl-2,5,7,8-tetramethyl Chhromane-2-carboxylic acid (Trolox)	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)

• Alpha-amylase from porcine pancreas type VI-B	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• Amyloglucosidase solution	Roche Diagnositics (IN, USA)
• Acetic acid	Merck (Darmstadt, Germany)
• Bile extracts porcine	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• Disodium phosphate (Na_2HPO_4)	Ajax finechem (Auckland, New Zealand)
• Ethanol	Merck (Darmstadt, Germany)
• Fluorescein sodium salt	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• Glucose oxidase-peroxidase (GOPOD) kit	HUMAN GmbH (Wiesbaden, Germany)
• Glucose	Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
• Hydrocholic acid (HCl)	Merck (Darmstadt, Germany)

- Iron (II) sulfate — Ajax finechem (Auckland, New Zealand)
- Iron (III) chloride hexahydrate — Ajax finechem (Auckland, New Zealand)
- Potassium sodium tartrate — Ajax finechem (Auckland, New Zealand)
- L-cysteine — Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
- Maleic acid — Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
- Malondialdehyde (MDA) — Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
- Monosodium phosphate (NaH_2PO_4) — Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
- Pancreatin from porcine pancreas — Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
- Pepsin from porcine gastric mucosa powder — Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)
- Porcine pancreatic α-amylase — Sigma-Aldrich Chemical Co. Ltd (St. Louis, MO, USA)

- Sodium acetate anhydrate — Ajax finechem (Auckland, New Zealand)
- Sodium chloride (NaCl) — Ajax finechem (Auckland, New Zealand)

3.1.2 Laboratory equipment

- Freezer -20°C — Sanyo (Osaka, Japan)
- HemoCue® Glucose 201 Analyzer — HemoCue AB (Ängelholm, Sweden)
- High speed refrigerated micro-centrifuge — Hettich (Tuttlingen, Germany)
- Hot air oven — Conthem Scientific (New Zealand)
- Microplate reader Infinite® 200 PRO — Tecan Trading (AG,Switzerland)
- pH meter — Thermo Scientific, Inc. (Waltham, MA, USA)
- pH meter — METTLER TOLEDO® (OH, USA)

• Pipette	Thermo Scientific, Inc. (Waltham, MA, USA)
• Refrigerator 4°C	Sharp (Kyoto, Japan)
• Spray dryer machine	Eyela world (Tokyo, Japan)
• Texture analyzer (TA.XT-Plus Texture analyzer)	Technologies Corp. and Stable Micro Systems Ltd. (MA, USA)
• Vortex	Gemmy industrial corp. (Taipei, Taiwan)

3.2 Methods

3.2.1 The effects of CTE on postprandial glycemic response and antioxidant status in healthy subjects.

3.2.1.1 Plant Preparation and Extraction

The *Clitoria ternatea* flower was purchased from a local herbal drug store, Bangkok, Thailand (Figure 3.1). The herbarium number of *Clitoria ternatea* was authenticated at the Princess Sirindhorn Plant Herbarium, Plant Varieties Protection Division, Department of Agriculture, Bangkok, Thailand, Voucher specimen: BKU066793. The flowers were dried and then boiled twice with distilled water in 1:20 w/v ratio at 90-95°C for 4 hours. After filtering with Whatman No.1, the aqueous extract was dried by using a spray dryer SD-100 (Eyela world, Tokyo Rikakikai Co., LTD, Japan) with a specific condition including inlet temperature at 178 °C, outlet temperature at 85-95°C, blower at 0.60-0.65 m^3/min and atomizing at 90 kPa. The powder of *Clitoria ternatea* flower extract (CTE) was immediately kept in a laminated aluminum foil vacuum bag at room temperature before use.

Figure 3.1 The photograph of dried *Clitoria ternatea* L. flower

3.2.1.2 Participants

Sample size calculation

The sample size was calculated according to Vuksan *et al*. (2010), considering the incremental area under the curve (iAUC) of postprandial glucose response as the main variable. A statistical power of 80 % and an expected difference of 21 % in the baseline values were adopted to form a total sample of at least 13 individuals. After calculation for 50% drop out rate, twenty-two healthy men were screened and recruited by advertisement at Chulalongkorn University.

Inclusion criteria

- 20-40 years old men
- Body mass index (BMI) in the range of 18.5 – 22.9 kg/m^2

- Fasting plasma glucose < 100 mg/dL
- Fasting plasma triglyceride < 150 mg/dL
- Total cholesterol concentration < 200 mg/dL
- Blood urea nitrogen (BUN) in range of 5 - 20 mg/dL
- Creatinine in range of 0.6 - 1.2 mg/dL
- AST < 40 U/L
- ALT < 40 U/L
- Non-smokers and non-alcohol users

Exclusion criteria

- Diagnose with any chronic diseases such as diabetes mellitus etc.
- Take any medication or used any dietary supplements related to antioxidants and glycemic response

3.2.1.3 Ethic approval

The study protocol was approved by the office of Ethics Review Committee for Research Involving Human Research Subjects, Human Science Group, Chulalongkorn University (COA No. 187/2558 and No.061/2560). All subjects gave their written informed consent to participate. All information of participants was kept confidential. There were no major changes in the study protocol after initiation of the study.

3.2.1.4 Study design and protocol

The study was randomized with 5 crossover trials with 1 week of washout period (Figure 3.2). The participants were randomly assigned to 1 of 5 intervention drinks. Randomization was achieved by a researcher using a computer to generate random numbers, simple randomization was used. Randomization numbers were assigned to participants after their screening assessments. Treatment allocation occurred when the participants met the inclusion criteria and signed the informed consent form. Randomization sequence and allocation was concealed to all study participants until completion of the study.

On the test day, the participants were asked to fast for 10-12 h overnight and avoid consumption of phytochemical-rich foods such as tea, berries, soy, red grape and orange etc. starting 3 days before each intervention period until completion of the study. After participants arrived at the study, the participants were seated for 10 min to rest. An intravenous catheter was then inserted into a peripheral arm vein for repeated blood collection by a registered nurse. After collection of blood for baseline, an assigned drink was consumed within 5 min. The subjects consumed five different beverages:

(1) 50 g sucrose in 400 mL water

(2) 1 g CTE in 400 mL of water

(3) 2 g CTE in 400 mL of water

(4) 50 g sucrose and 1 g CTE in 400 mL of water

(5) 50 g sucrose and 2 g CTE in 400 mL of water.

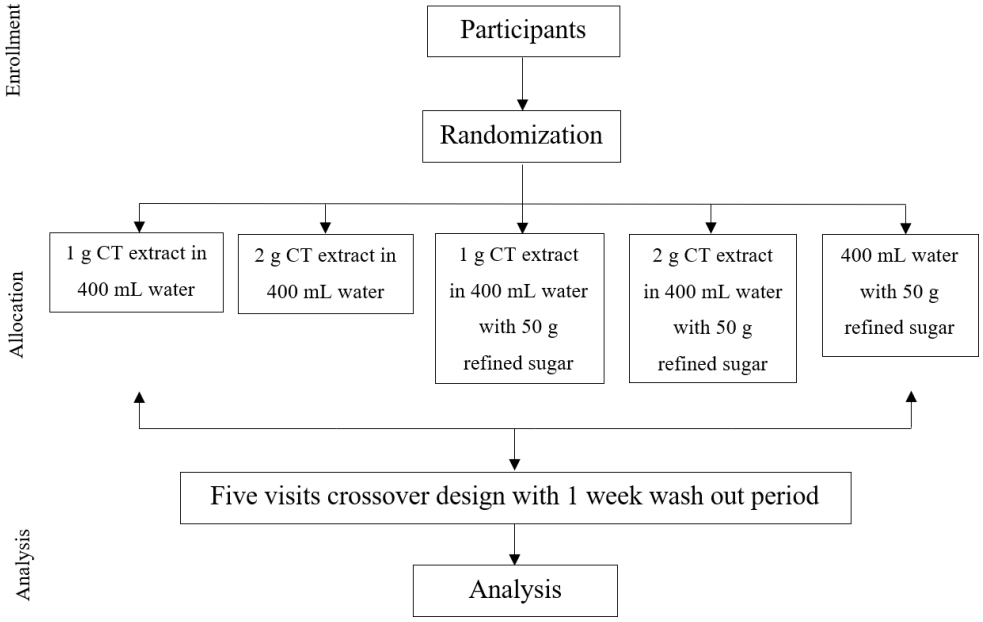


Figure 3.2 Intervention scheme

The beverages were packaged in identical containers at the kitchen at Chula 3 building, Faculty of Allied Health Sciences before distribution to participants, and numbered sequentially according to randomization schedule. The beverages have been coded, participants were blind to the randomization sequence and treatment allocation until the completion of the study as shown in Figure 3.3. No other food or drink was allowed after consuming the beverage. Food record was collected for 3 days (2 weekdays and 1 weekend) during the study period. Participants were also introduced to maintain their usual diet and physical activity until the end of the study.

Figure 3.3 The prepared beverages for participants

3.2.1.5 Blood collection and measurements

In the morning of the testing day, venous blood samples were obtained from the forearm by registered nurses (RN). An intravenous catheter was placed in the peripheral arm vein of the participants for repeated blood draws. Blood collection was performed into NaF- and EDTA-treated tubes at intervals over 180 min before and after administration of the beverage. Blood samples were centrifuged at 3,000 rpm for 10 min at 4 °C and the plasma samples were then kept at -20 °C before analysis.

Determination of plasma glucose, insulin and uric acid

Plasma glucose was determined by using a glucose oxidase method (HUMAN GmbH, Germany). The Human Insulin ELISA kit was used for the quantitative determination of plasma insulin (GenWay Biotech Inc., San Diego, CA, USA). The Enzymatic Colorimetric Teat for Uric Acid with Lipid Clearing Factor (LCF) was performed to measure plasma uric concentration (HUMAN GmbH, Wiesbaden, Germany).

Determination of plasma antioxidant capacity

- ***Plasma Ferric reducing ability of plasma (FRAP)***

The plasma FRAP level was analyzed according to a previous report (Benzie & Strain, 1996). The plasma sample (10 µL) was mixed with FRAP reagent (90 µL) containing 0.3 M sodium acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$. After incubation in a dark room maintained at room temperature for

30 min, the reaction was measured at the absorbance at 595 nm. Plasma FRAP level was reported as $FeSO_4$ equivalents.

- ***Plasma Oxygen radical absorbance capacity (ORAC)***

The plasma ORAC level was determined according to a previous study (Cao & Prior, 1999). The plasma sample (25 µL) was incubated with 4.8 nM sodium fluorescein in 75 mM PBS at 37°C for 10 min. Then, 64 mM AAPH solution was added and the mixture was measured the fluorescence intensity ($\lambda_{excitation}$= 485 mM and $\lambda_{emission}$= 535 nm) every 2 min for 1 h. The ORAC value was calculated from the calibration curve of net AUC against Trolox concentration and expressed as µM Trolox equivalent.

- ***Plasma trolox equivalent antioxidant capacity (TEAC)***

The plasma trolox equivalent antioxidant capacity (TEAC) level was determined according to a previous study (Wang et al., 2004). The 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) free radical ($ABTS^{\bullet+}$) solution was prepared by the mixture of 7 mM ABTS in 0.1 M PBS (pH 7.4) and 2.45 mM $K_2S_2O_8$ in distilled water (1:1, v/v). After 16 h of the incubation at room temperature in the dark, $ABTS^{\bullet+}$ solution was diluted with 0.1 M PBS (pH 7.4) to adjust the absorbance between 0.900 and 1.000 at 734 nm. The adjusted $ABTS^{\bullet+}$ solution was added in the 1:5 diluted plasma and then incubated for 6 min. The reaction was measured at 734 nm and plasma TEAC was expressed as mM Trolox equivalents.

- ***Plasma thiol***

The plasma thiol group level was measured using an Ellman's assay (Ellman, 1959) with slightly modification. The plasma sample (90 µL) was mixed with 2.5 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in 0.1 M PBS (pH 7.4). After incubation at room temperature for 15 min, the reaction was measured the absorbance at 410 nm. The plasma thiol level was calculated and expressed as µM L-cysteine equivalent.

- ***Plasma malondialdehyde (MDA)***

The plasma MDA level was determined according to a previously described report (Wolff & Dean, 1987). Plasma malondialdehyde (MDA) was quantified using a method based on the formation of thiobarbituric acid reactive substances (TBARS). The plasma sample (150 µL) was mixed with 10% thichloroacetic acid (TCA) and 50 mM 2,6-di-tert-butyl-4-methylphenol (BHT). After centrifugation at 13,000 rpm at 4°C for 10 min, 0.67% thiobarbituric acid (TBA) was added to the supernatant. The mixture was heated at 95 °C for 10 min and measured the absorbance at 532 nm. The plasma MDA level was expressed as nmol/L MDA equivalent.

3.2.1.6 Statistical analysis

The results were reported as mean ± SEM. For each test, the incremental data of plasma glucose, insulin, and antioxidant were analyzed by using a Kolmogorov-Smirnov test for normality testing. Statistical analysis of the incremental data was carried out using two-way repeated-measures (two-factor repetition) ANOVA, with beverage and time as within-subject factors. The Duncan post hoc comparison ($P<0.05$) was utilized to assess both mean differences among the beverages within a single time point and intervention effects at different time points within the treatments with respect to baseline. The incremental area under the curve (iAUCs) was calculated by using according to the trapezoidal method. Then, one-way repeated measures ANOVA with Duncan post hoc comparison ($P<0.05$) was evaluated.

3.2.2 The effect of CTE on the inhibition of pancreatic α-amylase, *in vitro* starch hydrolysis and predicted glycemic index of different type of flours including potato, cassava, rice, corn, wheat and glutinous rice flour and the application in a bakery product

3.2.2.1 Plant Preparation and Extraction

The *Clitoria ternatea* flower was purchased from a local herbal drug store, Bangkok, Thailand. The herbarium number of *Clitoria ternatea* was authenticated at the Princess Sirindhorn Plant Herbarium, Plant Varieties Protection Division, Department of Agriculture, Bangkok, Thailand, Voucher specimen: BKU066793. The flowers were dried and then boiled twice with distilled water in 1:20 w/v ratio at 90-95°C for 4 hours. After filtering with Whatman No.1, the aqueous extract was dried by using a spray dryer SD-100 (Eyela world, Tokyo Rikakikai Co., LTD, Japan) with a specific condition including inlet temperature at 178 °C, outlet temperature at 85-95°C, blower at 0.60-0.65 m^3/min and atomizing at 90 kPa. The powder of *Clitoria ternatea* flower extract (CTE) was immediately kept in a laminated aluminum foil vacuum bag. The content of phenolic compounds and total anthocyanins in CTE was 53.08 ± 0.08 mg gallic acid equivalents/g extract and 1.08 ± 0.12 mg delphinidin-3-glucoside equivalents/g extract, respectively.

3.2.2.2 Preparation of flour and extraction

Commercial flours including potato, rice, glutinous rice, wheat, corn and cassava flours were purchased from a local supermarket. Flour (0.25 g) was dissolved with 50 mL of boiled water at 100 °C and stirred for 10 min. The flour solution was allowed to cool at room temperature for 10 min. Furthermore, the CTE powder was dissolved in 0.1 M phosphate buffer saline (PBS) or 0.2 M sodium acetate buffer. The CTE solution was vortexed for 10 min. The CTE solution was added into flour solution (final concentration: 0.5%, 1% and 2% w/v or 5:1, 10:1 and 20:1 (w/w)) and subjected to *in vitro* digestion.

3.2.2.3 Inhibition of pancreatic α-amylase

The activity of pancreatic α-amylase was carried outed using a modified procedure of Adisakwattana et al. (Adisakwattana et al., 2012). Fifty microliters of flour solution were mixed with 100 µL of CTE in 0.1 M phosphate buffer saline (PBS). Fifty microliters of porcine pancreatic α-amylase (15 U/mL) in 0.1 M PBS, pH 6.9 was then added and the mixture was made to 250 µL with 0.1 M PBS. After incubation at 37°C for 10 min, the reaction was terminated by adding 250 µL of DNS reagent (1% DNS, 0.2% phenol, 0.05% Na_2SO_3 and 1% NaOH in distilled water) and heated at 100°C for 10 min. Then, 40% potassium sodium tartrate (250 µL) was added to stabilize the color. After cooling at room temperature, the absorbance was measured at 540 nm. The pancreatic α-

amylase inhibitory activity was calculated as percentage inhibition. A control was prepared using the same procedure replacing the CTE solution with 0.1 M PBS.

3.2.2.4 *In vitro* starch digestibility and predicted glycemic index (pGI)

The *in vitro* digestion of flour and CTE was performed according to a previous method with some modifications (Englyst, Kingman, & Cummings, 1992; Gularte, Gómez, & Rosell, 2012). Fifty microliters of flour solution were mixed with 100 µL of CTE in 0.2 M sodium acetate buffer. The mixture was incubated with 50 µL of porcine pancreatic α-amylase (15 U/mL) and 50 µL of amyloglucosidase (31.25 µg/mL) in 0.2 M sodium acetate buffer, pH 6.0 at 37oC for 180 min. After heating at 100^{o}C for 10 min for stopping the reaction, the supernatant was measured for glucose content using a glucose oxidase-peroxidase (GOPOD) kit. The values were plotted a graph and the area under the curve (AUC) was calculated by trapezoidal rule. The hydrolysis index (HI) was calculated from the percentage of the area under the hydrolysis curve of the sample to that the area under the curve of standard glucose. The predicted glycemic indices (pGI) of samples were estimated according to the followed equation (Goñi et al., 1997): pGI = 39.71 + 0.549 HI. A control was prepared using the same procedure replacing the CTE solution with 0.2 M sodium acetate buffer.

3.2.2.5 Estimation of starch fraction

Starch fraction was calculated based on *in vitro* starch digestibility of samples. Rapidly digestible starch (RDS) was calculated as the amount of glucose present in the

sample at 20 min of *in vitro* digestion, whereas slowly digestible starch (SDS) was calculated as the difference between the amount of glucose measured at 120 min and 20 min (Englyst et al., 1992; Mishra & Monro, 2009). Undigested starch was calculated as the amount of glucose that was not digested within 120 min. The conversion factor from glucose to starch was 0.9.

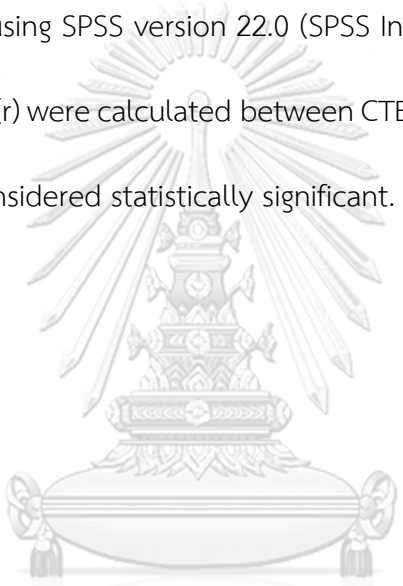
3.2.2.6 Bread preparation

Wheat flour and other dry ingredients as % on weight of flour basis including sugar (5%), salt (1.8%), yeast (3%), Benecel methylcellulose and hydroxypropylmethylcellulose (2%) (Ashland, Covington, KY, USA) were mixed using a Kitchen-Aid bowl mixer at speed 53 rpm for 1 min. The levels of CTE incorporated into this formulation were 5%, 10% and 20% (w/w) of wheat flour basis by adding to mix with other dry ingredients. Based on wheat flour, vegetable oil (6%), white egg (40%) and milk (70%) were added and then mixed together at speed of 160 rpm for 10 min. After that, batter was poured into a mold, placed at 30 °C and 90% relative humidity for 50 min and baked at 150 °C for 40 min. The loaf was removed from the mold and cooled at room temperature. The bread sample was packed in a sealed polyethylene bag until analysis. After baking, the in vitro starch hydrolysis of bread with or without CTE was determined to indicate the *in vitro* starch digestibility and pGI. Bread samples were weight (1g) and mixed with distilled water (10 mL) with stirring for 10 min. The in

vitro starch digestion of bread was performed according to the above-mentioned method. A control bread was prepared using the same procedure without CTE.

3.2.2.7 Statistical analysis

The data were expressed as mean ± standard error of the mean (SEM). The statistical significance of results was evaluated by one-way ANOVA using Duncan multiple comparisons using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Pearson's correlation coefficients (r) were calculated between CTE concentration and undigested starch. $P < 0.05$ was considered statistically significant.



3.2.3 The effectiveness of CTE incorporation into rice on *in vitro* and *in vivo* starch digestibility of cooked rice using domestic cooking methods

3.2.3.1 Preparation of *Clitoria ternatea* flower

The flower of *Clitoria ternatea* was purchased from a local herbal drug store, Bangkok, Thailand. The herbarium number of *Clitoria ternatea* was authenticated at the Princess Sirindhorn Plant Herbarium, Plant Varieties Protection Division, Department of Agriculture, Bangkok, Thailand, Voucher specimen: BKU066793. The dried flower was boiled with distilled water in 1.25% and 2.5% (w/v) at 90-95 °C for 30 min. Thereafter, the aqueous solution of *Clitoria ternatea* (CTE) was filtered and kept at 4 °C until usage. The total polyphenolic and anthocyanin content in CTE solution was determined using the Folin-Ciocalteau method and the pH differential method, respectively (Chayaratanasin et al., 2015). Total phenolic compounds in CTE solution (1.25% and 2.5% w/v) were 391.14 ± 22.51 and 451.62 ± 14.01 µg gallic acid equivalents/mL, respectively. Total anthocyanins in CTE solution (1.25% and 2.5% w/v) were 15.05 ± 0.14 and 56.27 ± 8.15 µg delphinidin-3-glucoside equivalents/mL, respectively.

3.2.3.2 Preparation of rice sample

Jasmine white rice (100%) was purchased from the supermarket and kept at dried place. The rice was cooked with or without CTE solution at 1.25 % and 2.5% (w/v) by using an electric rice cooker and a microwave oven. In the electric rice cooker method,

rice was washed and drained twice with clean water and then placed into an electric rice cooker with rice and water ratio 1:1.3 (w/v). After rice was automatically cooked and holed for 10 min at the warming setting, cooked rice was left to cool down at room temperature until further analysis. In the microwave oven method, rice was washed and drained thrice with clean water. Then, water was added to rice with a ratio of rice and water 1:2.3 (w/v) and cooked in a microwave oven at 800 W for 11 min. The cooking time was set in accordance with the manufacturer's recommendations. The cooked rice was left to cool down at room temperature until further analysis.

3.2.3.3 *In vitro starch digestibility of cooked rice*

The starch digestibility of rice was determined according to a previous study (Kaur, Ranawana, Teh, & Henry, 2015). Rice samples (2.5g) with 30 mL of distilled water were digested in specimen pots and inserted into a 12-position aluminum block placed in a circulating water bath maintained at 37 °C with stirring at 130 rpm. After stirring for 30 min, the oral phase was initiated by the addition of 100 µL of 10% α-amylase ($\geq$10 units/mg solid) in distilled water for 1 min. Then, 200 µL of 1 M HCL was added to the samples in quick succession to adjust pH 2.5 (±0.2) measured by the pH meter (SevenCompact™, METTLER TOLEDO®, OH, USA). Gastric phase was then initiated by addition of 1 mL of 10% pepsin ($\geq$250 units/mg solid) dissolved in 0.05 M HCl. The mixture was continuously stirred at 37 °C for 30 min to complete the gastric digestion phase. After which, 2 mL of 1 M $NaHCO_3$ and 5 mL of 0.2 M maleate buffer (pH 6) were

added into the mixture to neutralize the gastric HCl. Five milliliters of 10% bile extract solution in distilled water was added to the mixture and then distilled water was filled into the pots to adjust the volume to 55 mL. After holding for 15 min to reach 37 °C, the intestinal phase was started by adding 100 µL of amyloglucosidase (≥260 U/mL, aqueous solution) and 1 mL of 5% pancreatin in 0.2 M maleate buffer. An aliquot of 250 µL from baseline, the end of oral and gastric phase, and at 20, 60, 90, 120 and 180 min from the start of pancreatic phase was drawn and transferred into the tubes containing 1 mL of ethanol to end enzymatic digestion. The ethanolic digesta samples were then stored in a refrigerator until analysis of reducing sugar.

