กระบวนการสกัดและคุณสมบัติการลดคอเลสเตอรอลของใยอาหารจากกากมันสำปะหลัง

นางสาวพรอริยา ฉิรินัง

มหาวิทยาลัยเทคโนโลยีสุรนารี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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มหาวิทยาลัยเทคโนโลยีสุรนารี
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EXTRACTION PROCESS AND CHOLESTEROL-LOWERING PROPERTY OF DIETARY FIBER FROM CASSAVA PULP

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มหาวิทยาลัยเทคโนโลยีสุรนารี

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Food Technology

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EXTRACTION PROCESS AND CHOLESTEROL-LOWERING PROPERTY OF DIETARY FIBER FROM CASSAVA PULP

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

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พรอริยา ฉิรินัง : กระบวนการสกัดและคุณสมบัติการลดคอเลสเตอรอลของใยอาหารจากกากมันสำปะหลัง (EXTRACTION PROCESS AND CHOLESTEROL-LOWERING PROPERTY OF DIETARY FIBER FROM CASSAVA PULP) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.รัชฎาพร อุ่นศิวิไลย์, 175 หน้า.

กากมันสำปะหลังเป็นผลพลอยได้ที่มีมูลค่าสูงสำหรับการผลิตใยอาหารซึ่งประกอบด้วยใยอาหารที่สกัดด้วยสารละลายที่เป็นกลางร้อยละ 31.40 (w/w) จากการประยุกต์ใช้วิธีการพื้นผิวตอบสนองในการหาตัวแปรที่เหมาะสมในการสกัดโดยตัวแปรตามที่ใช้คือ ร้อยละของใยอาหารที่สกัดด้วยสารละลายที่เป็นกลางพบว่า สภาวะที่เหมาะสมโดยการย่อยด้วยเอนไซม์ที่มีใยอาหารที่สกัดด้วยสารละลายที่เป็นกลางมีค่าสูงสุดคือ ที่ปริมาณเอนไซม์แอลฟา-อะไมเลสร้อยละ 0.1 (w/v) นิวเทรสร้อยละ 1 (v/v) และอะไมโลกลูโคซิเดสร้อยละ 0.1 (v/v) ใยอาหารจากกากมันสำปะหลังประกอบด้วยใยอาหารที่สกัดด้วยสารละลายที่เป็นกลางร้อยละ 79.03 และเซลลูโลสร้อยละ 58.55 เมื่อทำการประเมินความสามารถในการเชื่อมเกาะกับน้ำมันหมู คอเลสเตอรอล และกรดน้ำดี 3 ชนิด ได้แก่ กรดโคลิก กรดดีออกซีโคลิก และกรดทอโรโคลิก พบว่า ความคงตัวต่อการย่อยของใยอาหารจากกากมันสำปะหลังเท่ากับร้อยละ 87.98 มีความสามารถในการเชื่อมเกาะกับน้ำมันหมู คอเลสเตอรอล และกรดน้ำดีมากกว่าเซลลูโลส การเชื่อมเกาะกับกรดโคลิก กรดดีออกซีโคลิก และกรดทอโรโคลิกมีค่าเท่ากับร้อยละ 38.50, 42.71 และ 40.84 ตามลำดับ ใยอาหารจากกากมันสำปะหลังแสดงกิจกรรมการเป็นพรีไบโอติกสำหรับกลุ่มแลคติกแบคทีเรียสายพันธุ์ *Lactobacillus plantarum* TISTR 1465 สูงกว่าเซลลูโลส แต่น้อยกว่าใยอาหารที่ละลายน้ำได้บางกลุ่ม (อินนูลิน แลคทูโลส และฟรุคโตโอลิโกแซคคาไรด์)

จากการทดสอบความเป็นพิษแบบเฉียบพลัน (14 วัน) ของใยอาหารจากกากมันสำปะหลังพบว่า ไม่พบการตายหรือพฤติกรรมที่ผิดปกติของหนูทดลองต่ออาหารที่มีใยอาหารจากกากมันสำปะหลังที่ระดับความเข้มข้นร้อยละ 2.5 และ 15 (w/w) ตลอดการสังเกตอาการทางคลีนิค ค่า no-observed-adverse-effect-level (NOAEL) ของใยอาหารจากกากมันสำปะหลังในหนูทั้งสองเพศคือที่ความเข้มข้นร้อยละ 15 (w/w) (เพศผู้ 10.01 กรัมต่อกิโลกรัมน้ำหนักตัวต่อวัน และเพศเมีย 11.21 กรัมต่อกิโลกรัมน้ำหนักตัวต่อวัน) สำหรับการศึกษาคุณสมบัติการลดคอเลสเตอรอลโดยใช้หนูทดลองสายพันธุ์ Wistar แบ่งออกเป็น 5 กลุ่ม กลุ่มที่ 1 ได้รับอาหารปกติ (กลุ่มปกติ) กลุ่มที่ 2 ได้รับอาหารไขมันสูงปราศจากใยอาหาร (กลุ่มควบคุม) กลุ่มที่ 3 ได้รับอาหารไขมันสูงปราศจากใยอาหารร่วมกับการป้อนยา simvastatin ปริมาณ 10 มิลลิกรัมต่อกิโลกรัมต่อวัน (กลุ่มเปรียบเทียบกับยาทางการค้า) กลุ่มที่ 4 ได้รับอาหารไขมันสูงที่มีใยอาหารจากกากมันสำปะหลังในปริมาณร้อยละ 5

(กลุ่มศึกษาผลของใยอาหารจากกากมันสำปะหลังต่อการลดลงของระดับคอเลสเตอรอล) และกลุ่มที่ 5 ได้รับอาหารไขมันสูงที่มีเซลลูโลสในปริมาณร้อยละ 5 (กลุ่มเปรียบเทียบกับใยอาหารทางการค้า) หนูทดลองทุกกลุ่มได้รับอาหารเป็นเวลา 30 วัน ผลการทดลองพบว่า กลุ่มใยอาหารจากกากมันสำปะหลังมีการลดระดับไตรกลีเซอไรด์ในซีรัม คอเลสเตอรอลในซีรัม ลิพิดในตับ และคอเลสเตอรอลในตับอย่างมีนัยสำคัญทางสถิติ (p<0.05) ในขณะเดียวกันมีการเพิ่มระดับลิพิดและคอเลสเตอรอลในอุจจาระอย่างมีนัยสำคัญทางสถิติ (p<0.05) เมื่อเปรียบเทียบกับกลุ่มควบคุม นอกจากนี้ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของระดับไตรกลีเซอไรด์ในซีรัม คอเลสเตอรอลในซีรัม ลิพิดในตับ และคอเลสเตอรอลในตับระหว่างกลุ่มที่ได้รับยา simvastatin และกลุ่มที่ได้รับใยอาหารจากกากมันสำปะหลัง (p>0.05) ผลการลดระดับคอเลสเตอรอลและไขมันในเลือดของใยอาหารจากกากมันสำปะหลังอาจจะเกี่ยวข้องกับการกระตุ้นการขับถ่ายของไขมันและคอเลสเตอรอล กล่าวโดยสรุปใยอาหารจากกากมันสำปะหลังมีแนวโน้มในการใช้เป็นส่วนประกอบในอาหารเพื่อลดคอเลสเตอรอลได้

มหาวิทยาลัยเทคโนโลยีสุรนารี

สาขาวิชาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2559 ลายมือชื่ออาจารย์ที่ปรึกษา_____

PORNARIYA CHIRINANG : EXTRACTION PROCESS AND CHOLESTEROL-LOWERING PROPERTY OF DIETARY FIBER FROM CASSAVA PULP. THESIS ADVISOR : ASST. PROF. RATCHADAPORN OONSIVILAI, Ph.D., 175 PP.

CASSAVA PULP/ DIETARY FIBER/ NEUTRAL DETERGENT FIBER/ *IN VITRO* BINDING CAPACITIES/ CHOLESTEROL-LOWERING PROPERTY

Cassava pulp is a high value by-product for dietary fiber production that contains a high amount of neutral detergent fiber (NDF) at 31.40% (w/w). Response surface methodology was applied for optimization of the extraction parameters which the percentage of NDF was the selected dependent variable. The optimum condition for the highest NDF by enzymatic digestion was 0.1% of α-amylase (w/v), 1% of neutrase (v/v) and 0.1% of amyloglucosidase (v/v). Dietary fiber from cassava pulp (CDF) contains 79.03% (w/w) of NDF and a high content of cellulose at 58.55% (w/w). The CDF was evaluated for its *in vitro* binding capacities for lard, cholesterol and bile acids: cholic acid (CA); deoxycholic acid (DCA); and taurocholic acid (TA). The digestive stability of CDF is 87.98%. The CDF showed a higher binding capacity for lard, cholesterol and bile acids compared to cellulose. Binding with CA, DCA and TA were 38.50%, 42.71% and 40.84%, respectively. The CDF showed prebiotic activity for *Lactobacillus plantarum* TISTR 1465 was higher than cellulose, but lower than some soluble dietary fibers (Inulin, Lactulose and Fructooligosaccharide).

With regard to the acute toxicity test (14 days) of CDF, the results showed that there were no deaths or abnormal behaviors of the rats fed on a diet containing 2.5%

and 15% (w/w) of CDF during clinical observation. The no-observed-adverse-effect-level (NOAEL) for CDF was 15% for both genders (male 10.01 g/kg body weight/day and female 11.21 g/kg body weight/day). For the study of cholesterol-lowering property, thirty Wistar rats were assigned to five groups: Group 1 was fed a basal diet (Normal); Group 2 received a high-fat diet without fiber (Control); Group 3 received a high-fat diet without fiber together with gavage of 10 mg/kg/day simvastatin (Simvastatin); Group 4 received a high-fat diet containing 5% (w/w) cassava dietary fiber (CDF); and Group 5 received a high-fat diet containing 5% (w/w) cellulose (Cellulose). All groups were fed with these diets for 30 days. The results illustrated that CDF significantly (p<0.05) decreased serum triglyceride, serum total cholesterol, liver total lipids, and liver cholesterol levels, while it also significantly (p<0.05) increased the levels of fecal total lipids and cholesterol when compared with the Control group. Moreover, there were no significant (p>0.05) differences in terms of serum triglyceride, serum total cholesterol, liver total lipids, and liver cholesterol between Simvastatin and CDF. The hypocholesterolemic and hypolipidemic effects of CDF might be correlated to enhancing fat and cholesterol excretion. In conclusion, these research results suggest that CDF could be a potential cholesterol-lowering food ingredient.

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Pornariya Chirinang

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LIST OF ABBREVIATIONS

mg/mL	=	Milligram per milliliter
g/g	=	Gram per gram
RSM	=	Response surface methodology
NDF	=	Neutral detergent fiber
ADF	=	Acid detergent fiber
ADL	=	Acid detergent lignin
WHC	=	Water holding capacity
SWC	=	Swelling capacity
WRC	=	Water retention capacity
CEC	=	Cation exchange capacity
NOAEL	=	No-observed-adverse-effect-level
ANOVA	=	Analysis of variance
SD	=	Standard deviation
v/v	=	volume by volume
w/v	=	weight by volume
CVD	=	Cardiovascular disease
EDTA	=	Ethylenediamine-tetraacetic acid
HCT	=	Hematocrit
RBC	=	Red blood cells
HGB	=	Hemoglobin

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LIST OF ABBREVIATIONS (Continued)

MCV	=	Mean corpuscular volume
MCH	=	Mean corpuscular hemoglobin
MCHC	=	Mean corpuscular hemoglobin concentration
WBC	=	Total leukocyte count
PLT	=	Platelet count
PMN	=	Polymorphonuclear neutrophils
ALT	=	Alanine aminotransferase
AST	=	Aspartate aminotransferase
ALP	=	Alkaline phosphatase
GLU	=	Glucose
TC	=	Total cholesterol
TG	=	Triglycerides
CREA	=	Creatinine
BUN	=	Blood urea nitrogen
H&E	=	Hematoxylin and eosin stains
HDL	=	High-density lipoprotein
LDL	=	Low-density lipoprotein
mg/dL	=	Milligram per deciliter
h	=	Hepatocyte
CV	=	Central vein
s	=	Sinusoid
cell/mm^3	=	Cell per cubic millimeter

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LIST OF ABBREVIATIONS (Continued)

fl.	=	Femtoliter
pg.	=	Picogram
SCFAs	=	Short-chain fatty acids

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CHAPTER I

INTRODUCTION

1.1 Introduction

Cassava, tapioca or manioc, is the common name of *Manihot esculenta* (L.) Crantz which grows in tropical and subtropical area. It is the third-largest important source of food carbohydrates in the world. Thailand is currently one of the world's biggest exporters of cassava products with the major competitors Indonesia, Brazil and Vietnam. Cassava were planted in all regions of Thailand except the South. In 2015/16, more than 50% was planted in the Northeast which the main province is Nakhon Ratchasima, followed by the Central plain (33%) and the North (15%). These planted areas included 48 provinces or around 8.8 million rais and the totally production is around 33 million tons (Thaitapiocastarchassociation, 2016). About fifty percent of pulp is used as raw material for the production of tapioca or cassava starch and other as cassava chips, cassava pellets and cassava flour. For cassava starch industry has market value approximately 1.4 million tons/year in the world. The cassava starch production is one of the most important agro-industries in Thailand. Thailand is the third largest producer of cassava starch, which yielded 2.9 million tons per year and has value around 41.2 billion baht (Centre of Agricultural Information and Office of Agricultural Economics, 2016). Thus, there are a lot of by-products from the processing of cassava which the important one is cassava pulp. At least 1 million tons of pulp is generated annually in Thailand. Cassava pulp represents

approximately 10-15% by weight of the original cassava roots. The starch remaining in the pulp is approximately 50-60% of its dry weight and, for the most part, is trapped inside ligno-cellulose. The fiber content of dried cassava pulp is reportedly in the form of insoluble fiber. Moreover, the pulp also contains pectin, cellulose, fiber (10-15%), protein (1.5-5%), and fat (0.1-4%). Historically, it has been used in the animal feed industry, bio-gas production, and ethanol production (Sriroth, Chollakup, Chotineeranat, Piyachomkwan and Oates, 2000; Thailandtopiocastarch, 2010). Due to its high content of fiber, cassava pulp could be used as a fiber-rich ingredient in food products. Wandee et al. (2014) studied the potential of cassava pulp and pomelo peel as sources of dietary fiber in dried rice noodles. The results show that a combination of cassava pulp and pomelo peel in rice noodles at a total amount of 20% exhibited an obvious increase in cooking weight and its highest total dietary fiber content was 14.4%. Thus, the alternative way to add value of cassava pulp is dietary fiber production with a mild and highly specific method to obtain a higher amount of dietary fiber content from cassava pulp might be very useful.

Dietary fiber has many health benefits for humans including laxation, fermentable by colonic micro flora, reducing the risk of colon cancer and reducing blood glucose and cholesterol levels (Brownlee, 2011; Kendall, Esfahani and Jenkins, 2010; Thebaudin, Lefebvre, Harrington and Bourgeois, 1997; Tungland and Meyer, 2002). Furthermore, because dietary fiber is not digested by the digestive system in the human body, dietary fiber can be used as a non-caloric ingredient for the replacement of caloric ingredients, such as fats, carbohydrates and protein in many food products (de Moraes Crizel, Jablonski, de Oliveira Rios, Rech and Flôres, 2013; Soukoulis, Lebesi and Tzia, 2009). Consequently, the consumption rate of fiber-rich products has increased. Many by-products from the fruit and vegetable industry are of

particular interest due to their low cost and availability in large quantities. Indeed, some of the agricultural by-products, such as apples, citrus fruits, grapes, carrots and Brassica vegetables have already been used in dietary fiber production (Figuerola, Hurtado, Estévez, Chiffelle and Asenjo, 2005; Grigelmo-Miguel and Martín-Belloso, 1999; Hsu, Chien, Chen and Chau, 2006). Dietary fiber is supplied as well as bioactive compounds such as polyphenols and essential oils, thus providing economic benefits to the food, cosmetic, and pharmaceutical industries. With regard to the food industry, dietary fiber can also incorporate some of the functional properties of foods, such as increasing water and oil holding capacity, emulsification, and gel formation. Dietary fiber can be incorporated into many varieties of food products, including bakery and dairy products, as well as jams, meats, soups etc. (Elleuch et al., 2011). However, various methods and different sources for obtaining dietary fiber might adjusted their chemical components and physicochemical properties which could subsequently affect their function as food ingredients in food applications (Chau and Huang, 2003).

Cholesterol is a primary risk factor that leads to cardiovascular disease (CVD) which is the leading cause of death in the developing countries. The possibility to delay and prevent hypercholesterolemia of CVD development through improvement of diets (Lin, Tsai, Hung and Pan, 2011). Many reports showed consumption of dietary fiber could effectively decrease serum cholesterol and lower the risk of cardiovascular disease (Chau, Huang and Lin, 2004; German et al., 1996; Hsu et al., 2006; Martín-Carrón, Goñi, Larrauri, García-Alonso and Saura-Calixto, 1999). The reduction in plasma cholesterol and lipid levels of dietary fiber has been associated with inhibited reabsorption of bile acids, cholesterol and dietary fats (Arjmandi, Ahn, Nathani and Reeves, 1992; Chau, Chen and Lee, 2004; C. F. Chau et al., 2004).

Similarly, increasing in the hepatic synthesis of bile acids in humans and rats, as well as a direct interaction with lipoprotein metabolism (by increased the number of hepatic LDL receptors, has been suggested as possible mechanisms of action (Jenkins et al., 1993; Matheson and Story, 1994). The effect from short-chain fatty acids such as acetate, propionate and butyrate which produced by fermentation in the colon may influence lipid metabolism (Theuwissen and Mensink, 2008). Soluble dietary fiber is known to be an effective hypocholesterolemic agent. Nowadays, some recent findings demonstrated that insoluble dietary fibers derived from some fruits, vegetables, and pomace could also effectively reduce serum cholesterol concentrations (C. F. Chau et al., 2004; ErkkilaÄ, Sarkkinen, Lehto, Pyörälä and Uusitupa, 1999; Knopp et al., 1999; Rosamond, 2002). It has been reported that the cholesterol-lowering actions of insoluble fibers might be correlated to some of their physicochemical properties such as water-holding capacity and cation-exchange capacity (Chau and Cheung, 1999). The aims of this study were to optimize the extraction process and determine the physicochemical and cholesterol-lowering properties of dietary fiber from cassava pulp.

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1.2 Research objectives

The objectives of this research were as follows:

(1) To investigate the optimum condition of dietary fiber production from cassava pulp.

(2) To characterize the structure and physicochemical properties of dietary fiber from cassava pulp.

(3) To determine the effect of dietary fiber from cassava pulp on lowering cholesterol level property in *in-vitro* test.

(4) To determine the effect of dietary fiber from cassava pulp on lowering cholesterol level property in animal test.

1.3 Research hypotheses

The residual from starch production such as cassava pulp could be source of dietary fiber. The dietary fiber has functional properties and affects cholesterol blood level reduction in animal test.

1.4 Scope of the study

Cassava pulp was purchased from tapioca starch industry in Maung district, Nakhon Ratchasima province, Thailand. Firstly, optimization of dietary fiber extraction was determined by using response surface methodology (RSM). Concentration of α-amylase, neutrase and amyloglucosidase concentration used as independent variables were optimized. Neutral detergent fiber (%) was used as criteria for the selection of the dietary fiber preparation. Secondly, the physicochemical and functional properties of dietary fiber were investigated. Finally, *in vitro* model and physiological property in term of "lowering cholesterol level" *in vivo* were studied.

1.5 Expected results

The outcomes from this research can provide the alternative way of value added to cassava by-product from starch production for dietary fiber preparation. Moreover, it will lead to understand both physicochemical and physiological properties of the

dietary fiber preparation from cassava pulp and could be apply as functional ingredient in food products.

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CHAPTER II

LITERATURE REVIEWS

2.1 Cassava and cassava starch production

Cassava, tapioca or manioc, is the common name of *Manihot esculenta* (L.) Crantz which grows in tropical and subtropical area in the world. It was originated in South America and first introduced into Africa. Today, it is the staple source of carbohydrate in the world. In 2006, Cassava planted areas around the world was approximately 113.8 million rais with an average yield of 1.92 tons/rai. Moreover, Nigeria is the world's largest cassava productions, followed by Brazil and Thailand. However, Thailand had a higher yield per area than Nigeria and Brazil. Thailand is currently one of the world's biggest cassava products exporters with the major competitors as Indonesia, Brazil and Vietnam. Cassava were planted in all regions of Thailand except the South. During the year 2015 to 2016, more than 50% was planted in the Northeast which the main province is Nakhon Ratchasima, followed by the Central (33%) and the North area of Thailand (15%). These planted areas included 48 provinces or around 8.8 million rais and the total production is around 33 million tons (Thaitapiocastarchassociation, 2016). Fifty percent of cassava area used as raw material for the tapioca or cassava starch and others as cassava chips, cassava pellets and cassava flour production.

The cassava starch production is the most important agro-industries in Thailand. Also, Thailand is the third largest cassava starch producer that yielded 2.9 million tons

per year and had value around 41.2 billion baht (Centre of Agricultural Information and Office of Agricultural Economics, 2016). There are 2 types of cassava starch production to produce native starch and modified starch. Normally, cassava starch production follows many steps such as cassava root receiving, cassava root preparing, washing, rasping, extraction, separation, drying and packing (Figure 2.1). From this cassava starch processing, the important by-product is cassava pulp.

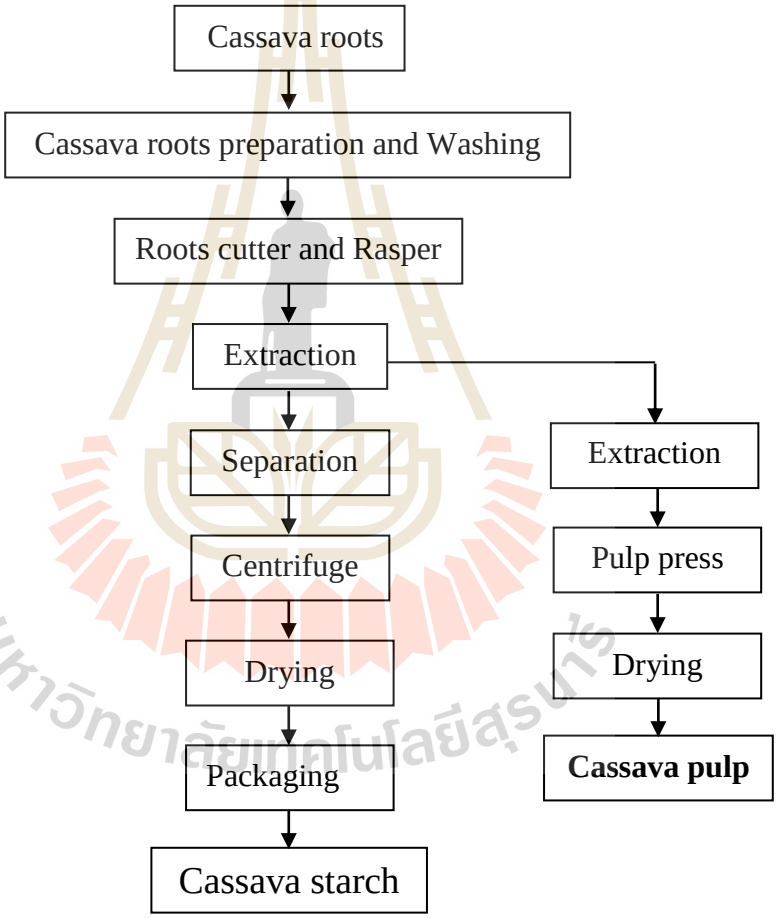

Figure 2.1 Process diagram of cassava starch production and its by-product; cassava pulp.