3.2.3.4 Analysis of reducing sugars released during *in vitro* digestibility

Reducing sugars released from the rice samples during *in vitro* digestion was measured using the dinitrosalicylic acid (DNS) colorimetric method (Englyst & Hudson, 1987; Kaur et al., 2015). The ethanolic digesta were centrifuged at 1000 rpm for 10 min. Fifty microliter aliquots of the supernatant were removed and mixed with 250 µL of 0.1 M acetate buffer (pH 5.2) containing 1% amyloglucosidase (≥260 U/mL, aqueous solution). After incubation at 37 °C for 10 min, 750 µL of DNS mixture (0.5 mg/mL of glucose: 4 M NaOH: DNS reagent mixed in ratio at 1:1:5) was added. The mixture was heated at 95-100 °C for 15 min and then 4 mL of distilled water was added to each sample. Absorbance was read after cooling at room temperature at 530 nm (Infinite® 200 PRO, Tecan Trading AG, Switzerland). Glucose (10 mg/ml) and distilled water were

used as standard and blank, respectively. The results were expressed as mg glucose equivalent/ g rice.

3.2.3.5 Determination of instrumental textural properties

Textural profile analysis (TPA) of cooked rice was determined using a texture analyzer. Thirty kernels of each cooked rice sample were selected and placed into Whatman No. 1 as a single layer before placing them on the base of the instrument (Figure 3.4). A texture analyzer (TA.XT-Plus Texture analyzer) with a 35 mm cylindrical probe attachment (Technologies Corp. and Stable Micro Systems Ltd., MA) was used with a standard two-cycle compression method. The probe was allowed to descend at 1 mm/s, return and then repeat the compression cycle. Compression was set to 75% strain. Textural parameters of hardness, stickiness, adhesiveness, springiness, cohesiveness, gumminess and chewiness were recorded from the test curves. For each cooking sample, the textural measurements were repeated 10 times.

Figure 3.4 Single layer of thirty kernels of cooked rice before TPA analysis

3.2.3.6 Determination of sensory properties

The panelists was male and female aged 20-50 year old without problems with the sensory perception such as color blindness, cold or mouth and throat diseases. Moreover, the panelists with allergies to CTE and food related the study or women who are pregnant or breastfeeding were excluded from the study. The study protocol was approved by the office of Ethics Review Committee for Research Involving Human Research Subjects, Human Science Group, Chulalongkorn University (COA No. 097/2018). All subjects gave their written informed consent to participate. All information of participants was kept confidential.

Panelists were seated in individual sensory booths under white light and water was provided to cleanse the palate between evaluations. All samples were coded with

random 3-digit numbers for identification and presented separately in a randomized order. A 9-point hedonic scale (1: dislike extremely, 5: neither like nor dislike, 9: like extremely) was used and selected quality parameters were taste, aroma, appearance, texture, color, hardness, stickiness and overall acceptability.

3.2.3.7 Human study

This study was designed and conducted according to the Singapore Good Clinical Practice guideline and principles of Declaration of Helsinki. Singapore's National Healthcare Group (NHG) Domain Specific Review Board (NHG DSRB Reference: 2016/00926) reviewed and approved the protocol of this study. All subjects provided written consent before participation in this study.

Sample size calculation

The sample size was calculated according a previous study (Robert, Ismail, & Rosli, 2016). The considering change in the iAUC postprandial blood glucose concentrations was 954 ± 910 mg/dL.min with a 80% power of test and 95% confidence interval. Therefore, a sample size at least 8 participants was necessary for this study.

Inclusion criteria

- Chinese male
- Age between 21 to 60 years old
- BMI between 18.5 to 22.9 kg/m^2
- Fasting blood glucose < 100 mg/dL

- Blood pressure < 140/90 mmHg
- General good health

Exclusion criteria

- Regularly smoking
- Diagnose with metabolic diseases such as diabetes and hypertension etc.
- Take any medications known to affect glycemic response such as glucocorticoids, thyroid hormones and thiazide diuretics.
- Have intolerance or allergies to any food study
- Partake in sports at the competitive and/or endurance levels
- Intentionally restrict food intake

Study design and protocol

The randomized, crossover trial, non-blind design was conducted for this study. Participants were introduced to fast 10-12 h. and also to avoid alcoholic beverages and avoid strenuous physical activity in 24 h before the test. Participants were asked to visit at the Clinical Nutrition Research Center (CNRC), the 7th floor of MD-6 building, National University of Singapore, Singapore in the morning of each test session. Each participant completed to consume three reference food before two test foods in random order with 1-week washout to minimize carry-over effects. The cooked rice with or without 2.5% (w/v) of CTE were served each time with 65.3 g rice using an electric rice cooker. They were required to consume within 10-12 min. The participants

was allowed to consume 200 mL of drinking water during the study period. The amount of anhydrous glucose (50 g) was dissolved in 250 mL of water as a reference food. All reference and test foods served in portions containing 50 g available carbohydrate and they were freshly prepared by a researcher in the morning of each test day. Participants were advised to keep physical activity to a minimum during testing and remain seated. Work tables, reading areas and a television were provided for their use. Blood glucose concentration were measured at before and after 15, 30, 45, 60, 90 and 120 min the start of the test foods (Figure 3.5). Finger-prick capillary blood samples were taken and analyzed using the HemoCue Glucose 201 Analyzer (HemoCue® Glucose 201 RT) with intra- and inter-assays CVs < 2%.

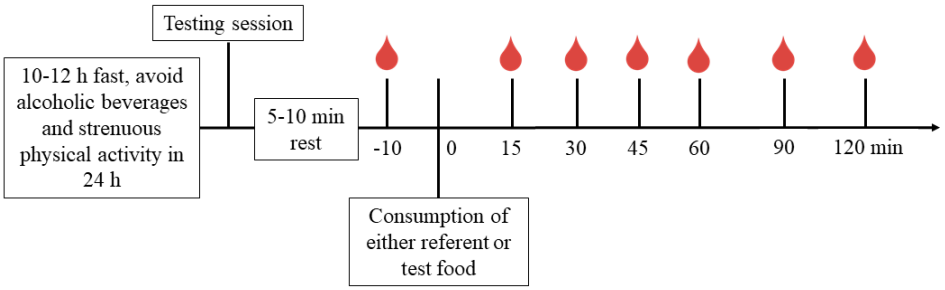


Figure 3.5 Study session protocol

3.2.3.8 Statistical analysis

The results were assessed for normality using the Shapiro-Wilk test. Parametric data were expressed as mean ± SEM and non-parametric data were expressed as mean ± SD. For normally distributed data, the statistical analysis of in vitro starch digestibility and the instrument texture profile analysis (TPA) and sensory evaluation were performed using One-way ANOVA following by Duncan multiple comparisons. For non-normally distributed data, the Friedman test was used to analyze the differences in the incremental postprandial blood glucose response and incremental area under the glucose curve. The Wilcoxon singed-rake test was used as the post hoc test. The statistical analysis was carried out using SPSS version 22.0 (SPSS Inc., USA). $P < 0.05$ was considered statistically significant.

CHAPTER IV

RESULTS

4.1 The effects of CTE on postprandial glycemic response and antioxidant status in healthy subjects.

Between September 2016 and January 2017, twenty-two subjects were recruited for this study according to the flow chart (Figure 4.1). Four subjects were excluded from the study as they did not meet the inclusion criteria of the study. The eighteen remaining subjects were randomly assigned into 5 groups. Three subjects withdrew during the study due to the reasons unrelated to the study. Fifteen subjects finally completed the study. The baseline characteristics of the fifteen subjects are shown in Table 4.1. No adverse events after consumption of beverages were observed.

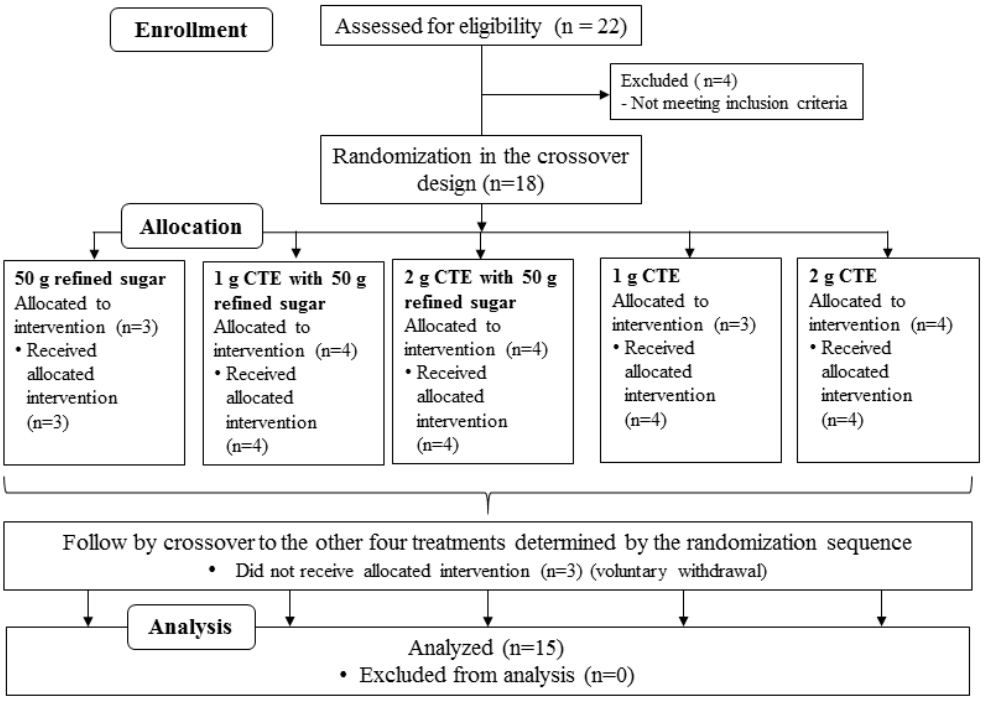


Figure 4.1 The flowchart describing the trial

Table 4.1 Baseline characteristics of the study participants

	Mean ± SEM
Age (years)	22.53 ± 0.30
Height (cm)	173.65 ± 1.46
Weight (kg)	65.16 ± 2.05
BMI (kg/m^2)	21.57 ± 0.54
Fasting plasma glucose (mg/dL)	84.77 ± 1.94
Total cholesterol (mg/dL)	183.22 ± 6.29
Serum triglyceride (mg/dL)	81.00 ± 6.23
LDL-C (mg/dL)	120.56 ± 6.44
Creatinine (mg/dL)	0.95 ± 0.04
BUN (mg/dL)	10.53 ± 0.48
ALT (U/L)	21.55 ± 0.99
AST (U/L)	15.27 ± 2.35
Systolic blood pressure (mmHg)	115.54 ± 1.42
Diastolic blood pressure (mmHg)	68.21 ± 1.51

All values are means ± SEM, n=15.

4.1.1 Plasma glucose concentration

The results of postprandial glucose concentration are presented in Figure 4.2a. At the individual time points, consumption of 1 and 2 g CTE did not significantly change in the baselines of glucose concentration. Consumption of sucrose caused a rapid rise of glucose, with the peak concentration at 30 min, followed by a rapid fall below the baseline level within 120 min. Ingestion of 1 g and 2 g CTE together with sucrose significantly decreased postprandial plasma glucose concentration at 30 and 60 min ($P < 0.05$). As shown in Figure 4.2b, 1g and 2 g CTE plus sucrose resulted in 0.67-and 0.60-fold lower iAUCs for plasma compared with sucrose, respectively.

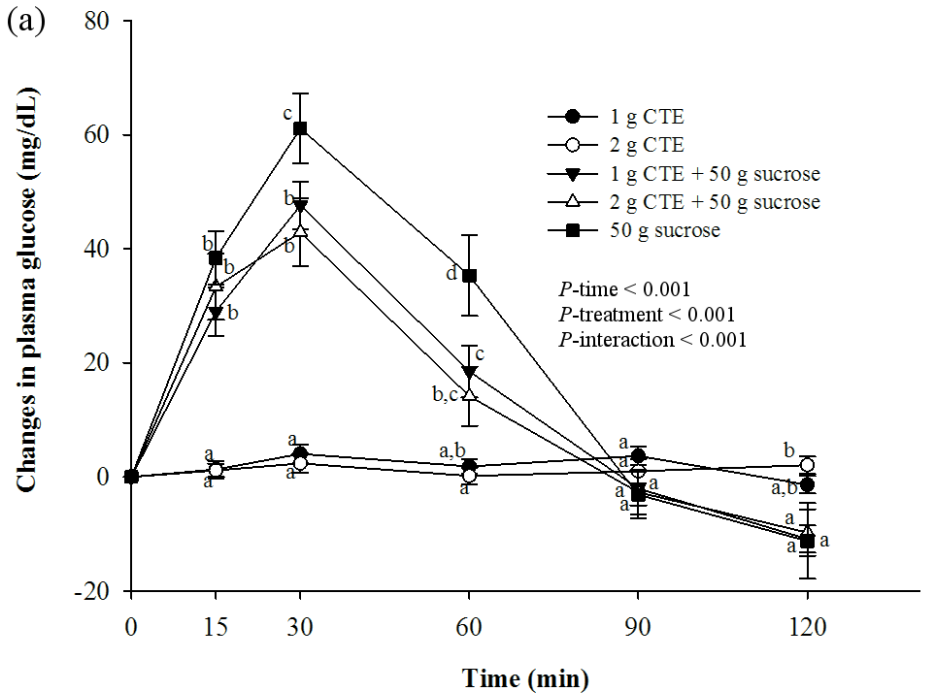


Figure 4.2 Postprandial plasma glucose response of five different beverages. (a) Changes in glucose concentrations during 120 min of five different beverages; (b) Glucose changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different (P < 0.05).

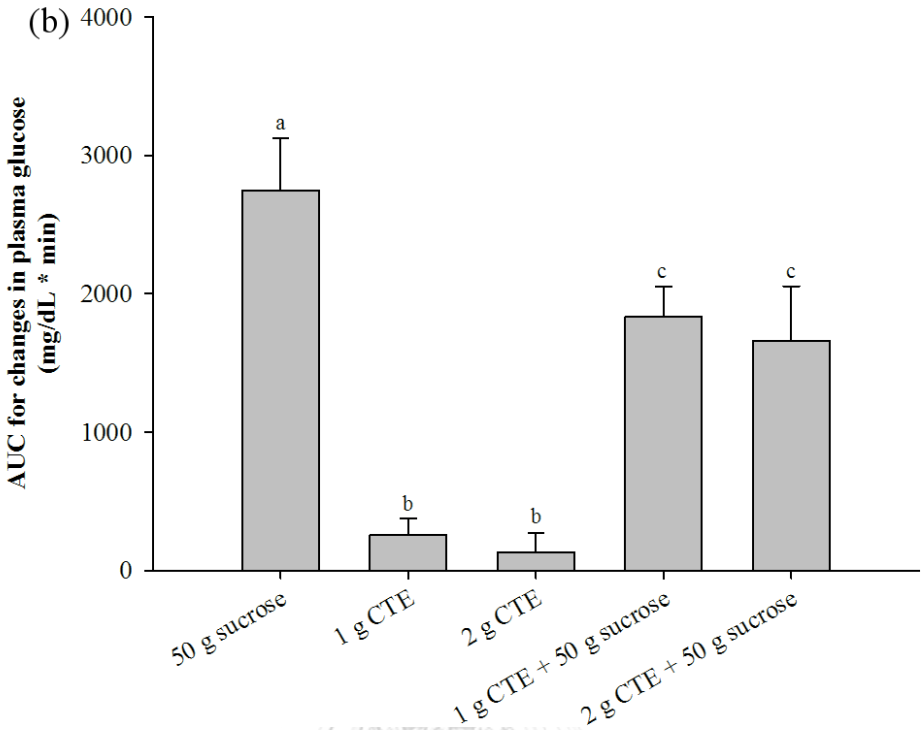


Figure 4.2 Postprandial plasma glucose response of five different beverages. (a) Changes in glucose concentrations during 120 min of five different beverages; (b) Glucose changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

4.1.2 Plasma insulin concentration

As shown in Figure 4.3a, there was significant effect on plasma insulin concentration for time, treatment and time x treatment interaction ($P < 0.001$). The results demonstrated that consumption of 1 g and 2 g CTE did not alter the plasma insulin concentration. After consumption of sucrose, the peak of plasma insulin concentration was observed at 30 min and returned to the baseline within 90 min. The results showed that only consumption of 2 g CTE with sucrose significantly suppressed a rise in postprandial plasma insulin at 60 min compared to sucrose ($P< 0.05$). In Figure 4.3b, the iAUCs for plasma insulin of 2 g CTE plus sucrose were 0.67 times less than that of sucrose. However, the iAUCs for plasma insulin had no significant difference between 1 g and 2 g of CTE without sucrose.

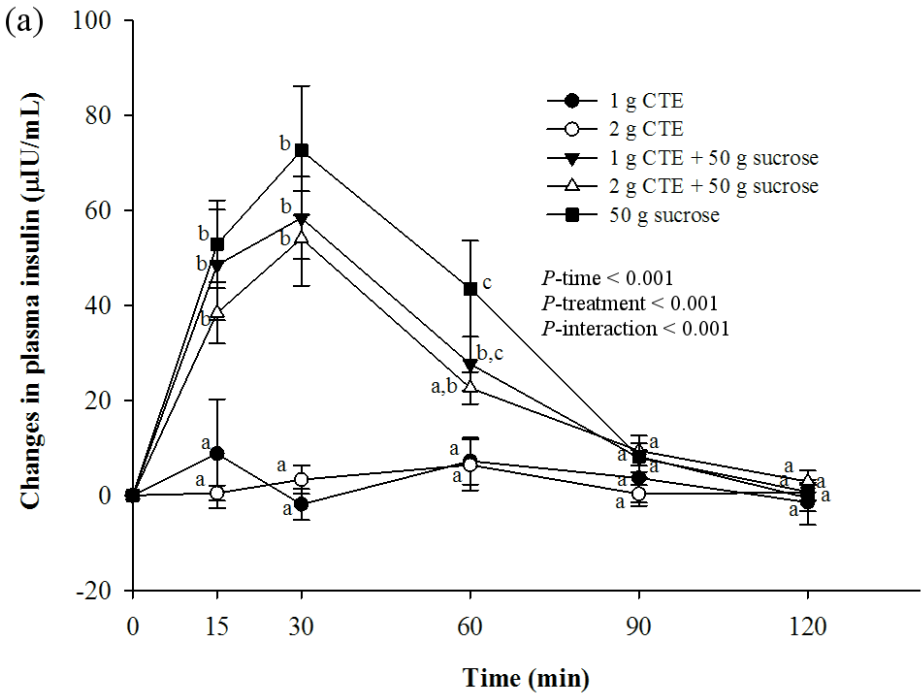


Figure 4.3 Postprandial plasma insulin response of five different beverages. (a) Changes in insulin concentrations during 120 min of five different beverages; (b) Insulin changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

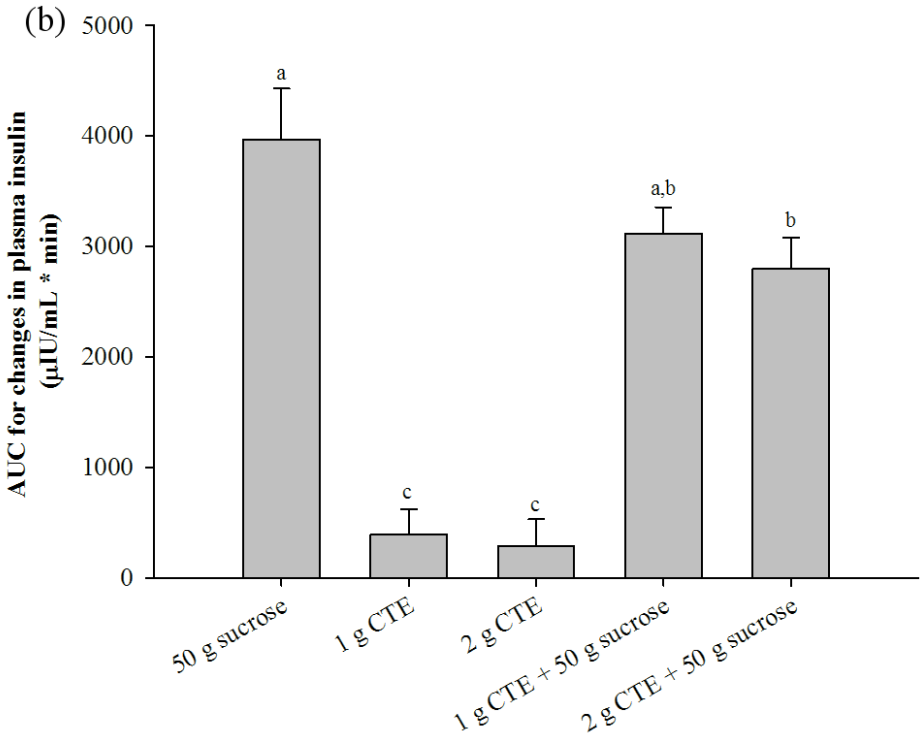


Figure 4.3 Postprandial plasma insulin response of five different beverages. (a) Changes in insulin concentrations during 120 min of five different beverages; (b) Insulin changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

4.1.3 Plasma uric acid concentration

Postprandial plasma uric acid increased rapidly at 60 min after ingestion of sucrose. No main effects for time, treatment and their interactions were observed. In contrast, CTE ingestion with or without sucrose did not significantly increase plasma uric acid (Figure 4.4a). Postprandial iAUCs for plasma uric acid did not differ between all beverages, as shown in Figure 4.4b.

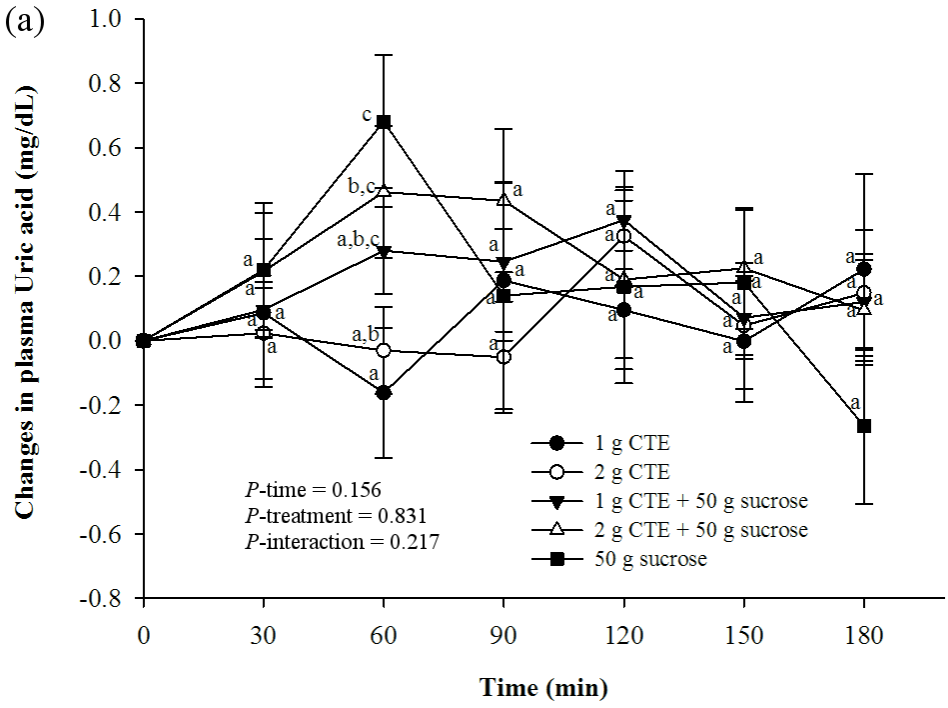


Figure 4.4 Postprandial plasma uric acid of five different beverages. (a) Changes in uric acid concentrations during 180 min of five different beverages; (b) Uric acid changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

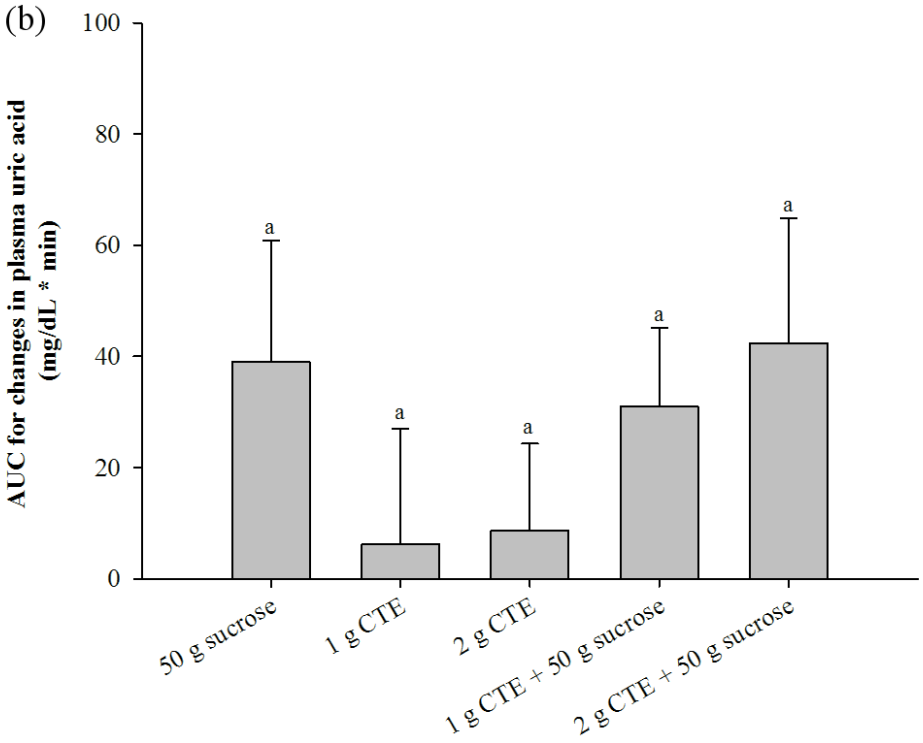


Figure 4.4 Postprandial plasma uric acid of five different beverages. (a) Changes in uric acid concentrations during 180 min of five different beverages; (b) Uric acid changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

4.1.4 Plasma antioxidant status

Plasma FRAP

Figure 4.5a shows the changes in postprandial plasma FRAP level after consumption of CTE. The postprandial plasma FRAP was slightly increased after 60 min consumption of sucrose. In addition, 1 g and 2 g CTE plus sucrose showed a maximum peak at 60 min, whereas the significant results were observed at 120 and 150 min when compared to sucrose group ($P< 0.05$). In contrast, 1 g and 2 g CTE could increase the postprandial plasma FRAP at 30 min and maintained its effect throughout the postprandial period. When compared to the group which received sucrose, the iAUCs for plasma FRAP were significantly higher 3.71- and 5.48-fold after consumption of sucrose with 1 g and 2 g CTE, respectively (Figure 4.5b). Furthermore, consumption of 1 g and 2 g CTE were 3.74 and 4.73-fold higher iAUCs for plasma FRAP than that of sucrose.

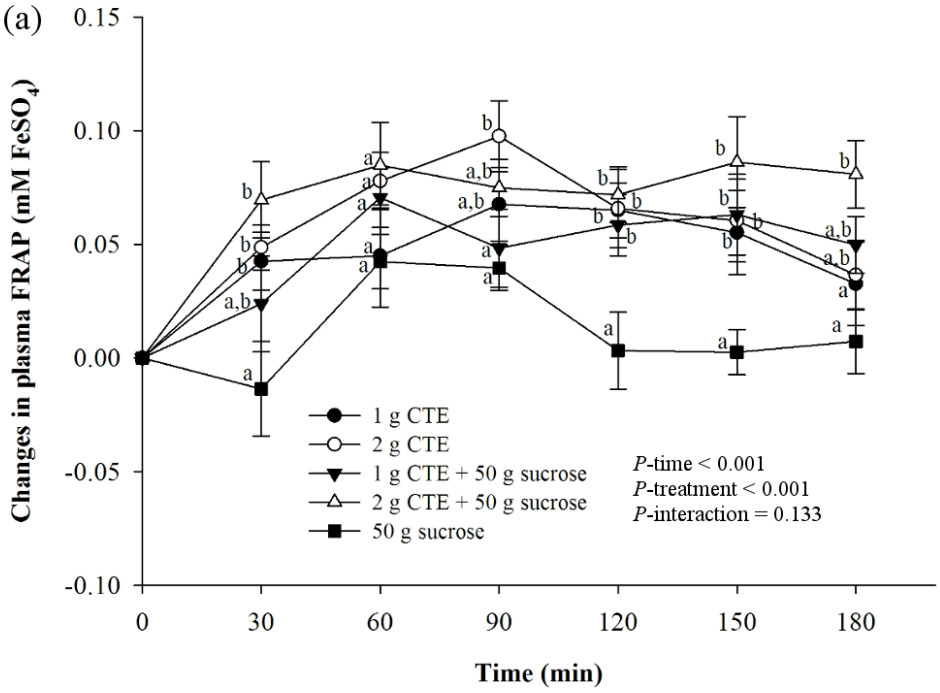


Figure 4.5 Postprandial plasma FRAP of five different beverages. (a) Changes in FRAP concentrations during 180 min of five different beverages; (b) FRAP changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

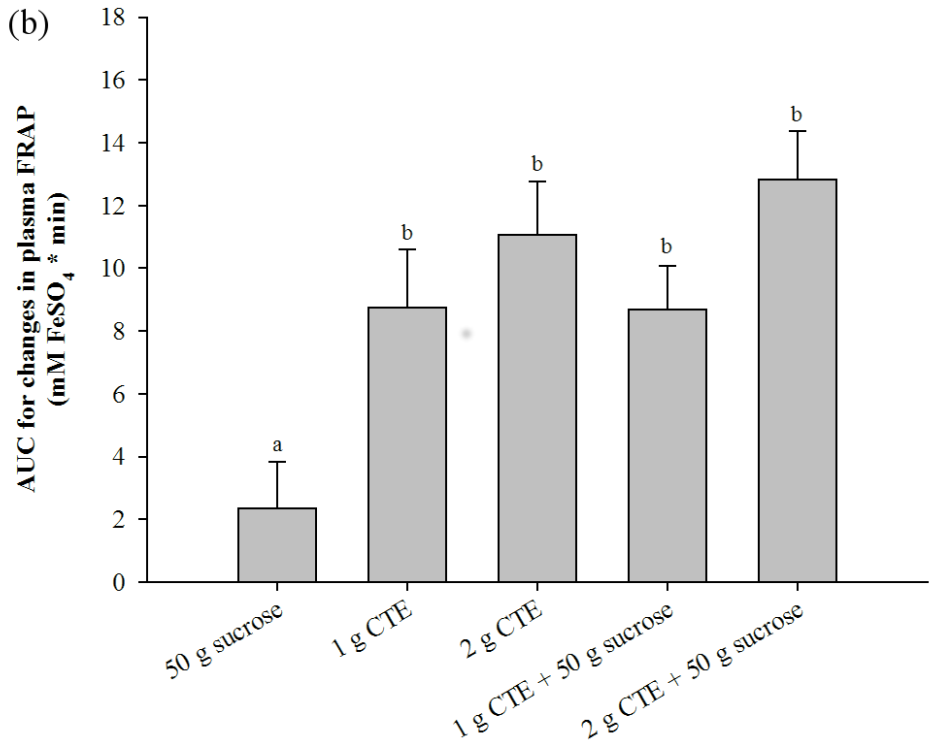


Figure 4.5 Postprandial plasma FRAP of five different beverages. (a) Changes in FRAP concentrations during 180 min of five different beverages; (b) FRAP changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

Plasma TEAC

As shown in Figure 4.6a, ingestion of sucrose showed significantly lower postprandial plasma TEAC than 1 g and 2 g CTE with or without sucrose during the postprandial period ($P< 0.05$). Compared with sucrose, iAUCs for plasma TEAC after consumption 1 g and 2 g CTE with sucrose were both significantly higher 1.34-fold (Figure 4.6b). Additionally, iAUCs for plasma TEAC was 1.38- and 1.46-fold higher after 1 g and 2 g CTE, respectively.

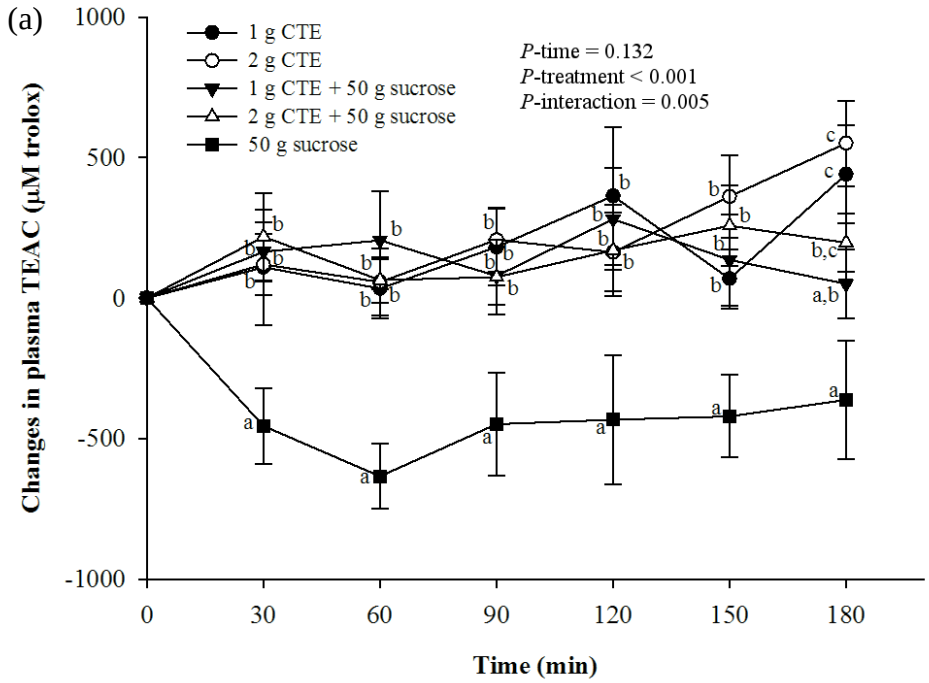


Figure 4.6 Postprandial plasma TEAC of five different beverages. (a) TEAC concentrations during 180 min of five different beverages; (b) TEAC a changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

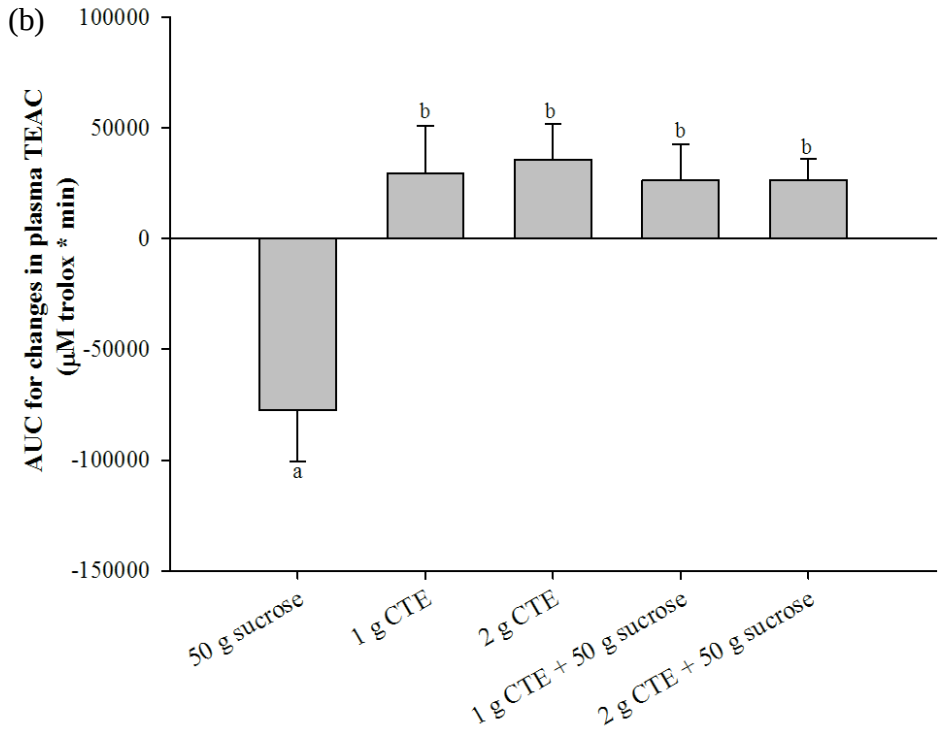


Figure 4.6 Postprandial plasma TEAC of five different beverages. (a) TEAC concentrations during 180 min of five different beverages; (b) TEAC a changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

Plasma ORAC

The decreased postprandial plasma ORAC was seen after 30 min of sucrose ingestion (Figure 4.7a). By contrast, the reduction in plasma ORAC was suppressed after CTE consumption compared with that of sucrose. Furthermore, Figure 4.7b shows the iAUCs for plasma ORAC after consumption CTE. After ingestion of 1 g and 2 g CTE with sucrose, iAUCs for plasma ORAC were 2.09- and 2.49-fold significantly increased compared with that in the sucrose. The iAUCs for plasma ORAC after 1 g and 2 g CTE were 1.93- and 2.69-fold, respectively ($P < 0.05$).

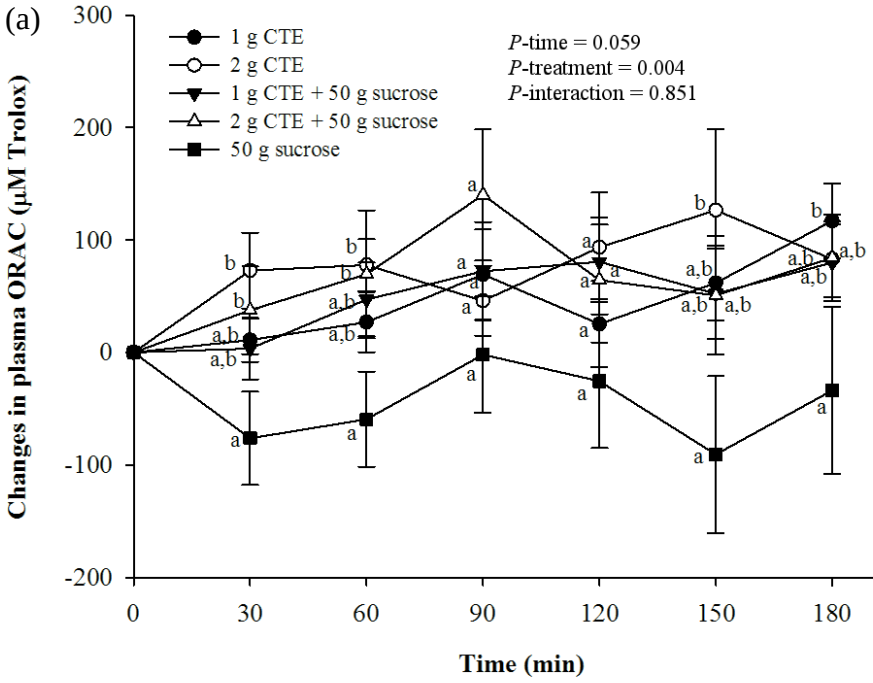


Figure 4.7 Postprandial plasma ORAC of five different beverages. (a) ORAC concentrations during 180 min of five different beverages; (b) ORAC changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

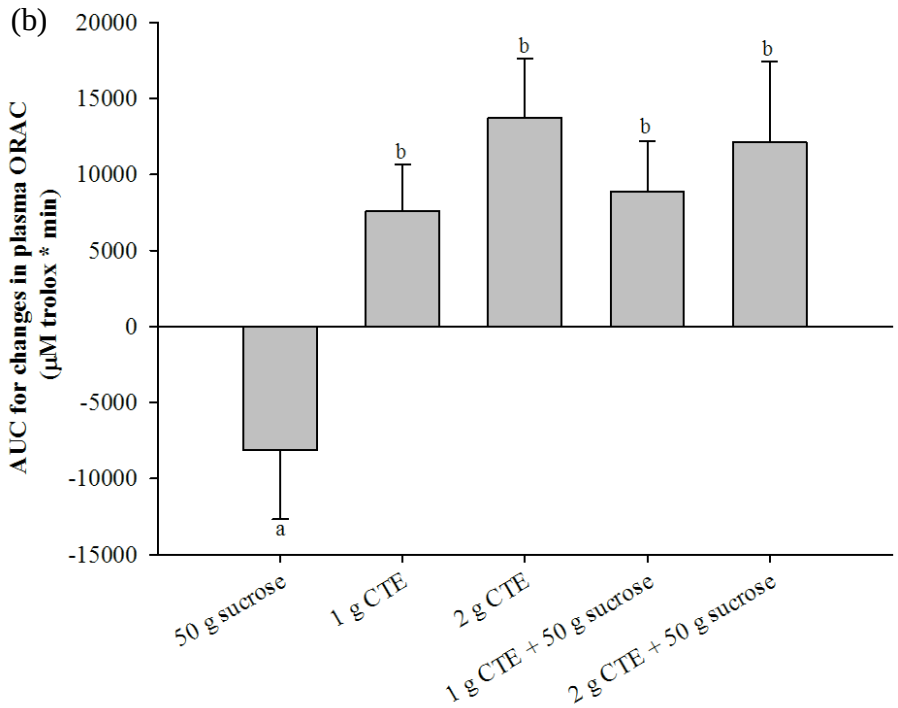


Figure 4.7 Postprandial plasma ORAC of five different beverages. (a) ORAC concentrations during 180 min of five different beverages; (b) ORAC changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters are significantly different ($P < 0.05$).

Plasma thiol

Postprandial plasma thiol level was significantly reduced at 30 min after sucrose consumption as shown in Figure 4.8a. A significant effect for treatment (P = 0.001) and time (P < 0.001) was observed. Consumption of 1 g and 2 g CTE with or without sucrose caused a significant rise in postprandial plasma total thiol at 30 and 180 min after (P < 0.05). The iAUCs for plasma thiol concentration after CTE consumption were significantly different from that with sucrose (Figure 4.8b). There were 2.87- and 3.15-fold significantly higher after 1 g and 2 g CTE, respectively. The iAUCs for plasma thiol after both 1 g and 2 g CTE with sucrose were resulted in 2.63-fold higher than the corresponding iAUCs after sucrose (P < 0.05).

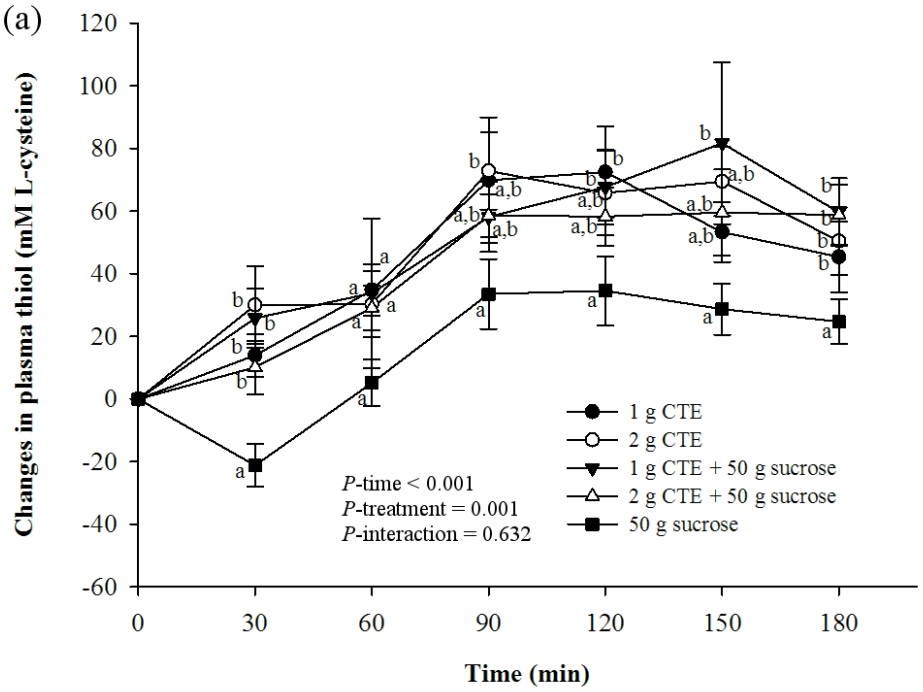


Figure 4.8 Postprandial plasma thiol of five different beverages. (a) Changes in plasma thiol concentrations during 180 min of five different beverages; (b) Thiol changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters denote statistically significant differences (P<0.05).

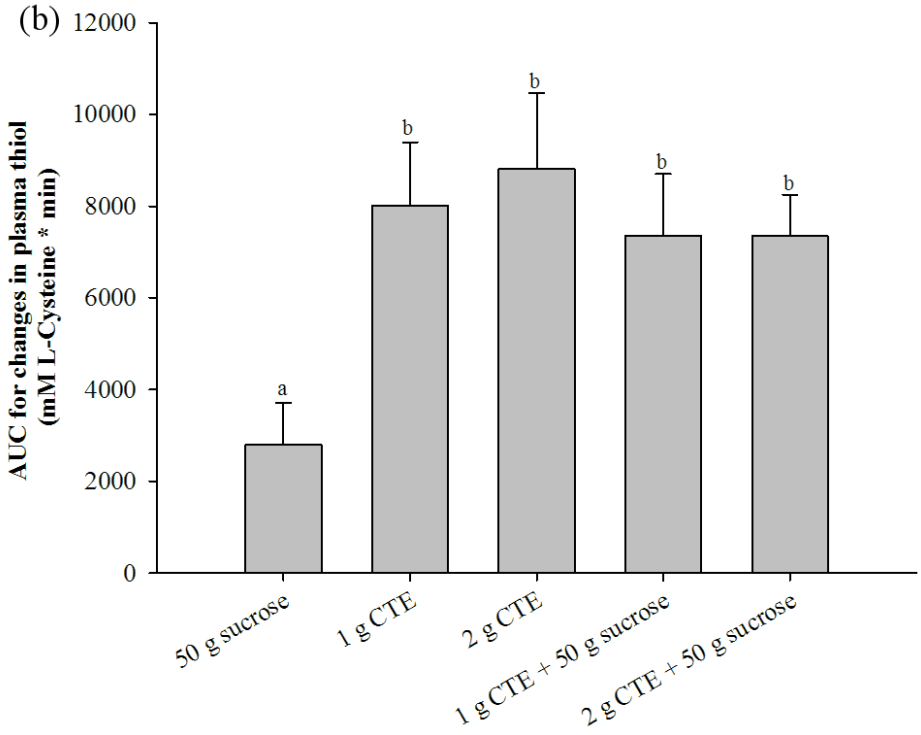


Figure 4.8 Postprandial plasma thiol of five different beverages. (a) Changes in plasma thiol concentrations during 180 min of five different beverages; (b) Thiol changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters denote statistically significant differences (P<0.05).

Lipid peroxidation

The postprandial plasma MDA significantly increased after 30, 60, 150 and 180 after ingestion of sucrose (Figure 4.9a). The results showed that 1g and 2 g CTE with sucrose significantly reduced plasma MDA at all individual time points ($P<0.05$). Ingestion 1 g and 2 g CTE also reduced plasma MDA at 30, 60, 150 and 180 min ($P<0.05$). There were significant lower in iAUCs for plasma MDA after CTE consumption with and without sucrose (Figure 4.9b).