2.2 Cassava pulp

Cassava stem, soil, sand, and pulp are solid wastes from the cassava starch production. Cassava pulp is the main by-product from the production process and around 10-15% of the original root weight (Figure 2.2). Form starch production, 10 million tons of fresh roots could be generate at least 1 million tons of the cassava pulp annually (Sriroth et al., 2000). Nowadays, the solid wastes are most used as soil additive. Pulp is fine and white with moisture content up to 75%. The main composition is carbohydrate around 55-56%. Starch remaining in the pulp may be approximately 50-60% of its dry weight, in which the starch are most trapped inside ligno-cellulose which refers to cellulose, hemicellulose and lignin (Figure 2.3). The pulp also contains pectin, cellulose, and fiber at approximately 10-15% including protein and fat at 1.4-5% and 0.1-5%, respectively. Other components are minerals such as Fe^{2+} (155 ppm), Mn^{2+} (40 ppm), Mg^{2+} (1100 ppm), Cu^{2+} (4 ppm), and Zn^{2+} (21 ppm) per kg-dry pulp and all data are shown in Table 2.1 (Thailandtopiocastarch, 2010).

Table 2.1 Chemical composition of cassava pulp (dry weight).

Compositions	**% of dry weight**			
	Lohwongwatthana (1982)	**Nawinwan (1999)**	**Sriroth et al. (2000)**	**Laohapatthanalert (2016)**
moisture	9.25	11.63	-	-
carbohydrate	48.72-70.07	67.46	-	-
fiber	12.15-24.13	11.58	27.75	15.39
protein	1.46-2.53	1.85	1.55	1.81
fat	0.16-0.52	4.18	0.12	5.07
ash		3.30	1.70	2.00
starch	59.77	-	68.89	56.18
minerals				
Fe^{+2} (mg/kg)	155	-	-	-
Mn^{+2} (mg/kg)	4	-	-	-
Cu^{+2} (mg/kg)	4	-	-	-
Zn^{+2} (mg/kg)	21	-	-	-
Mg^{+2} (mg/kg)	1100			

Figure 2.2 Fresh cassava pulp.

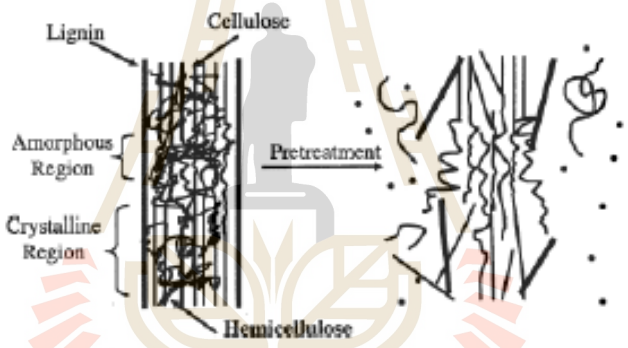

Figure 2.3 Structure and chemical composition of ligno-cellulose.

Source: Lee et al. (2008)

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2.2.1 Utilization of cassava pulp

From its high advantage compositions, the pulp could be utilized in many ways, for example; used for animal feed industry, bio-gas production, ethanol production and soil additive. There were many researches applied cassava pulp as the substrate for production of many products. Microbial conversion in bioprocessing is an important approach for the production value-added products from cassava pulp. In

conventional bioprocessing of cassava pulp, the pulp is subjected to high temperature pre-gelatinization in the presence of a thermostable α-amylase to release the trapped starch granules, followed by saccharification with glucoamylase (Pandey et al., 2000). Cassava pulp hydrolysate has been used for various fermentation products production such as citric acid, fumaric acid and lactic acid (Rattanachomsri et al., 2009). Carta et al. (1999) showed that cassava pulp was applied as the sole carbon source for fumaric acid production by submerged fermentation using several *Rhizopus* strains. Six different nitrogen sources and six different compositions of the enzymatic hydrolysate were used and the media cultivation was optimized using an experimental design. *Rhizopus formosa* MUCL 28422 was selected as the best strain for fumaric acid production, yield 21.28 g/l in a media containing cassava bagasse as the sole carbon source, KNO_3 as nitrogen source (C/N ratio of 168), 20 g/l of $CaCO_3$, 10 µg/l of biotin, 0.04 g/l of $ZnSO_4.7H_2O$, 0.25 g/l of $MgSO_4.7H_2O$, 0.15 g/l of KH_2PO_4 and 15 ml/l of methanol. Moreover,Vandenberghe et al. (2000) studied citric acid production efficiency by a culture of *Aspergillus niger* in sugar cane bagasse, coffee husk and cassava pulp. Cassava pulp is the best support for mould's growth and also perform the highest yield of citric acid and all tested substrates. In this study, the fungal strains showed good adaptation and high the protein content 23 g/kg in the fermentation process which used cassava pulp as substrate. The highest citric acid (88 g/kg dry matter) was performed within 120 h (initial moisture of cassava pulp is 62% at 26°C).

Nawinwan (1999) studied the production of xanthan gum from cassava pulp by *Xanthomonas campestris* TISTR 840. The results showed that the xanthan gum extract has the same chemical and physical character as commercial xanthan, except less viscous at the same concentration.

Cassava pulp is a good raw material for ethanol production. Kosugi et al. (2009) studied the ethanol production from cassava pulp via fermentation with a surface engineered yeast strain displaying gluco-amylase without amylolytic enzyme addition. The results revealed that the theoretical yield of ethanol production by yeast *Saccharomyces cerevisiae* strain K7G was higher up to 91% and 80% from 5% and 10% cassava pulp, respectively.

Panichnumsin et al. (2010) studies the production of methane by co-digestion of cassava pulp (CP) with various concentration of pig manure (PM). Co-digestion of CP with PM showed high methane production while volatile solids (VS) and buffering capacity are reduced. However, the digestion of PM alone showed higher the specific methane yield (41%) higher when co-digestion that combine with CP in concentrations up to 60% of the incoming VS. Moreover, the increasing of CP of the feedstock led to a decrease in methane yield and solid were decreased.

2.3 Enzymes application in carbohydrate and protein breakdown

2.3.1 α-Amylase (α- 1,4-glucan 4-glucanohydrolase, EC 3.2.1.1)

The commercial name of α-amylases is Termamyl®, common name is Diastase. The α-amylases form is found in plants, fungi and bacteria. Also, it can be found in human physiology which both the salivary and pancreatic amylases are α-amylase form. Alpha-amylase is calcium metalloenzymes that means it is completely unable to function in the absence of calcium. Alpha-amylases could hydrolyze α-1,4 glycosidic bond in starch molecule by random cleavage, eventually yielding maltotriose and maltose from amylose, or maltose, glucose and "limit dextrin" from amylopectin with α-configuration form. This enzyme is known as endo-splitting

enzyme and cannot hydrolyze α-1,6 glycosidic bond in amylopectin. Since it can act position in the substrate, α-amylase tends to faster-acting when compares with β-amylase. It is a major digestive enzyme in animals and its optimum pH range is 6.7–7.0 (Anprung, 2000; Wikipedia, 2016).

2.3.2 γ-Amylase or Amyloglucosidase or Glucoamylase (α-1,4-glucan glucohydrolase, , EC 3.2.1.3)

The γ-amylases form is commonly found in microorganism such as fungi and bacteria. The optimum pH for this enzyme is 4.0-4.4. Amyloglucosidase is known as exo-splitting enzyme which hydrolyze terminal of 1,4-linked α-D-glucose residues consecutively from the non-reducing ends of maltooligo- and polysacharides with release of β-D-glucose. Most forms of this enzyme can speedily hydrolyze 1,6-α-D-glucosidic bonds when the next bond in the sequence is 1,4-, while some forms of this enzyme can hydrolyze 1,6- and 1,3-α-D-glucosidic bonds in polysaccharides. Amyloglucosidase is importance in food fermentation industries for starch saccharification and other related oligosaccharides. A variety of microorganisms can be used to produce this enzyme, especially *Aspergillus niger*, *Aspergillus awamori* and *Rhizopus oryzae* have been considered for industrial application more than other microorganisms (Anprung, 2000; Coutinho and Reilly, 1997; Pavezzi et al., 2008).

2.3.3 Proteases

Proteases, also called peptidase or proteinase or proteolytic enzyme, refers to a group of enzymes which hydrolyze peptide bonds that link amino acids together in a polypeptide chain of proteins. They can be found in animals, plants, fungi, bacteria, archaea and viruses. Proteases are very important in digestive system since they breakdown the peptide bonds in protein foods and release the amino acids

that are needed by the body. Additionally, this enzymes have been used for a long time in various forms of therapy including in medication as carcinology, inflammatory conditions, blood rheology control, and immune regulation. Proteases is the important enzyme in human digestive system such as pepsin, trypsin and chymotrypsin. They have many advantages in food processing such as gluten development in baking products, rennet for cheese production, papain for tenderizing meats, generation of bio-active peptides and improved functionality in plant protein processing etc. (Anprung, 2000; Barrett et al., 2012).

Neutrase® is a bacterial protease produced by a selected strain of *Bacillus amyloliquefaciens*. It breaks down proteins to peptides. According to its name, Neutrase® is a neutral protease with an optimum working condition at pH 5.5-7.5 and temperature 45-55°C. It is a metallo proteinase (Zn) which requiring zinc ions for its activity. It is stabilized with the presence of calcium ions (Ca^{2+}) and consequently inhibited by EDTA. The stability of Neutrase® at a certain temperature is influenced by the type and concentration of the proteins present. Moreover, Neutrase® can be inactivated by heat treatment, e.g. at 85 °C for 2 minutes (National Center for Biotechnology Education, 2016).

2.4 Prebiotics

The term of prebiotic is defined as "nondigestible food ingredient that beneficially affects the host by selective stimulating the growth or the activity of one bacteria in the colon, that has the potential to improve health" (Gibson and Roberfroid, 1995). Prebiotics often referred to nondigestible oligosaccharides which are extracted from natural sources or synthesized from oligosaccharides. Recently, the well-known

prebiotics are inulin, fructose-containing oligosaccharides (FOSs), galacto-oligo-saccharides (GOSs) and dietary fibers (e.g. from wheat, maize, rice, fruits). To be an effective prebiotic, the following characteristic must be found: (1) cannot be hydrolyzed and absorbed in the upper part of the gastrointestinal tract and (2) be selective fermented by intestinal microbiota that is altered towards a healthier compositions (Rastall et al., 2001). According to the concept of a prebiotic, it proposes to increase the number of target bacteria in the colon, mainly on the increase of *Lactobacillus* and *Bifidobacterium* as the two main groups of "friendly bacteria" (Farnworth, 2007). Feeding the prebiotics for increasing the number of selected bacteria and produce various short-chain fatty acids (SCFAs; acetate, butyrate and propionate) will have the impact on the lower gut environment, metabolism, and disease prevention (Farnworth, 2007; Jenkins et al., 1999). The SCFAs are quickly absorbed and could serve as energy source for the host, especially between meals. Moreover, the generation of SCFAs during prebiotic fermentation by the gut microflora has been suggested to be one of the important mechanisms that responsible for their lipid-lowering effects via enzymes inhibition involved in *de novo* lipogenesis (Lovegrove and Jackson, 2001).

Mechanism of lipid lowering by prebiotics

Prebiotics have been illustrated to be an ideal substrate for health- promoting bacteria in the intestine, especially bifidobacteria and lactobacilli (Gibson and McCartney, 1998). The main by-products during fermentation process are gases (H_2S, CO_2, H_2 and CH_4), lactate and SCFAs (acetate, butyrate and propionate). These end products are both absorbed by the colon and excreted via the stool (Figure 2.4). SCFAs which are acetate and propionate will be absorbed into the bloodstream portal where they are used by the liver. Acetate is the major SCFAs produced, converted to acetyl

CoA in the liver and serves as a lipogenic precursor for *de novo* lipogenesis, but also stimulates gluconeogenesis. Meanwhile, propionate is mainly metabolized in the liver and reported to lower plasma cholesterol and lipid levels by inhibiting hepatic cholesterogenesis and lipid synthesis. (Anderson and Bridges, 1984; Chen et al., 1984; Demigné et al., 1995; Wolever et al., 1989). Butyrate is the main energy substrate and can be absorbed by the large intestinal cells (colonocytes). Its metabolized products are glucose and glutamine which are about 70% of the total energy demand of the colonic mucosa (A. Henningsson et al., 2001; Scheppach, 1994). It has been shown to prevent tumors formation in the colon. The type of SCFAs that are produced during the fermentation process would depend on the microflora that could be stimulated by prebiotic. Inulin has been reported to increase acetate and butyrate concentrations while synthetic prebiotics such as GOSs increases the level of acetate and propionate, xylo-oligosaccharides increased acetate only. (Lovegrove and Jackson, 2001; Van Loo et al., 1999).

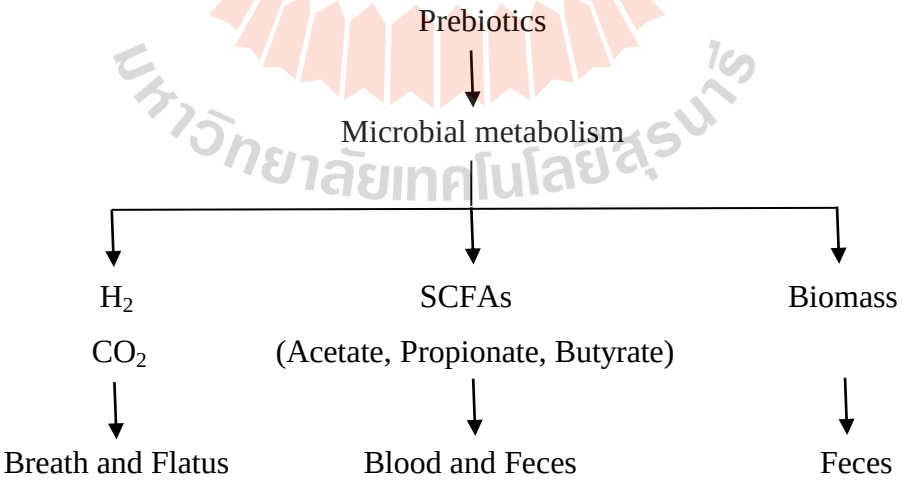

Figure 2.4 Prebiotics fermentation in the human colon.

Source: Adapted from Asa Henningsson et al. (2001)

2.5 The definition of dietary fiber

The term of dietary fiber was first used in 1953 for replacing of crude fiber which refer to the nondigestible residue in foods (Potty, 1996). In 1974, Burkitt, Walker and Painter first purposed that the prevalence of a range of diseases, including cardiovascular disease, colon cancer and diabetes, in developed communities related to low dietary fiber consumption. Many studies had demonstrated its chemical and nutritional importance (Kritchevsky, 1988)

Trowell (1974) defined dietary fiber as the portion of foodstuffs, derived from plant cells that resistant to hydrolytic digestion with the digestive enzyme system in human body and consists of hemicelluloses, celluloses, lignins, oligosaccharides, pectins, gums and waxes. Nowadays, some researchers define dietary fiber as an indigestible fraction which contains oligosaccharides and resistant starches, resistant proteins, and associated compounds such as polyphenols (Davidson and McDonald, 1998).

The American Association of Cereal Chemists (AACC, 2001) defines dietary fiber as ‘‘the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber comprises polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber promotes beneficial physiological effects including laxation, blood cholesterol attenuation, and blood glucose attenuation”. The sum of soluble and insoluble dietary fiber could be called total dietary fiber (Prosky and DeVries, 1992). Normally, dietary fiber could be classified into two main components related to the solubility property that are soluble and insoluble dietary fiber.

2.5.1 Soluble dietary fiber

The type of fiber which dissolved in water to form a gel-like material, showed lowering blood cholesterol and glucose levels. Soluble fiber is found in oats, peas, beans, apples, citrus fruits, carrots, barley and psyllium. The example of soluble dietary fibers are as in the following.

2.5.1.1 Pectins are the polysaccharides cell walls of plants especially in the outer skin and rind of fruits and vegetables, for example orange contains 30% pectin, apple peel 15%, and onion skin 12%. The main chemical chains of pectin is 1,4 linked α-D-galacturonic acid and the units of rhamnose, arabinose, xylose and fucose is a branch chain. Pectic substance is normally found between cellulose and hemicellulose, but almost in middle lamella area (Mudgil and Barak, 2013; Ridley et al., 2001). They are soluble in hot water and forming gels with cooling thus it could be used as gelling and thickening agents in various food products. Lowering effects of serum cholesterol and sugar of pectin are due to its strong gel-forming capacity. Pectin reduce serum cholesterol by cholesterol and bile acids binding in the intestine and promoting their excretion via stools (Mahalko et al., 1984; Schwab et al., 2006).

2.5.1.2 Gums and mucilages are soluble fibers that compose of hexose and pentose monomers. These compounds are not a component of the cell wall, but the physical structure and properties of these compounds are similar to pectin and hemicellulose. Gums are polysaccharides that synthesized by plants at the site of trauma and appear to function in a manner similar to scar tissue in humans (Jalili et al., 2007). The main chemical chains are galactose, glucuronic acid-mannose and galacturonic acid-rhamnose with xylose and galactose are the branch chain. Guar gums or galactomannan is the primary marketable and well-known product of plants. The fiber consist of a 1,4-linked-β-D-mannopyranose backbonewith 1,6-linked-α-D-

galactose side chains. Guar gum is an economical thickener and stabilizer for food industry. Due to its ability to easy hydrate in cold water, thus giving a highly viscous solution. Some studies revealed that guar gum could decrease serum cholesterol concentrations and use to treat constipation (Brown et al., 1999; Theuwissen and Mensink, 2008). While, mucilages are the production of plant secretory cells to preclude excess loss of water through transpiration. The main chemical chain of mucilages are galactose-mannose, glucose-mannose, arabinose-rhamnose with galactose is branch chain. This fiber was less used in food industry (Jalili et al., 2007).

2.5.1.3 β-glucan is mostly found in cereals, especially in oats and barley, also in mushrooms, bacteria, yeast and algae. β-glucan is a polysaccharides that composed of glucose molecules. In bacteria and algae, glucose molecules are linked by β-(1,3)-glycosidic bonds forming linear chain, meanwhile in yeast and mushrooms, glucose molecules are joined by β-(1,3)- and β-(1,6) glycosidic bonds, in oats and barley by β-(1,3)- and β-(1,4) glycosidic bonds (Theuwissen and Mensink, 2008). Recently, β-glucan has been widely used as functional food ingredients to lower serum total cholesterol and LDL cholesterol (Anderson, Spencer, et al., 1990; Kerckhoffs et al., 2003; Önning et al., 2000; Ripsin et al., 1992).

2.5.2 Insoluble dietary fiber

Normally, this type of fiber is the component of cell wall of plants which are cellulose, hemicellulose and lignin. This type of fiber might provide bulking or metabolically are fermented in the large intestine and act as a prebiotic fiber. They are unable to dissolve in water, however could absorb water and move through the digestive system. Fermentation of insoluble fibers are gently promote regularity. But they could be readily fermented in colon and converted into gases and physiological active by-products. Whole-wheat flour, wheat bran, nuts and many vegetables are

good sources of insoluble fiber. The example of insoluble dietary fibers are as in the following.

2.5.2.1 Cellulose is known to be the most abundant polysaccharide in nature and the important composition of plant cell wall that about 10-25%. The molecular structure is similar to amylose that made up with repeating units of the hexose glucose. However, glucose molecules of cellulose are linked by β-(1,4)-glycosidic bonds consisting of up to 10,000 glucose monomer units per molecule. (Jalili et al., 2007). Cellulose is a straight chain polymer unlike starch (Figure 2.5). Cellulose is also more crystalline when compared to starch. In water, starch becomes a crystalline to amorphous transition when heated to 60–70 °C, whereas cellulose requires a temperature up to 320 °C and pressure of 25 MPa (Deguchi et al., 2006). The linear molecules of cellulose are packed firmly together as long fiber, very insoluble and resistant to human enzymes digestion. Due to its insolubility in water, it has binding water capacity which helps increasing fecal volume. Thus, it could promote regular bowel movements. Although cellulose is not digested by human enzymes but partial digested in the gut by beneficial microflora. Cellulose is degraded by natural fermentation in colon about 50% and produces significant amount of short-chain fatty acids that feed our intestinal cells (Mudgil and Barak, 2013). Wheat bran is a rich source of cellulose, moreover, it is also found in grains and fruits around one fourth of the dietary fiber, and one third in vegetables and nuts (Ferguson et al., 2001).

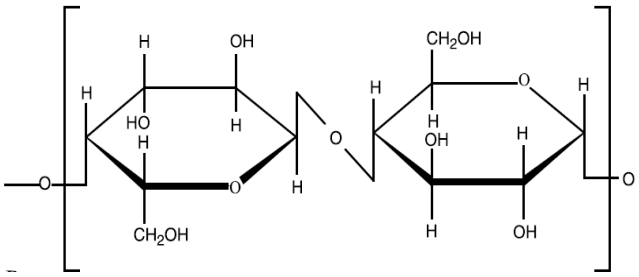

Figure 2.5 The β1–4 linkage between glucose units in cellulose.

Source: Jalili et al. (2007)

2.5.2.2 Hemicellulose are a polysaccharide component of plant cell wall like cellulose. But it is different from cellulose because of its monomers are heterogeneous. Hemicellulose contains varied amounts of 50-200 pentose units (xylose and arabinose) and hexose units (glucose, galactose, mannose, rhamnose, glucuronic and galacturonic acids) with a covalently bound in a 1-4 linkage, as well as both linear and branched molecules that is smaller than cellulose. Commonly, some of the familiar monosaccharide in hemicelluloses are xylose, mannose, and galactose (Figure 2.6). Hemicelluloses are the composition about one third of the dietary fiber in vegetables, fruits, legumes and nuts. Hemicellulose promotes regular bowel movement by increasing hydration of the stool since it has water holding capacity. The other important physical property of hemicellulose is cation exchange capacity. Hemicelluloses could prevent cholesterol absorption by directly binding cholesterol in the intestine. Moreover, microflora in the gut could digest hemicelluloses affect increasing the number of beneficial bacteria, creating short-chain fatty acids which colon cells use as fuel and also cholesterol reduction (Jalili et al., 2007; Mudgil and Barak, 2013; Mudgil et al., 2012).

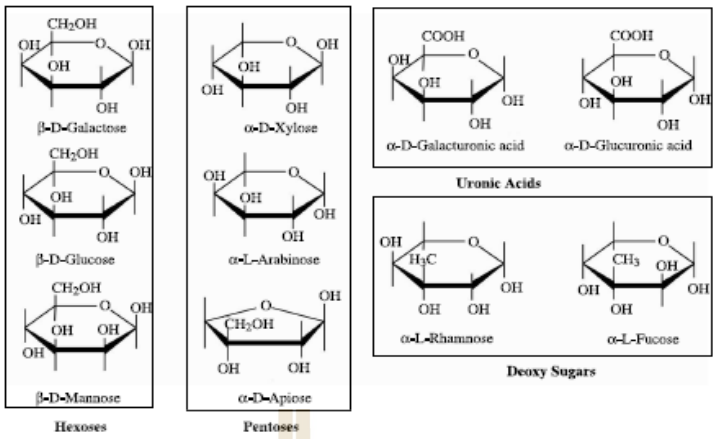

Figure 2.6 Carbohydrate monomers common to polysaccharide fibers.

Source: Jalili et al. (2007)

2.5.2.3 Lignin is considered as an insoluble dietary fiber although it is not a polysaccharide. It is chemically bound with hemicellulose in the plant cell wall. Lignin molecules are variable polymers and highly complex with the chain of oxygenated phenyl propane. They are composed of three major aromatic alcohols: coumaryl, coniferyl, and sinapyl. A typical lignin monomer is presented in Figure 2.7 (Jalili et al., 2007). The molecules of lignin are insoluble in both strong acid and alkaline solution and resistant to human enzyme (Anderson and Chen, 1979; Schneeman, 1986). It also influences gastrointestinal physiology. The major physical properties of lignin are; bile acid absorption and delay nutrient adsorption time in small intestine (Prosky and DeVries, 1992). It can be found in foods with a 'woody' component such as celery and in the outer layers of cereal grains (Anderson and Bridges, 1988).

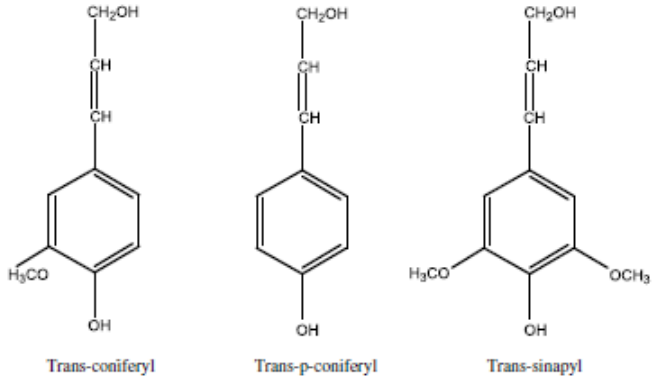

Figure 2.7 Typical phenolic monomer of lignin molecule.

Source: Jalili et al. (2007)

2.6 Chemical structure of dietary fiber

Dietary fibers are complex of chemical nature and constituted of a mixture of chemical entities. We also know that dietary fiber is composed of lignin, nondigestible carbohydrate and other substances of plant and animal fibers origin and modified or synthetic nondigestible carbohydrate polymers. The nondigestible carbohydrates are consisted of polysaccharides as β-glucan, hemicellulose, cellulose, gums, mucilage, pectin, inulin, resistant starch; oligosaccharides: fructo-oligosaccharides, oligo-fructose, galacto-oligosaccharides, polydextrose; and soybean oligosaccharides raffinose and stachyose, hemicelluloses and lignin, whereas the primary sources of pectin were found in fruits and vegetables. Chitosan is an example of fiber from animal origin, derived from the chitin containing in the exoskeletons of crustaceans and squid pens and its molecular structure is similar to plant cellulose (Borderías et al., 2005). Cereals are the main source of cellulose, hemicellulose and lignin while the primary sources of gums pectin and mucilage were found in many fruits and vegetables (Normand et al., 1987). Regarding to polysaccharides, each type of dietary

fiber is characterized by its sugar residues and by the nature of the bond between them (Table 2.2).

Dietary fiber is a complex mixture materials with different chemical nature, degree of polymerization, presence of oligosaccharide and polysaccharide, and the mixture varies with sources. Nevertheless, no method of analysis could correlate precisely with any specific physiological function. There are various analytic methods for the dietary fiber quantification such as non-enzymatic-gravimetric method, enzymatic-chemical method and enzymatic-gravimetric method, for precisely determination its composition in food products. Finally, the procedures which apply for analyzing dietary fiber contents have already been adopted as official analytical methods within the AOAC (AOAC, 2005).

Table 2.2 Chemical composition of dietary fibers.

Fibers	Main chain	Branch units
Cellulose	β-(1,4) glucose	
β-glucans	β-(1,4) glucose and β-(1,3) glucose	
Hemicelluloses		
Arabinoxilans	Arabinoxilans	Arabinose
Xiloglucoses	Xyloglucoses	Xylose
Galactomannans	Galactomannans	Galactose, glucose
Xylans	β-D-(1,4) xylose	
Arabinoxylans	β-D-(1,4) xylose	Arabinose
Mannans	β-D-(1,4) mannose	
Glucomanns	β-D-(1,4) mannose and β-D-(1,4) glucose	
Galactoglucomannans	β-D-(1,4) mannose, β-D-(1,4) glucose	Galactose
Galactomannans	β -(1,4) mannose	α -D-galactose
Xyloglucans	β-D-(1,4) glucose	α -D-xylose
Pectin	α-galacturonans	Arabinose, Galactose
Homogalacturonan	α-(1,4)-D-galacturonic acid (some of the carboxyl groups are methyl esterified)	

Table 2.2 Chemical composition of dietary fibers (Continued).

Fibers	Main chain	Branch units
Chitosan	β-(1-4)-linked D-glucosamine and Nacetyl-D-glucosamine	
Oligofructose (enzymatic hydrolysis of inulin)	β-(2-1)-D-fructosyl-fructose	
Polydextrose (synthetic)	D- Glucose	
Resistant maltodextrins (heat and enzymatic treatment of starch)	α (1-4)-D- Glucose	α (1-6)-D- Glucose
Lignin	Polyphenols: Syringyl alcohol (S), Guaiacyl alcohol (G) and p-coumaryl alcohol (H)	
Rhamnogalacturonan-I	(1,4) galacturonic acid, (1,2) rhamnose and 1-, 2-, 4-rhamnose	Galactose, arabinose, xylose, rhamnose, galacturonic acid
Rhamnogalacturonan-II	α-(1-4) galacturonic acid	Unusual sugar such as: apiose, aceric acid, fucose
Arabinanes	α-(1-5)-L-arabinofuranose	α-arabinose
Galactanes	β-(1-4)-D-galactopyranose	
Arabinogalactanes-I	β-(1-4)-D-galactopyranose	α-arabinose
Arabinogalactanes-II	β-(1-3)- and β-(1-6)-D-galactopyranose	α-arabinose
Xylogalacturonan	α-(1-4) galacturonic acid	xylose
Inulin	β-(2-1)-D-fructosyl-fructose	
Gum[a]		
Carrageenan	Sulfato-galactose	
Alginate	β-(1,4)-D-mannuronic acid or α–(1-4)-L-guluronic acid	
e.g. 1: seed gum from *Abutilon indicum*	β-(1,4)-D-mannose	D-(1,6) galactose
e.g. 2: seed gum from *Lesquerella fendleri*	Rhamnose, arabinose, xylose, Mannose, galactose, glucose, galacturonic acid	

[a] Chemical structure of gums depends of its source.

Source: Adapted from Jiménez et al. (2000) and Elleuch et al. (2011)

2.7 Fiber content in foods and consumption

Dietary fiber is naturally found in cereals, vegetables, fruits, algae, fungi and nuts, and composed of fibers that differ from food to food. Several non-starch foods, starch-contained foods including fruits and vegetables provide fiber at 20–35, 10 and 1.5–2.5 g/100 g of dry weight, respectively. Among the different rich in fiber-foods, cereals are the main sources of fiber, contributing to about 50% of the fiber intake in western countries (Lambo et al., 2005) also 30–40% of dietary fiber might come from vegetables and 16% from fruits and the remained 3% from other minor sources (Rodriguez et al., 2006).

Recently, plant-based products has recommended to eat higher amounts of these foods, which are involved in maintaining or improving the consumer health. The intake of dietary fiber is different in each country. Meanwhile, UK proposes 18 g/day of dietary fiber expressed as non-starch polysaccharides (NSP), the amount proposed is increased to 30 g/day in Germany, and the intake should be 38 g/day for men and 26 g/day for women in USA (Miller, 2004). A mediterranean diet, typically in Spain, Italy and Greece, provides a significant content of dietary fiber as it is rich in vegetables, fruits, legumes and cereals which recommended for men in these countries should be intake 20 g/day and 15.7 g/day for women (Capita and Alonso-Calleja, 2003). In Thailand, the Thai Recommended Daily Intakes (Thai RDI) suggest the intake of dietary fiber should be 25-30 g/day.

2.8 Physicochemical properties of dietary fiber related with physiological effects

Different type of fiber molecules showed different physical properties to human body, as shown in Table 2.3.

Table 2.3 Some physical properties of different dietary fiber types**.**

Dietary fiber	Type of action
Soluble dietary fiber (e.g., pectin, gum, mucilage)	Slow gastric emptying, bind bile acids, increase colonic fermentation
Cellulose	Holds water, reduces colonic pressure, reduced transit time of digestion
Hemicellulose	Holds water, increases stool bulk, may bind bile acids and cholesterol, reduced colonic pressure, reduces transit time
Lignin	Holds water, may bind bile acid and trace minerals and increase excretion, may increase fecal steroid levels

Source: Jalili et al. (2007)

The physiological effects of dietary fibers mainly depend on their physicochemical properties such as solubility, hydration property, viscosity and gel forming capacity, absorption of organic materials, cation binding capacity and fermentability.

2.8.1 Solubility

According to the classification of dietary fiber based on solubility in water, dietary fiber are two types: soluble and insoluble fiber. Both soluble and insoluble nature of dietary fibers showed the different action in their technological functionality and physiological property (Jiménez et al., 2000; Roehrig, 1988). The relationship between solubility and physiological property is shown as Table 2.4.

Polysaccharides structure is the factor influencing solubility that could be set regularly (insoluble) or irregularly (soluble) on the backbone or as side chains. The adding of a substitution group such as COOH or SO_4^{2-} help increasing solubility (Elleuch et al., 2011). Moreover, solubility is affected by temperature and ionic strength (Bertin et al., 1988; Fleury and Lahaye, 1991; Manas et al., 1994). Soluble fibers are characterized by their capacity to increase viscosity, reduce the glycemic response and plasma cholesterol (Elleuch et al., 2011; McCarty, 2005; Slavin and Greenberg, 2003; Theuwissen and Mensink, 2008). In addition, insoluble fibers are characterized by their porosity, low density and ability to increase stool bulk and decrease intestinal transit time (Olson et al., 1987; Roehrig, 1988). The incorporation of soluble fiber in food products during food processing operations is more beneficial than insoluble fiber, as it provides viscosity, ability to form gels and/or act as emulsifiers (Mudgil and Barak, 2013).

2.8.2 Hydration properties

When dietary fiber are used as a food ingredient could offer physiological functionalities for each hydration properties, as shown in Table 2.4.

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Table 2.4 Physicochemical and physiological properties of dietary fiber products.

Physicochemical properties	Physiological functionality
Water holding capacity	Laxative
Water swelling capacity	Reduction of blood cholesterol
Water retention capacity	Reduction of blood glucose
Water solubility	Reduction the risk of chronic disorder e.g. CHD, diabetes, obesity and some forms of cancer

Source: Elleuch et al. (2011)

Hydration properties of dietary fiber refer to water holding capacity (WHC), swelling capacity (SWC) and water retention capacity (WRC). WHC represents the volume of a hydrated sample under centrifugal force, while SWC represents the volume of hydrated sample under gravity forces (López et al., 1996). WRC is defined as the quantity of water that remains bound to the hydrated fiber when subjected to an external force such as pressure of centrifugation (Ma and Mu, 2016). The hydration properties of dietary fibers are correlated to the chemical structure of the component polysaccharides, and other factors such as particle size, porosity, ionic form, pH, temperature, ionic strength, type of ions in solution and stresses upon fibers. The ability of dietary fibers to hold water is strongly related to the source of the dietary fiber (Table 2.5) (Elleuch et al., 2011). Furthermore, hydration properties are correlate with both physiological and technological aspects and could influence the incorporation of fiber-enriched ingredients into food products (Femenia et al., 1999). There are many factors that effect to hydration property as mention above. WHC and SWC of dietary fiber were strongly affected by particle size. Huang et al. (2009) determined that the decreasing of WHC and SWC of mung bean hulls correlated with a reduction in particle size. Smaller fiber particles are considered to have a higher bulk density and might lower the ability to absorb water and oil of fiber (Huang et al., 2009). On the other hand, surface area and pore volume of dietary fiber also effect to WHC (Auffret et al., 1994) and pH also affects the WHC. Gorecka et al. (2000) illustrated that lupin hulls showed maximum WHC at pH 8.7, and minimum WHC at pH 1.8. Furthermore, the ratio of lignin to polysaccharides in legume fibers influence WHC. The lignin in legume fibers involves hydrophobic properties and binds a significantly lower amount of water compared to hydrophilic polysaccharides (Bell and Shires, 1982; Yang et al., 2014). Water holding capacity of dietary fiber could be

Table 2.5 Effect of processing on the hydration properties of some sources of dietary fiber.

Sources of fibers	Treatments	WHC (g /g)	SWC (mL/g)	WRC (g/g)	References
Carrot insoluble fiber	- Control (123 μm)	12.5	18	-	Chau et al. (2007)
	- After micronisation Ball milling (12.4 μm)	13	18.3	-	
	Jet milling (28.3 μm)	12.6	25.7	-	
	High-pressure micronisation (7.23 μm)	42.5	62.2	-	
Coconut fiber	Particle size (μm) :				Raghavendra et al. (2006)
	- 1127	5.56	17	-	
	- 550	7.21	20	-	
	- 390	4.42	18	-	
Deoiled cumin fiber	Alkaline extraction	-	3.75	3.30	Ma and Mu (2016)
	Enzymatic hydrolysis	-	3.49	5.48	
	Shear emulsified assisted enzymatic hydrolysis	-	6.76	6.26	
Onion bagasse	Control	6.7	21.0	-	Benítez et al. (2011)
	Sterilized	6.4	19.0	-	
Peach - peel	drying at 30 °C for 7 h	25	39	14	de Escalada Pla et al. (2012)
	freeze drying	47	43	31	
- pulp	drying at 30 °C for 7 h	24	29	14.3	
	freeze drying	59	47	33	
Polygonatum odoratum	Steam processing	21.14	9.81	-	Lan et al. (2012)
	Drying in sunshine	23.94	17.54	-	
Maca (*Lepidium meyenii*) liquor residue	Acid-alkaline chemical method	8.39	12.33		Chen et al. (2015)
	Enzymatic method	16.29	26.17		

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occurred in two types. First, soluble fiber especially pectin and some hemicelluloses have high water binding capacity because they have free polar group at sugar residues. Thus, they can swell and form gels, resulting higher in viscosity and reduce the rate of absorption of nutrients in small intestine. Second, cellulose and hemicellulose (insoluble fiber) have high water holding capacity that could absorb water molecule at hydrogen bond to form intermolecular bond. Consequently, they could increase fecal bulk and decrease intestinal transit time (Attavanich, 2001).

2.8.3 Viscosity

Viscosity is defined as the ratio of shear stress (Γ) to shear rate (γ). Most polysaccharide solutions show non-Newtonian flow and increasing in shear rate could increase or decrease viscosity (Sanderson, 1981). Viscosity is a physicochemical property correlated with dietary fibers especially soluble dietary fibers (Dikeman and Fahey Jr, 2006). Water soluble fibers such as gums, pectins, psyllium, and β-glucans are the major element that would increase solution viscosity (Abdul-Hamid and Luan, 2000). As mention above, soluble fibers have high water binding capacity, hence they can swell and form gels, resulting increase viscosity in the gastrointestinal tract. Gels seem to respond more like solids than liquids in the colon. Also, gels may provide lubrication to stool (Mudgil and Barak, 2013). Moreover, a high viscosity fiber impedes nutrient absorption since its retard the enzymatic digestion and micelle formation which may reduce lipid absorption, resulting in lower body weight gain (Davidson and McDonald, 1998; Dvir et al., 2000). In term of technological application, high viscosity fiber would be the important factor for light product foods because it may increase viscosity of the liquid phase aim to mimic viscosity and sensorial impression of oil (Matz, 1996).

2.8.4 Absorption of organic materials

Dietary fiber can absorb organic substances such as bile acids, toxic compounds and micelle components, such as monoglycerides, free fatty acids and cholesterol in the small intestine, and increase the fecal excretion of these entities. Normally, soluble fibers e.g. pectin, β-glucan etc., are known to decrease serum cholesterol through its ability to reduce the amount of bile reabsorbed in the small intestines. Soluble fibers able to trap bile acids in the small intestine with its gel forming capacity. The gel matrix are eventually excreted in the feces may entrap some of the bile acids. This physical entrapment occurs in the terminal ileum where bile acids are usually reabsorbed (Cho et al., 2007; Elleuch et al., 2011; Martinez-Flores et al., 2004). Recently, some researches revealed that insoluble fibers can also decrease blood cholesterol and triglycerides. Furthermore, some in vitro studies showed that lignin can bind to bile acids as well. However, cellulose hardly binds bile acids (Attavanich, 2001).

2.8.5 Cation exchange capacity

Cation exchange capacity (CEC) refers to ability of plants to attract and bind hydrogen ions which have a positive charge. Cellulose has a relatively low cation exchange capacity while pectin has a high cation exchange capacity (McBurney et al., 1983). Cation exchange capacity partly depend on the presence of uronic acid in the nonesterified form. The method to prepare fiber material may reduce the number of nonesterified carboxyl groups and the apparent cation exchange capacity. Comparison of cation exchange capacity from different sources of fiber is difficult, except it is expressed per edible portion (Mongeau and Brooks, 2016). From this property, dietary fiber can be used as functional food to help prevent several diseases. Chemical side chain groups in the structure of dietary fiber, such as carboxyl and hydroxyl act as the

cation exchange resin (Baghurst et al., 1996; McDougall et al., 1996). Many studies illustrated that many cations especially some toxic cations can be exchanged by dietary fiber (Hosig et al., 1996; Truswell, 1995; Yang et al., 2007). Therefore, such absorbed toxic ions can be excreted via the feces. Dietary fiber able to absorb some of the harmful substances in animals which effect to prevent many disease (Pandolf and Clydesdale, 1992). For instance, some researches showed that adsorption of NO_2 and bile acid can prevent cancer (Kaczmarczyk et al., 2012), high blood pressure (Behall et al., 2006; Mudgil and Barak, 2013) and cardiovascular disease (Lovegrove and Jackson, 2001; Merchant et al., 2003). Moreover, wheat bran fibers can permanently bind heavy metal ions to decrease their toxicity. Nevertheless, the negative effect of consumption of high fiber diets is considered. With regard to the affinity of minerals for carboxylic acid groups, cabbage and coarse wheat bran show a high cation exchange capacity compared with pectin. Some *in vitro* studies reveal that the ability of dietary fiber to bind minerals may lead to mineral deficiencies in individuals consuming high-fiber diets which may be from phytic acid in each plants (Attavanich, 2001; Mongeau and Brooks, 2016).

2.8.6 Fermentability

Fermentability of dietary fiber by bacteria normally occurs in colon. Bacterial degradation of polysaccharide is dependent on fiber source, chemical structure and physical structure of dietary fiber, microflora and its ability, and duration of food in the colon (Guillon et al., 2001). Also, fermentability of dietary fibers by intestinal microflora are varied. Normally, soluble fiber such as pectin, β-glucan and gums seem to be almost completely fermented, while insoluble fiber are partly fermented and lignin are totally unfermented (Elleuch et al., 2011). Plants contain varying proportions of fermentation process which are rapidly, slowly and

unfermentable. Fruits; such as apples and bananas and vegetables; such as potatoes and beans able to ferment rapidly and may conduce less of fecal bulking than other fibers. Meanwhile, psyllium and wheat bran able to ferment slowly and help build up the fecal mass through fermentation, which occur along the entire length of the colon (Mudgil and Barak, 2013). Fermentation of soluble fibers may play an important role in some physiological effects to the colon. Some fiber sources such as fructoligosaccharides and inulin, known as prebiotics, can selectively stimulate the growth of health-promoting bacteria, including bifidobacteria and lactobacilli (Salminen et al., 1998; Van Loo et al., 1999). They may be the main genera responsible for the protective barrier function and for stimulating healthy immune response in adults.

The major by-products of fermentation process include gases (H_2, CO_2 and CH_4), lactate and short chain fatty acids (SCFAs), mainly acetate, butyrate and propionate (Figure 2.4). The amount and molar ratios of the three main SCFAs vary considerably, depending on the substrate type (Table 2.6). The major SCFA produced is acetic acid, whatever the substrate. Starch is mainly associated with a large proportion of butyrate while arabinogalactan, guar gum and galacto-oligosaccharides are observed with the highest proportion of propionic acid (Guillon et al., 2001). SCFAs are beneficial effect to human body such as reduction risk of some diseases including irritable bowel syndrome, inflammatory bowel diseases, cardiovascular disease, and cancer (Jenkins et al., 1999; Wong et al., 2006).

Table 2.6 Short chain fatty acid profile of some dietary fibers.

Substrates rich in	Example	Short chain fatty acid profiles
Cellulose		High amount of acetate
Mixed β-glucans	Oat bran	High amount of butyrate and relatively low amount of acetate
Resistant starch	Retrograded or native Eurylon®, Hylon®, Crystalean®	High amount of butyrate and relatively low amount of acetate
Fructo-oligosaccharides	Actilight	High amount of lactate and relatively high amount of acetate
Inulin		High amount of propionate and acetate
Pectins		High amount of acetate
Galactomannans		High amount of propionate
Wheat bran		High amount of butyrate
Guar gum		High amount of acetate
Sugar-beet fiber		High amount of acetate

Source: Adapted from Botham et al. (1998); Guillon et al. (2001); Hara et al. (1996)

2.9 Nutritional and health effects of dietary fiber

The main sources of dietary fiber including vegetables, wheat and most other grains. Foods rich in soluble fiber include fruits, oats, barley and beans. The nutritional and health benefits of dietary fiber is still the subject of much study and have been reviewed by many authors. High fiber foods is continue to be the interesting topics because of their consistency, encourage mastication and stimulate the secretion of digestive juices. The soluble fiber cause an increase in the viscosity of the stomach contents, thereby retarding gastric emptying (Leclere et al., 1994). This property affects the rate of digestion and the uptake of nutrients, after that creates a feeling of satiety. Soluble fiber has also been shown to lower serum LDL cholesterol level, improving glucose metabolism and insulin response (Glore et al., 1994). In

addition, dietary fiber tends to increase faecal bulking in the colon due to increased water retention, meanwhile insoluble fiber reduces transit time. This is particularly important because the conversion of sterols to carcinogenic polycyclic aromatic hydrocarbons is well-known to appear with time. Epidemiological evidence revealed that low fecal weights are related with increasing in risk of colon cancer (Burkitt and Trowell, 1975). Dietary fiber could also bind toxins, bile acids and carcinogens (Callegaro et al., 2009; Hu et al., 2010). In vitro studies have illustrated that the carboxyl groups of the uronic acids in NSP can bind divalent cations such as calcium, iron, copper and zinc (Hu et al., 2010; James et al., 1978). There are many epidemiological evidences of the function of dietary fiber in disease prevention such as obesity, heart disease, colon cancers and gastrointestinal disorders (Ahmad, 1995; Bingham et al., 2003; Park et al., 2005). Moreover, it may be beneficial in weight reduction and in the control of diseases such as hypolipidaemia and diabetes (Anderson and Tietyen-Clark, 1986; Slavin, 2005).

2.9.1 Lowering blood cholesterol level

Cardiovascular disease (CVD) is the leading cause of death in the United States and most Western countries after cancer (Anderson, Deakins, et al., 1990).The most important risk factors of developing CVD are high total-cholesterol levels and low-density lipoprotein (LDL) cholesterol levels (Jalili et al., 2007; Theuwissen and Mensink, 2008). One way to reduce serum total and LDL cholesterol levels by making dietary changes. Besides to decrease saturated fat and cholesterol intake, and increase *cis*-unsaturated fat intake, the importance approaches are increasing the consumption of water-soluble dietary fibers has become increasingly recognized. Because soluble fibers, in particular, appear to have the ability to lower serum cholesterol levels. However, the exact mechanism of water-soluble fibers to lower serum LDL

cholesterol levels is not already known. Some evidence suggests that water-soluble fibers may interfere with lipid and/or bile acid metabolism. Other mechanisms are the inhibition of hepatic cholesterol synthesis by end-products from fermentation and delayed the macronutrients absorption that resulting in increased insulin sensitivity (Theuwissen and Mensink, 2008). Thus, the possible hypocholesterolemic mechanisms of water-soluble fibers are as following.

2.9.1.1 Interference with lipid and/or bile acid metabolism

Soluble fibers decrease absorption of cholesterol by forming a viscous matrix with cholesterol within the intestinal lumen (Figure 2.8). Regarding to the mechanism, cholesterol absorption is retarded and more cholesterol is removed through the ileum into the large bowel via feces. The net result is reduced cholesterol uptake into the body parts (Jones, 2008). In addition, it has been suggested that soluble fiber decreases plasma cholesterol through its ability to bind bile acids in the gastrointestinal tract. As this reason, the formation of micelle is altered and bile acids reabsorption is subsequently impaired, leading to the excretion of the fiber-bile complex through the feces. There are two classes of bile acids which are primary and secondary. Primary bile acids (cholic and chenodeoxycholic acid) are those synthesized directly from the liver, meanwhile secondary bile acids (deoxycholic and lithocholic acid) are produced after modification of primary bile acids by bacterial action in the colon (Jalili et al., 2007). According to excretion of cholesterol and bile acids via feces, hepatic conversion of cholesterol into bile acids increases whereas hepatic pools of free cholesterol decrease.