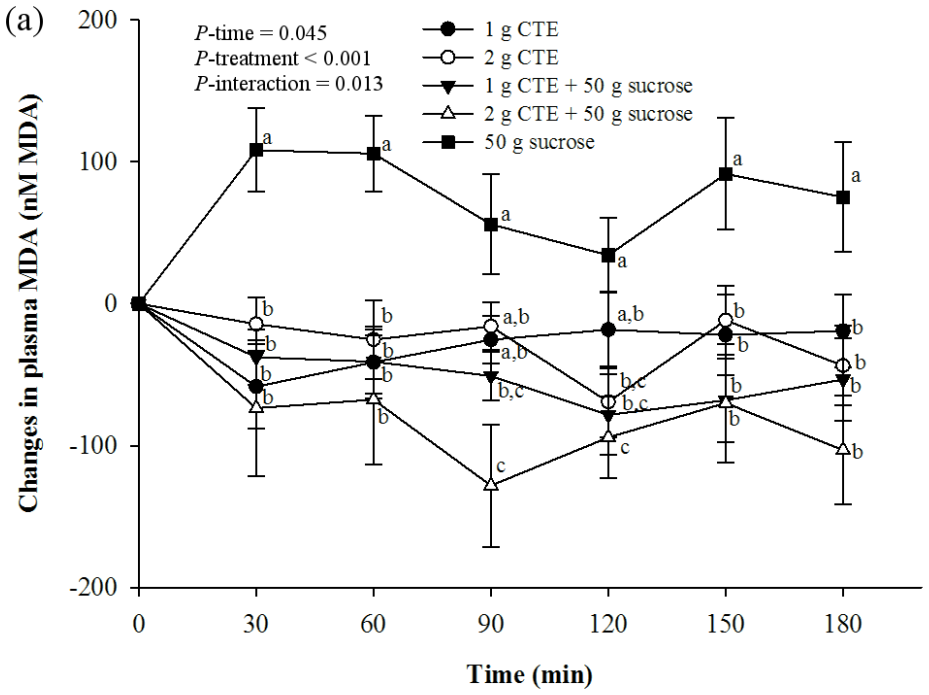


Figure 4.9 Postprandial plasma MDA of five different beverages. (a) Changes in MDA concentrations during 180 min of five different beverages; (b) MDA changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters (a c) denote statistically significant differences (P<0.05).

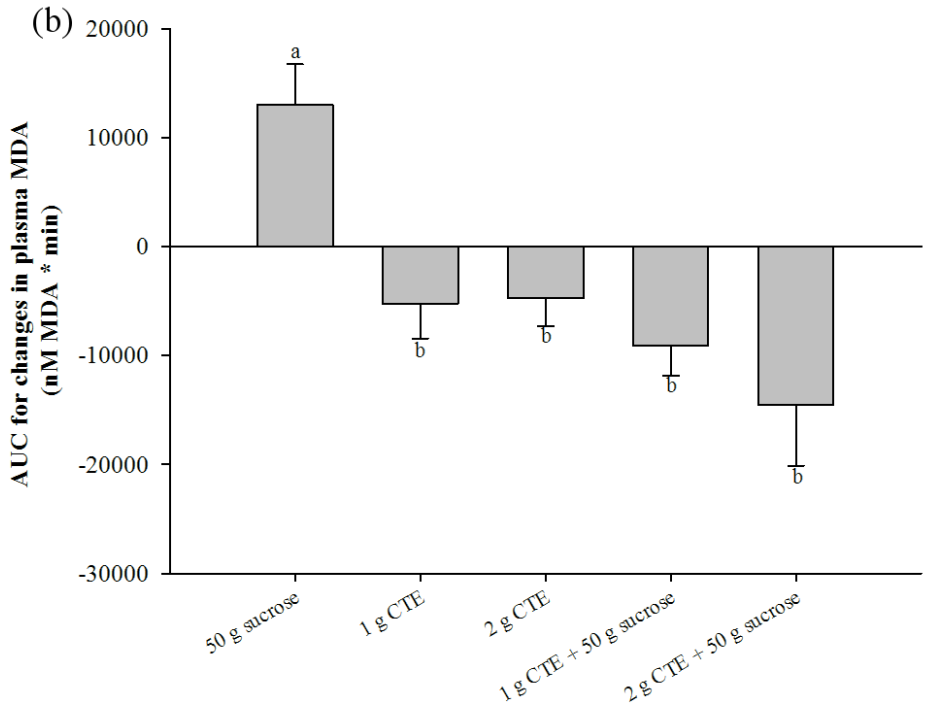


Figure 4.9 Postprandial plasma MDA of five different beverages. (a) Changes in MDA concentrations during 180 min of five different beverages; (b) MDA changes area under the curves (AUCs) of five different beverages. Values are means ± SEM, n=15. Different letters (a c) denote statistically significant differences (P<0.05).

4.2 The effect of CTE on the inhibition of pancreatic α-amylase, *in vitro* starch hydrolysis and predicted glycemic index of different type of flours including potato, cassava, rice, corn, wheat and glutinous rice flour and the application in a bakery product

4.2.1 Inhibition of pancreatic α-amylase

As shown in Figure 4.10, the amount of maltose released from all flours was observed after 10 min of incubation. There was a significant reduction for the release of maltose after mixing the potato, rice, glutinous rice, wheat, corn and cassava flour with 1% and 2% (w/v) CTE compared to the control ($P<0.05$).

The percentage of pancreatic α-amylase inhibitory activity after mixing CTE into flour is shown in Table 4.2. The increased percentage of pancreatic α-amylase inhibitory activity was concomitant with the increased concentration of CTE. The results demonstrated that the mixture of potato, rice, glutinous rice, wheat, corn and cassava flours with 1% and 2% (w/v) CTE resulted in a higher pancreatic α-amylase inhibitory activity than that of the 0.5% (w/v) of CTE ($P<0.05$). At 2 % (w/v) CTE, the potato flour had the highest percentage of pancreatic α-amylase inhibitory activity, followed by glutinous rice, rice, wheat, corn and cassava, respectively.

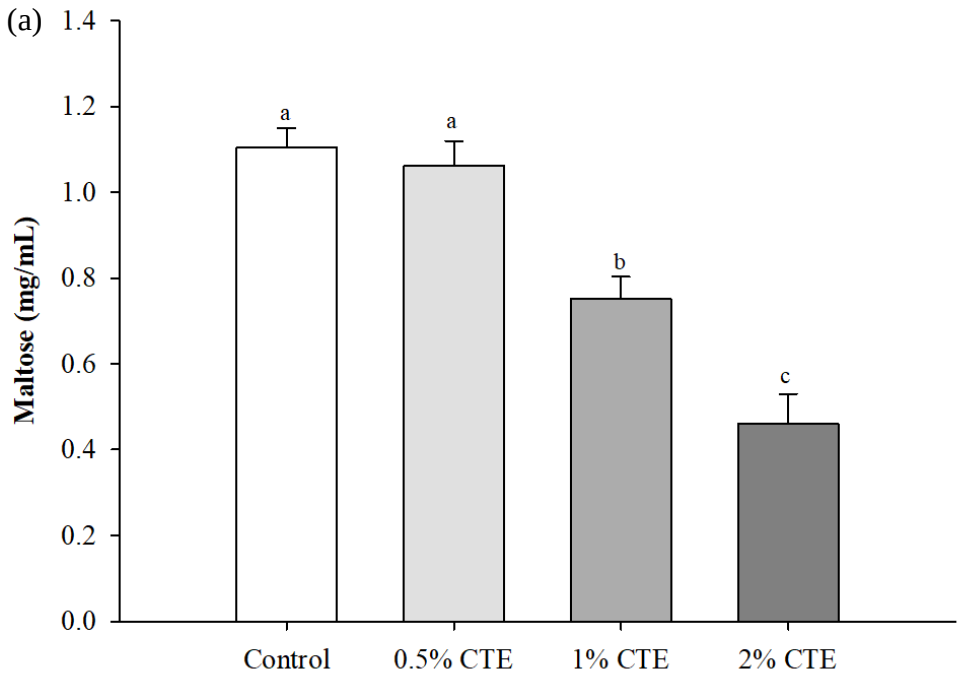


Figure 4.10 The amount of maltose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE against pancreatic α-amylase activity at 10 min. The results are expressed as mean ± S.E.M., n=4. Different letters denote statistically significant differences in mean values. (P<0.05). Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

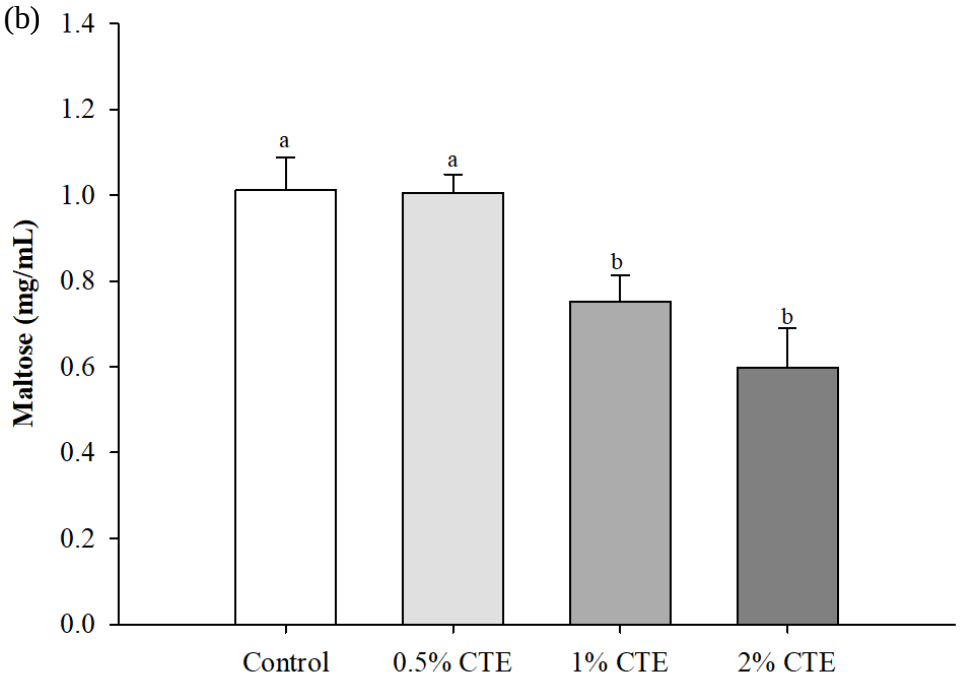


Figure 4.10 The amount of maltose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE against pancreatic α-amylase activity at 10 min. The results are expressed as mean ± S.E.M., n=4. Different letters denote statistically significant differences in mean values. (P<0.05). Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

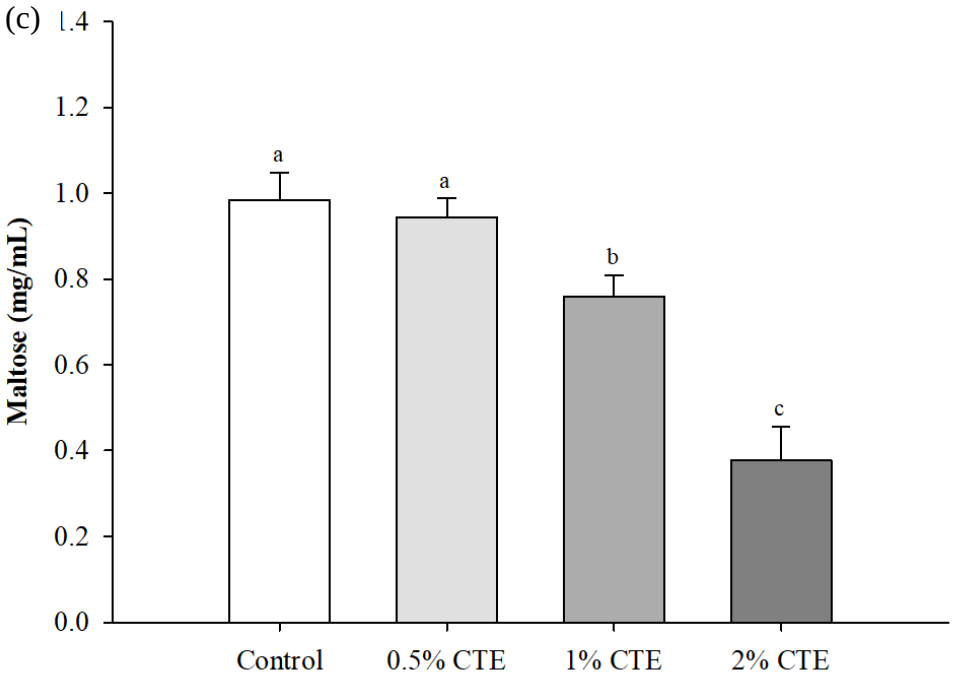


Figure 4.10 The amount of maltose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE against pancreatic α-amylase activity at 10 min. The results are expressed as mean ± S.E.M., n=4. Different letters denote statistically significant differences in mean values. (P<0.05). Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

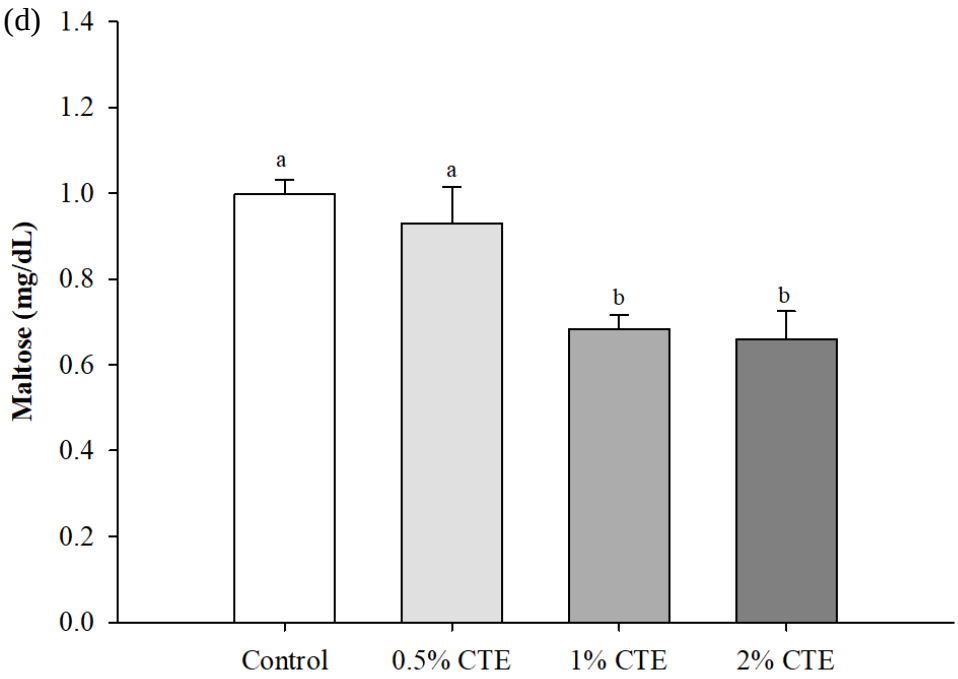
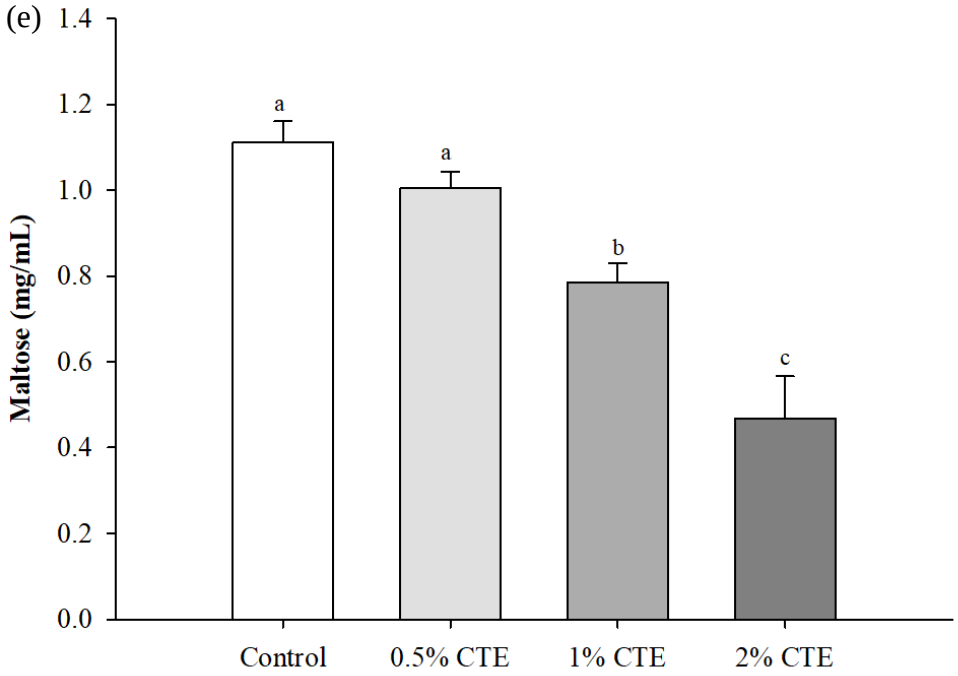


Figure 4.10 The amount of maltose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE against pancreatic α-amylase activity at 10 min. The results are expressed as mean ± S.E.M., n=4. Different letters denote statistically significant differences in mean values. (P<0.05). Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.



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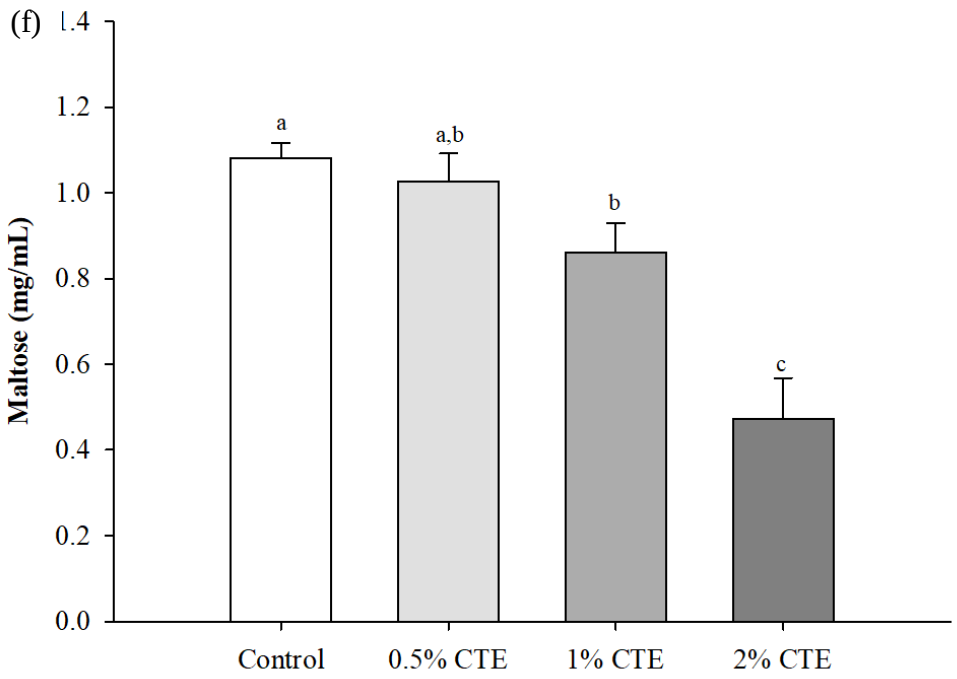


Figure 4.10 The amount of maltose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE against pancreatic α-amylase activity at 10 min. The results are expressed as mean ± S.E.M., n=4. Different letters denote statistically significant differences in mean values. (P<0.05). Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

Table 4.2 The percentage of pancreatic α-amylase inhibitory activity of CTE.

CTE	% Inhibition					
	Potato	Rice	Glutinous rice	Wheat	Corn	Cassava
0.5% (w/v)	7.1 ± 4.7[a]	23.8 ± 9.2[a]	20.8 ± 9.2[a]	17.4 ± 6.5[a]	24.1 ± 9.5[a]	12.9 ± 1.4[a]
1% (w/v)	56.7 ± 7.8[b]	82.1 ± 8.1[b]	51.7 ± 8.5[b]	50.1 ± 7.7[b]	48.3 ± 8.2[b]	34.5 ± 8.9[b]
2% (w/v)	93.4 ± 5.7[c]	87.3 ± 13.4[b]	89.7 ± 11.9[c]	85.2 ± 4.3[c]	81.6 ± 10.6[c]	79.9 ± 19.7[c]

The results are expressed as mean ± S.E.M., n=4. Different letters denote statistically significant differences in mean values. ($P<0.05$).

4.2.2 *In vitro* starch digestibility and predicted glycemic index (pGI)

The results of *in vitro* starch digestibility of flour at different concentration of CTE are shown in Figure 4.11. The incorporation of CTE decreased the glucose released from flour. The results demonstrated that the amount of glucose released from the mixture of CTE and flours was lower than the control. The addition of 2% (w/v) CTE into flour significantly caused the highest inhibition of starch digestibility.