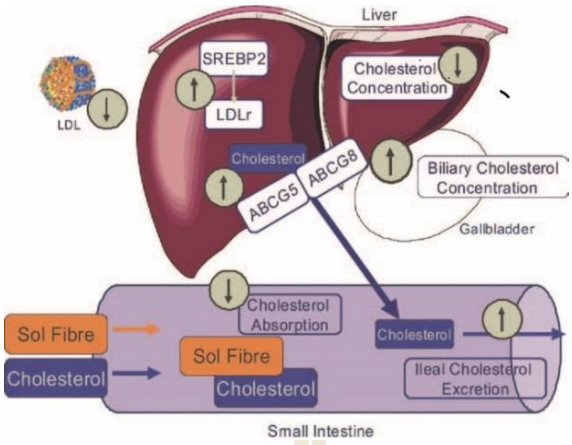

Figure 2.8 The effect of soluble fibers in the diet on decreasing cholesterol absorption. Source: Jones (2008)

Consequently, to reach a new steady-state, endogenous cholesterol synthesis will increase, leading to increased activities of 7-α-hydroxylase and HMG-CoA reductase to compensate for the losses of bile acids and cholesterol from the liver stores. Moreover, receptors of hepatic LDL cholesterol become upregulated to restore hepatic cholesterol stores, which will ultimately lead to decreased serum LDL cholesterol concentrations (Figure 2.9) (Theuwissen and Mensink, 2008).

Research has shown that increasing consumption of soluble fiber by 5 to 10 g per day can reduce LDL cholesterol by about five percent. Oat bran and oatmeal, as well as psyllium and barley, are the source which rich in beta-glucan, a soluble form of fiber, have been shown to lower total cholesterol and LDL cholesterol. Evidence suggests that more than 11 g of beta-glucan from oats can lower cholesterol up to 14.5 percent. According to the United States of Food and Drug Administration (FDA), the consumption of β-glucan soluble fiber from oats and barley (3 g/d) and psyllium (7

g/d) can state on their label that they may reduce the risk of heart disease, likewise a diet low in cholesterol and saturated fat (Kendall et al., 2010; Wikipedia, 2012).

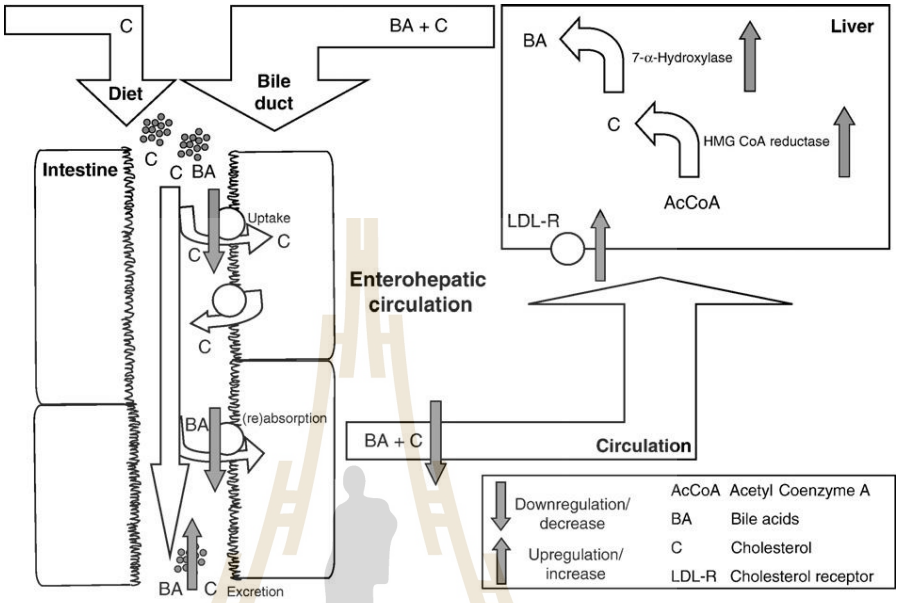

Figure 2.9 Postulated hypocholesterolemic mechanism of water-soluble fibers. The viscous water-soluble fibers form a thick unstirred water layer in the intestinal lumen, thereby decreasing the reabsorption of cholesterol and bile acids. This leads to an increased fecal output of these two components. As a result hepatic conversion of cholesterol into bile acids increases, hepatic pools of free cholesterol decrease and endogenous cholesterol synthesis increases. In addition, hepatic LDL cholesterol receptors are upregulated to re-establish hepatic free cholesterol stores. These processes will ultimately lead to decreased serum LDL cholesterol concentrations.

Source: Theuwissen and Mensink (2008)

2.9.1.2 Effects of short-chain fatty acids produced by fermentation in the colon

Dietary fibers are fermented by colonic bacteria in the large intestine. One of the important end-products from this fermentation are short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate. Acetate and propionate are rapidly absorbed into the bloodstream portal and are used by the liver, while butyrate is primarily metabolized by colonic mucosal cells. Acetate is the major SCFAs produced, converted to acetyl CoA in the liver and serves as a lipogenic precursor for *de novo* lipogenesis, but also stimulates gluconeogenesis. While, propionate is mainly metabolized in the liver and was reported to lower plasma cholesterol and lipid levels by inhibiting hepatic cholesterogenesis and lipid synthesis. Furthermore, it has been hypothesized that in particular changes in the propionate:acetate ratio may influence lipid metabolism. (Anderson and Bridges, 1984; Chen et al., 1984; Demigné et al., 1995; Jalili et al., 2007; Theuwissen and Mensink, 2008; Wolever et al., 1989). However, this hypothesis mainly studied in animal model, while properly controlled intervention studies in humans are limited. Besides, data on the effects of SCFA production in the large bowel on serum cholesterol in humans are still conflicting.

2.9.1.3 Reduced insulin stimulation of hepatic lipogenesis

The rate of glucose absorption in the intestine may be lower, due to increased intestinal viscosity of water-soluble fibers (Würsch and Pi-Sunyer, 1997). After consumption of soluble fiber, the concentrations of postprandial glucose is lowered, resulting in lowered postprandial insulin concentrations and decreased insulin-stimulated hepatic HMG-CoA activity and hence cholesterol synthesis (Theuwissen et al., 2008). Thus, the effects of soluble fiber on LDL metabolism is consideration from many studies that soluble fiber also may decrease insulin levels.

The consumption of soluble fibers may retard the glycemic response, reduce circulating insulin levels, and therefore inhibit the synthesis of cholesterol as well as fat. In conclusion, soluble fiber affects hepatic and intestinal cholesterol metabolism and decreases cholesterol concentrations (Jones, 2008). However, the result of hormonal changes in lowered fasting LDL cholesterol concentrations never been demonstrated in humans

2.9.1.4 Lowering blood cholesterol level of water-insoluble fibers

Recently, insoluble fiber also have been suggests to lower serum cholesterol levels. The ability of cholesterol and bile acid absorption, and able to enhance the excretion via feces, is the important property of water-insoluble fibers to lower cholesterol concentration. Normally, adsorption of bile acids by dietary fibers depend on the composition of fibers, the chemistry of the sterol, pH and osmolality of the surrounding medium. Lignin (the water-insoluble fiber) is the most potent bile acid adsorbent and binding capacity is influenced by molecular weight, pH, and the presence of methoxyl and carbonyl groups on the lignin molecule that adsorption is greatest at low pH (Vahouny and Cassidy, 1985). Chi-Fai Chau et al. (2004) studied about water-insoluble fiber-rich fraction (WIFF) which was isolated from the pomace of *Averrhoa carambola*, which is popular in Asia. The effects of WIFF-added diet on the lipid and cholesterol metabolism in hamsters were investigated and compared with those of cellulose-added and fiber-free diet as controls. Experimental results showed that the consumption of carambola WIFF decreased (p<0.05) the concentrations of serum triacylglycerol, serum total cholesterol, and liver cholesterol, and increased (p<0.05) the concentrations of fecal total lipids, fecal cholesterol, and fecal bile acids. The intake of WIFF also increased the fecal bulk and moisture. These pronounced cholesterol and lipid-lowering effects of WIFF might be attributed to its ability to

enhance the excretion of cholesterol and bile acids via feces. These results suggested that carambola WIFF is used as a promising cholesterol-lowering ingredient in human diets or new formulations of fiber-rich functional foods.

As the same result of C. F. Chau et al. (2004) investigated a water-insoluble fiber-rich fraction (WIFF). It was isolated from the peel of *Citrus sinensis* L. cv. Liucheng. The effects of a WIFF containing diet on lipid and cholesterol absorption in hamsters were investigated and compared with those of a cellulose-containing diet and fiber-free diet, as controls. Results demonstrated that WIFF could significantly (p<0.05) decrease the levels of serum triglyceride, serum total cholesterol, liver total lipids, and liver cholesterol, while it could also significantly (p<0.05) increase the levels of fecal total lipids, fecal cholesterol, and fecal bile acids, as well as the fecal bulk and moisture. The pronounced hypocholesterolemic and hypolipidemic effects of WIFF might be attributed to its ability to enhance cholesterol and bile acids excretion. These results suggest that WIFF could be a potential cholesterol-lowering ingredient in human diets or new formulations of fiber-rich functional foods.

Hsu et al. (2006) investigated potential effects of the insoluble fiber-rich fractions (IFRF) prepared from carrot pomace on lipid and cholesterol absorption in hamsters. As compared with the cellulose-added and fiber-free diets, IFRF diet significantly (p<0.05) lowered the levels of serum triglyceride, serum total cholesterol, and liver cholesterol, and meanwhile resulted in a significantly (p<0.05) higher HDL: total cholesterol ratio as well as higher levels of fecal lipids, cholesterol, and bile acids. Significant correlations were observed among the levels of serum triglyceride, serum total cholesterol, liver cholesterol, fecal lipids, fecal cholesterol, and fecal bile acids, implying that the hypolipidemic and hypocholesterolemic effects of IFRF were partly attributed to the reduced absorption of lipid and cholesterol. The

results suggested that IFRF could be a potential functional ingredient in the fiber-rich diets for controlling serum cholesterol concentration.

2.9.2 Lowering blood glucose level

The hydration of dietary fiber will allow for the formation of a gel matrix in the small intestine. Theoretically, the gel formation of fiber in the small intestine could increase viscosity of the food derived contents and slow the rate of nutrients absorption. It has been proposed that this mechanism may slow the rate of carbohydrate absorption and decrease the magnitude of the postprandial spike in blood glucose (Jalili et al., 2007). According to the American Diabetes Association (ADA) dietary strategies that the regulation of blood glucose levels is the primary objective in management of type 2 diabetes (Sheard et al., 2004). Moreover, glycaemic control is also the important factor in management of type 2 diabetes. In general, most of dietary fibers such as all-bran, oats and legumes are defined as most medium and low GI foods. Some data indicated high fiber and low GI foods can be beneficial for management and prevention of type 2 diabetes (Kendall et al., 2010). Many clinical studies in type 1 and 2 diabetics have demonstrated that consumption of low GI, high fiber diets improved glycated proteins level which are glycaemic control markers (Kendall et al., 2006). Sievenpiper et al. (2009) studied the effect of legumes on glycaemic markers, they concluded that legumes as part of a high fiber diet significantly decrease fasting blood glucose and glycated proteins. As the same result of Anderson et al. (2004) who studies on the effect of high fiber diets vs. low fiber diets on markers of glycemia. The results showed that high fiber diets significantly improve glycaemic markers including fasting blood glucose, postprandial plasma glucose levels and HbA1c. In addition, consumption of high fiber and low GI diets is correlated to improved management of body weight and effecting in weight loss. The

possible mechanism for this effect is through increased satiety (Kendall et al., 2010; Ludwig, 2000). Appleby et al. (1998) revealed that higher fiber consumption is related with lower body mass index (BMI) in both men and women.

2.9.3 Reduce risk of colonic cancer

Dietary fiber could help reduce the risk of some cancers, especially colon cancer. This notion is based on information that higher intake of insoluble fiber could increase stool weight and frequency of defecation which may contribute to the prevention of large bowel disorders such as constipation, diverticulitis and large bowel cancers (Elleuch et al., 2011; Rodriguez et al., 2006). This means the body may have less exposure to toxic substances produced during digestion process. In addition, the by-product from intestinal microbiota, butyrate is the main energy substrate and can be absorbed by the large intestinal cells (colonocytes). Its metabolized products are glucose and glutamine, about 70% of the total energy demand of the colonic mucosa (Henningsson et al., 2001; Scheppach, 1994). It has been also shown to prevent the formation of tumors in the colon. However, more recent studies have not confirmed the protective effects of dietary fiber in developing of colon cancer.

2.10 Rat

Rat is the common name for any large member of rodent family, mostly with dull-colored, coarse fur, long tails, relatively large ears and pointed snouts. Also, the laboratory rat (*Rattus norvegicus*) is one of the most commonly used as experimental animals, representing it does the best functionally characterized mammalian model system. The rat play an important role as a model organism for the analysis of a number of considerable biomedical traits, such as metabolic disorders, cardiovascular

diseases, metabolic disorders, neurological disorder, diabetes mellitus, neurobehavioural studies, cancer susceptibility and renal diseases. It proposes a number of unique advantages for modelling human diseases, developing new therapeutic agents and in studying responses to environmental agents. The most popular rats used experimentally are derived from Wistar and Sprague-Dawley colonies, irrespective of the fact that their genetic make-up is often extremely variable. They served over a long period of time as the major source in animal experimentation which primarily in toxicity studies (Hedrich, 2000).

The strong advantages of rats are as following. Handled with practice of rats is easy. Although they are relatively small in size, 8-10 times larger than the mouse which allow them to be treated with the test article via a variety of routes such as gavage, mixed in the diet, intravenous, subcutaneous as well as intraperitoneal. Small amounts of blood can be easily repeated withdrawn, and surgical operations for retrieving blood and other body fluids, and to remove small organs, such as adrenals and pituitaries at necropsy are not a problem. Rats can be housed in large numbers in a restricted animal room, and they breed easily and continuously during their normal reproductive life-span. However, the results of these safety studies are frequently complicated by various factors such as species differences in sensitivity between the experimental animal and humans, which may result in differences in anatomical, physiological, reproductive and behavioral features (Mutai, 2000).

Although hematological data in the rats are limited, some reference intervals of Wistar rats including serum and urine biochemical constituents are available provided in Table 2.7 and 2.8. However, reference intervals should be used as a tool, but not as

Table 2.7 Hematologic parameters in diets-restricted 8-16 week old Wistar Han rats collected under Isoflurane Anesthesia.

Parameter	Unit	Male range (2.5-97.5%)	Mean (males)	Female range (2.5-97.5%)	Mean (females)
Red blood cells (RBCs)	$\times 10^6/\mu L$	7.27 - 9.65	8.39	7.07 - 9.03	8.02
Hemoglobin (Hgb)	g/dL	13.7 - 17.6	15.7	13.7 - 16.8	15.2
Hematocrit (Hct)	%	39.6 - 52.5	45	37.9 - 49.9	43.3
Mean cell volume (MCV)	fL	8.9 - 57.9	53.5	49.9 - 58.3	53.8
Mean cell hemoglobin (MCH)	pg	17.1 - 20.4	18.7	17.8 - 20.9	19
Mean cell hemoglobin concentration (MCHC)	g/dL	32.9 - 37.5	34.9	33.2 - 37.9	35.3
Red cell distribution width (RDW)	%	11.1 - 15.2	12.7	10.5 - 14.9	12.2
Absolute reticulocytes	$\times 10^6/\mu L$	152.3 - 381.5	238	152.3 - 381.5	216.6
Platelets	$\times 10^3/\mu L$	638 - 1177	904	680 - 1200	929
White blood cells (WBCs)	$\times 10^3/\mu L$	1.96 - 8.25	4.52	1.13 - 7.49	3.12
Neutrophils	$\times 10^3/\mu L$	0.22 - 1.57	0.68	0.15 - 1.5	0.46
Lymphocytes	$\times 10^3/\mu L$	1.41 - 7.11	1.56	0.82 - 5.66	2.5
Monocytes	$\times 10^3/\mu L$	0.03 - 0.18	0.08	0.02 - 0.16	0.06
Eosinophils	$\times 10^3/\mu L$	0.01 - 0.16	0.04	0.01 - 0.15	0.05
Basophils	$\times 10^3/\mu L$	0 - 0.05	0.01	0 - 0.03	0.01
Large unstained cells	$\times 10^3/\mu L$	0 - 0.06	0.02	0 - 0.04	0.01

Source: Bolliger et al. (2010)

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Table 2.8 References values for hematology of blood and urine in Wistar rats.

Parameter	Reference values		Unit
	Blood	Urine	
Red blood cell (RBC)			
Male	7.69	-	$\times 10^6/\mu L$
Female	7.77	-	$\times 10^6/\mu L$
White blood cell (WBC)			
Male	11.6	-	$\times 10^3/\mu L$
Female	8.8	-	$\times 10^3/\mu L$
Hematocrit			
Male	45.1	-	%
Female	46.0	-	%
Hemoglobin			
Male	16.6	-	g/dL
Female	16.6	-	g/dL
Platelet			
Male	12.91		$\times 10^5/\mu L$
Female	12.81		$\times 10^5/\mu L$
Total protein	5.9-8.4	-	g/dL
Albumin	3.2-4.3	-	g/dL
Globulin	2.9-4.8	-	g/dL
Glucose	89.5-183.3	-	mg/dL
Creatinine	42.5	6	μmol/L
Blood urea nitrogen	6.9	-	mmol/L
Sodium	135	200	mmol/L
Potassium	4.9	150	mmol/L
Calcium	2.6	0.7	mmol/L
Chloride	97-110	-	mEq/L
Phosphate	2.3	-	mmol/L

Source: Matsuda et al. (2000)

the sole guide to determine if values are normal or abnormal, or to determine whether or not hematologic changes are the result of an experimental procedure (Bolliger et al., 2010). Besides, extensive online reference intervals are also available (Charles River Laboratories, 2016).

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2.11 References

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CHAPTER III

OPTIMIZATION OF ENZYMATIC DIGESTION AND PHYSICOCHEMICAL PROPERTIES OF DIEATARY FIBER FROM CASSAVA PULP

3.1 Abstract

Cassava pulp is a high value by-product from cassava starch industry that contain high amount of neutral detergent fiber (NDF) around 31.40% (w/w). Response surface methodology was applied for optimization of the extraction parameters which the percentage of NDF was the selected dependent variable. Three parameters, namely: α-amylase concentration, neutrase concentration, and amyloglucosidase concentration including three level for each parameter were studied. The optimum condition for the highest NDF by enzymatic digestion was at 0.1% of α-amylase (w/v), 1% of neutrase (v/v) and 0.1% of amyloglucosidase (v/v). Dietary fiber from cassava pulp contain 40.24% (w/w) crude fiber, 79.03% (w/w) neutral detergent fiber (NDF), 70.14% (w/w) acid detergent fiber (ADF) and a high content of cellulose at 58.55% (w/w). In addition, the hydration properties of the dietary fiber prepared were investigated. The results show that the dietary fiber exhibited 4.82 mL/g swelling capacity, 8.36 g/g water retention capacity and 8.17 g/g water holding capacity. Bulk density, oil holding capacity and cation-exchange capacity of dietary fiber were 0.28 g/mL, 3.97 g/g and 0.47 meq g^{-1}, respectively. The major monosaccharide constituent of dietary fiber was glucose, together with other neutral sugars identified by ion

exchange chromatography. The FTIR spectrum of dietary fiber prepared was similar to the cassava pulp spectrum which showed a sharp peak at 1028-1003 cm^{-1} that is the usual fingerprint of polysaccharides. Finally, scanning electron microscopy (SEM) of the cassava pulp revealed a large amount of starch granules embedded within the cell wall material of the cassava pulp. Otherwise, no starch granules appeared in the dietary fiber after enzymatic digestion. In conclusion, as a result of the physicochemical properties of the dietary fiber prepared from cassava pulp by enzymatic digestion described above, cassava pulp can be used as a rich source of useful dietary fiber and applied to many food products.

3.2 Introduction

Dietary fiber (DF) is defined as a non-digestible carbohydrate and lignin, that are intrinsic and intact in plants, along with isolated non-digestible carbohydrates which have beneficial physiological effects in humans; some researchers propose that they are non-digestible carbohydrate polymers with three or more monomers while others maintain that dietary fibers are non-digestible carbohydrates and lignins found in plant sources, along with additional synthetic non-digestible carbohydrates. Non-digestible matter cannot be digested or absorbed in the small intestine of humans. An even broader definition of dietary fibers may include fibers of animal origin and modified or synthetic non-digestible carbohydrate polymers (Polymerisation Degree ≥ 3), e.g., polydextrose, fructo-oligosaccharides (Borderías, Sánchez-Alonso and Pérez-Mateos, 2005). Dietary fiber functional food can help prevent several diseases. They absorb toxic ions and can be excreted with the feces. Dietary fiber can also absorb some of the harmful substances in animals (Yan et al., 2011), which play a role in disease

prevention. For example, it has been proved that the adsorption of NO_2 and bile acid prevents cancer, high blood pressure, heart disease (Bourdon et al., 2001) and cardio-vascular disease (Merchant et al., 2003). Moreover, it can help reduce the rate of glucose absorption, the levels of total cholesterol (Brown et al., 1999; Tucker and Thomas, 2009) and triglycerides, which inhibit hepatic fatty acid synthesis and maintain stable blood sugar levels after meals which is very important for diabetics (Brownlee, 2011). Furthermore, because dietary fiber is not digested by the digestive system in the human body, dietary fiber can be used as a non-caloric ingredient for the replacement of caloric ingredients, such as fats, carbohydrates and protein in many food products. Consequently, the consumption rate of fiber-rich products has increased. Many by-products from the fruit and vegetable industry are of particular interest due to their low cost and availability in large quantities. Indeed, some of the agricultural by-products, such as apples, citrus fruits, grapes, carrots and Brassica vegetables have already been used in the production of dietary fiber (Figuerola et al., 2005; Grigelmo-Miguel and Martín-Belloso, 1999; Hsu et al., 2006). They supply dietary fiber as well as bioactive compounds such as polyphenols and essential oils, thus providing economic benefits for the food, cosmetic, and pharmaceutical industries. With regard to the food industry, dietary fiber can also incorporate some functional properties of foods, such as increasing water and oil holding capacity, emulsification, and gel formation. Dietary fiber can be incorporated into many varieties of food products, such as bakery, dairy products, jams, meats and soups etc. (Elleuch et al., 2011). However, various methods and different sources for obtaining dietary fiber might alter their chemical composition and physicochemical properties that subsequently affect their function as food ingredients in food applications (Chau and Huang, 2003).

Cassava (*Manihot esculenta* Crantz) is the third-largest source of food carbohydrates in the tropics. Cassava is mostly grown in tropical countries with 70% of the world's cassava production coming from Nigeria, Brazil, Thailand, Indonesia and the Democratic Republic of the Congo (Khempaka, Molee and Guillaume, 2009). The cassava starch industry has a global market value of approximately 1.4 billion tons/year. Cassava starch production in Thailand is one of the most important agro-industries. Thailand is the third largest producer of cassava starch, yielding 22.2 million tons per year. Cassava pulp is an important by-product of many by-products obtained from processing cassava. At least 1 million tons of pulp are generated annually in Thailand. Cassava pulp represents approximately 10-15% by weight of the original cassava roots. The starch remaining in the pulp is approximately 50-60% of its dry weight and, for the most part, is trapped inside ligno-cellulose. The fiber content of dried cassava pulp is reported in the form of insoluble fiber. Moreover, the pulp also contains pectin, cellulose, fiber (10-15%), protein (1.5-5%), and fat (0.1-4%). Historically, it has been used in the animal feed industry, bio-gas production, and ethanol production (Sriroth, Chollakup, et al., 2000; Thailandtopiocastarch, 2010). Due to its high content of fiber, cassava pulp can be used as a fiber-rich ingredient in food products. Wandee et al. (2014) evaluated the potential of cassava pulp and pomelo peel as a source of dietary fiber in dried rice noodles. The results show that a combination of cassava pulp and pomelo peel in rice noodles at a total amount of 20% exhibited obvious increases in cooking weight and its highest total dietary fiber content was 14.4%. Thus, a purification method with mild and highly specific conditions to obtain a higher amount of dietary fiber content from cassava pulp should be of interest. Also, an alternative way to add value of cassava pulp is to be good source for dietary fiber preparation.

The aims of this study were to investigate the optimum conditions for obtaining neutral detergent fiber from cassava pulp, also its characteristic structure and physicochemical properties were evaluated.

3.3 Materials and Methods

3.3.1 Materials

Cassava pulp was collected from Sanguan Wongse Industries Co., Ltd. in the area of Nakhon Ratchasima province, Thailand. Heat-stable α-amylase (EC 3.2.1.1, Merk, Darmstadt, Germany), amyloglucosidase AMG 300L from *Aspergillus niger* (EC 3.2.1.3, Bray, Co. Wicklow, Ireland) and neutrase® (EC 3.4.24.28 from *Bacillus amyloliquefaciens*, Novozymes Co., Bagsvaerd, Denmark) were used. All chemicals used were of reagent grade.

3.3.2 Sample preparation and compositional analysis

Cassava pulp was dried at 60°C in a tray dryer (Kluaynumtaitowop, Bangkok, Thailand) overnight. Before use, the dried cassava pulp was finely ground (GmbH & Co.KG D-42781, Haan, Germany) and stored at room temperature in a vacuum-packed container.

3.3.2.1 Proximate analysis

Dried cassava pulp and dietary fiber were analyzed for moisture, ash, crude protein, fat and crude fiber contents according to the methods of AOAC (2005) as the following; moisture (AOAC 925.10), ash (AOAC 900.02A), protein (AOAC 928.08), fat (AOAC 963.15) and crude fiber (AOAC 978.10). Starch content was analyzed by Anthrone method (Hansen and Moller, 1975) with modification. Briefly, sample and standard glucose solution (1 mL) were added by 4 mL of anthrone reagent

and mixed the contents as well. The test tube was transferred to water bath for 10 min. Then, the test tube was cooled to the room temperature. The concentration of glucose in the tested sample was determined based on the standard curve of standard glucose solution within the range of concentrations was 0.2 to 1 mg/mL. Absorbance was measured using a spectrophotometer (Biochrom Libra S22 S/N 97765, UK) at a wavelength of 630 nm.

3.3.2.2 Determination of neutral detergent fiber (NDF)

The determination of neutral detergent fiber (NDF) was carried out according to the method of Van Soest, Robertson and Lewis (1991). One gram (W_0) of the sample was accurately weighed in a dried crucible. One hundred milliliter of neutral detergent solution at room temperature with 0.5 g of sodium sulfite and 50 µl of heat resistant amylase were added to the crucible. The crucible was transferred to hot extraction unit of Fibertec System 2010 apparatus (Foss Tecator, Höganäs, Sweden) and heated to boiling for 60 min (constant degree of boiling). The solution was filtered and washed with boiled distilled water until pH was neutral and then the crucible was transferred to the cold extraction unit and washed with acetone at least twice. After that, the crucible was dried at 100˚C for 5 h and transferred to desiccators and weighed after cooling (W_1). Finally, the crucible was ashed in a muffle at 550 ˚C for 2 h and transferred to desiccators and weighed after cooling (W_2). NDF was calculated from the equation below.

$$NDF\ (\%) = ((W_1 - W_2)/ W_0) \times 100$$

3.3.2.3 Determination of acid detergent fiber (ADF)

Acid detergent fibers (ADF) were analyzed according to the AOAC Method 973.18 (AOAC, 2005). One gram (W_3) of the sample was accurately weighed in a dried crucible and 100 mL of acid detergent solution were added. The crucible

was transferred to a hot extraction unit of Fibertec System 2010 apparatus (Foss Tecator, Höganäs, Sweden) and heated to boiling for 60 min (constant degree of boiling). The solution was filtered and washed with boiled distilled water until pH was neutral. The crucible was then transferred to the cold extraction unit and washed with acetone at least twice. After that, the crucible was dried at 100˚C for 5 h and transferred to desiccators and weighed after cooling (W_4). Finally, the crucible was ashed in a muffle at 550 ˚C for 2 h and transferred to desiccators and weighed after cooling (W_5). ADF was calculated from the equation below.

$$ADF\ (\%) = ((W_4 - W_5)/ W_3) \times 100$$

3.3.2.4 Determination of acid detergent lignin (ADL)

Acid detergent lignin (ADL) was analyzed followed by the AOAC Method 973.18 (AOAC, 2005). The crucible of ADF residue was placed on a plate and filled (3/4) with 72% H_2SO_4 solution. The crucible was placed for 3 h with stirring every hour and refilled with 72% H_2SO_4 solution as necessary. After that, the sample was filtered and washed with hot distilled water until pH was neutral. Then, the crucible was dried at 100˚C for 5 h and transferred to desiccators and weighed after cooling (W_6). Finally, the crucible was ashed in a muffle at 550 ˚C for 2 h and transferred to desiccators and weighed after cooling (W_7). ADL was calculated from the equation below.

$$ADL\ (\%) = ((W_6 - W_7)/ W_3) \times 100$$

The components that were determined by these tests are summarized below:

NDF = Cellulose + Hemicellulose + Lignin + Mineral Ash

ADF = Cellulose + Lignin + Mineral Ash

ADL = Lignin + Mineral Ash

Cellulose content = ADF – ADL

Hemicellulose content = NDF – ADF

3.3.3 Optimization of dietary fiber extraction

The aim of this study is the determination of the factors affecting the optimized yield of NDF. Response Surface Methodology was used to investigate the optimum points. All experimental data were statistically analysed with Design Expert® software (Version 8.0.7.1, Stat-Ease, Inc. Minneapolis, MN). The independent variables were the concentration of α-amylase, *X1* (0.1-0.3% w/v), neutrase, *X2* (0.5-1.5% v/v) and amyloglucosidase, *X3* (0.1-0.5% v/v) which were optimized using 3-factors with 3-levels (Table 3.1). The selected dependent variable was the cumulative percentage of NDF (*Y1*) because this research needs to focus on extraction process to get the highest percentage yield of insoluble dietary fiber. The Box-Behnken design requires fewer 15 runs in a 3-factor experimental design.

Cassava pulp solution was prepared at 4% (w/v) with a phosphate buffer (50 mM, pH 6) and heated for 30 min at 60°C (with gentle shaking at 5 min intervals). Heat-stable α-amylase (0.1%, 0.2% and 0.3% w/v) was added. After cooling down to room temperature, the pH was adjusted to 7.5 with sodium hydroxide solution; neutrase (0.5%. 1.0% and 1.5% v/v) was added, and the solution incubated for 30 min at 60°C with continuous shaking. After cooling down to room temperature, the pH was adjusted to 4.5 with hydrochloric acid solution, an amyloglucosidase (0.1%, 0.3% and 0.5% v/v) was added and then the solution was incubated at 60°C for another 30 min with continuous shaking. The supernatant was discarded and the pellet was washed three times with distilled water following centrifugation (Hettich, Universal 32R, DJB labcare Ltd) at 10000×g for 10 min. The residue (dietary fiber) was dried at 60°C in a

tray dryer and then finely ground. The dietary fiber powder was kept in a sealed container at 4°C until used.

Table 3.1 Codes, ranges and level of independent variables in RSM design.

Symbols	Independent variables	Coded levels		
		Low	Medium	High
X_1	α-amylase (%w/v)	0.1	0.2	0.3
X_2	neutrase (%v/v)	0.5	1.0	1.5
X_3	amyloglucosidase (%v/v)	0.1	0.3	0.5

3.3.4 Determination of hydration properties

3.3.4.1 Water holding capacity (WHC)

Water holding capacity of the dietary fiber was determined using the method of Robertson et al. (2000). A dry sample (1 g) was accurately weighed and mixed with an excess of distilled water (30 mL), then allowed to hydrate at an ambient temperature for 18 h. The supernatant was removed by being passed through a sintered glass crucible under vacuum. The hydrated residue was weighed and dried at 105 °C for 2 h. WHC is defined as follows:

$$\text{WHC (g/g)} = \frac{\text{Residue hydrated weight} - \text{Residue dry weight}}{\text{Residue dry weight}}$$

3.3.4.2 Water retention capacity (WRC)

Water retention capacity was determined following the method of Robertson et al. (2000). A dry sample (1 g) was accurately weighed and hydrated in 30 mL distilled water at room temperature. After equilibration (18 h), the sample was centrifuged at 3000×g for 20 min and the excess supernatant was removed by being

passed through a sintered glass crucible under vacuum. The hydrated residue weight was recorded and then the sample was dried at 105 °C for 2 h to obtain its dry weight. WRC is defined as the amount of water retained by the sample (g/g dry weight).

$$\text{WRC (g/g)} = \frac{\text{Residue hydrated weight} - \text{Residue dry weight}}{\text{Residue dry weight}}$$

3.3.4.3 Swelling capacity (SWC)

Swelling capacity is defined as the ratio of the volume occupied when the sample is immersed in excess of water after equilibration to the actual weight (Robertson et al., 2000). Dry samples (0.2 g) were accurately weighed and then hydrated in 10 mL of distilled water in a calibrated cylinder at room temperature. After equilibration for 18 h, the increase in the volume of the sample was recorded and expressed as mL water/g of the original dry weight of the sample.

$$\text{SWC (mL/g)} = \frac{\text{Volume occupied by sample (ml)}}{\text{Original sample dry weight (g)}}$$

3.3.5 Oil holding capacity

Oil holding capacity was determined by the method of Caprez et al. (1986) with slight modifications. A dried sample (1 g) was mixed with soybean oil in a centrifugal tube and left for 1 h at room temperature. The mixture was then centrifuged at 3000×g for 30 min and the supernatant was decanted and the pellet was recovered by filtration through a sintered glass crucible under vacuum. The OHC is expressed as follows:

$$\text{OHC} = \frac{\text{pellet weight} - \text{dry weight}}{\text{dry weight}}$$

3.3.6 Bulk density

Bulk density (BD) was determined using a graduated cylinder (10 mL),

after being weighed previously, which was filled with the sample up to 10 mL by constant tapping, until there was no further change in volume. The content was weighed and the bulk density of the sample was calculated from the differences in weight, the bulk density of sample was expressed in grams per milliliter (Chau et al., 2003).

3.3.7 Cation exchange capacity

Cation exchange capacity (CEC) was determined according to Jiménez et al. (2000) with slight modifications. Dry samples (500 mg) were suspended in 25 mL of 2 N hydrochloric acid. After continuous stirring for 24 h, the suspension was centrifuged for 15 min at 12000×g. The residue was washed extensively with distilled water to remove any acid left on the samples until the pH of the supernatant was above 4. The acidic residue was suspended in 25 mL of 0.3 M sodium chloride together with a blank with distilled water. After stirring and centrifugation as above, the supernatant was titrated with 0.01 N sodium hydroxide. The CEC is expressed as the number of milliequivalents per gram dry weight.

3.3.8 Monosaccharide analysis

A monosaccharide analysis was also carried out. The sample was first hydrolyzed by 72% H_2SO_4 at 30°C for 1 h. The residue was hydrolyzed with 1 M H_2SO_4 at 100 ºC for 4 h, to give constituent monosaccharides. The monosaccharide hydrolysate was cooled, diluted with water, and filtered through a 0.45 µm filter before injection (Wood, Weisz and Blackwell, 1994). A volume of 25 µl was injected into the High Performance Anion Exchange Chromatography (HPAEC) system (DIONEX) (Archemica international, Co. Ltd., Sunnyvale, CA, USA) and differentiated by a CaboPac PA1 column connected with pulsed amperometric

detection (PAD). Gradient elution was carried out using 250 mM NaOH and deionized water at a flow rate of 0.2 mL/min, with 20/100 to 0/100 of a linear gradient. The monosaccharide content was quantitatively analyzed by comparison with known individual standard curves (rhamnose, arabinose, galactose, glucose, xylose and mannose) at concentrations varying from 10 to 100 ppm.

3.3.9 Fourier transform infrared spectroscopy (FTIR) analysis

Dried cassava pulp, dietary fiber and commercial cellulose powder were dried and stored in desiccators prior to a Fourier transform infrared spectroscopy (FTIR) analysis. FTIR spectra were recorded using a golden-gate diamond single reflectance ATR on a Bruker T27/Hyp 2000 FTIR spectrophotometer (Germany). The spectra were recorded in the transmittance mode from 4000 to 400 cm^{-1} (mid-infrared region) at a resolution of 4 cm^{-1} and 64 scans were collected. At least triplicate spectra readings for each sample were obtained.

3.3.10 Scanning electron microscope

Dried cassava pulp and dietary fiber powder were spread on a double sided conducting adhesive tape, pasted on to a metallic stub, coated with gold in a sputter coating unit for 8 mins and observed in a JEOL JSM-6010LV electron microscope (JEOL ltd., Tokyo, Japan).

3.3.11 Statistical analysis

All experiments were performed in triplicate and the mean values (on a dry basis) with standard deviations are reported. The experimental data were analyzed using analysis of variance (ANOVA). SPSS® software (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical calculations.

3.4 Results and Discussion

3.4.1 Chemical compositions of dried cassava pulp

The chemical compositions of dried cassava pulp powder is shown in Table 3.2. The composition of cassava pulp containing crude fiber, carbohydrate, starch, NDF, ADF, ADL, cellulose and hemicellulose was: 17.23%, 70.15%, 58.11%, 31.40%, 25.08%, 4.16%, 20.92%, and 6.32%, respectively. It was clear that the main constituents of cassava pulp was carbohydrates which is starch. Cassava pulp still contained a high starch content of more than 50% because the starch granules which are called “bound starch” are bound inside a complex structure of the pulp which still remains and is difficult to separate after the rasping step (Saengchan et al., 2015). Many studies report that cassava pulp is composed mainly of starch followed by cellulose, hemicellulose and lignin, respectively (Ali et al., 2011; Kosoom et al., 2009; Rattanachomsri et al., 2009; Suksombat, Lounglawan and Noosen, 2007). Kosoom et al. (2009) reported that cassava pulp from many cassava starch manufacturing plants in Thailand consist of average crude fiber, NDF, ADF, lignin and starch at 13.99, 40.62, 27.65, 3.60 and 50.19%, respectively. Sriroth, Piyachomkwan et al. (2000) revealed that the major constituents of cassava pulp were cellulose, hemicellulose, lignin and protein matrix, which form lignocellulosic material. Pandey et al. (2000) showed that cassava pulp is composed of approximately 50–70% starch and 20–30% fiber, on a dry weight basis; these fibers contain mainly cellulose and other non-starch polysaccharides. On the contrary, the pulp contains small amounts of protein, ash, lipid and lignin. The results are similar to those observed by Sriroth, Piyachomkwan et al. (2000), who reported that cassava pulp is commonly found with less amounts of protein, pectin and lignin. The differences in the chemical composition of cassava pulp

depend on the source, species and storage conditions (Laohapatthanalert, 2016).

The definition of NDF indicates that cassava pulp fiber has a high content of insoluble dietary fiber which consists of cellulose, hemicellulose and lignin. In many agricultural byproducts of fruits and vegetables, insoluble fiber is also revealed to be the major fiber fractions (Chau et al., 2003; Chau and Huang, 2004; Figuerola et al., 2005; Grigelmo-Miguel et al., 1999). Moreover, the cellulose content of cassava pulp fiber is higher than those shown by other food industry residues, such as malt bagasse, oat hull, rice hull and fibrous residue of banana pseudo-stems, but is lower in hemicellulose and lignin content (Jacometti et al., 2015). The beneficial technological functionality and physiological effects of insoluble fibers are characterized by their porosity, low density and ability to increase fecal bulk and reduce intestinal transit time (Elleuch et al., 2011). While the physiological effects of soluble fibers reduce serum LDL cholesterol levels and improve glucose metabolism and insulin response (Glore et al., 1994). However, some recent research illustrates that insoluble fibers isolated from some fruits, vegetables, and pomace can also effectively decrease serum cholesterol and triglyceride (Chau, Huang and Lin, 2004; ErkkilaÄ et al., 1999; Knopp et al., 1999; Rosamond, 2002). Thus, cassava pulp can possibly be used in the preparation of dietary fiber to lower blood cholesterol levels.

Because of the high starch content of cassava pulp, the removal of starch is necessary for dietary fiber preparation and it is essential to remove protein. An enzymatic hydrolysis method was chosen to remove these constituents in this case since it has many advantages, as it is a highly specific method and it has less effect on the chemical structure of the product than chemical hydrolysis and it is also hydrolyzed under mild conditions which are safe for further application in food products (Adler-Nissen, 1986; Madmarn and Prasertsan, 2002). Therefore, α-amylase

and amyloglucosidase are used to digest starch, while neutrase is used for protein digestion.

Table 3.2 Chemical compositions of dried cassava pulp powder.

Components	Content (% dry weight basis)
Crude protein	2.02 ± 0.19
Fat	0.21 ± 0.08
Moisture	6.63 ± 0.11
Ash	3.76 ± 0.05
Crude fiber	17.23 ± 0.13
Carbohydrate	70.15
Starch	58.11 ± 0.06
Neutral detergent fiber (NDF)	31.40 ± 0.58
Acid detergent fiber (ADF)	25.08 ± 0.17
Acid detergent lignin (ADL)	4.16 ± 0.10
Cellulose[a]	20.92
Hemicellulose[b]	6.30

[a]ADF-ADL, [b]NDF-ADF

3.4.2 Effect of enzyme concentration on NDF extraction

The effect of three independent parameters on the percentage yield of NDF from cassava pulp was investigated and the results were shown in Table 3.3. The condition that gave the highest NDF percentage yield was at 0.1% (w/v) with 1% (v/v) and 0.1% (v/v) of α-amylase, neutrase and amyloglucosidase concentration, respectively.

Table 3.3 Effects of extraction conditions on percentage NDF (mean ± SD) of dietary fiber from cassava pulp.

Trt.*	Independent variables			NDF (g/100 g dry basis)
	α-amylase (%w/v) X_1	Neutrase (%v/v) X_2	Amyloglucosidase (%v/v) X_3	
1	0.2	0.5	0.5	58.53 ± 0.25^{fg}
2	0.3	0.5	0.3	54.22 ± 1.49^{g}
3	0.3	1.0	0.1	69.49 ± 1.96^{bc}
4	0.3	1.0	0.5	69.44 ± 2.26^{bc}
5	0.2	1.5	0.5	65.15 ± 2.62^{cde}
6	0.2	0.5	0.1	68.45 ± 1.10^{bcd}
7	0.2	1.0	0.3	57.50 ± 1.95^{fg}
8	0.2	1.5	0.1	63.06 ± 2.01^{def}
9	0.3	1.5	0.3	61.39 ± 1.51^{ef}
10	0.2	1.0	0.3	64.74 ± 1.51^{cde}
11	0.1	0.5	0.3	62.19 ± 4.95^{ef}
12	0.2	1.0	0.3	60.60 ± 4.44^{ef}
13	0.1	1.0	0.5	70.95 ± 0.42^{b}
14	0.1	1.5	0.3	70.86 ± 0.59^{b}
15	0.1	1.0	0.1	79.03 ± 0.51^{a}

[a,b] Data in the same column with different superscripts are significantly different (p<0.05).

* Experiments were conducted in a random order.

The statistical analysis indicated that the second-order polynomial model correlated with three variables in this study which were obtained in Eq. (1) below:

$$NDF = 100.67969 - 257.69167X_1 + 26.25667X_2 - 160.17500X_3 - 7.50000X_1X_2 + 108.50000X_1X_3 + 30.02500X_2X_3 + 490.54167X_1^2 - 14.74833X_2^2 + 163.44792X_3^2 \quad (1)$$

ANOVA was used to analyze the model for significance and appropriateness; a statistical summary is shown in Table 3.4. The statistical significance of the model

was checked by the F-test and F-value of 59.79 of the model implies that the model is statistically significant ($p< 0.05$). The results show that the F value of 5.22 of the model has a low probability *P* value ($p<0.05$) which implies the model is significant. The coefficient of determination (R^2) is used to analyze how differences in one variable can be explained by a difference in a second variable. For the model fitted, the coefficient of determination (R^2) was 0.9038, indicating that 9.62% of the total variations was not explained by the model. The regression coefficient of the experimental model suggested that the model is a good fit for NDF extraction. The model validation was carried out which the correlation between experimental design and the predictive model are non-significant difference ($p>0.05$). The value of the adjusted determination coefficient (adjusted $R^2 = 0.7305$) also confirms that the model is significant. The statistical analysis indicates that the proposed model is adequate, since there is no significant lack of fit. Concentration of α-amylase is the most effective parameter to obtain the highest NDF yield ($p<0.05$). Oonsivilai et al. (2011) studied on enzymatic digestion of dietary fiber from cassava pulp by using three factors: heating temperature (°C) after adding α-amylase, α-amylase concentration (%v/v) and amyloglucosidase concentration (%v/v). The results showed that in the optimum condition, the NDF content of dietary fiber is 13.96%.

The effect of concentration of α-amylase, neutrase and amyloglucosidase on the percentage of NDF of cassava pulp is shown in Figures 3.1A-C, respectively. The concentration of α-amylase is the major factor affecting the yield of extraction.

Figure 3.1A presents the effect of concentration of α-amylase and neutrase on the NDF yields at a concentration of 0.1% amyloglucosidase. The interaction between the concentration of α-amylase and neutrase results in higher NDF yields at low α-

amylase and medium neutrase concentrations. Figure 3.1B displays the effect of the concentration of α-amylase and amyloglucosidase on the NDF yields at a concentration of 1% neutrase. The higher NDF yields were obtained at low concentrations of α-amylase and amyloglucosidase. Figure 3.1C reveals the effect of concentrations of neutrase and amyloglucosidase on NDF yields at a concentration of 0.1% α-amylase. A higher yield was obtained at a medium concentration of neutrase and a low concentration of α-amylase and amyloglucosidase.

Table 3.4 Analysis of variance for the fitted quadratic polynomial model of extraction of cassava pulp.