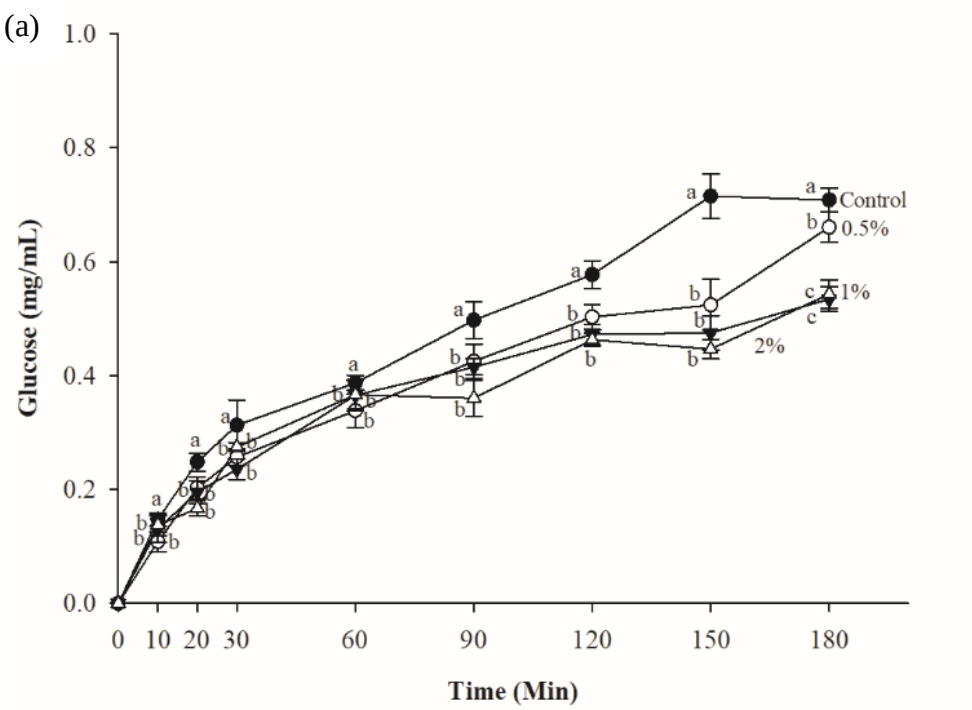


Figure 4.11 The amount of glucose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when combination with the different concentration of CTE under in vitro digestibility during 180 min. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

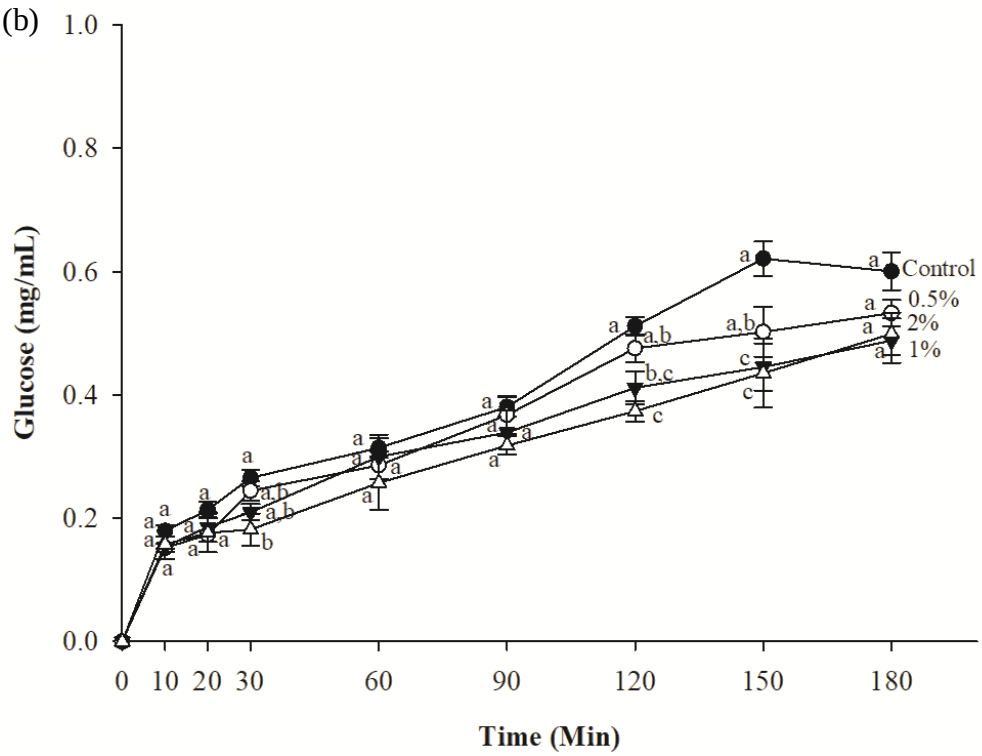


Figure 4.11 The amount of glucose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when combination with the different concentration of CTE under *in vitro* digestibility during 180 min. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

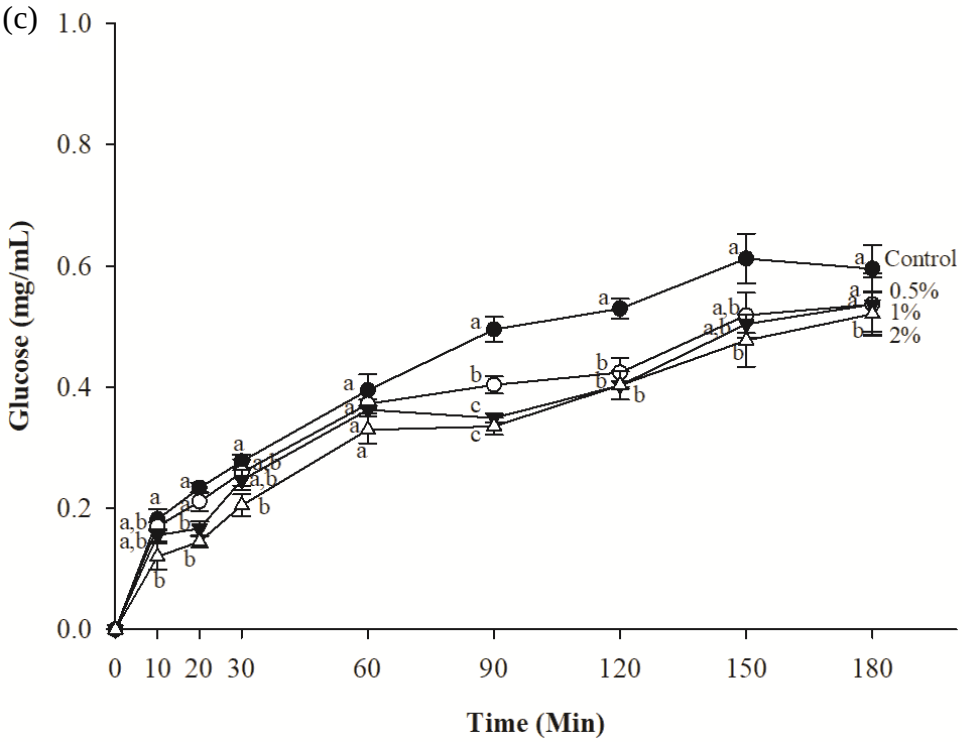


Figure 4.11 The amount of glucose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when combination with the different concentration of CTE under *in vitro* digestibility during 180 min. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

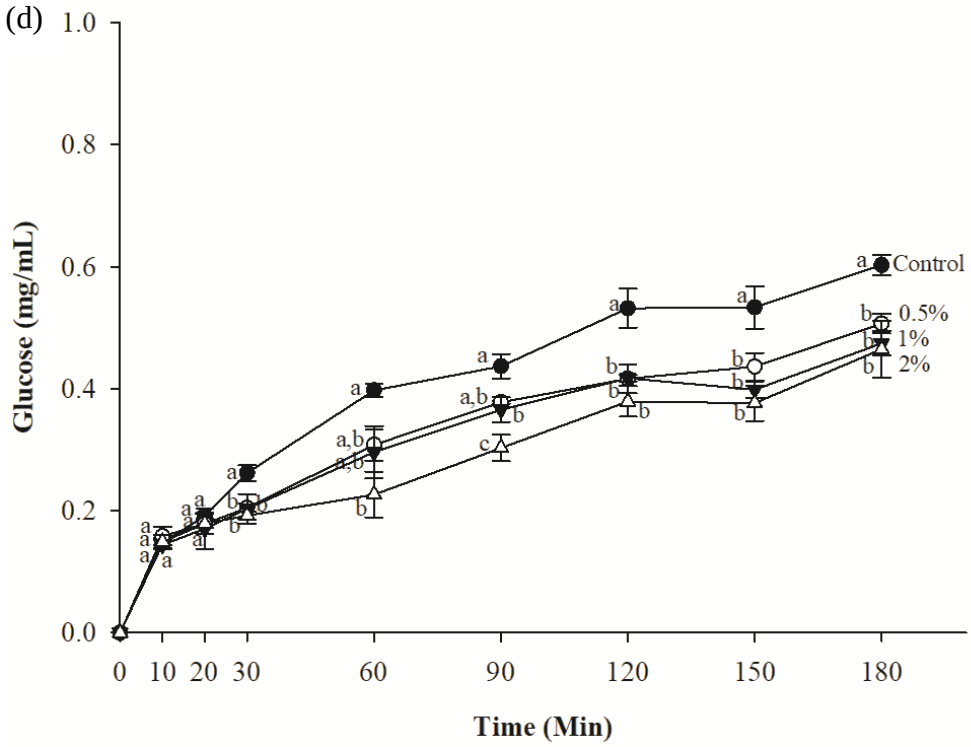


Figure 4.11 The amount of glucose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when combination with the different concentration of CTE under *in vitro* digestibility during 180 min. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

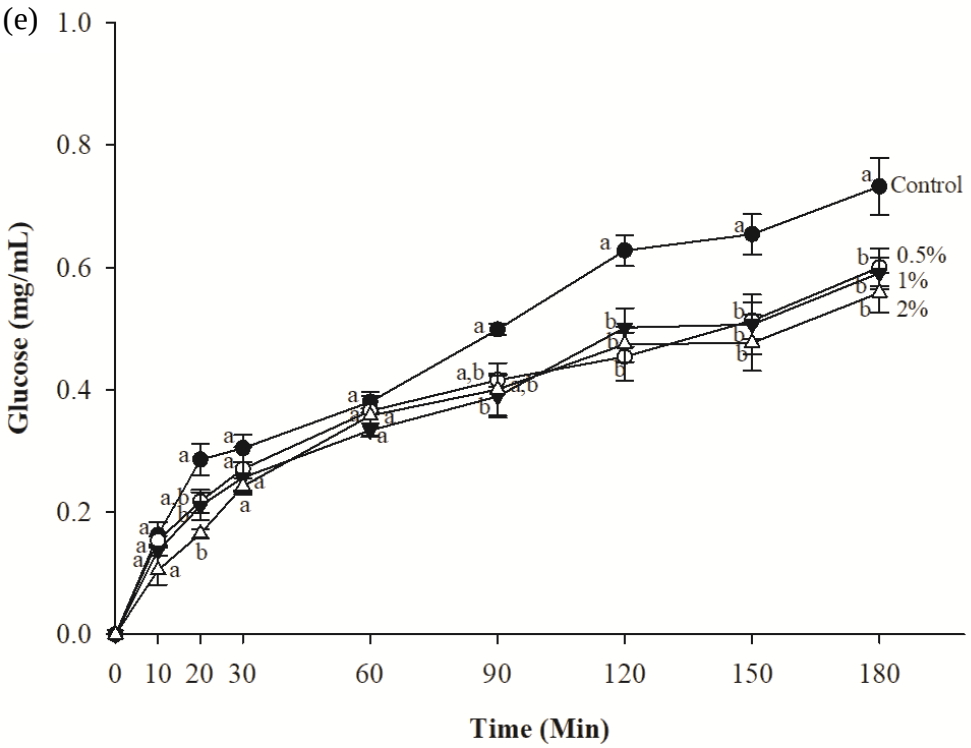


Figure 4.11 The amount of glucose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when combination with the different concentration of CTE under *in vitro* digestibility during 180 min. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

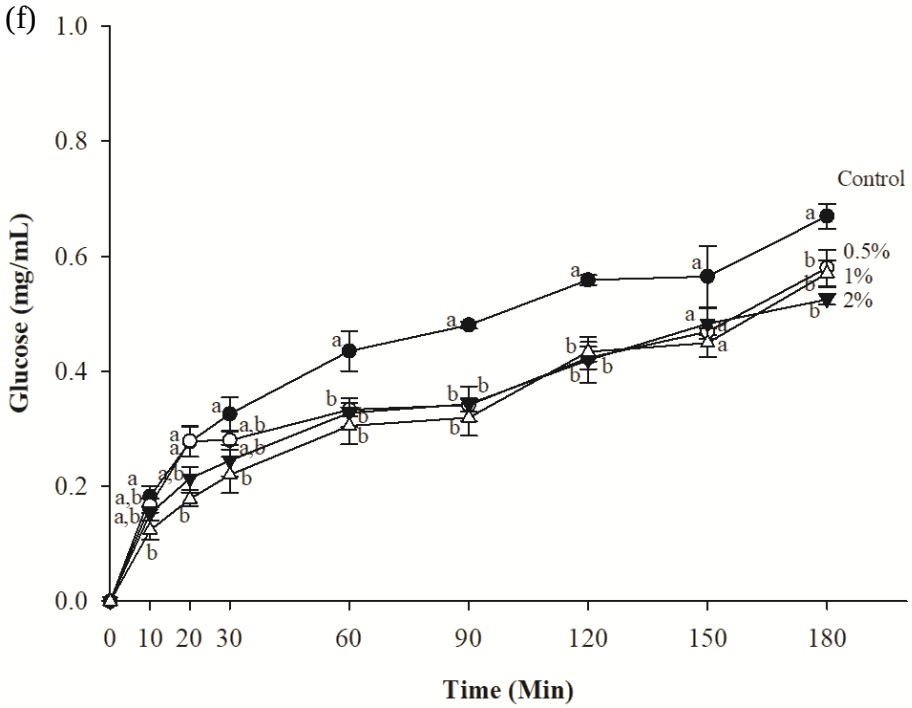


Figure 4.11 The amount of glucose released from (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when combination with the different concentration of CTE under *in vitro* digestibility during 180 min. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a or b) were similar and no statistically significant differences were observed for these samples.

Table 4.3 represents an interpretation of the hydrolysis index (HI) and the predicted glycemic index (pGI) of all of the samples. The HI and pGI of potato, rice, glutinous rice, wheat, corn and cassava flour with 0.5%, 1% and 2% (w/v) of CTE were significantly lowered when compared with the control (P<0.05). Interestingly, the addition of 2% (w/v) CTE caused the reduction of pGI of the glutinous rice, wheat, and cassava flour from the high value to the medium value (GI<70).

Table 4.3 The hydrolysis index (HI) and predicted glycemic index (pGI) of the flours with CTE.

CTE	Hydrolysis index (HI)					
	Potato	**Rice**	**Glutinous rice**	**Wheat**	**Corn**	**Cassava**
Control	85.2 ± 0.5^{a}	74.1 ± 4.3^{a}	86.9 ± 2.4^{a}	74.3 ± 2.5^{a}	94.4 ± 3.6^{a}	81.0 ± 1.3^{a}
0.5% (w/v)	71.0 ± 1.6^{b}	63.4 ± 2.4^{a,b}	65.5 ± 4.1^{b}	62.4 ± 3.7^{b}	69.5 ± 3.2^{b}	65.5 ± 2.2^{b}
1% (w/v)	68.8 ± 3.7^{b,c}	57.2 ± 4.9^{b}	63.7 ± 5.2^{b}	61.0 ± 1.4^{b}	68.5 ± 2.2^{b}	61.3 ± 3.4^{b,c}
2% (w/v)	62.2 ± 2.1^{c}	55.2 ± 3.3^{b}	50.3 ± 5.1^{c}	50.4 ± 4.8^{c}	59.0 ± 2.3^{c}	51.5 ± 6.0^{c}
CTE	**Predicted glycemic index (pGI)**					
	Potato	**Rice**	**Glutinous rice**	**Wheat**	**Corn**	**Cassava**
Control	86.5 ± 0.3^{a}	80.4 ± 2.4^{a}	87.2 ± 1.3^{a}	80.5 ± 1.4^{a}	91.2 ± 2.0^{a}	84.2 ± 0.7^{a}
0.5% (w/v)	78.7 ± 0.9^{b}	74.5 ± 1.3^{a,b}	75.7 ± 2.2^{b}	74.0 ± 2.0^{b}	77.9 ± 1.8^{b}	75.7 ± 1.2^{b}
1% (w/v)	77.5 ± 2.0^{b,c}	71.1 ± 2.7^{b}	74.7 ± 2.8^{b}	73.2 ± 0.8^{b}	77.3 ± 1.2^{b}	73.4 ± 1.9^{b,c}
2% (w/v)	73.8 ± 1.1^{c}	70.0 ± 1.8^{b}	67.4 ± 2.8^{c}	67.4 ± 2.6^{c}	72.1 ± 1.3^{c}	68.0 ± 3.3^{c}

The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values (P<0.05). Mean values with the same superscript letters (a, b, or c) were similar and no statistically significant differences were observed for these samples.

4.2.3 Starch fraction

The RDS, SDS, and undigested starch of flours are presented in Figure 4.12. The RDS content of six flours mixed with at 2% (w/v) CTE significantly decreased when compared with the control (P<0.05). The addition of CTE at 0.5%, 1% and 2% (w/v) caused a reduction in the SDS content of glutinous rice, corn and cassava flour (P<0.05). However, CTE did not alter the SDS content of potato flour. The observed results also found that only the glutinous rice flour significantly increased the undigested starch with the addition of CTE (P<0.05). Table 4.4 shows the correlation between the concentration of CTE and undigested starch of flour. The undigested starch of the wheat and cassava flour correlated significantly and positively with the concentration of CTE (r=0.650 and 0.758, respectively; P<0.05). However, no significant correlation was observed between the concentration of CTE and other flours, including potato flour, rice flour, glutinous rice flour and corn flour.

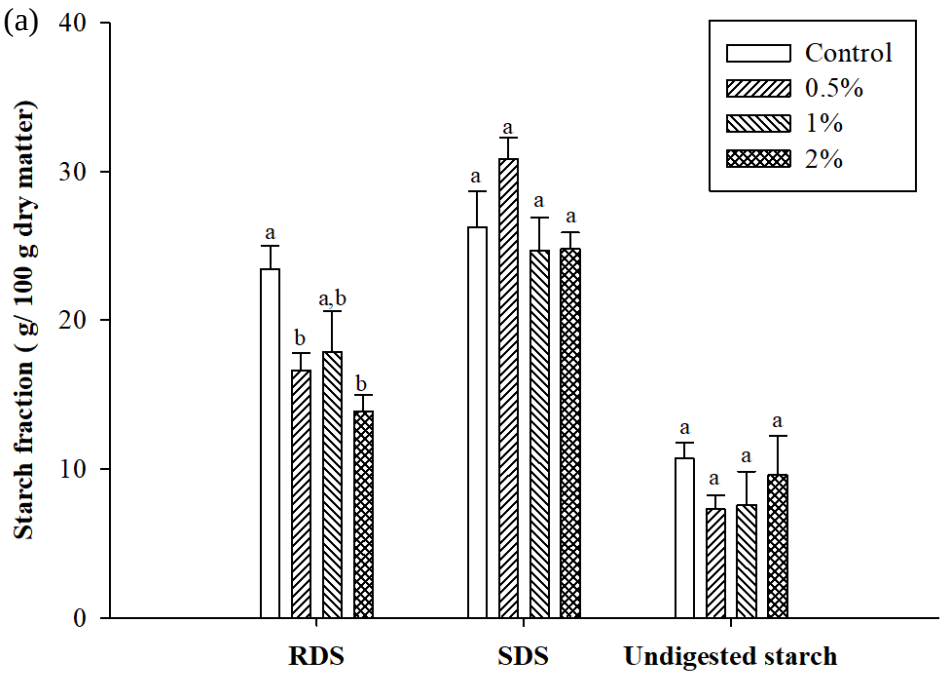


Figure 4.12 Starch fraction after *in vitro* digestibility of (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a, b, or c) were similar and no statistically significant differences were observed for these samples. RDS—rapidly digestible starch; SDS—slowly digestible starch.

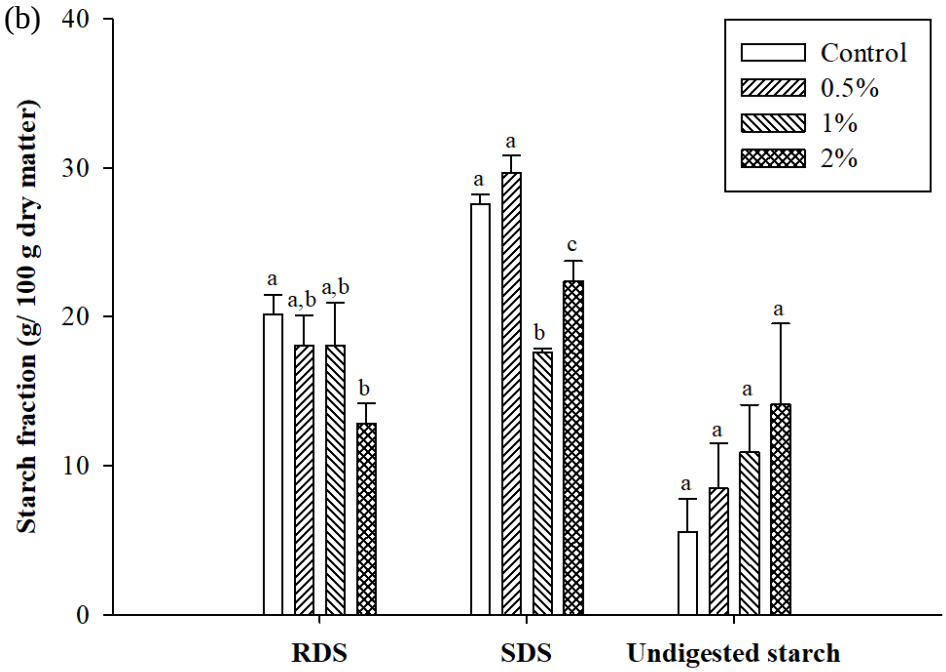
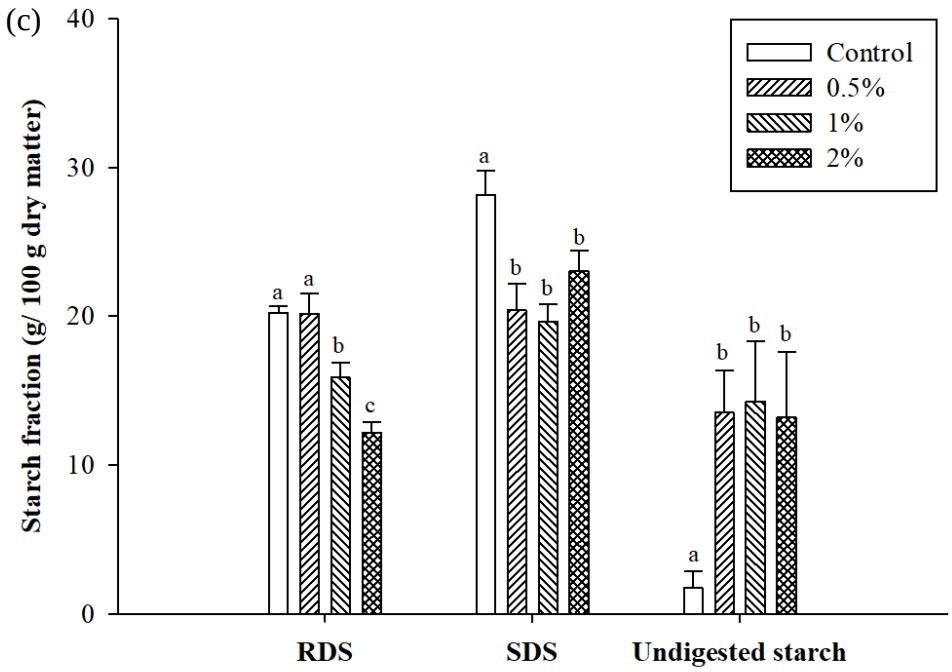


Figure 4.12 Starch fraction after *in vitro* digestibility of (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a, b, or c) were similar and no statistically significant differences were observed for these samples. RDS—rapidly digestible starch; SDS—slowly digestible starch.



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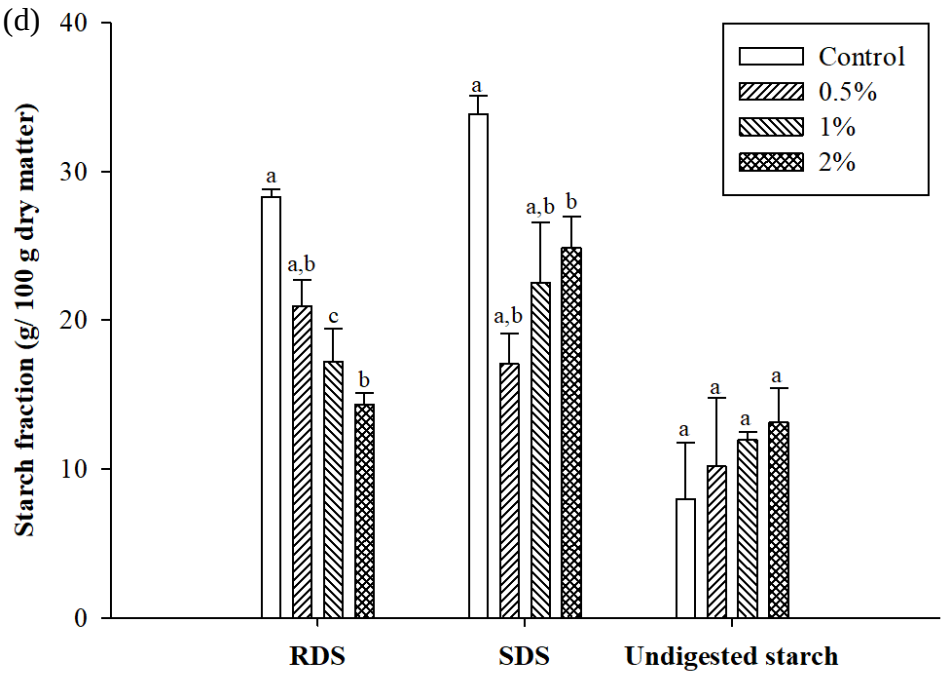

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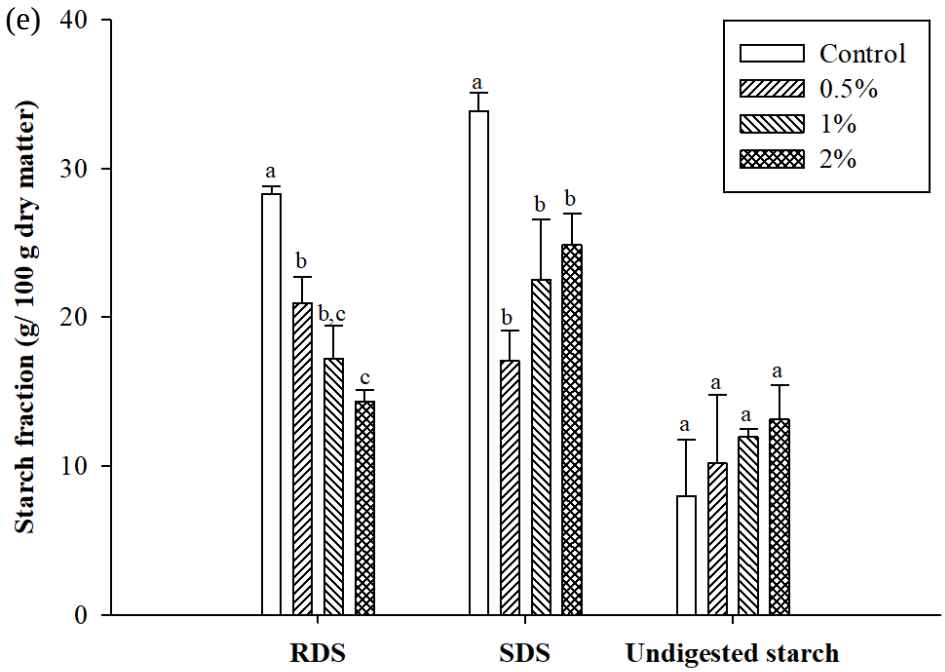

Figure 4.12 Starch fraction after *in vitro* digestibility of (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a, b, or c) were similar and no statistically significant differences were observed for these samples. RDS—rapidly digestible starch; SDS—slowly digestible starch.