Source	SS	DF	MS	F-value	Prob > F
Model	538.08	9	59.79	5.22	0.0418
A-amylase	106.14	1	106.14	9.26	0.0286
B-neutrase	36.42	1	36.42	3.18	0.1347
C-amyloglucosidase	34.49	1	34.49	3.01	0.1433
AB	0.56	1	0.56	0.049	0.8334
AC	18.84	1	18.84	1.64	0.2561
BC	36.06	1	36.06	3.15	0.1363
A^2	88.85	1	88.85	7.75	0.0387
B^2	50.20	1	50.20	4.38	0.0906
C^2	157.83	1	157.83	13.77	0.0138
Residual	57.30	5	11.46		
Lack of fit	30.91	3	10.30	0.78	0.6038
Pure Error	26.39	2	13.19		
Cor Total	595.38	14			
	R^2 = 0.9038	R_{adj} = 0.7305	CV = 5.20		

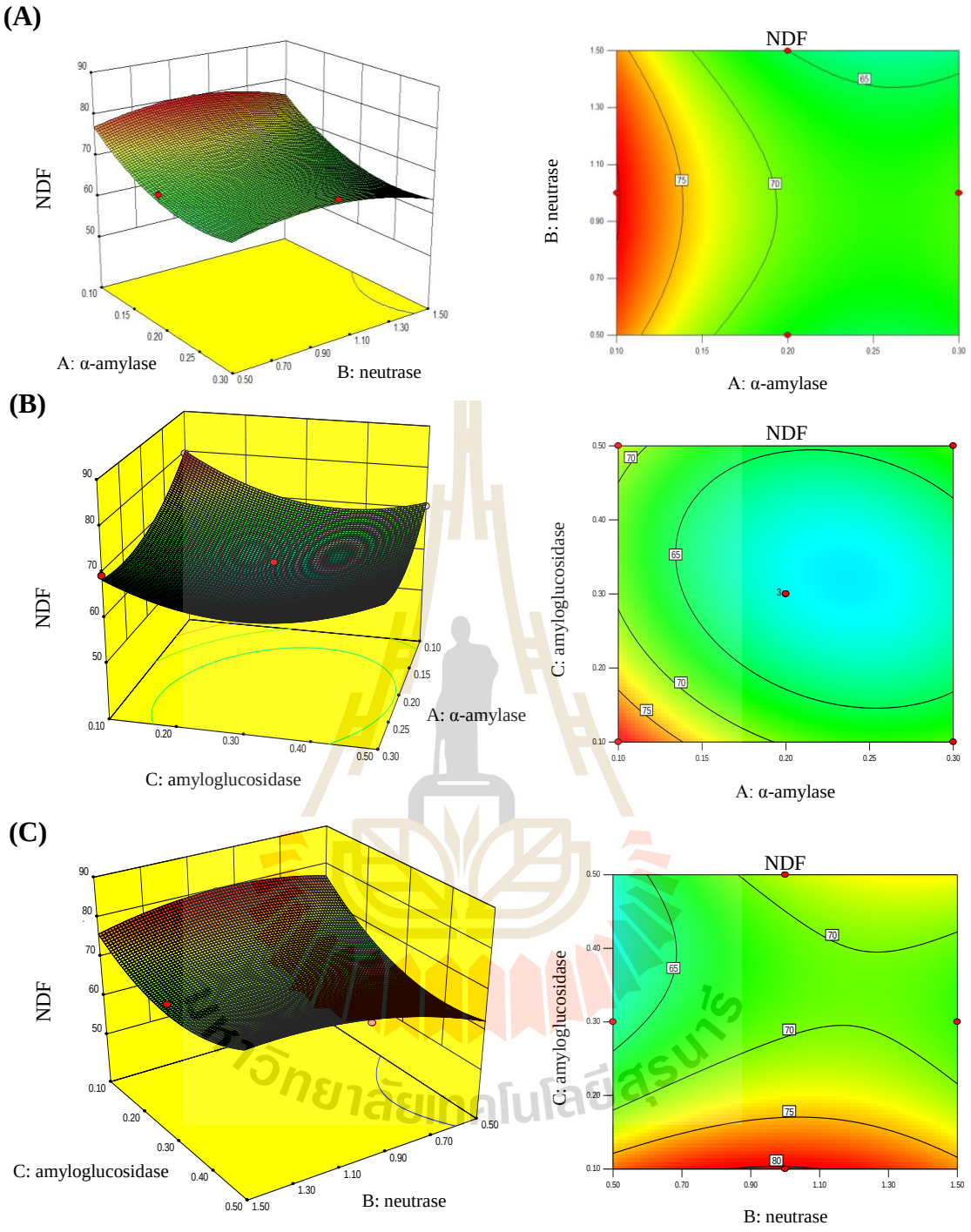

Figure 3.1 Response surface and contour plots of the effect of extraction conditions on NDF yield. (A) The effect of concentration of α-amylase and neutrase on the NDF at concentration of 0.1% amyloglucosidase. (B) The effect of concentration of α-amylase and amyloglucosidase on the NDF at concentration of 1% neutrase. (C) The effect of concentration of neutrase and amyloglucosidase on the NDF at concentration of 0.1% α-amylase.

After enzymatic hydrolysis of cassava pulp, the results clearly showed an increase in content of NDF range from ~31% to ~79%. The removal of starch for dietary fiber production was carried out by using α-amylase and amyloglucosidase. This process starts from cassava pulp which is subjected to high temperature pre-gelatinization by using a thermostable α-amylase to release the trapped starch granules, followed by saccharification with amyloglucosidase which cleaves glucose from α-1,4-linked and α-1,6-linked polysaccharides (Laohapatthanalert, 2016; Pandey et al., 2000). Lacourse et al. (1994) revealed that when cassava fiber is treated by using enzymes which depolymerize the starch, it results in a depolymerized starch residue which can be easily eliminated from the fiber by washing with water. This destarching process of the enzymes can release trapped starch granules in cassava pulp from the fibrous cell wall structure (Chaikaew et al., 2012), thus the NDF content increases markedly. Neutrase is a bacterial protease which breaks down the peptide bonds in protein foods to release amino acids (Barrett, Woessner and Rawlings, 2012). Generally, amino acids are soluble in water and insoluble in non-polar organic solvents such as hydrocarbons (Clark, 2016). Therefore, they are discarded from the fiber by washing in water. We can conclude that this enzymatic hydrolysis by α-amylase, nuetrase and amyloglucosidase could be appropriate for dietary fiber preparation.

3.4.3 Chemical compositions of dietary fiber

A proximate analysis of dietary fiber is shown in Table 3.5. The composition of the dietary fiber containing crude fiber, carbohydrate, starch, NDF, ADF, ADL, cellulose and hemicellulose was: 40.24%, 48.46%, 8.50%, 79.03%, 70.14%, 58.55, and 8.89%, respectively. The major constituent of dietary fiber was

crude fiber. The dietary fiber contained high cellulose in an insoluble form. It was clear that the main constituent of dietary fiber is fiber material. Moreover, dietary fiber showed a higher content of NDF, ADF, ADL, cellulose and hemicellulose when it was passed through the extraction process due to the hydrolysis properties of enzymes used. To purify starch, thermostable α-amylase is subjected to a high temperature in the cassava pulp solution to release the trapped starch granules. This enzyme hydrolyzes α-1,4 glycosidic bonds of starch by random cleavage, but does not hydrolyze α-1,6 glycosidic bonds of amylopectin in the starch granules. Amyloglucosidase is used to hydrolyze α-1,4 glycosidic bonds from the non-reducing end of starch. This enzyme can hydrolyze α-1,6 glycosidic bonds of amylopectin at a slow rate (Pandey et al., 2000). While the protein in the raw material is eliminated by neutrase. However, protein content in dietary fiber still high, it may be due to some proteins will be trapped in fiber structure, resulting still remains in dietary fiber. Moreover, ash content was increased, it may be due to partly come from phosphate buffer and NaOH which using in digestion process. Lacourse et al. (1994) reveal that removing starch content from tapioca fiber by enzymatic treatment, especially when carried out with an alpha-amylase may decrease starch from about 60% to about 15% and preferably 5%, by weight. With regard to starch content, the results show that starch still remains in the dietary fiber which may be due to the starch granules which are bound inside the pulp complex structure and which are difficult to separate (Saengchan et al., 2015). Thus, some pretreatment methods before the use of enzymatic hydrolysis provide an interesting topic for further study. Another reason was due to Anthrone method for starch determination. Based on the principle of this method, Anthrone reagent react with dextrins, monosaccharides, disaccharides,

polysaccharides, starch, gums and glycosides. Therefore, the measurement of starch content in dietary fiber might over estimation.

Table 3.5 Chemical compositions of dietary fiber.

Components	Content (% dry weight basis)
Crude protein	1.01 ± 0.10
Fat	0.25 ± 0.06
Moisture	5.52 ± 0.09
Ash	4.52 ± 0.04
Crude fiber	40.24 ± 2.22
Carbohydrate	48.46
Starch	8.50 ± 0.31
Neutral detergent fiber (NDF)	79.03 ± 0.51
Acid detergent fiber (ADF)	70.14 ± 0.40
Acid detergent lignin (ADL)	11.59 ± 0.01
Cellulose[a]	58.55
Hemicellulose[b]	8.89

[a]ADF-ADL, [b]NDF-ADF

Cassava dietary fiber contains a high amount of insoluble fiber which is composed of cellulose, hemicellulose and lignin. Recently, it has also been suggested that insoluble fiber is related to lower serum cholesterol levels. The ability of cholesterol and bile acid absorption and the ability to enhance excretion via feces are important properties of water-insoluble fibers leading to lower cholesterol

concentration. Normally, adsorption of bile acids by dietary fibers depends on the composition of the fibers, the chemistry of the sterol, pH and osmolality of the surrounding medium. Lignin (a water-insoluble fiber) is the most potent bile acid adsorbent (Prosky and DeVries, 1992) and its binding capacity is influenced by molecular weight, pH, and the presence of methoxyl and carbonyl groups on the lignin molecules where adsorption is greatest at low pH (Vahouny and Cassidy, 1985). In addition, hemicelluloses can prevent cholesterol absorption by directly binding cholesterol in the intestine. Moreover, microflora in the gut can digest hemicelluloses by increasing the number of beneficial bacteria and creating short-chain fatty acids which the colon cells use as fuel and which decrease cholesterol (Jalili, Medeiros and Wildman, 2007; Mudgil and Barak, 2013; Mudgil, Barak and Khatkar, 2012).

3.4.4 Monosaccharide profile

The sugar profile of cassava pulp and dietary fiber showed that glucose is the most abundant constituent (Table 3.6). In dietary fiber, glucose is present in the highest amounts followed by galactose, rhamnose, xylose, arabinose and mannose, respectively. Chaikaew et al. (2012) indicated that the main monosaccharide of cassava pulp is glucose followed by galacturonic acid, xylose, galactose, arabinose, mannose and rhamnose. Rattanachomsri et al. (2009) also reported that monosaccharides of cassava pulp is composed mainly of glucose, followed by galacturonic acid, xylose, galactose, arabinose, mannose, and rhamnose. Kosugi et al. (2009) showed that the monosaccharide analysis of non-starch polysaccharides in cassava pulp is composed of glucan, xylan, arabinan, galactan and mannan. Glucans are the most abundant, such as cellulose, which are the major polysaccharides. Similar to cassava pulp, starch and cellulose are the main constituents which are the main

chain of these materials, namely, α-(1,4) glucose and β-(1,4) glucose, respectively (Mongeau and Brooks, 2016).

The results reveal that the glucose content of dietary fiber clearly decreases when compared with the raw material of cassava pulp. This might be due to the reduction of starch content and, secondly, to the effects of enzyme activity. Other increases in monosaccharides are correlated to increases of hemicellulose and lignin content (Table 3.5) of which the main chain of hemicellulose is arabinose and xylose (Mongeau et al., 2016).

Table 3.6 Monosaccharide compositions (relative %) of dried cassava pulp powder and dietary fiber.

Monosaccharides	Cassava pulp (%)	Dietary fiber (%)
Galactose	6.95 ± 0.02	14.90 ± 0.10
Glucose	76.66 ± 0.40	48.85 ± 0.38
Xylose	2.34 ± 0.13	10.56 ± 0.06
Mannose	0.64 ± 0.11	2.81 ± 0.12
Rhamnose	7.92 ± 0.08	12.89 ± 0.16
Arabinose	5.49 ± 0.07	9.99 ± 0.12

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3.4.5 Functional properties of dietary fiber

The functional properties of dietary fiber compared with cellulose which are the most used for food fiber as listed in Table 3.7. The results show the values for swelling, water retention capacity and water holding capacity of dietary fiber are 4.82 mL/g, 8.36 g/g dry weight and 8.17g/g, respectively. The bulk density and oil holding capacity of dietary fiber are 0.28 g/mL and 3.97 g/g, respectively. The bulk density of dietary fiber is lower than cellulose which makes cassava dietary fiber less dense. This

means that this material shows high porosity (Benítez et al., 2011). The results obtained are similar to those of onion by-products, but higher than *Polygonatum odoratum*, and lower than passion fruit seed, malt bagasse, oat hull, rice hull and fibrous residue from banana pseudo-stem (Benítez et al., 2011; CF Chau et al., 2004; Jacometti et al., 2015; Lan et al., 2012). Bulk density depends on the structural characteristics of each product, the particle size and their distribution, and it is related with other physicochemical properties (Benítez et al., 2011).

The hydration properties of dietary fiber refer to water holding capacity (WHC), swelling capacity (SWC) and water retention capacity (WRC). The hydration properties of dietary fibers are correlated to the chemical structure of the component polysaccharides, and other factors such as particle size, porosity, ionic form, pH, temperature, ionic strength, type of ions in solution and stresses upon fibers. The ability of dietary fibers to hold water is strongly related to the source of the dietary fiber (Elleuch et al., 2011). Furthermore, hydration properties correlate with both physiological and technological aspects and can influence the incorporation of fiber-enriched ingredients into food products (Femenia et al., 1999). WHC represents the volume of a hydrated sample under centrifugal force, while SWC represents the volume of hydrated sample under gravity forces (López et al., 1996). Dietary fibers with high WHC can be used as functional ingredients to avoid syneresis and they modify the viscosity and texture of some formulated foods (Grigelmo-Miguel et al., 1999). The results obtained for WHC, SWC and WRC were higher than cellulose. Moreover, the WHC of cassava fiber was higher than that of onion by-product, malt bagasse, oat hull, rice hull and fibrous residue from banana pseudo-stems, passion fruit seeds, but lower than *Polygonatum odoratum*, the peel of citrus fruit and peach pulp and peel (Benítez et al., 2011; Chau et al., 2003; CF Chau et al., 2004; de Escalada Pla

et al., 2012; Jacometti et al., 2015; Lan et al., 2012). Insoluble dietary fibers which have a high WHC can increase faecal bulk and reduce the gastrointestinal transit time, which may be linked to the prevention and treatment of different intestinal disorders, including constipation, diverticulitis, haemorrhoids and other bowel conditions (Goñi and Martin-Carrón, 1998). Regarding swelling capacity (SWC), the result obtained was similar to that for malt bagasse, oat hull, rice hull, fibrous residue from banana pseudo-stem, pea and chickpeas (Jacometti et al., 2015; Tosh and Yada, 2010). The main factors effecting SWC and WHC are particle size, polysaccharide composition and the intermolecular organization of plant cell walls (López et al., 1996; Serena and Knudsen, 2007). WRC is defined as the quantity of water that remains bound to the hydrated fiber when subjected to an external force such as pressure of centrifugation (Ma and Mu, 2016). The WRC obtained was higher than that of deoiled cumin, grapefruits, citrus fruits, apples and bananas (Figuerola et al., 2005; Ma et al., 2016).

Table 3.7 Functional properties of dietary fiber.

Functional properties	Dietary fiber	Cellulose
Water holding capacity (g/g dry weight)	8.17 ± 0.40	4.92 ± 0.33
Water retention capacity (g/g dry weight)	8.36 ± 0.20	5.95 ± 0.49
Swelling capacity (mL/g)	4.82 ± 0.15	1.13 ± 0.15
Bulk density (g/mL)	0.28 ± 0.00	0.45 ± 0.04
Oil holding capacity (g/g)	3.97 ± 0.14	2.66 ± 0.09
Cation exchange capacity (meq g^{-1})	0.47 ± 0.01	0.03 ± 0.00

The results obtained for oil holding capacity (OHC) were similar to that of maca residue, although higher than that for peel and pulp of peach, soybean DF and some orange DF products (Chen et al., 2015; de Escalada Pla et al., 2012; Grigelmo-Miguel et al., 1999). The different in OHC among various DF might be attributed to their chemical and physical differences (Chau et al., 2003). The above results show high levels of WHC, SWC, WRC and OHC of dietary fiber prepared from cassava pulp which have potential use as food ingredients to reduce caloric levels in the food industry.

Cation exchange capacity (CEC) refers to ability of plants to attract and bind hydrogen ions which have a positive charge. Cellulose has a relatively low cation exchange capacity while pectin has a high cation exchange capacity. CEC partly depend on the presence of uronic acid in the nonesterified form. The method to prepare fiber material may reduce the number of nonesterified carboxyl groups and the apparent cation exchange capacity. Comparison of cation exchange capacity from different sources of fiber is difficult, except it is expressed per edible portion (McBurney et al., 1983; Mongeau and Brooks, 2016). From this property, dietary fiber can be used as functional food to help prevent several diseases. The CEC of dietary fiber was higher than that of cellulose (Table 3.7). Other dietary fiber products has different CEC values and the extraction methods could effect this functional property. From the result of Lan et al. (2012) who studied different extraction process to prepare dietary fiber from *Polygonatum odoratum*, it was found that drying in sunshine had a higher CEC than steam processing. With regard to the study of Benítez et al. (2011), their results revealed that the sterilization method produced a decrease in CEC with a reduction range of 41% to 50 % depending on the by-product. The stronger ion binding effect of DF is related to uronic acid content (CF Chau et al., 2004; Gordon,

1989). The cation-exchange capacity of dietary fiber have a great impact on their physiological functions. According to Furda (1990), the fibers with higher cation-exchange capacity can entrap, destabilize and break down emulsion of lipid, subsequently decreasing the dispersion and absorption of cholesterol and lipids in the small intestine.

Some authors illustrate that the technological properties of dietary fiber products (water holding capacity, swelling capacity, water retention capacity and oil holding capacity) affect their physiological functionality (Elleuch et al., 2011; Jenkins et al., 2004). In addition, some properties such as water holding capacity, swelling capacity, and oil holding capacity are significant characteristics of fiber preparations as food additives. These characteristics can be adjusted by mechanical, chemical, and thermal processes (Elleuch et al., 2011).

3.4.6 Fourier transform infrared spectroscopy (FTIR) studies

The FTIR spectrum of cassava pulp, dietary fiber and commercial cellulose is shown in Figure 3.2. The FTIR spectrum of carbohydrates is used for the identification of its chemical structure. Normally, the spectrum at wave numbers between 1200 and 950 cm^{-1} is called the molecular fingerprint of which the position and intensity of the bands are specific for each major chemical group in polysaccharides (Černá et al., 2003). The FTIR spectrum of dietary fiber exhibits similarities to the absorption pattern for the raw material. When compared with cellulose, the dietary fiber spectrum is more similar to cellulose than to cassava pulp, confirming the preliminary result from the chemical composition analysis (Table 3.5) that the most important component in dietary fiber is cellulose which is insoluble. Peaks at 895 cm^{-1} are indicative of stretching vibrations of β-glycosidic linkages in polysaccharides (Ma et al., 2016). The sharp peak appearing at around 1028, 1020 and

1003 cm^{-1} is indicative of the stretching vibration of pyranose (Ying, Han and Li, 2011). The band at 1240 cm^{-1} of dietary fiber indicates the presence of acetyl group substitution of some of the –OH groups present (Mathlouthi and Koenig, 1986). The bands at 1370 cm^{-1} point to ring breathing with C–H stretching. Both cassava pulp and dietary fiber are composed of protein which usually has specific absorption bands in the 1700-1500 cm^{-1} region. The carbonyl (C=O) stretching was at 1735 and 1605 cm^{-1}. A shoulder peak at 1735 and 1730 cm^{-1} was found in both the dietary fiber and the cassava pulp, but it was not found in cellulose powder. This peak could indicate hemicellulose (Himmelsbach, Khalili and Akin, 2002). The bands at 3000-2800 cm^{-1} were indeed characteristic of C-H vibrations from some methylene groups of polysaccharides (Ma et al., 2016) and can be associated with the ring hydrogen atoms in lignocellulosic components such as cellulose, hemicellulose and lignin (Ibrahim, Osman and Mahmoud, 2011). There was an intense peak at 3331 and 3317 cm^{-1} which

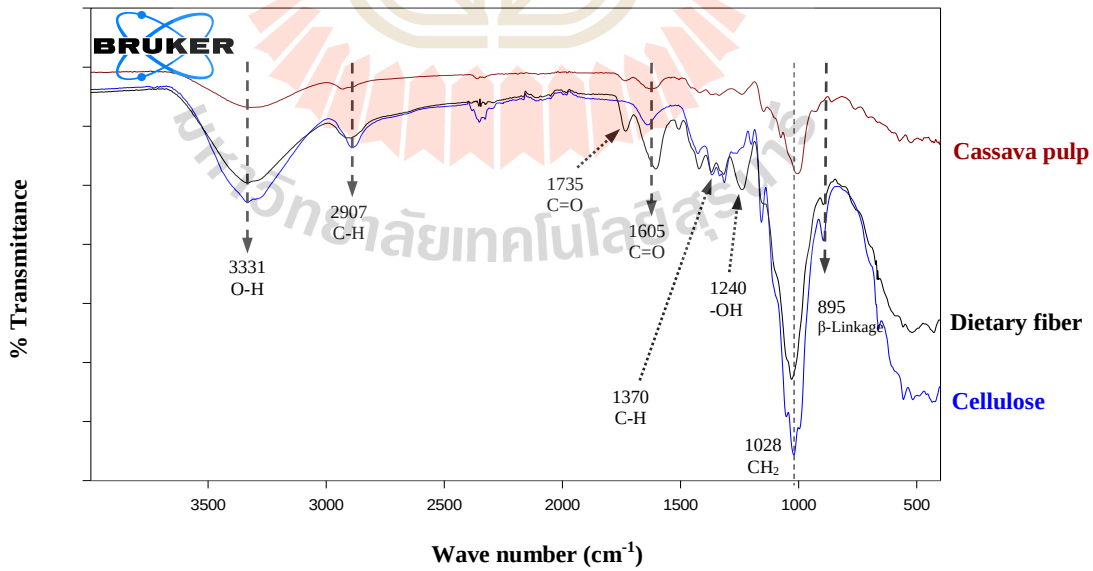

Figure 3.2 Fourier transform infrared spectrum of cassava pulp, dietary fiber and cellulose.

can be attributed to O–H stretching of the hydrogen bound to the hydroxyl groups originating mainly from cellulose, hemicellulose and lignin (Jacometti et al., 2015; Ma et al., 2016).

3.4.7 Scanning electron microscopy study

From the SEM results, the pulp shows huge starch granules embedded well inside the matrix which is indicative of starch content as mentioned above (Figure 3.3a,b). This result correlates to the chemical composition of the pulp as in Table 3.2 which shows a high content level of starch. Sriroth, Chollakup, et al. (2000) report that cassava pulp or fiber acts as a fibrous network holding the starch granules together. For the extraction steps of starch factories, the fibrous matrix of pulp is incompletely stripped and some starch granules still remain bound inside, resulting in a loss of starch in the cassava pulp. Normally, starch might be lost with pulps of up to 10% dry basis of starch input (Saengchan et al., 2015).

With regard to dietary fiber, after hydrolysis by α-amylase and amyloglucosidase enzyme, the SEM results show that there are no starch granules embedded inside the fiber matrix (Figure 3.3c, d), although starch still remains at about 8.50% (Table 3.5), it might be due to the over estimation of starch determination method (Anthrone method). However, this dietary fiber produced from cassava pulp shows higher purification from starch and its fiber structure seems to have high porosity. Dietary fiber prepared from cassava pulp tends to be decontaminated by starch granules. Sowbhagya et al. (2007) extracted dietary fiber from spent residue cumin. They found that the fiber matrix appeared to have a typical “honeycomb” structure, which was almost void of starch granules. Ma et al. (2016) showed that enzymatic

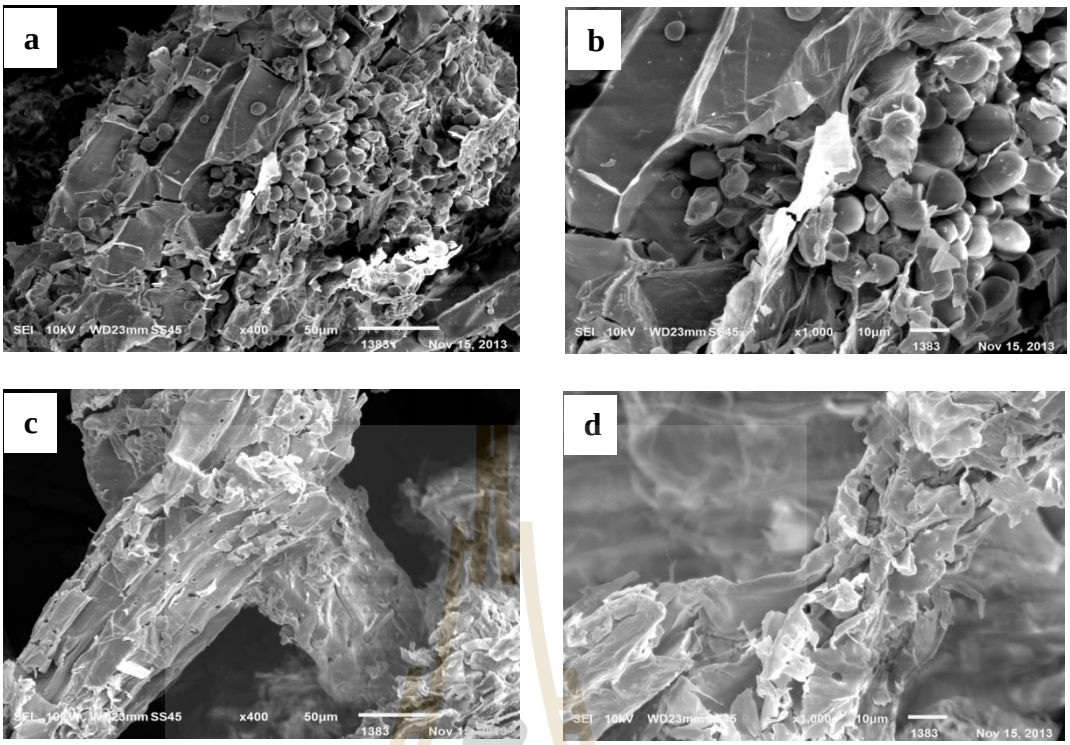

Figure 3.3 Scanning electron micrographs of cassava pulp (a&b) and dietary fiber (c&d) at 400 and 1000x.

hydrolysis of dietary fiber production from deoiled cumin had a characteristic honeycomb structure with a greater number of cracks and holes and yet still retains the microstructure better than the alkali process.

3.5 Conclusions

Cassava pulp could be a new source of dietary fiber production by enzymatic extraction at the optimal conditions of 0.1% w/v, 1% v/v and 0.1% v/v for concentration of α-amylase, neutrase and amyloglucosidase enzymes, respectively. In addition, dietary fiber prepared from cassava pulp contained 79.03% and a high content of cellulose. The physical properties of hydration of dietary fiber such as

swelling, water retention capacity and water holding capacity showed values of 4.82 mL/g, 8.36 g/g dry weight and 8.17g/g, respectively. Finally, there was no starch granule embedded inside the fiber matrix that suggests a high porosity of the fiber structure. The application of dietary fiber in food products should provide an interesting topic for future study.

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CHAPTER IV

IN-VITRO BINDING CAPACITY FOR LARD, CHOLESTEROL, BILE ACIDS AND ASSESSMENT OF PREBIOTIC POTENTIAL OF DIETARY FIBER FROM CASSAVA PULP

4.1 Abstract

Water-insoluble dietary fiber from cassava pulp (CDF) was evaluated for its functional properties such as binding capacities for lard, cholesterol and bile acids: cholic acid (CA); deoxycholic acid (DCA); and taurocholic acid (TA). The digestive stability of CDF is 87.98%. CDF showed a higher binding capacity for lard, cholesterol and bile acids compared to cellulose. Binding with CA, DCA and TA were 38.50%, 42.71% and 40.84%, respectively. Prebiotic activity of five *Lactobacillus* strains: 3C2-10, 21C2-10, 21C2-12 and OC4-4 which isolation from cassava pulp and *Lactobacillus plantarum* TISTR 1465 were investigated. The CDF showed prebiotic activity for *Lactobacillus plantarum* TISTR 1465 was higher than cellulose, but lower than some soluble dietary fibers (inulin, lactulose and fructooligosaccharide). Meanwhile, there was no prebiotic activity for the rest of *Lactobacillus* strains tested.

4.2 Introduction

Cassava pulp is an important by-product from cassava starch production, which is a major industry in Nakhon Ratchasima province and also Thailand. During year

2015 to 2016, more than 50% was planted in the Northeast which the main province is Nakhon Ratchasima, followed by the Central plain (33%) and the North of Thailand (15%) (Thaitapiocastarchassociation, 2016). There are many cassava starch factories in this province. The composition of the pulp suitable to be dietary fiber source, because cassava pulp remain high content of ligno-cellulose that refer to cellulose, hemicellulose and lignin. The cassava pulp also contains pectin, cellulose, and fiber at approximately 10-15%, protein 1.4-5%, fat 0.1-5%. Other components are minerals: i.e., Fe^{2+} of 155 ppm, Mn^{2+} of 40 ppm, Mg^{2+} of 1100 ppm, Cu^{2+} of 4 ppm, and Zn^{2+} of 21 ppm per kg-dry pulp (Thailandtopiocastarch, 2010).

We have already known that dietary fibers act as a potential "functional food". The consumption of foods containing high fiber has many advantages to the human body including reduces the incidence of cardiovascular diseases (CVDs) which is the most common causes of death and disability worldwide, by reducing the risk of type-2 diabetes, body weight, and serum cholesterol levels, and improve in large bowel function (Gibson and Roberfroid, 1995; Mudgil and Barak, 2013; Viuda-Martos et al., 2010). Furthermore, dietary fiber can bind organic substances such as bile acids, toxic compounds and cholesterol in the small intestine, and increase the fecal excretion of these entities (Mudgil and Barak, 2013; Zhang, Huang and Ou, 2011). Normally, bile acids are derived from cholesterol and essential for the lipids digestion in the small intestine. Dietary fiber decreases plasma cholesterol through its ability to bind bile acids in the gastrointestinal tract. From this reason, the formation of micelle is altered and bile acids reabsorption is subsequently impaired, leading to the excretion of the fiber-bile complex through the feces, thus increasing the hepatic synthesis of bile

acids from blood cholesterol (Dongowski, 2007; Jalili, Medeiros and Wildman, 2007; Jones, 2008).

The term of prebiotic is defined as “nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth or the activity of one or a number of bacteria in the colon, that has the potential to improve health” (Gibson and Roberfroid, 1995). Prebiotics often referred to nondigestible oligosaccharides which are extracted from natural sources or synthesized from oligosaccharides. Purpose of prebiotics consumption is increasing number of selected bacteria for production of various short-chain fatty acids (SCFAs; acetate, butyrate and propionate) because of their impact on the lower gut environment, metabolism, and disease prevention (Farnworth, 2007; Jenkins, Kendall and Vuksan, 1999). The SCFAs are quickly absorbed and could be served as energy source for the host, especially between meals. Moreover, the generation of SCFAs during fermentation of the prebiotic by the microflora in gut suggested that one of the important mechanisms responsible for their lipid-lowering effects is enzymes inhibition involved in *de novo* lipogenesis (Lovegrove and Jackson, 2001). Therefore, the aim of this study was to determine the binding capacity of dietary fiber from cassava pulp for lard, cholesterol, bile acids including potential to be prebiotic for lactic acid bacteria.

4.3 Materials and Methods

4.3.1 Materials and samples preparation

Cassava pulp was collected from Sanguan wongse industries Co., Ltd. in the local area of Nakhon Ratchasima province, Thailand. Heat-stable α-amylase (EC 3.2.1.1, Merk, Darmstadt, Germany), amyloglucosidase AMG 300L from *Aspergillus*

niger (EC 3.2.1.3, Bray, Co. Wicklow, Ireland) and neutrase® (EC 3.4.24.28 from *Bacillus amyloliquefaciens*, Novozymes Co., Bagsvaerd, Denmark) were used. All chemicals using were reagent grade.

Cassava pulp was dried at 60°C in tray dryer (Kluaynumtaitowop, Bangkok, Thailand) for 24 h. The dried cassava pulp was finely ground (GmbH & Co.KG D-42781, Haan, Germany) and stored at room temperature in a vacuum-packed container before use.

4.3.2 Dietary fiber preparation

Cassava pulp solution was prepared at 4% (w/v) with phosphate buffer (50 mM, pH 6). Heat-stable α-amylase (0.1% w/v) was added and heated for 30 min at 95°C (with gentle shaking at 5 min intervals). After cooling to room temperature, the pH was adjusted to 7.5 with sodium hydroxide solution; neutrase (1.0% v/v) was added, and the solution incubated for 30 min at 60°C with continuous shaking. After cooling down to room temperature, the pH was adjusted to 4.5 with hydrochloric acid solution, an amyloglucosidase (0.1% v/v) was added and then the solution was incubated at 60°C for another 30 min with continuous shaking. The supernatant was discarded and the pellet was washed three times with distilled water following centrifugation (Hettich, Universal 32R, DJB labcare Ltd) at 10000×g for 10 min. The residue (dietary fiber) was dried at 60°C in a tray dryer and then finely ground. The dietary fiber powder was kept in a sealed container at 4°C until used.

4.3.3 *In vitro* digestion for determination of digestive stability

Simulated digestion of cassava dietary fiber was performed according to Garrett, Failla and Sarama (1999) and Ferruzzi, Failla and Schwartz (2001) with

modification. The *in vitro* digestion consisted of simulated gastric and small intestinal phases of digestion.

4.3.3.1 Gastric phase

Briefly, 1 g of dried sample was homogenized with 20 mL of 120 mM NaCl in 50 mL polypropylene tube by homogenizer (model T25D, Germany), then adjusted pH to 2.0 ± 0.1 with 1 M HCl and 2 mL of porcine pepsin (40 mg/mL in 100 mM HCl) was added. Then the volume was adjusted to 40 mL with 120 mM NaCl, filled with nitrogen gas, tightly cap and sealed with parafilm and incubated in a shaking water bath (JULABO, SW22, USA) for 1 hr at 37ºC, 150 rpm.

4.3.3.2 Small intestinal phase

At the end of gastric phase, the small intestinal phase was followed by raising the pH to 6.0 ± 0.1 with 1 M sodium bicarbonate ($NaHCO_3$). Crude bile extract (40 mg/mL in 100 mM $NaHCO_3$) 3 mL, pancreatin (12 mg/mL in 100 mM $NaHCO_3$) 2 mL were added to the reaction tube. Next, the pH of sample was increased to 7.0 ± 0.1 with 1 M $NaHCO_3$. Then the tube was filled with nitrogen gas tightly cap and sealed with parafilm before putting in a shaking water bath (JULABO, SW22, USA) for 2 h at 37ºC, 150 rpm to complete the intestinal phase of the *in vitro* digestion process. The mixture was centrifuged which induce precipitation and the sediment was analyzed for neutral detergent fiber (NDF).

$$\text{Digestive stability (\%)} = \frac{\text{Amount of NDF in digesta}}{\text{Amount of NDF in pre-digested sample}} \times 100$$

4.3.4 Binding capacity of dietary fiber for bile acids

The bile acids binding capacity was determined by colorimetry according

to the method of Kahlon and Woodruff (2003) and Dziedzic et al. (2012) with minor modification. The factor triggering the color reaction in this method is a 5% aqueous solution of furfural (Sigma-Aldrich®, MO, USA). Three bile acids were selected for analysis, i.e. cholic acid (CA, AMRESCO, Ohio, USA), deoxycholic acid (DCA, ACROS Organics, New Jersey, USA) and taurocholic acid (TA, ACROS Organics, New Jersey, USA) at a concentration of 1 mM. The principle of measurement was to determine concentrations of bile acids in the supernatant after incubation at a temperature of 37°C and gastric juice pH. The analytical sample of CA, DCA and TA was dissolved in 25 mL absolute ethanol, using ultrasounds, and next made up with phosphate buffer of pH 6.9. The sample of 0.5 g and 20 mL bile acids dissolved in the phosphate buffer was placed in a flask and incubated in a shaking water bath at temperature of 37°C for 2 h. Additionally, 0.5 g analysed material in phosphate buffer without bile acids was used for a blank. After incubation in a water bath at 37°C for 2 h, the sample was filtered. The supernatant was kept for further analysis.

The amount of 5 mL supernatant was mixed with 5 mL of 70% sulphuric acid and left stand at room temperature for 2 min. After that, 1 mL of 5% furfural solution was added (after 5 min pink coloring appeared), and the absorbance was measured. The amount of bile acids absorbed by dietary fiber was determined based on the difference of concentrations before and after incubation. The concentration of bile acids in the tested sample was determined based on the standard curve for a given acid within the range of concentrations was 0.1 to 1 mM. Absorbance was measured using a spectrophotometer (Biochrom Libra S22 S/N 97765, UK) at a wavelength of 510 nm.

Calculation of bile acid binding capacity of dietary fiber (BA):

$$\text{BA (\%)} = \left(1 - \frac{\text{BA after incubation}}{\text{BA before incubation}}\right) \times 100$$

4.3.5 Binding capacity of dietary fiber for lard

The binding capacity of dietary fibers for lard was determined according to the method proposed by Zhang, Huang and Ou (2011) with slightly modification. Dietary fiber (3 g) was mixed with melted lard (prepared from fresh pork) in a centrifugal tube and left undisturbed for 1 h at 37°C. Then, the mixture was centrifuged at 10000×g for 10 min. The supernatant was decanted, and the pellet was recovered by filtration. The binding capacity (BOC) was calculated as follows:

$$BOC = (W_2 - W_1)/W_1$$

Where W_1 and W_2 are the weights of the dietary fibers before and after adsorbing lard, respectively.

4.3.6 Binding capacity of dietary fiber for cholesterol in egg yolk

In this research, egg yolk was used as a model system for binding capacity of cholesterol since cholesterol is difficult to dissolve in water even after addition of emulsifiers. The method was determined as described by Zhang, Huang and Ou (2011) with slightly modification. Fresh egg yolk was diluted with 9 times volumes of deionized water. Adjusted the pH of the mixtures of 2.0 g dietary fiber in 50 mL of the diluted yolk at pH 2.0 and 7.0, respectively (similar to the pH conditions prevailing in the stomach and small intestine, respectively), then the mixture were shaken at 120 rpm for 2 h in a water-bath maintained temperature at 37°C, diluted yolk without dietary fiber added was used as a blank. At the end of adsorption, 4 mL of the sample was collected, and 16 mL of absolute ethanol was added to precipitate the dietary fibers sedimentation and centrifuged at 10000×g for 20 min. The ethanol

in the supernatant was removed with a vacuum rotary evaporator (Buchi Rotavapor R-114, USA). One mL of the concentrate was diluted with 5 mL of 90% acetic acid. Color was developed according to the method of Park (1999) by adding 0.1 mL of o-phthaladehyde reagent and 2mL of concentrated H_2SO_4. The mixture was left to stand for 20 min and the absorbance was measured at 550 nm against a reagent blank. The cholesterol concentration in the samples was determined against a standard curve generated by a standard cholesterol (Sigma Aldrich®, Saint Louis, MO, USA) within the range of concentrations was 0.025, 0.050, 0.075, 0.100 and 0.125 mg/mL.

The binding capacity (BC) was calculated as follows:

$$BC = [C_{blank} - (C_{supernatant}) \times F] \times (50/w)$$

where C_{blank} and $C_{supernatant}$ are the cholesterol concentrations in the yolk without dietary fiber and the supernatant mixed with dietary fibers, respectively; F is the dilution factor; 50 is the adsorption volume (mL); and w is the weight of the dietary fiber.

4.3.7 Determination of prebiotic activity

Prebiotic activity was tested followed method described by Huebner et al. (2008). Five *Lactobacillus* strains (3C2-10, 21C2-10, 21C2-12 and OC4-4) from Nawong (2015) and *Lactobacillus plantarum* TISTR 1465 (Thailand Institute of Scientific and Technological Research) were cultured in MRS-broth with 2% prebiotics. Cassava pulp dietary fiber (CDF), Fructooligosaccharide (FOS; Sigma Aldrich®, Saint Louis, MO, USA), Lactulose (LAC; ACROS Organics™, Thermo Fisher Scientific Inc, New Jersey, USA), Inulin (Sigma Aldrich®, Saint Louis, MO, USA) and Cellulose (HiMedia® Laboratories Pvt Ltd., Mumbai, India) were tested and incubated 37°C under anaerobic condition for 20 h. All samples were already

passed *in vitro* digestion process (section 4.3.3) before prebiotic activity determination. The prebiotic activity score was determined following equation:

$$\text{Prebiotic activity score} = \left[\frac{(\text{probiotic log CFU on the prebiotic at 24 h} - \text{probiotic log CFU on the prebiotic at 0 h})}{\text{probiotic log CFU on the glucose at 24 h} - \text{probiotic log CFU on the glucose at 0 h}}\right] - \left[\frac{(\text{enteric log CFU on the prebiotic at 24 h} - \text{enteric log CFU on the prebiotic at 0 h})}{\text{enteric log CFU on the glucose at 24 h} - \text{enteric log CFU on the glucose at 0 h}}\right]$$

4.3.8 Statistical analysis

All experiments were performed in triplicate and mean values (on dry basis) with standard deviations are reported. The experimental data were analyzed using an analysis of variance (ANOVA). SPSS® software (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical calculations.

4.4 Results and Discussion

4.4.1 Determination of digestive stability

Digestive stability is defined as the percentage of neutral detergent fiber (NDF: cellulose, hemicellulose, lignin) of dietary fiber recovered in the precipitate. The digestive stability of cassava pulp dietary fiber (CDF) is 87.98%. This value is seem high because CDF composed high amount of cellulose, hemicellulose and lignin which is insoluble form of dietary fiber (Table 3.5). Cellulose is the glucose residues joined by β-1, 4 linkages, these linkages allow greater inter- and intra-polymer hydrogen bonding which it resistance to the enzymes of the human digestive tract (Mongeau and Brooks, 2016). Moreover, the molecules of lignin are insoluble in both strong acid and alkaline solution and resistant to human enzyme (Anderson and Chen, 1979; Schneeman, 1986).

4.4.2 Binding capacity of dietary fiber for bile acid

Binding and excretion of bile acids in the small intestine were one of the main mechanisms for their cholesterol-lowering effects by other digestion-resistant polysaccharides (Gallaher et al., 2000; Zacherl, Eisner and Engel, 2011; Zhou et al., 2006). *In vitro* study has been widely used to predict the potential bile acid-binding capacity of many polysaccharides including chitosan, wheat bran, soy bean hull apple peel, citrus fruits and other dietary fibers (Wang et al., 2015; Zhang, Huang and Ou, 2011; Zhou et al., 2006). Insoluble dietary fiber from cassava pulp was studied for its direct binding capacity against bile acid including cholic acid, deoxycholic acid and taurocholic acid. Cholic acid-primary bile acid and deoxycholic acid-secondary bile acid were used as the representative of bile acid binding capacity of dietary fiber. Meanwhile, taurocholic acid was chosen because of its higher UV activity although both deoxycholic acid and lithocholic acid which are secondary bile acids, seemed to be appropriate standards for the determination of bile acid binding (Cornfine et al., 2010). In this experiment, cellulose was used as a reference for low bile acid binding capacity (Cornfine et al., 2010; Dongowski, 2007; van Bennekum et al., 2005). The CDF showed an *in vitro* bile acid binding capacity for CA, DCA and TA were 38.50%, 42.71% and 40.84%, respectively (Figure 4.1). CDF showed high bile acid binding capacity compared to cellulose, it may be due to chemical composition especially for lignin. The major physical property of lignin are; it can absorb bile acid and delay nutrient adsorption in small intestine (Prosky and DeVries, 1992). Other studies have reported similar bile acid binding capacity for lupins product, grape fruit, buckwheat hull and buckwheat bran (Cornfine et al., 2010; Dziedzic et al., 2012; Wang et al., 2015). However, some studies exhibited lower bile acid binding

capacities for insoluble dietary fiber preparations from soy beans or black eye bean, which could be due to differences in the *in vitro* bile acid binding assay, the different raw materials, or the composition and structure of the plant cell wall material (Cornfine et al., 2010; Kahlon and Shao, 2004).

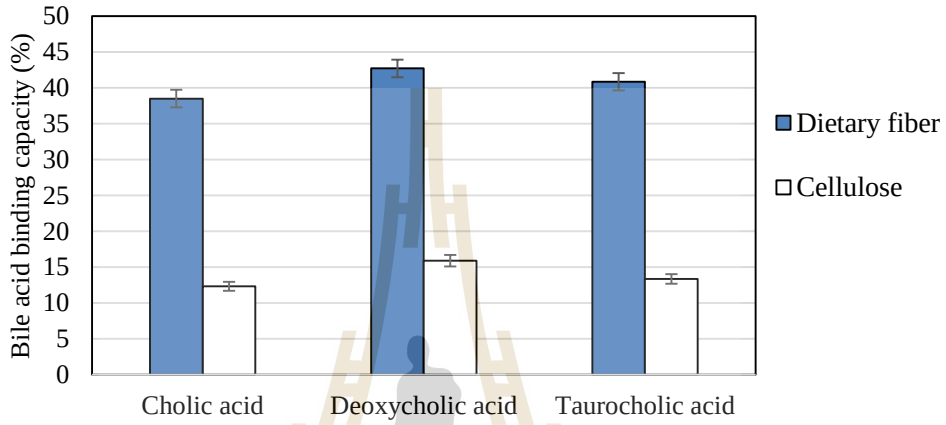

Figure 4.1 Bile acid binding capacity (%) of dietary fiber and cellulose.

4.4.3 Binding capacity of dietary fiber for lard and cholesterol

Blood-lipid profile has been proven that it correlate with dietary fat and dietary fiber (Zhang, Huang and Ou, 2011). Saturated fatty acids and cholesterol are considered as atherogenic fats (Heshmati and Khodadadi, 2009) which is the important risk factors of developing cardiovascular disease (Jalili, Medeiros and Wildman, 2007; Theuwissen and Mensink, 2008). One way to reduce serum total and LDL cholesterol levels by making dietary changes. Besides to decrease saturated fat and cholesterol intake, the importance approaches are increasing the consumption of dietary fibers. The result in Table 4.1 showed that the dietary fiber from cassava pulp had higher binding capacity for lard and cholesterol than cellulose. Binding capacity for lard of CDF was similar of apple peel fiber (4.57 g/g) (Zhang, Huang and Ou,

2011). While, binding capacity for cholesterol of CDF was similar to soybean hull fiber, ponkan fiber and orange fiber (Wang et al., 2015; Zhang, Huang and Ou, 2011). It is indicated that CDF had potential for cholesterol-lowering property.

Oil binding capacity of dietary fiber could effectively promote ability to absorb the carcinogenic mutations and cholesterol due to its chemical composition mainly is lipophilic (Yoshimoto, Yamakawa and Tanoue, 2005). The ability to bind oil depend on chemical composition of dietary fiber including lignin which is not a polysaccharide. Molecules of lignin are variable polymers and highly complex with the chain of oxygenated phenyl propane which has hydrophobic properties, but strongly binding with oil (Sosulski and Cadden, 1982). Sosulski and Cadden (1982) found that fiber sources rich in hemicellulose and lignin (wheat bran, sunflower hulls) had high fat absorption properties, digestibility and serum triglycerides. Pea hulls and cellulose showed less strong functional properties as sources of dietary fiber.

Table 4.1 Binding capacity of dietary fiber and cellulose for lard and cholesterol.

	Lard (g/g)	**Cholesterol (mg/g)**	
		pH 2	**pH 7**
Dietary fiber	3.85 ± 0.08	5.27 ± 0.15	7.16 ± 0.24
Cellulose	1.92 ± 0.03	1.55 ± 0.07	2.94 ± 0.04

4.4.4 Determination of prebiotic activity

Prebiotic activity indicates the ability of bacterial growth which is supported by non-prebiotic substrates, such as glucose compared with prebiotics as a particular oligosaccharide (Huebner, Wehling and Hutkins, 2007). Prebiotic activity scores of five *Lactobacillus* strains: 3C2-10, 21C2-10, 21C2-12, OC4-4 which isolation from cassava pulp and *Lactobacillus plantarum* TISTR 1465 are shown in

Figure 4.2. *Lactobacillus plantarum* TISTR 1465 was selected for this study because it is the probiotic strains (Chaipojjana, Phosuksirikul and Leejeerajumnean, 2014). Prebiotic activity of *Lactobacillus plantarum* TISTR 1465 of CDF shows higher value than prebiotic activity of cellulose, but lower than some soluble dietary fibers. In addition, there was no prebiotic activity of the rest *Lactobacillus* strains tested. This results indicated that CDF has potential to be a prebiotic source for some species of *Lactobacillus* strains. A positive prebiotic activity score illustrated well growth of probiotic strains. *Lactobacillus* spp. has ability to metabolized prebiotic carbohydrate and produces gases (H_2S, CO_2, H_2 and CH_4), lactate and SCFAs (acetate, butyrate and propionate) and other short chain organic acids during fermentation process (Huebner et al., 2008). The organic acids may be associated with antagonistic of intestinal competitors (Wang, 2009). Propionate is mainly metabolized in the liver and reported to reduce plasma cholesterol and lipid levels by inhibiting hepatic cholesterogenesis and lipid synthesis. (Anderson and Bridges, 1984; Chen, Anderson and Jennings, 1984; Demigné et al., 1995; Wolever et al., 1989). The types of SCFAs produced during the fermentation process depend on the microflora which could be stimulated by prebiotic.