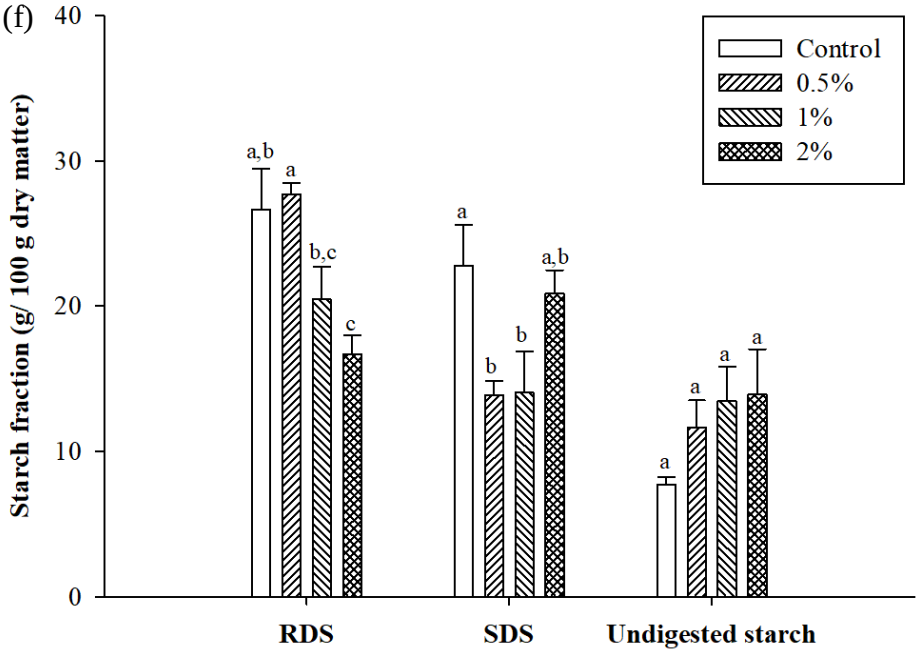

Figure 4.12 Starch fraction after *in vitro* digestibility of (a) potato flour, (b) rice flour, (c) glutinous rice flour, (d) wheat flour, (e) corn flour and (f) cassava flour when in combination with the different concentration of CTE. The value of 0.5%, 1% and 2% (w/v) represent the concentration of CTE, respectively. The results are expressed as mean ± S.E.M., n=4. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a, b, or c) were similar and no statistically significant differences were observed for these samples. RDS—rapidly digestible starch; SDS—slowly digestible starch.

Table 4.4 Correlation coefficients calculated between the concentration of *Clitoria ternatea* (CTE) and undigested starch contents after *in vitro* digestion

	Potato	Rice	Glutinous rice	Wheat	Corn	Cassava
CTE	-0.040	0.511	0.486	0.650*	0.373	0.758*

*Significant correlations (P<0.05).

4.2.4 *In vitro* digestibility of bread

Cross sections of bread made from wheat flour and CTE are shown in Figure 4.13. The *in vitro* starch digestibility of wheat bread with 5%, 10%, and 20 % (w/w) of the CTE are presented in Figure 4.14a. The amount of glucose released from bread with CTE was lower than that of the control. The addition of 5%, 10%, and 20% (w/w) CTE into wheat bread significantly reduced the rate of starch digestion after 120, 150 and 180 min of incubation ($P<0.05$). As shown in Figure 4.14b, the iAUCs for the glucose release of bread incorporated with 5-20% (w/w) CTE were 8,136 ± 82, 8,997 ± 42 and 7,363 ± 386 mg/dL.min, respectively (the control = 11,364 ± 172 mg/dL.min). The pGI of the bread with 5-20% (w/w) CTE was 65.40 ± 0.26, 68.11 ± 0.13 and 62.96 ± 1.22, respectively, whereas the wheat bread had the pGI of 75.58 ± 0.54.

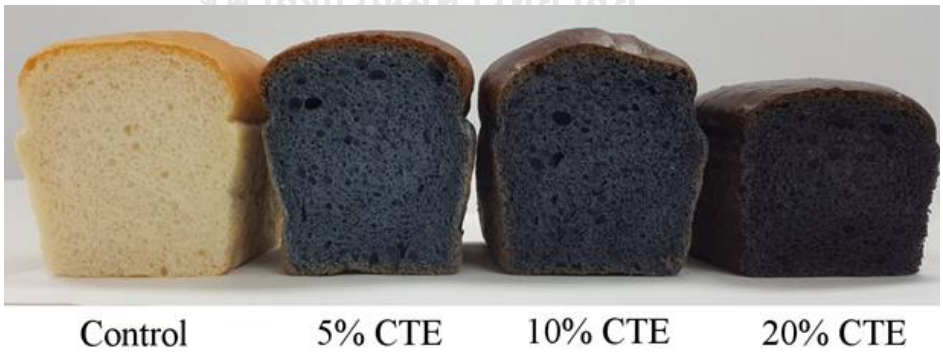

Figure 4.13 The cross section of bread made from wheat flour and CTE

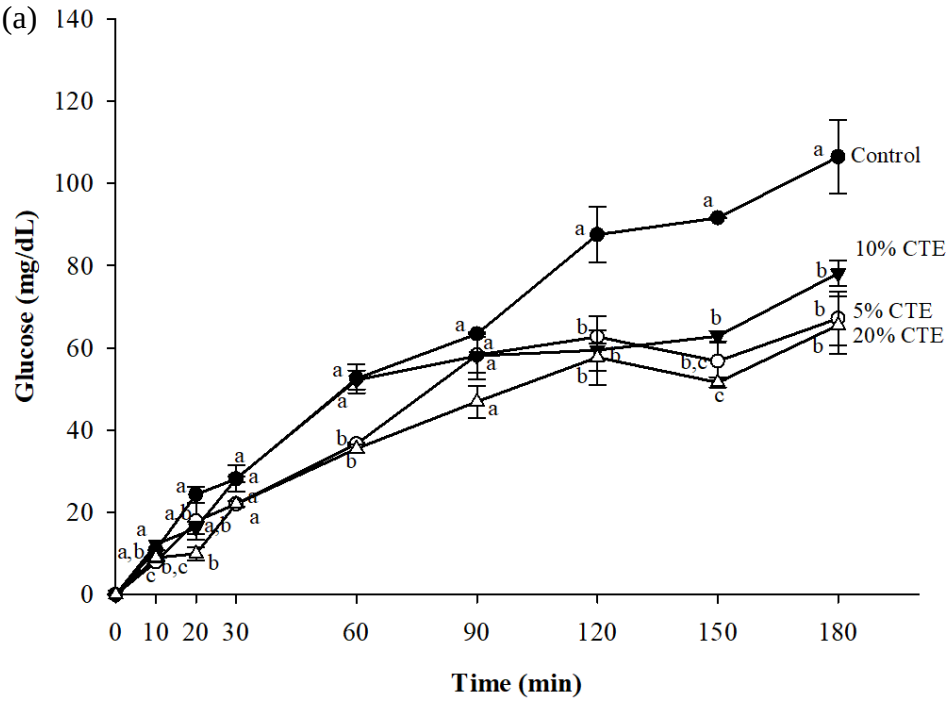

Figure 4.14 The amount of glucose released from wheat bread after in vitro digestibility (a) and incremental area under the curve (iAUC) for glucose release (b) when in combination with the different concentration of CTE. The values of 5%, 10% and 20% (w/w) represent the concentration of CTE, respectively. The results are expressed as mean ± SEM. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a, b, or c) were similar and no statistically significant differences were observed for these samples.

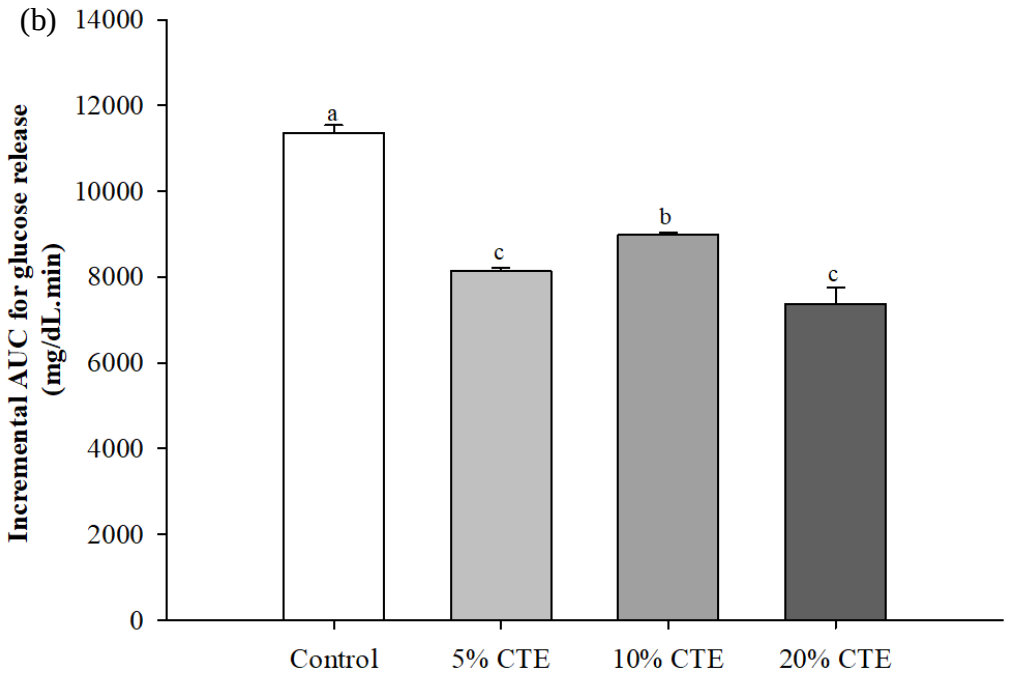

Figure 4.14 The amount of glucose released from wheat bread after *in vitro* digestibility (a) and incremental area under the curve (iAUC) for glucose release (b) when in combination with the different concentration of CTE. The values of 5%, 10% and 20% (w/w) represent the concentration of CTE, respectively. The results are expressed as mean ± SEM. The different letters denote statistically significant differences in mean values. (P<0.05) Mean values with the same superscript letters (a, b, or c) were similar and no statistically significant differences were observed for these samples.

4.3 The effectiveness of CTE incorporation into rice on *in vitro* and *in vivo* starch digestibility of cooked rice using domestic cooking methods

4.3.1 *In vitro* starch digestibility of cooked rice with CTE

The photographs of cooked rice with 1.25 % and 2.5% (w/v) CTE using an electric rice cooker and a microwave oven are presented in Figure 4.15. Figure 4.16a shows the incremental reducing sugars release of CTE incorporated with cooked rice using an electric rice cooker and a microwave oven during *in vitro* digestibility. The highest reducing sugars release was found in the control (cooked rice). In contrast, cooked rice with 1.25% and 2.5% CTE exhibited a significant reduction in the release of reducing sugars when compared with the control at every individual time points except 120 min. As shown in Figure 4.16b, the iAUCs for reducing sugars release of cooked rice with 1.25% and 2.5% CTE (81,797 ± 3,966 and 75,409 ± 1,680 mg glucose equivalents/ g rice.min, respectively) were lower than the control (10,3295 ± 5,562 mg glucose equivalents/ g rice.min; $P<0.05$). In the microwave method, the incremental reducing sugars release of cooked rice with 1.25% and 2.5% CTE were slightly lower than the control (Figure 4.16c). The iAUC for the reducing sugars release of cooked rice with 2.5% CTE (62,030 ± 1,770 mg glucose equivalents/ g rice.min) was lower than the control (80,332 ± 5,725 mg glucose equivalents/ g rice.min) (Figure 4.16d).

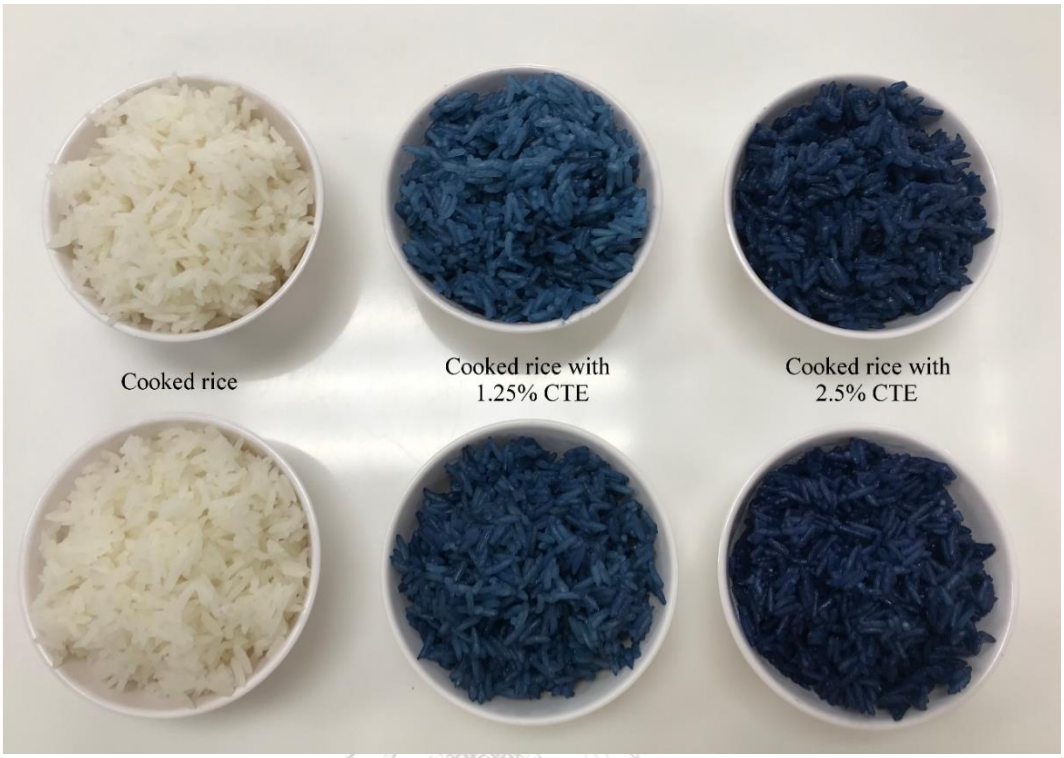

Figure 4.15 The photographs of Clitoria ternatea L. flower cooked rice by an electric rice cooker (top) and cooked rice by a microwave oven (bottom)

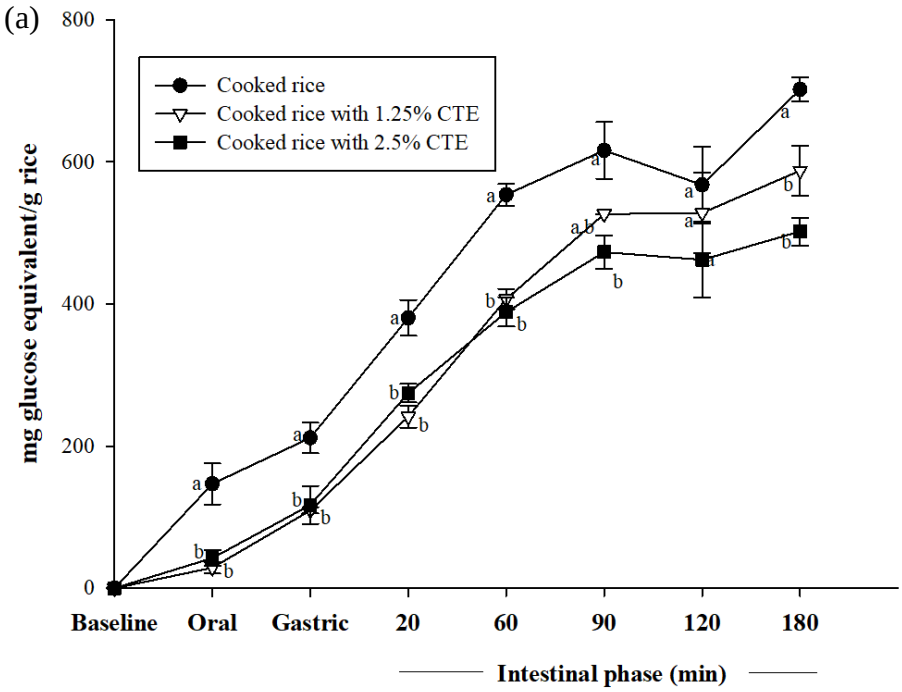

Figure 4.16 The incremental reducing sugars release of cooked rice incorporated with CTE using (a) an electric rice cooker and (c) a microwave oven during in vitro digestibility. The iAUCs for reducing sugars release of cooked rice with CTE using (b) an electric rice cooker and (d) a microwave oven. The results are expressed as mean ± S.E.M., n=4. Different letters (a c) denote statistically significant differences (P<0.05).

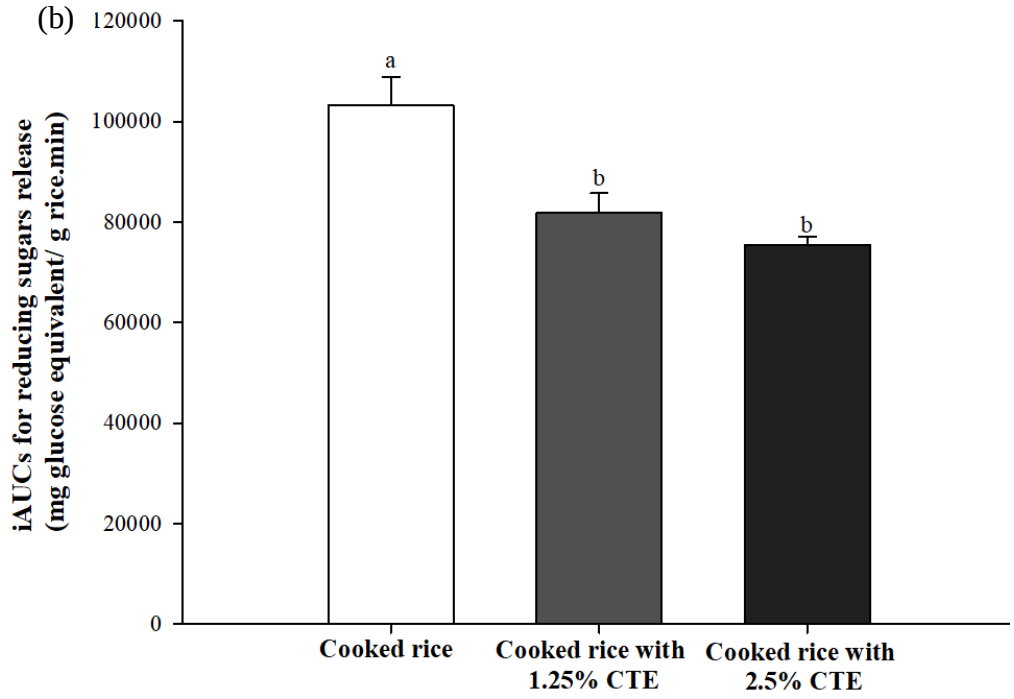

Figure 4.16 The incremental reducing sugars release of cooked rice incorporated with CTE using (a) an electric rice cooker and (c) a microwave oven during *in vitro* digestibility. The iAUCs for reducing sugars release of cooked rice with CTE using (b) an electric rice cooker and (d) a microwave oven. The results are expressed as mean ± S.E.M., n=4. Different letters (a c) denote statistically significant differences ($P<0.05$).

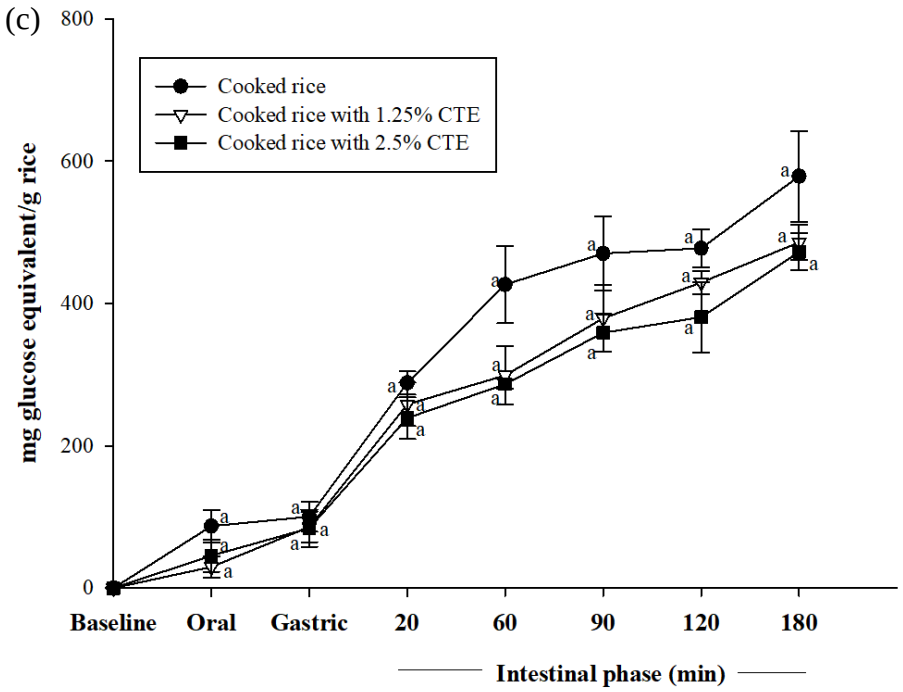

Figure 4.16 The incremental reducing sugars release of cooked rice incorporated with CTE using (a) an electric rice cooker and (c) a microwave oven during *in vitro* digestibility. The iAUCs for reducing sugars release of cooked rice with CTE using (b) an electric rice cooker and (d) a microwave oven. The results are expressed as mean ± S.E.M., n=4. Different letters (a c) denote statistically significant differences (P<0.05).

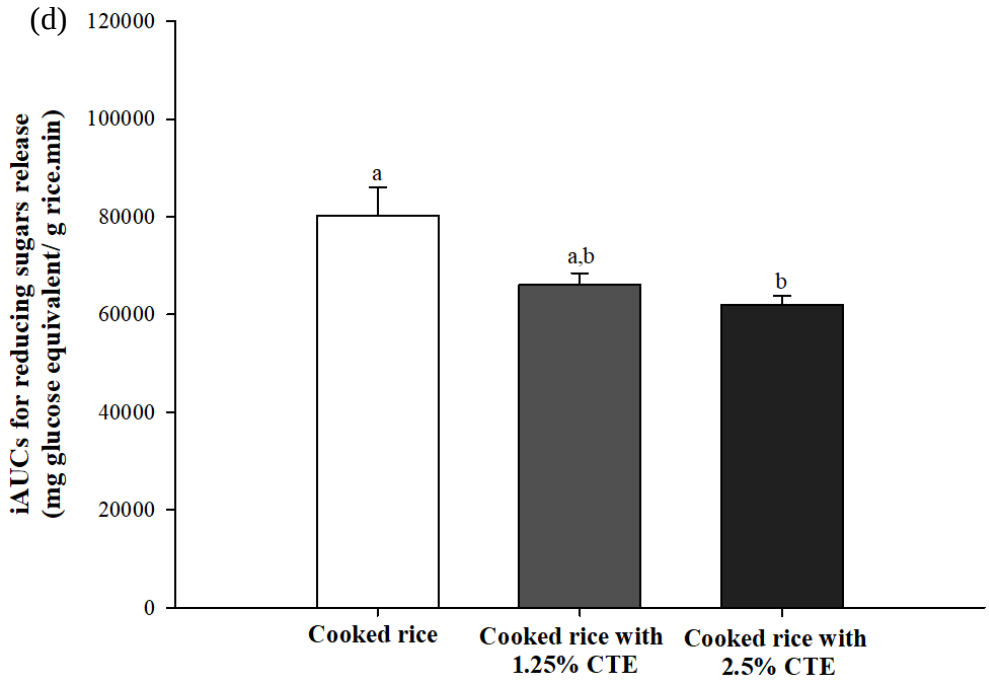

Figure 4.16 The incremental reducing sugars release of cooked rice incorporated with CTE using (a) an electric rice cooker and (c) a microwave oven during *in vitro* digestibility. The iAUCs for reducing sugars release of cooked rice with CTE using (b) an electric rice cooker and (d) a microwave oven. The results are expressed as mean ± S.E.M., n=4. Different letters (a c) denote statistically significant differences (P<0.05).

4.3.2 Texture profile analysis (TPA) of cooked rice with CTE

Table 4.5 presents mean intensities for instrumental texture attributes (hardness, stickiness, springiness, cohesiveness, gumminess and chewiness) of cooked rice containing CTE using an electric rice cooker and a microwave oven. In the electric rice cooker method, addition of CTE (1.25% and 2.5%) significantly resulted in decreased intensity attributes for stickiness and increased intensity attributes for cohesiveness (P = 0.003 and P=0.012, respectively). In the microwave method, there were no significant differences in all texture parameters between cooked rice with and without CTE. The hardness of cooked rice with CTE using both domestic cooking methods was similar to the control group.

Table 4.5 The intensities for instrumental texture profile analysis of cooked rice with CTE using an electric rice cooker and a microwave oven

Samples	Texture attributes						
	Hardness (kg)	Stickiness (g)	Adhesiveness (g.sec)	Springiness (mm)	Cohesiveness	Gumminess	Chewiness (kg)
Rice cooker							
Cooked rice	14.3 ± 0.5 [a]	-32.7 ± 0.6[a]	-9.9 ± 0.2 [a]	0.5 ± 0.0 [a]	0.6 ± 0.0[a]	9.3 ± 0.3 [a]	4.6 ± 0.1 [a]
Cooked rice with 1.25% CTE	14.5 ± 0.9 [a]	-23.2 ± 2.1[b]	-10.1 ± 0.1 [a]	0.5 ± 0.0 [a]	0.7 ± 0.0[b]	9.8 ± 0.6 [a]	5.2 ± 0.4 [a]
Cooked rice with 2.5% CTE	14.2 ± 0.6 [a]	-23.5 ± 1.5[b]	-10.2 ± 0.2 [a]	0.5 ± 0.0 [a]	0.7 ± 0.0[b]	9.8 ± 0.3 [a]	5.2 ± 0.2 [a]
Microwave oven							
Cooked rice	16.7 ± 0.2 [a]	-21.1 ± 3.8 [a]	-10.0 ± 0.4 [a]	0.5 ± 0.0 [a]	0.6 ± 0.0 [a]	10.8 ± 0.6 [a]	5.8 ± 0.5 [a]
Cooked rice with 1.25% CTE	15.3 ± 2.0 [a]	-22.7 ± 4.8 [a]	-10.1 ± 0.2 [a]	0.5 ± 0.0 [a]	0.6 ± 0.0 [a]	10.0 ± 1.7 [a]	5.4 ± 1.2 [a]
Cooked rice with 2.5% CTE	16.1 ± 0.2 [a]	-24.1 ± 4.6 [a]	-10.1 ± 0.3 [a]	0.5 ± 0.0 [a]	0.6 ± 0.0 [a]	9.8 ± 0.1 [a]	5.3 ± 0.1 [a]

The results are expressed as mean ± S.E.M., n=4. The textural measurements were repeated 10 times for each sample.

Values (a b) with different letters in a same column are significantly different ($P<0.05$).

4.3.3 Sensory evaluation of cooked rice with CTE

The sensory evaluation of cooked rice was performed using 65 untrained panel (41 females and 24 males, average aged 35.17 ± 1.86 and 33.83 ± 2.44 years old, respectively). Table 4.6 shows the effect of CTE in cooked rice on color, appearance, texture, flavor, taste, stickiness, hardness and overall acceptability using an electric rice cooker and microwave oven. After cooking with an electric rice cooker, color, appearance, texture and flavor scores significantly decreased when addition of 2.5% CTE into cooked rice ($P<0.05$). In cooking with a microwave oven, the sensory scores of color and appearance with respect to the cooked rice significantly decreased when addition of 2.5% CTE into cooked rice ($P<0.05$).

Table 4.6 Sensory attributes of cooked rice with CTE using an electric rice cooker and a microwave

Samples	Sensory attributes							
	Color	Appearance	Texture	Flavor	Taste	Stickiness	Hardness	Overall acceptability
Rice cooker								
Cooked rice	7.4 ± 0.2 [a]	7.3 ± 0.2 [a]	6.9 ± 0.2 [a]	7.2 ± 0.2 [a]	7.1 ± 0.2 [a]	6.7 ± 0.2 [a]	6.1 ± 0.3 [a]	7.1 ± 0.2 [a]
Cooked rice with 1.25% CTE	7.0 ± 0.2 [a]	6.7 ± 0.2 [b]	6.3 ± 0.2 [ab]	6.4 ± 0.2 [b]	6.4 ± 0.2 [b]	6.3 ± 0.2 [a]	5.8 ± 0.2 [a]	6.5 ± 0.2 [ab]
Cooked rice with 2.5% CTE	5.8 ± 0.3 [b]	6.0 ± 0.2 [c]	6.1 ± 0.2 [b]	6.2 ± 0.2 [b]	6.2 ± 0.2 [b]	6.2 ± 0.2 [a]	5.8 ± 0.2 [a]	6.2 ± 0.2 [b]
Microwave oven								
Cooked rice	7.3 ± 0.2 [a]	7.1 ± 0.2 [a]	6.5 ± 0.2 [a]	6.6 ± 0.2 [a]	6.8 ± 0.2 [a]	6.3 ± 0.3 [a]	5.8 ± 0.3 [a]	6.7 ± 0.2 [a]
Cooked rice with 1.25% CTE	6.7 ± 0.2 [ab]	6.6 ± 0.2 [a]	6.3 ± 0.2 [a]	6.5 ± 0.2 [a]	6.7 ± 0.2 [a]	6.2 ± 0.2 [a]	6.0 ± 0.2 [a]	6.5 ± 0.2 [a]
Cooked rice with 2.5% CTE	6.3 ± 0.2 [b]	6.0 ± 0.2 [b]	6.4 ± 0.2 [a]	6.2 ± 0.2 [a]	6.4 ± 0.2 [a]	6.4 ± 0.2 [a]	5.8 ± 0.2 [a]	6.3 ± 0.2 [a]

The results are expressed as mean ± S.E.M., n=64. Values (a b) with different letters in a same column are significantly different ($P<0.05$).

4.3.4 Glycemic response in human study

Thirteen subjects were recruited for this study. Four subjects withdrew during the study due to the reasons unrelated to the study. Nine subjects finally completed the study (Figure 4.17). The baseline characteristics of the fifteen subjects are shown in Table 4.7. No adverse events after consumption of tests were observed.

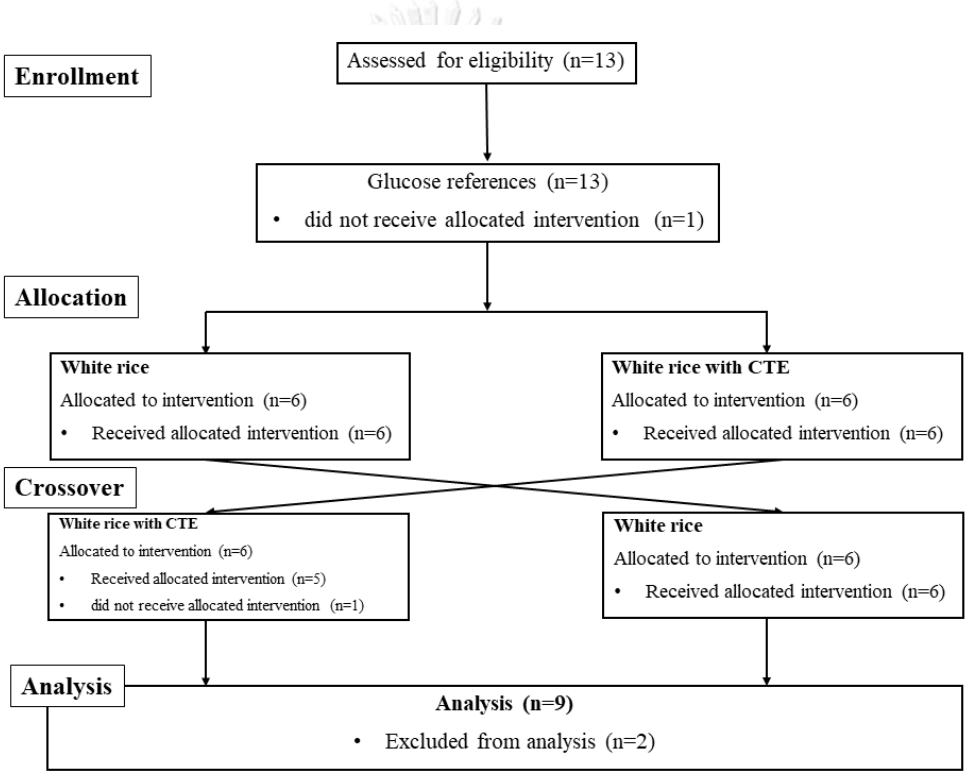

Figure 4.17 The flowchart describing the trial

Table 4.7 Baseline characteristics of the study participants (n=13).

	Mean ± SEM
Age (years)	25.15 ± 0.87
Height (cm)	174.96 ± 1.70
Weight (kg)	68.38 ± 2.13
BMI (kg/m^2)	22.32 ± 0.55
Body fat (%)	16.23 ± 1.31
Fasting blood glucose (mg/dL)	85.02 ± 1.84
Systolic blood pressure (mmHg)	117.23 ± 1.81
Diastolic blood pressure (mmHg)	72.15 ± 1.91

All values are means ± SEM, n=13.

The incremental postprandial blood glucose concentration of cooked rice with 2.5% CTE is represented in Figure 4.18. Consumption of cooked rice with or without CTE significantly lower the concentration of postprandial blood glucose than the glucose solution at 15, 30, 45 and 60 min (P<0.05). Consumption of cooked rice with 2.5% CTE had a significant reduction in iAUCs for glucose (3,678 ± 1,171 mg/dL.min) when compared to glucose solution (iAUC= 5,037 ± 860 mg/dL.min) and cooked rice (iAUC= 4,353 ± 1,188 mg/dL.min). The glycemic index (GI) of cooked rice with 2.5% CTE (~73) was significant lower than cooked rice (~86) when compared to the glucose solution (P<0.05).

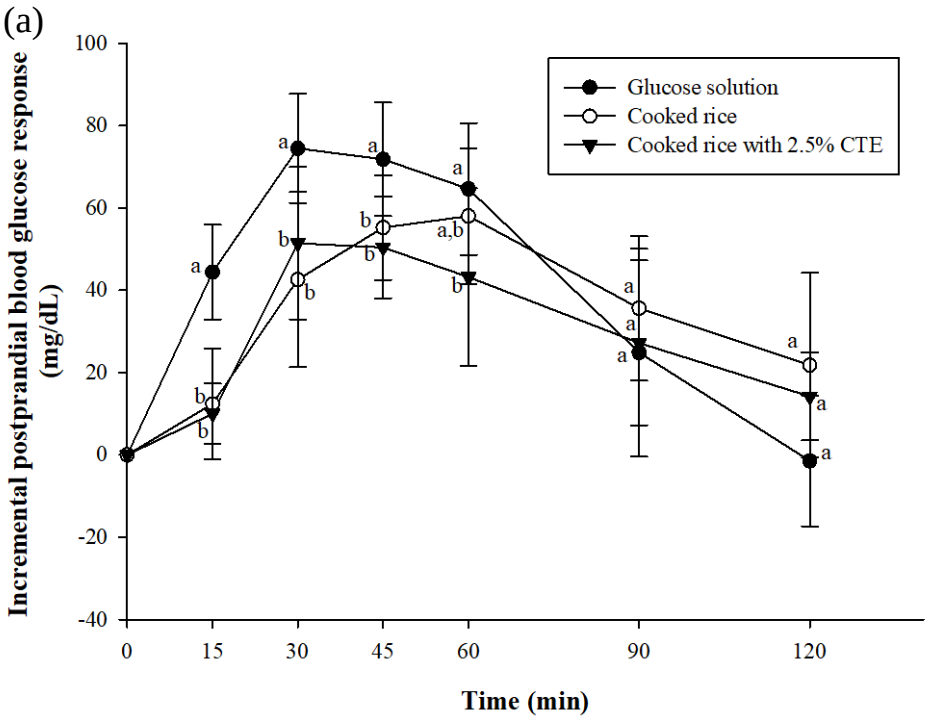

Figure 4.18 (a) the incremental postprandial blood glucose response and (b) the iAUCs for blood glucose concentration of healthy subjects after consuming glucose solution and cooked rice with 2.5% (w/v) CTE using an electric rice cooker. The results are expressed as mean ± SD, n=9. Different letters (a b) denote statistically significant differences ($P < 0.05$).

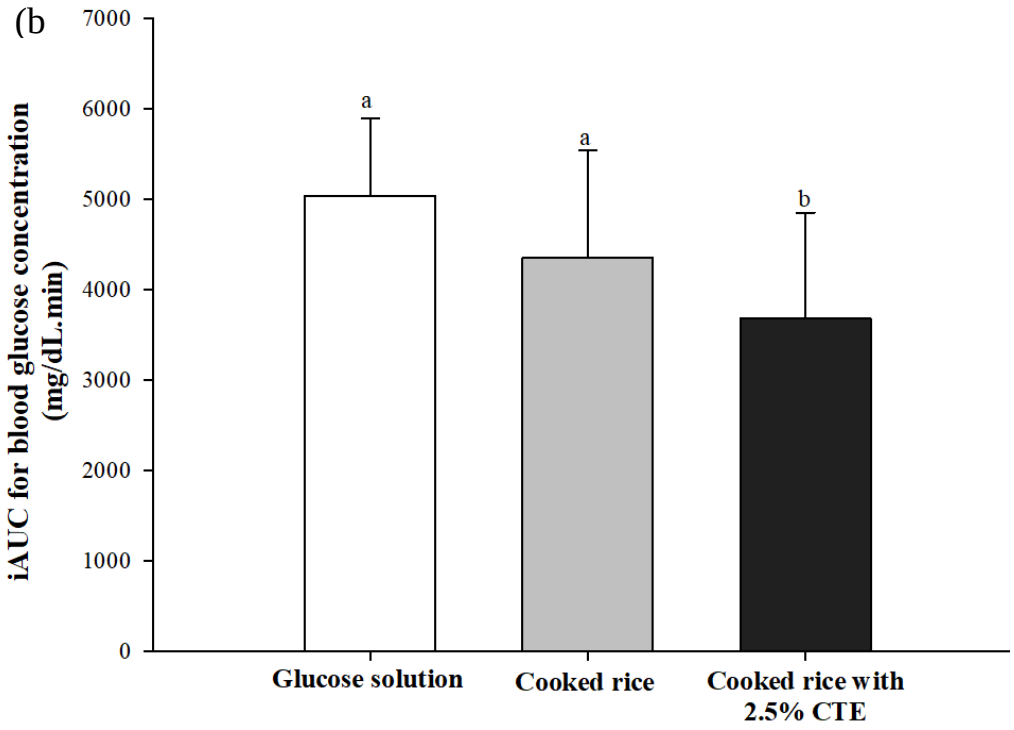

Figure 4.18 (a) the incremental postprandial blood glucose response and (b) the iAUCs for blood glucose concentration of healthy subjects after consuming glucose solution and cooked rice with 2.5% (w/v) CTE using an electric rice cooker. The results are expressed as mean ± SD, n=9. Different letters (a b) denote statistically significant differences ($P < 0.05$).

CHAPTER V

DISCUSSION

5.1 The effects of CTE on postprandial glycemic response and antioxidant status in healthy subjects.

Consumption of foods and beverages containing high sucrose markedly induces postprandial hyperglycemia. This effect may result in overstimulation of insulin from pancreatic β-cells causing hyperinsulinemia (Ludwig, 2002). Recently, hyperinsulinemia has been associated with the development of metabolic syndromes and gestational and type 2 diabetes (Crofts, Zinn, Wheldon, & Schofield, 2015). Furthermore, postprandial hyperglycemia and hyperinsulinemia play an important role in excessive generation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals and superoxide anion (Crofts et al., 2015; Crofts, Zinn, Wheldon, & Schofield, 2016). Overproduction of ROS predominates the endogenous antioxidant capacity, causing imbalance of antioxidative defenses and consequently oxidative damage to the protein, lipids and DNA (Dröge, 2002). Postprandial hyperglycemia can be controlled by retarding the absorption of *glucose* through inhibition of intestinal sucrase, the rate-limiting enzyme in the conversion of sucrose to glucose and fructose before absorption (Caspary, 1978). Previously, consumption of blackcurrants and lingonberries suppressed postprandial rise of plasma glucose in healthy participants

compared to sucrose reference due to the inhibitory effect of berries on α-glucosidase activity (Törrönen et al., 2012). The delayed digestion of sucrose and absorption of glucose after berries consumption contributed to lower postprandial insulin response. In addition, the reduced hyperinsulinemia has been associated with reduced the risk of insulin resistance, endothelial dysfunction, obesity, and metabolic syndromes (Crofts et al., 2016; Reaven, 1991). Our findings showed the suppression of the peak postprandial glucose and insulin concentration after consumption of CTE and sucrose. Previous findings indicate that CTE inhibited carbohydrate digestive enzymes such as intestinal α-glucosidase and pancreatic α-amylase *in vitro* (Adisakwattana et al., 2010). It suggests that CTE suppresses sucrose-induced postprandial glucose and insulin responses through the inhibition of intestinal sucrase. It is noted that phenolic compounds in edible plants can inhibit pancreatic α-amylase and α-glucosidase, resulting in the delay of postprandial glucose (Kumar, Narwal, Kumar, & Prakash, 2011). Major phenolic compounds in CTE composed of anthocyanins including delphinidin-3,5-glucoside, delphinidin-3-glucoside, malvidin-3β-glucoside, kaempferol, *p*-coumaric acid and six major ternatins (ternatins A1, A2, B1, B2, D1 and D2). These compounds could inhibit pancreatic α-amylase and α-glucosidase activity (Terahara et al., 1996). For example, delphinidin-3-glucoside and kaempferol showed the inhibitory effect against pancreatic α-amylase and α-glucosidase activity *in vitro* (Adisakwattana et al., 2012; Peng, Zhang, Liao, & Gong, 2016) which may be useful as a potential inhibitor for

delaying postprandial hyperglycemia. For this reason, the phenolic compounds in CTE may contribute to delay the hydrolysis of sucrose to glucose and fructose by inhibiting intestinal sucrase. We found that CTE alone did not alter postprandial glucose and insulin level, indicating that antihyperglycemic activity of CTE is not involved in the insulin secreting activity. Thus, drinking CTE may not produce hypoglycemia in the fasting state.

Reactive oxygen species (ROS) can be normally generated from nutrient metabolisms consumption of carbohydrate-containing foods and beverages (Gregersen et al., 2012). Increased production of ROS contributes to an imbalance condition between oxidative generation and antioxidant defense, resulting in a consequence of postprandial oxidative stress (Williams, 2012). Thiols, also called sulfhydryls, exist in proteins in the side-chain of cysteine (Cys) amino acids (Turell, Radi, & Alvarez, 2013). Albumin, the most abundant proteins, plays a major role in the total defense system against free radicals and oxidative damage in plasma through enzymatic and non-enzymatic mechanisms (Jones et al., 2000; Turell et al., 2013). A previous study reported that a high sucrose intake resulted in reduced antioxidant defense mechanisms and increased oxidative stress (Jones et al., 2000). The reduced plasma thiols were markedly observed in the participants receiving high carbohydrate diet (69% of total energy) (Chusak, Thilavech, & Adisakwattana, 2014). Malondialdehyde (MDA), a marker of lipid peroxidation, is produced from the oxidation process of polyunsaturated fatty acids (PUFA) in the membrane of red blood cell. During unstable

free radicals promoting chain reaction, double bounds of PUFA are cleaved and then released as bis-aldehyde MDA. Overproduction of ROS and free radicals have shown the positive correlation with the production of MDA (Gaweł, Wardas, Niedworok, & Wardas, 2003). Moreover, excessive intake of sucrose provokes the generation of peroxidation markers concomitant with reduced plasma antioxidant capacities.

Our findings are consistent with previous studies that reduced total antioxidant capacity concomitant with increased plasma MDA was detected after consumption of sucrose beverage (Ceriello et al., 1998; Moreto, de Oliveira, Manda, & Burini, 2014). The various methods have been utilized for the measurement of total antioxidant capacity; TEAC assay has been used for the assessment of antioxidant status to scavenge the ABTS radical cation compared to Trolox, a water soluble analogue of vitamin E; FRAP assay based on the reduction of ferric-TPTZ complex to its ferrous for the measurement of total reducing power of antioxidants; and ORAC assay measures the water-soluble antioxidants inhibition of peroxyl radical which can induce oxidations and thus reflects classical radical chain breaking antioxidant activity by hydrogen atom transfer (Prior et al., 2007). From our results, the observed postprandial alterations of antioxidant capacity were markedly attenuated when receiving sucrose and CTE. Interestingly, CTE could increase plasma antioxidant capacity and maintain the level of thiol group and subsequently reduced plasma MDA concentration. Several studies have shown the ability of edible plants to improve antioxidant capacity and decrease plasma MDA concentration. Accordingly, Micallef *et al*. (2007) demonstrated that

ingestion of 400 mL/day of red wine enriched with polyphenols significantly increased plasma thiol and decreased plasma MDA in young and old subjects, suggesting that polyphenols promoted the protection of both lipid and protein oxidation to avoid oxidative damage of the arterial walls and oxidative complication. Other authors have found that polyphenol-rich antioxidant containing pomegranate extract and green tea significantly reduced plasma MDA with increased antioxidant defense, indicating that supplementation of polyphenol-rich antioxidants has important against effects on oxidative stress and lipid peroxidation in type 2 diabetic patients (Fenercioglu, Saler, Genc, Sabuncu, & Altuntas, 2010). Similarly, consumption of foods rich in polyphenols with high carbohydrate diet have been reported to decrease lipid and protein oxidation in overweight subjects (Chusak et al., 2014). Thus, increased antioxidant capacity and decreased lipid peroxidation observed after CTE ingestion may be related to polyphenols containing in CTE which has an *in vitro* antioxidant activity such as 1,1-diphenyl 2-picrylhydrazyl (DPPH), hydroxyl radical scavenging activity (HRSA), superoxide radical scavenging activity (SRSA), FRAP and TEAC (Chayaratanasin et al., 2015). It is assumed that antioxidant activity of CTE is at least in the part directly responsible action for reduced oxidative imbalance mediated by sucrose.

Interestingly, it has demonstrated that consumption of sucrose and fructose have been linked to increased plasma uric acid (Lecoultre et al., 2013). In this pathway, uric acid is produced from fructokinase mediated metabolism to fructose-1-phosphate. It is well recognized that increased plasma uric acid may result in augmentation of

plasma antioxidant potential by increasing FRAP level (Godycki-Cwirko et al., 2010). In our study, the sucrose load increased in postprandial plasma uric acid and was paralleled by a rise in plasma FRAP levels at 60 min. This is consistent with the previous study of Lotito and Frei (2004) reporting the rise of FRAP after consumption due to sucrose induced-generation of uric acid in healthy subjects. However, ingestion of CTE and sucrose resulted in higher FRAP level without any change in plasma uric acid. Therefore, the rise of plasma FRAP level after consumption of CTE is not due to the sucrose load and its elevating effect on the plasma level of uric acid. The main limitation of this study is that the impact of CTE was observed in healthy men. This results cannot be generalized to all population, in particular to those with glucose intolerance, type 2 DM or different sex and ages. Future research will concentrate on how CTE may be used to modulate glycemia when co-ingested with complex carbohydrates such as white rice and bread. Any positive results that emerge from such studies will enable us to provide a public health advocacy on how such simple food based interventions may be used in our war against diabetes.

5.2 The effect of CTE on the inhibition of pancreatic α-amylase, *in vitro* starch hydrolysis and predicted glycemic index of different type of flours including potato, cassava, rice, corn, wheat and glutinous rice flour and the application in a bakery product

Starchy foods or ingredients are digested by amylolytic α-amylases and α-glucosidase enzymes including maltase-glucoamylase and sucrose-isomaltase at the brush border of the small intestine (Zhang & Hamaker, 2009). Thereafter, the absorbable glucose is transported into the bloodstream through glucose transporter in the small intestine. The high rate of digestion and absorption of these foods contributes to a rise in postprandial hyperglycemia related to health consequences. The physio-chemical properties of carbohydrate food are normally investigated by measurement of the rate and extent of glucose release after enzymatic digestion under controlled conditions (Englyst et al., 1999). Our findings demonstrated that a higher amount of maltose and glucose released from flours was observed after *in vitro* digestion. When the CTE was mixed with the flours, the release of maltose and glucose was significantly decreased. These findings suggest that CTE has a potential to reduce the release of maltose and glucose from flours, leading to a delay in the rate of starch digestibility. In agreement with another study, CTE inhibited the pancreatic α-amylase and intestinal α-glucosidase related to its phytochemical compounds (Suganya, Sampath Kumar, Dheeba, & Sivakumar, 2014). It has been revealed that the

phytochemical compounds in CTE are delphinidin-3,5-glucoside, delphinidin-3-glucoside, malvidin-3-glucoside, delphinidin-based ternatins (ternatins A1-A3, B1-B4, C1-C5 and D1-D3), kaempferol, quercetin-3-O-(2-rhamnosyl) rutinoside, and rutin (Terahara et al., 1996). In particular, rutin and kaempferol could inhibit the pancreatic α-amylase and intestinal α-glucosidase activity (Oboh, Ademosun, Ayeni, Omojokun, & Bello, 2015; Sheng et al., 2018). Moreover, the natural delphinidin and malvidin compounds have shown a competitive inhibiting effect against the intestinal α-glucosidase (Everette, Walker, & Islam, 2013; Homoki et al., 2016). A study conducted by Podsedek *et al*. (2017) observed that the degree of the inhibitory effect on the carbohydrate digestive enzymes is positively correlated with the concentration of anthocyanins. We suggest that the phytochemical compounds in CTE may contribute to delaying the hydrolysis of starch by inhibiting the carbohydrate digestive enzymes, including pancreatic α-amylase and intestinal α-glucosidase. Additionally, Zhu (2015) also explained other mechanisms of polyphenols on delayed starch digestibility. It found that polyphenol could interact with the starch chains to form the complex, resulting in the alteration of enzyme susceptibility. This evidence was supported from the higher content of undigested starch after *in vitro* digestion of starch and anthocyanins in blue maize (Camelo-Méndez et al., 2016). The interaction between polyphenols and starch was due to the non-covalent bonding and/or the hydrogen binding formation (Wu, Lin, Chen, & Xiao, 2011). Further studies are needed to define

the hypothesized mechanisms specific to the interaction between the anthocyanins in the CTE and flours, and the type of enzyme inhibition.

For nutritional purposes, the starch in food is generally classified into three categories, including rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 2007). In term of RDS, starch is readily and completely digested in the small intestine associated with more rise postprandial plasma glucose within first 20 min. SDS is a complete digestion of starch in the small intestine with slow rate (Jenkins et al., 1981). Furthermore, RS is defined as the dietary starch that resist digestion in the small intestine. According to a previous study, undigested starch contributes to the resistant starch, which is due to the inhibitory activity of α-amylase by antinutrients (e.g. polyphenols) (Calixto & Abia, 1991). The various amount of starch fractions in the different types of flour depends on their physical and chemical characteristics (Cone & Wolters, 1990). The observed results indicate that all flours had a higher level of RDS with a concomitant lower content of undigested starch (Zhang & Hamaker, 2009). The gelatinization is one of the factors affecting starch hydrolysis during the cooking process (Alcázar-Alay & Meireles, 2015). Thus, gelatinization of the starch granule induces an increase in the RDS response with the release of the glucose molecule. The results also showed that the flour with a high RDS content produced a significantly higher level of HI and pGI, whereas the higher SDS content with lower RDS reduced the levels of HI and pGI. A previous study

supported our findings, indicating that the pGI value was correlated with the parameters of the digestible starch fractions, including RDS and SDS. In particular, SDS is found to be the main contributing factor to the GI (Meynier et al., 2015). It has been reported that an intake of diet containing a high RDS level could induce a rapid hyperglycemic response and a subsequent glucose-induced insulin secretion from pancreatic β-cells (Jenkins et al., 1981). In contrast, undigested starch (RS) in human diets provides functional properties and applications for delaying postprandial glucose (Slavin, 2013) and improving postprandial insulin (Ells et al., 2005). Our findings demonstrated that the addition of CTE into glutinous rice, wheat, corn, and cassava flour causes the reduction of RDS and SDS, in relation to the increased content of undigested starch. Moreover, the undigested starch of wheat and cassava flours significantly and strongly correlated with the concentration of CTE. For a traditional use, glutinous rice flour, grinded from glutinous rice or sticky rice, is usually used as the ingredients for desserts, sweets, rice cakes, and puffed rice in Asia and Southeast Asia (Keeratipibul, Luangsakul, & Lertsatchayarn, 2008). Basically, glutinous rice has been classified as a high GI because of its high amylopectin content and rate of digestion (Hu, Zhao, Duan, Linlin, & Wu, 2004). The starch digestibility of glutinous rice produced more rapid and is more complete than other high amylose rice varieties. Chan *et al*. (2001) found an increased glycemic response and GI values in Caucasian and Asian population after the consumption of glutinous rice, which was similar to a

previous study of Ranawana *et al*. (2009). In the current study, the mixture of CTE into glutinous rice flour can reduce the pGI value, suggesting that CTE suppresses the digestive process of glutinous rice flour to absorbable monosaccharides. A combination of glutinous rice flour and CTE might have opportunities for flour applications to reduce the GI of the food products.

Several studies have reported that the plant-based diets containing polyphenols alter the glycemic index of various foods. The current study found that the addition of CTE caused the rate of carbohydrate digestion and pGI of wheat bread to slow down. Our results are in agreement with Reshmi *et al*. (2017), who reported the *in vitro* glycemic impact of bread fortified with pomelo fruit. Because of the action of phenolic compounds and flavonoids in pomelo, the bread fortification with pomelo caused a lower level of digestible starch with a concomitant increased level of undigested starch. Lemlioglu-Austin *et al*. (2012) also found that the incorporation of phenolic-rich sorghum bran extract into porridges contributes to slow starch digestion with a reduced GI and increase undigested starch. In addition, thermal processing also affects starch digestibility by the alteration of its granular structure (Dupuis, Lu, Yada, & Liu, 2016). The cooking process with heating and excess water induces the gelatinization of the starch granule, increasing in starch digestibility. The crumb portions of baked bread increased the starch digestibility when compared with the crust portion, because the starches in the crust portion are not completely gelatinization after baking. The

fortification of green tea polyphenol in baked bread reduced in glucose release for both the crumb and crust after *in vitro* digestion (Goh et al., 2015) and reduced the rapidly digestible starch of white bread samples (Coe, Fraser, & Ryan, 2013). During baking, the interaction between gelatinized starch granules and the gluten network occurs in crumb, causing a loss of kinetic energy and a subsequent increase in firmness (Sivam, Sun-Waterhouse, Quek, & Perera, 2010). Previous evidence revealed that polyphenols can form a complex with bread ingredients including protein and polysaccharides (Sivam et al., 2010). The formation of polyphenols and polysaccharides or protein as enzymes clearly indicates a reduced *in vitro* digestibility (Wu et al., 2011). Moreover, polyphenols affect the breadmaking quality by altering the flour protein properties (Han & Koh, 2011). The interaction of polyphenols and gluten proteins in wheat bread is associated with a reduction in protein cross-linking, resulting in decreased bread volume. Our study showed that the addition of CTE caused the bread volume to and air pocket in wheat bread to reduce. These findings are consistent with a study of Pathak et al. (2017), who reported that the addition of mango fruit peel powder could decrease the volume and height of loaf, whereas it increased the density of the loaf with less visible air pockets of bread, owning to the compact crumb structure. It could be explained that the gluten network was not completely formed, leading to the ineffectiveness to hold air during fermentation, which caused the decreased loaf volume (Huang, Guo, Wang, Ding, & Cui, 2016). According to our findings, the addition of CTE into various types of flours successfully

altered the parameters of starch digestibility and consequently decreased the level of HI and pGI. Further studies are warranted to elucidate whether the consumption of bread incorporated with CTE delays postprandial glucose in humans.

5.3 The effectiveness of CTE incorporation into rice on *in vitro* and *in vivo* starch digestibility of cooked rice using domestic cooking methods

Most Asian population usually consumed rice, especially white rice as a source of carbohydrate. Rice is subjected to various domestic cooking methods such as an electric rice cooker or a microwave oven. Our study found that cooking white rice by a microwave oven had lower the release of reducing sugars than cooking with an electric rice cooker. The various domestic cooking methods could change its physicochemical properties, resulting in alteration in starch digestibility of rice (Darandakumbura, Wijesinghe, & Prasantha, 2013). The microwave cooking uses a shorter time to make cooked foods than other cooking methods. It is suggested that decrease in the cooking time of microwaves is associated with the reduction of degree of starch gelatinization (Lee, Lee, Han, Lee, & Rhee, 2005). Lee *et al*. also found that the hydrolysis rate and kinetic constant of rice sample treated with microwave was lower than those of electric rice cooker. The addition of CTE into cooked rice decreased the rate of starch digestibility and the release of reducing sugars. Our findings are in agreement with a previous study indicating that cooking white rice with red grape extract exhibited the lowering effect on glucose release (Quek & Henry, 2015). The bioactive compounds in CTE could inhibit carbohydrate digestive enzymes such as pancreatic α-amylase and intestinal α-glucosidase (Adisakwattana et al., 2012; Chayaratanasin et al., 2015). The inhibitory activity of these enzymes leads to slow

down the digestion of carbohydrate and the release of glucose. We suggest that the reduced starch digestibility of rice through inhibiting carbohydrate digestive enzymes is attributed to the polyphenols and anthocyanins in CTE. Other reports suggest that anthocyanins might inhibit starch digestibility through the interaction with starch components restricting the amylolytic attack (Camelo-Méndez et al., 2016). The reaction between starch component, mainly amylose and polyphenol could form the complex and further interrupt and resist to the carbohydrate digestion (Camelo-Méndez et al., 2016).

The instrumental texture profile analysis (TPA) mimics the conditions to which the material is subjected throughout the mastication process (Bourne, 2002). Although there are several textural attributes recognized after the analysis, but the hardness and stickiness are most important characteristics of cooked rice in Asian (Okabe, 1979). The hardness and stickiness of cooked rice related to the eating quality of cooked rice, affecting the consumers' acceptability (Srisawas & Jindal, 2007). Jasmine rice is most popular rice in Thailand and other countries as its soft texture, slightly sticky and aroma (Payakapol, Moongngarm, Daomukda, & Noisuwan, 2011). However, cooked rice is generally kept warm in an electric rice cooker for a day. Park *et al*. (2012) reported that storage of cooked rice at high temperature resulted in the increase in hardness and decrease in stickiness. Moreover, the texture of rice is also influenced by several factors such as variety of amylose content, gelatinization temperature, processing

factors, cooking methods and polyphenols (Bourne, 2002; Wang, Zhou, & Isabelle, 2007). It has been revealed that hardness of rice starch decreased with concomitant increase in concentration of polyphenols (Wu, Che, & Chen, 2014). In present study, the hardness of cooked rice with CTE remained unchanged, suggesting that CTE did not affect the hardness of cooked rice during storage at high temperature. In contrast, addition of CTE into cooked rice significantly decreased the level of stickiness. Ayabe *et al*. (2009) suggested that the difference in the amount of leached materials from the surface of cooked rice contributed to the differences in stickiness. We suggest that CTE may coat on the surface of rice leading to the reduction in stickiness of cooked rice. Moreover, the effect on cohesiveness of cooked rice is very complicated as the extent of cohesive behavior depends on the nature of the food and external factors like moisture and temperature (Adhikari, Howes, Bhandari, & Truong, 2001). Our results are in agreement with a previous study indicating that cohesiveness of cooked rice was increased with the increasing levels of polyphenol green tea powder (Shin & Lee, 2004). There was an increase in cohesiveness of cooked rice with increasing concentration of medicinal herb extracts (Cho et al., 2010).

The nine-point hedonic scale is a rating scale to evaluate the degree of likeness of products and to determine acceptance of the food product (Stone, 2012). Our findings indicate that the presence of CTE in cooked affects the liking scores of acceptance, color, texture, flavor, taste and overall acceptability. In addition, consumer acceptance

for natural derived colorants is related to their image of health quality and food products (Wissgott & Bortlik, 1996). The colored of cooked rice was darkened with the increasing amount of added CTE and significantly impact on the lower score of color on sensory properties. Similarly, Bing and Chun (2015) reported that increasing amount of the wide grape pomace powder resulted in a decrease in the lightening color of rice chiffon cake. Rice chiffon cake with incorporation of the wild grape pomace powder had lower the color scores and overall acceptability compared to the control. However, a previous study revealed that anthocyanin-rich products such as juice and smoothies were well accepted by children and adolescents (Drossard et al., 2012). In the present study, the overall acceptability of cooked rice with CTE both using an electric rice cooker and a microwave oven had mean scores between 6 and 7 equivalents to the terms “like slightly” to “like moderately” on the 9-point hedonic scale. Lucia *et al*. (2013) suggested that the higher acceptability means of food products should be between 6 (“liked slightly”) and 8 (“liked very much”). It could be referred to the cooked rice with CTE that the panelists showed the acceptability from sensory point of view.

The electric rice cooker method was selected for the human study. Actually, the electric rice cooker is programmed to prepare the beat quality cooked rice from the most common white rice. The results from human study are consistent with *in vitro* digestion of rice, suggesting that CTE could lower GI of rice, resulting in a decrease in

postprandial glucose in the subjects. Similarly, pigment of anthocyanins in red basmati rice could lower GI of white basmati rice (Somaratne et al., 2017). In addition to rice consumption, CTE decreased postprandial plasma glucose when consuming with sucrose. The dietary strategy to control postprandial hyperglycemia could promote to improve glucose homeostasis and insulin resistance and reduce the risk of type 2 diabetes (Castro-Acosta et al., 2017). As a result, cooked rice with 2.5% of aqueous CTE were highly recommended for consumption to control postprandial hyperglycemia.

CHAPTER VI

CONCLUSION

Anthocyanins are recognized as a major phytochemical compound that involves their biological and pharmacological properties such as antioxidant and antihyperglycemic effects. Consumption of CTE beverage increases plasma antioxidant capacities without hypoglycemia in healthy subjects. Furthermore, CTE reduces postprandial plasma glucose and insulin concentration concomitant with improved antioxidant status in the subjects when consumed with sucrose.

The present study demonstrated that CTE could inhibit pancreatic α-amylase activity, leading to a reduction of maltose release from flour. The addition of CTE to flour alters *in vitro* starch digestibility, resulting in a reduction in the amount of glucose released from various types of flour and a subsequent reduction of HI and pGI. Moreover, the addition of the CTE reduced the starch digestibility rate and pGI of wheat bread. The findings indicate that the addition of CTE into flour can inhibit the starch digestibility of flour through the inhibition of carbohydrate digestive enzymes, including pancreatic α-amylase and intestinal α-glucosidase. We suggest that CTE may be a potent ingredient for the reduced glycemic index of flours.

The digestibility of cooked rice was significantly reduced by incorporating aqueous extract of CTE using the domestic cooking methods including an electric rice cooker and a microwave oven. Moreover, the addition of CTE affected the sensory attributes

and textural properties of cooked rice (stickiness and cohesiveness). The results also showed that CTE could decrease GI of cooked rice, resulting in reduction of postprandial blood glucose in the subjects. Overall, our findings support future research on developing low GI starchy foods using CTE.

To sum up, CTE may potentially be a useful ingredient to reduce glycemic response in high carbohydrate foods and beverages.

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APPENDIX

AF 01-12

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย
254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330
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COA No. 187/2558

ใบรับรองโครงการวิจัย

โครงการวิจัยที่ 140.1/58 : ผลของสารสกัดอัญชันต่อระดับน้ำตาลกลูโคส ระดับอินซูลินภายหลังรับประทานอาหารและการต้านการเกิดออกซิเดชันในอาสาสมัครเพศชายที่มีสุขภาพดี

ผู้วิจัยหลัก : รองศาสตราจารย์ ดร.สิริชัย อดิศักดิ์วัฒนา

หน่วยงาน : คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย ได้พิจารณา โดยใช้หลัก ของ The International Conference on Harmonization – Good Clinical Practice (ICH-GCP) อนุมัติให้ดำเนินการศึกษาวิจัยเรื่องดังกล่าวได้

ลงนาม.....
(รองศาสตราจารย์ นายแพทย์ปรีดา ทัศนประดิษฐ)
ประธาน

ลงนาม.....
(ผู้ช่วยศาสตราจารย์ ดร.นันทรี ชัยชนะวงศาโรจน์)
กรรมการและเลขานุการ

วันที่รับรอง : 6 ตุลาคม 2558 **วันหมดอายุ** : 5 ตุลาคม 2559

เอกสารที่คณะกรรมการรับรอง

1) โครงการวิจัย
2) ข้อมูลสำหรับอาสาสมัครหรือผู้มีส่วนร่วมในการวิจัยและใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย
3) ผู้วิจัย

เลขที่โครงการวิจัย.....140.1/58.....
วันที่รับรอง.....- 6 ต.ค. 2558.....
วันหมดอายุ.....- 5 ต.ค. 2559.....

เงื่อนไข

1. ข้าพเจ้ารับทราบว่าจะปฏิบัติตามข้อกำหนดของคณะกรรมการพิจารณาจริยธรรมการวิจัยในคน หากดำเนินการเก็บข้อมูลการวิจัยก่อนได้รับการอนุมัติจากคณะกรรมการพิจารณาจริยธรรมการวิจัยฯ
2. หากใบรับรองโครงการวิจัยหมดอายุ การดำเนินการวิจัยต้องยุติ เมื่อต้องการต่ออายุต้องขออนุมัติใหม่ล่วงหน้าไม่ต่ำกว่า 1 เดือน พร้อมส่งรายงานความก้าวหน้าการวิจัย
3. ต้องดำเนินการวิจัยตามที่ระบุไว้ในโครงการวิจัยอย่างเคร่งครัด
4. ใช้เอกสารข้อมูลสำหรับกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย ใบยินยอมของกลุ่มประชากรหรือผู้มีส่วนร่วมในการวิจัย และเอกสารชี้ชวนเข้าร่วมวิจัย (ถ้ามี) เฉพาะที่ประทับตราคณะกรรมการเท่านั้น
5. หากเกิดเหตุการณ์ไม่พึงประสงค์ร้ายแรงในสถานที่เก็บข้อมูลที่ขออนุมัติจากคณะกรรมการ ต้องรายงานคณะกรรมการภายใน 5 วันทำการ
6. หากมีการเปลี่ยนแปลงการดำเนินการวิจัย ให้ส่งคณะกรรมการพิจารณารับรองก่อนดำเนินการ
7. โครงการวิจัยไม่เกิน 1 ปี ส่งแบบรายงานสิ้นสุดโครงการวิจัย (AF 03-12) และบทคัดย่อผลการวิจัยภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น สำหรับโครงการวิจัยที่เป็นวิทยานิพนธ์ ให้ส่งบทคัดย่อผลการวิจัย ภายใน 30 วัน เมื่อโครงการวิจัยเสร็จสิ้น

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel/Fax: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 097/2018

Certificate of Approval

Study Title No. 055.1/61 : BIOACTIVE *CLITORIA TERNATEA* L. FLOWER EXTRACT FOR THE GLYCEMIC CONTROL TO CARBOHYDRATE FOODS AND SUGARY BEVERAGES

Principal Investigator : MISS CHAROONSRI CHUSAK

Place of Proposed Study/Institution : Faculty of Allied Health Sciences, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP) **Part 3 of Research protocol: Sensory evaluation only**.

Signature: ....Prida Tasanapradit..... Signature: ....Nuntaree Chaichanawongsaroj.....
(Associate Professor Prida Tasanapradit, M.D.) (Assistant Professor Nuntaree Chaichanawongsaroj, Ph.D.)
Chairman Secretary

Date of Approval : 17 April 2018 Approval Expire date : 16 April 2019

The approval documents including

1) Research proposal
2) Patient/Participant Information Sheet and Informed Consent Form
3) Researcher
4) Questionnaire

Protocol No. 055.1/61
Date of Approval 17 APR 2018
Approval Expire Date 16 APR 2019

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the RECCU for any serious adverse events within 5 working days*
5. *Report to the RECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

VITA

Charoonsri Chusak was born in September 20th, 1988 in Chanthaburi, Thailand. She graduated from Sriyanusorn Chanthaburi School in 2006 and completed her Bachelor's degree with the first class honor (Gold medal) in Nutrition and Dietetics at Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok in 2010. After that, she decided to attend graduate school at Faculty of Allied Health Sciences where she achieved her Master's degree in Applied Food and Nutrition in 2012. Her thesis dealt with the effect of Chinese Mesona (Mesona chinensis benth) extract on postprandial glycemia and antioxidant status in overweight men. In 2013, she desired to continue her study in a doctoral course in Food and Nutrition in Faculty of Allied Health Sciences, Chulalongkorn University. Her dissertation focused on bioactive Clitoria ternatea L. flower extract for the glycemic control to carbohydrate foods and sugary beverages. Her advisor was Associate Professor Sirichai Adisakwattana, Ph.D.