The main chemical compositions of CDF are high cellulose and hemicellulose. Although cellulose is not digested by human enzymes but can be partial digested in the gut by beneficial microflora. Cellulose is degraded by natural fermentation in colon about 50% and produce significant amount of short-chain fatty acids which feed our intestinal cells (Mudgil and Barak, 2013). Moreover, microflora in the gut can digest hemicellulose increasing the number of beneficial bacteria and produce short-chain fatty acids which colon cells use as fuel and affect cholesterol reduction (Jalili,

Medeiros and Wildman, 2007; Mudgil and Barak, 2013; Mudgil, Barak and Khatkar, 2012).

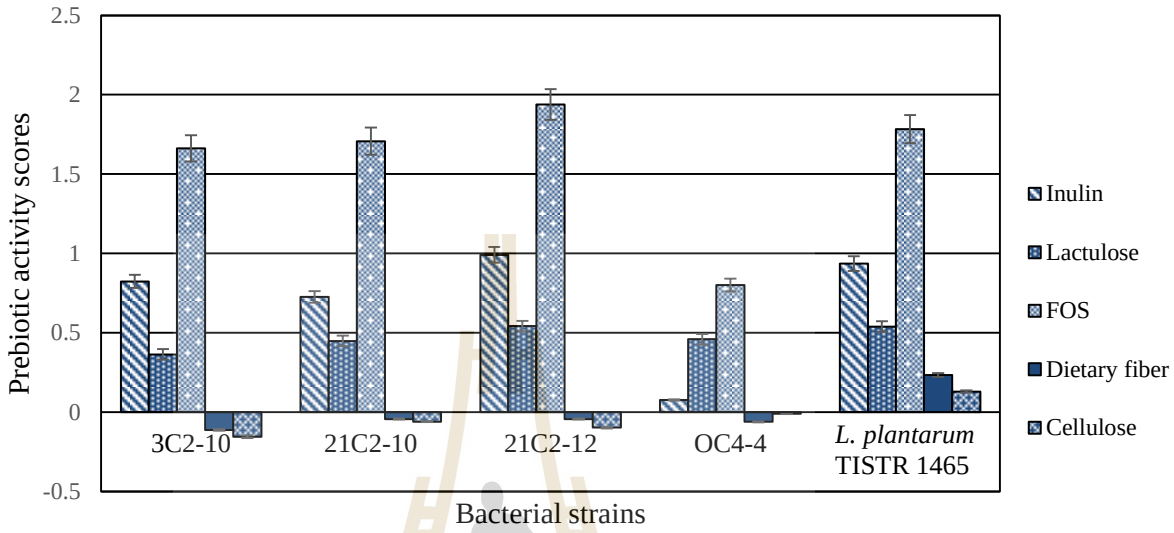

Figure 4.2 Prebiotic scores of *Lactobacillus* strains (3C2-10, 21C2-10, 21C2-12, OC4-4 and *Lactobacillus plantarum* TISTR 1465).

4.5 Conclusions

Dietary fiber from cassava pulp had potential to bind lard, cholesterol and bile acid which correlated to cholesterol-lowering property. Moreover, assessment of prebiotic potential for some *Lactobacillus* strains concluded that CDF had prebiotic activity for *Lactobacillus plantarum* TISTR 1465 and might be act as prebiotic substance. *In vivo* study of CDF is the interesting topics for further study.

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CHAPTER V

TESTING FOR ACUTE TOXICITY AND CHOLESTEROL-LOWERING PROPERTY OF DIETARY FIBER FROM CASSAVA PULP IN WISTAR RATS

5.1 Abstract

Cassava pulp is a high value by-product from cassava starch. After enzymatic digestion, dietary fiber from cassava pulp (CDF) contains a high content of neutral detergent fiber and cellulose. With regard to an acute toxicity test (14 days) of CDF, male and female rats were fed with basal diets as the control group. The tested group was fed a basal diet containing 2.5% and 15% (w/w) of CDF, respectively. Clinical observations and measurements of food consumption were carried out daily and body weights were recorded every two days. Blood was collected to measure hematology and biochemical parameters. The results showed that there were no deaths or abnormal behaviors of the rats fed on a diet containing 2.5% and 15% (w/w) of CDF during clinical observation. Diets with CDF added did not produce any mortal effects or changes in the gross morphology of the rats' internal organs. The CDF diet decreased the concentration of total cholesterol (TC) and total triglyceride (TG) in serum when compared with the control group (p<0.05). The no-observed-adverse-effect level (NOAEL) for CDF was 15% for both genders (male 10.01 g/kg body weight/day; female 11.21 g/kg body weight/day).

For this study of the cholesterol-lowering property, thirty Wistar rats were assigned to five groups: Group 1 was fed a basal diet (Normal); Group 2 received a high-fat diet without fiber (Control); Group 3 received a high-fat diet without fiber together with gavage of 10 mg/kg/day simvastatin (Simvastatin); Group 4 received a high-fat diet containing 5% (w/w) cassava dietary fiber (CDF); and Group 5 received a high-fat diet containing 5% (w/w) cellulose (Cellulose). All groups were fed with these diets for 30 days. The results illustrate that CDF significantly (p<0.05) decreases serum triglyceride, serum total cholesterol, liver total lipids, and liver cholesterol levels, while it also significantly (p<0.05) increased the levels of fecal total lipids and cholesterol when compared with the Control group. Moreover, there were no significant (p>0.05) differences in terms of serum triglyceride, serum total cholesterol, liver total lipids, and liver cholesterol between modern medicine (Simvastatin) and CDF. The hypocholesterolemic and hypolipidemic effects of CDF might be correlated to enhancing fat and cholesterol excretion. However, the gross pathology of liver in rats fed a high fat diet showed a larger size (p<0.05) and a paler color (by sight) than the rat livers' group fed a basal diet. Also, the liver tissue haematogram for the CDF group looked normal similar to that of the Normal group. In conclusion, these research results suggest that CDF could be a potential cholesterol-lowering food ingredient.

5.2 Introduction

Cholesterol is an important factor that leads to cardiovascular disease (CVD). Many reports show that the consumption of dietary fiber effectively decreases serum cholesterol and lowers the risk of CVD (Chau, Huang and Lin, 2004; German et al., 1996; Hsu et al., 2006; Martín-Carrón et al., 1999). The reduction in plasma

cholesterol levels of dietary fiber has been associated with restrained reabsorption of bile acids, cholesterol and dietary fats (Arjmandi et al., 1992; Schectman, Hiatt and Hartz, 1993). Similarly, an increase in the hepatic synthesis of bile acids in humans (Jenkins et al., 1993) and rats (Matheson and Story, 1994), as well as a direct interaction with lipoprotein metabolism (by increasing the number of hepatic LDL receptors (la)), has been suggested as a possible mechanism of action. Soluble dietary fiber is known to be an effective hypocholesterolemic agent. Recently, some researches illustrated that insoluble fibers isolated from some fruits, vegetables, and pomace could also effectively decrease serum cholesterol and triglyceride (Chau, Huang and Lin, 2004; ErkkilaÄ et al., 1999; Knopp et al., 1999; Rosamond, 2002). Many reports reveal that the cholesterol-lowering actions of insoluble fibers might be correlated to their physicochemical properties, such as water-holding capacity and cation-exchange capacity (Chau and Cheung, 1999).

Dietary fiber has many health benefits for humans such as reducing the risk of colon and rectal cancer and reducing blood glucose levels etc. Furthermore, because dietary fiber is not digested by the digestive system in the human body, dietary fiber can be used as a non-caloric ingredient for replacement of caloric ingredients, such as fats, carbohydrates and protein in many food products. Consequently, the consumption rate of fiber-rich products has recently increased. Many by-products from the fruit and vegetable industry are of particular interest due to their low cost and availability in large quantities. Indeed, some agricultural by-products, such as apples, citrus fruits, grapes, carrots and Brassica vegetables have already been used in the production of dietary fiber (Figuerola et al., 2005; Grigelmo-Miguel and Martín-Belloso, 1999; Hsu et al., 2006). They supply dietary fiber as well as bioactive compounds such as polyphenols and essential oils which provide economic benefits to

the food, cosmetic, and pharmaceutical industries. With regard to the food industry, dietary fiber can also incorporate some functional properties of foods, such as increasing water and oil holding capacity, emulsification, and gel formation. Dietary fiber can be incorporated into many varieties of food products including bread, dairy, jams, meats, soups etc. (Elleuch et al., 2011). However, various methods and different sources for obtaining dietary fiber might affect their chemical composition and physicochemical properties which could subsequently affect their function as food ingredients in food applications (Chau and Huang, 2003).

Cassava (*Manihot esculenta* Crantz) is the third-largest source of food carbohydrates in the tropics. Cassava is mostly grown in tropical countries with 70% of the world's cassava production coming from Nigeria, Brazil, Thailand, Indonesia and the Democratic Republic of the Congo (Khempaka, Molee and Guillaume, 2009). The cassava starch industry has a global market value of approximately 1.4 billion tons/year. Cassava starch production in Thailand is one of the most important agro-industries. Thailand is the third largest producer of cassava starch, yielding 22.2 million tons per year. Cassava pulp is an important by-product of the many by-products during the processing of cassava. At least 1 million tons of pulp is generated annually in Thailand. Cassava pulp represents approximately 10-15% by weight of the original cassava roots. The starch remaining in the pulp is approximately 50-60% of its dry weight and, for the most part, is trapped inside ligno-cellulose. The fiber content of dried cassava pulp is reportedly in the form of insoluble fiber. Moreover, the pulp also contains pectin, cellulose, fiber (10-15%), protein (1.5-5%), and fat (0.1-4%). Historically, it has been used in the animal feed industry, bio-gas production, and ethanol production (Sriroth et al., 2000; Thailandtopiocastarch, 2010). Due to its

high content of fiber, cassava pulp can be used as a fiber-rich ingredient in food products.

Acute toxicity refers to the adverse effects occurring following oral or dermal administration of a substance that results either from a single exposure or from multiple exposures in a short period of time which is usually less than 24 hours. Moreover, the adverse effects of acute toxicity should occur up to 14 days after the administration of the substance in animal models which are typically mice or rats (IUPAC, 2006). Research about the possible toxicity of cassava pulp fiber has not been carried out. Furthermore, there are no published studies on the cholesterol-lowering properties of dietary fiber in cassava pulp. Therefore, the purpose of this study was to determine the safety of dietary fiber obtained from cassava pulp after acute oral administration in Wistar rats and to evaluate the potential hypocholesterolemic action of dietary fiber from cassava pulp on rats that were fed a high-fat diet.

5.3 Materials and Methods

5.3.1 Materials and sample preparation

Cassava pulp was provided from Sanguan wongse industries Co., Ltd. in the local area of Nakhon Ratchasima province, Thailand. Heat-stable α-amylase (EC 3.2.1.1, Merk, Darmstadt, Germany), amyloglucosidase AMG 300L from *Aspergillus niger* (EC 3.2.1.3, Bray, Co. Wicklow, Ireland) and neutrase® (EC 3.4.24.28 from *Bacillus amyloliquefaciens*, Novozymes Co., Bagsvaerd, Denmark) were used. All chemicals using were reagent grade. Normal rat diet (082G/15, C.P.mice Feed, Perfect Companion Group, Thailand) was used as normal diets.

Cassava pulp was dried at 60°C in a tray dryer (Kluaynumtaitowop, Bangkok, Thailand) overnight. Before use, the dried cassava pulp was finely ground (GmbH & Co.KG D-42781, Haan, Germany) and stored at room temperature in a vacuum-packed container.

5.3.2 Dietary fiber preparation

Cassava pulp solution was prepared at 4% (w/v) with a phosphate buffer (50 mM, pH 6). Heat-stable α-amylase (0.1% w/v) was added and heated for 30 min at 95°C (with gentle shaking at 5 min intervals). After cooling to room temperature, the pH was adjusted to 7.5 with sodium hydroxide; neutrase (1% v/v) was added, and the solution incubated for 30 min at 60°C with continuous shaking. After cooling to room temperature, the pH was adjusted to 6.0 with hydrochloric acid, an amyloglucosidase (0.1% v/v) was added and then the solution was incubated at 60°C for another 30 min with continuous shaking. The supernatant was discarded and the pellet was washed three times with distilled water following centrifugation (Hettich, Universal 32R, DJB labcare Ltd) at 10000×g for 10 min. The residue (dietary fiber) was dried at 60°C in a tray dryer and then finely ground. The dietary fiber powder was kept in a sealed container at 4°C until used.

5.3.3 Acute toxicity study of dietary fiber

5.3.3.1 Diet preparation

Normal rat diet (082G/15, C.P. Mice Feed, Perfect Companion Group, Thailand) was ground and mixed with dietary fiber (2.5% and 15% (w/w)). The mixed diet was prepared for rat diets by an extruder (Tricool engineering Ltd., Hants, U.K.) through a process of weighing, milling, mixing, adding water, extruding and then re-drying.

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5.3.3.2 Experimental protocol

The oral acute toxicity study was carried out according to the guideline No.423 provided by the Organization of Economic Co-Operation (OECD) for the acute toxicity class method (ATC) procedure (OECD, 2001a). All animals were handled according to Guidelines of the Animal Care and Use of Laboratory Animals from the Center for Laboratory Animal Science of Suranaree University of Technology. Healthy male (260-290 g) and female (190-220 g) Wistar rats were bred in The Experimental Animal job, Suranaree University of Technology, Nakhon Ratchasima province. They were acclimatized to the laboratory environment for one week prior to experimentation. The rats were randomLy assigned to three groups (five males, five females per group). The first group fed with basal diet alone defined as control group. The second and third groups were administered daily diets containing 2.5% and 15% (w/w) cassava pulp dietary fiber (CDF). Animals were housed in individual screen-bottomed, stainless steel cages in a room maintained at 22 ± 3°C with a 12 h light: dark cycle. Throughout the whole experimental period, food and water were provided with ad libitum access. Food consumption was recorded every day and body weights were recorded every two days. The rats were examined daily for clinical signs of toxicity such as mortality, respiratory pattern, changes in general behavior, skin, eyes, fur and somato-motor activity. After the experimental period (14 days), the rats were sacrificed by using carbon dioxide after fasting for 12 h. Blood sample was collected by cardiac puncture, and serum was prepared for biochemical tests. The effects from CDF on all groups of body weight gain, food consumption, relative organ weight and organ histopathology were examined. All procedures were in accordance with the Guidelines of the Animal Care and Use of Laboratory Animals

from the Center for Laboratory Animal Science of Suranaree University of Technology.

5.3.3.3 Hematological assay

The rats were fasted overnight before blood collection. Blood samples were drawn into separate tubes containing ethylenediamin-tetraacetic acid (EDTA). A XS-800i Sysmex (Sysmex Corporation, Kobe, Japan) was used to determine hematocrit (HCT), red blood cells (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte count (WBC), lymphocytes, monocytes, eosinophil, basophil, platelet count (PLT) and polymorphonuclear neutrophils (PMN).

5.3.3.4 Blood Chemistry

Blood samples were centrifuged to separate plasma. Clinical chemistry parameters analyzed using VITROS® 5600 Chemistry System (Ortho-Clinical Diagnostics, Inc., New York, USA) included: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glucose (GLU), total cholesterol (TC), triglycerides (TG), creatinine (CREA) and blood urea nitrogen (BUN).

5.3.3.5 Organ weights

The liver, heart, lungs, kidneys, adrenal glands, spleen, and testes/ovaries organs were clipped of any adherent tissue, as appropriate, and were washed with normal saline solution to eliminate blood from the organs. Paired organs were weighed individually. Relative organ weights (%) were calculated against fasting body weight.

5.3.3.6 Histopathological examination

The livers were dissected, weighed, and then immerse immediately in neutral buffer formalin (Formaldehyde neutral buffer; 10% Formalin 238 mL,

Distilled water 762 mL, pH 7.4) at room temperature for no more than 24 h. Liver sections were dehydrated and then embedded in paraffin. Sections with 5-mm thickness were cut and then deparafinized, rehydrated, and stained in hematoxylin-eosin (H&E). The sections were subsequently subjected to photomicroscope examination (Nikon Corporation, Japan) coupled with Olympus DP72 (Hollywood International group, Thailand).

5.3.3.7 Statistical analysis

SPSS® software (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical calculations. The experimental data were analyzed using an analysis of variance (ANOVA) to compare the control and treatment groups. A *P*-value was less than 0.05 which is considered statistically significant.

5.3.4 Cholesterol-lowering property of dietary fiber

5.3.4.1 Diet preparation

The experiments were divided into five groups: Group 1 was fed a basal diet (Normal) which was prepared by C.P. Perfect companion group, Thailand (No. 082G/15); Group 2 received a high-fat diet without fiber (Control); Group 3 received a high-fat diet without fiber together with gavage of 10 mg/kg/day simvastatin (Simvastatin); Group 4 received a high-fat diet containing 5% (w/w) cassava dietary fiber (CDF); and Group 5 received a high-fat diet containing 5% (w/w) cellulose (Cellulose). The diets of Groups 2 to 5 were prepared by Research Diets, Inc. (New Brunswick, NJ. USA) and supplemented with cholesterol (1.25 g/100 g) and sodium cholic acid (0.5 g/100 g) to induce hypercholesterolemia in the Wistar rats. The ingredients and chemical composition of the diets are listed in Table 5.1.

5.3.4.2 Experimental protocol

Thirty male rats (250-290 g) were obtained from the Laboratory Animal Center of Suranaree University of Technology, Thailand. After an acclimatization period of 7 days, the rats were randomLy allotted to the five diet groups with six animals in each group. Animals were housed in individual stainless steel cages in a room maintained at 22±3°C with a 12 hour light:dark cycle. For the whole experimental period (30 days), food and water were provided with ad libitum access. In addition, feces were collected and weighed daily, and food intakes and body weights were recorded every day. Fecal samples were lyophilized, weighed, milled, and stored at -20°C until analyzed. Before the experiment, blood samples were taken from the tail vein. At the end of the experiment, the rats were sacrificed using carbon dioxide after fasting for 12 hours. Blood was drawn from the left atrium of the heart, and serum was prepared for biochemical tests. Rat organs were removed and weighed. The liver was removed, weighed, and kept at -80°C before a pathological analysis. All procedures were in accordance with the Guidelines of the Animal Care and Use of Laboratory Animals from the Center for Laboratory Animal Science of Suranaree University of Technology.

5.3.4.3 Biochemical assays of serum cholesterol, triglyceride and glucose

The concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride and glucose in the serum samples were determined enzymatically by VITROS® 350 Chemistry System (Ortho-Clinical Diagnostics, Inc., CA, USA) at the Biochemical Laboratory at Suranaree University of Technology Hospital.

Table 5.1 Formulations of the experimental diets.

Ingredients[a]	**Control (Fiber free)**	**Cassava DF**	**Cellulose**
Casein	140	140	140
DL-Methionine	2	2	2
Corn starch	435	435	435
Maltodextrin	150	150	150
Sucrose	100	100	100
Cassava DF	-	50	-
Cellulose	-	-	50
Soybean oil	50	50	50
Coconut oil	35	35	35
Mineral mix S10001	35	35	35
Calcium carbonate	5.5	5.5	5.5
Sodium chloride	8	8	8
Potassium citrate	10	10	10
Vitamin mix V10001	10	10	10
Choline bitartrate	2	2	2
Cholesterol	12.5	12.5	12.5
Sodium cholic acid	5	5	5
FD&C blue dye #1	0.1	-	-
FD&C Yellow dye #5	-	0.1	-
FD&C red dye #40	-	-	0.1

[a]The ingredients are expressed as g/kg of diet (dry weight).

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5.3.4.4 Liver total lipids and cholesterol

Following the method described by Folch, Lees and Stanley (1957), total lipids were extracted from livers (1–2 g) with a chloroform:methanol mixture (2:1 v/v). The concentration of total lipids in the liver tissue was determined gravimetrically by vanishing the organic solvents in the liver lipids extract. Liver cholesterol concentration in the liver lipids extract was determined colorimetrically following the method of Searcy and Bergquist (1960). Lipid extract, cholesterol standard*, and blank (chloroform:methanol mixture, 2:1, v/v only) amount 0.2 mL were added into a series of test tubes. After that, 3 mL of $FeSO_4$–acetic acid solution** and 1 mL of concentrated sulfuric acid were added. The mixture was left to stand for 10 min at room temperature (25°C) and the absorbance of the mixture was measured at 490 nm against the blank. The cholesterol concentration in the samples was determined against a standard curve generated by a standard cholesterol (Sigma Aldrich®, Saint Louis, MO, USA).

*Cholesterol standard: Sixty grams of cholesterol was dissolved in 100 mL chloroform:methanol mixture (2:1, v/v).

**Saturated $FeSO_4$–acetic acid solution: Three grams of $FeSO_4.7H_2O$ was stirred in 500 mL acetic acid for 5 min. The mixture was then filtered to obtain a clear saturated solution.

5.3.4.5 Fecal total lipids and cholesterol

According to the method of Folch, Lees and Stanley (1957), fecal total lipids and cholesterol in the dried fecal samples were extracted with a chloroform:methanol mixture (2:1 v/v). The total lipids concentration in the dried fecal sample was then quantified gravimetrically by evaporating off the organic

solvent in the fecal lipids extract. The concentration of cholesterol in the fecal lipids extract was determined colorimetrically at 490 nm (Searcy and Bergquist, 1960).

5.3.4.6 Relative organ weights

The liver, heart, lungs, kidneys, adrenal glands, spleen, and testes/ovaries organs were trimmed of any adherent tissue, as appropriate, and were washed with normal saline solution to eliminate blood from the organs. Paired organs were weighed individually. Relative organ weights (%) were calculated against fasting body weight.

5.3.4.7 Histopathological examination

The livers were dissected, weighed, and then immerse immediately in Formaldehyde neutral buffer (Formaldehyde neutral buffer; 10% Formalin 238 mL, Distilled water 762 mL, pH 7.4) at room temperature. Liver sections were dehydrated and then enclosed in paraffin. The tissues were sectioned and stained with hematoxylin and eosin stains (H&E). Photographs were taken in a Nikon eclipse 80i microscope (Nikon Corporation, Japan) coupled with Olympus DP72 (Hollywood International group, Thailand).

5.3.4.8 Determination of short-chain fatty acid (SCFA) in feces

Short-chain fatty acid in feces was analyzed following the method of de Almeida Jackix et al. (2013) with slight modifications. A 150 mg of dried feces was homogenized with 2.5 mL of methanol by vortexing; the mixer was then incubated for 15 mins. Samples were then transferred to a vial by filtering through a 0.45 µm membrane. Following this step, 1 µL of aliquots was injected into a gas chromatography (Hewlett Packard HP 6890 series GC system, USA) equipped with an Agilent J&W GC capillary column (DB-FFAP 30 m, ID: 0.32 mm; film: 0.25 µm; Agilent technologies, USA). The chromatographic conditions were the following:

Injector and detector temperature of 250ºC and a flow of 1.8 mL/min; the oven temperature was set to 80ºC during the first 5 mins and was increased to 170ºC at a rate of 10ºC/min; the temperature was maintained at 170ºC for 1 min and was increased to 250ºC at a rate of 30ºC/min; the temperature was then maintained at 250 ºC for 5 mins. Helium was used as a carrier gas and the split ratio was 30:1. The SCFAs were quantitatively determined by a comparison of the retention times and peak areas of standards (acetic acid, propionic acid and butyric acid). All samples were analyzed in triplicate.

5.3.4.9 Statistical analysis

All experiments were performed in triplicate and mean values (on dry basis) with standard deviations are reported. The experimental data were analyzed using an analysis of variance (ANOVA). SPSS® software (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical calculations.

5.4 Results and Discussion

5.4.1 Acute toxicity study of dietary fiber

5.4.1.1 Clinical observation, feed consumption and body weight

In this experiment, we compared the results between the control group and diets containing 2.5% and 15% (w/w) of CDF. After oral administration of test substances for 14 days, the rats moved freely. They behaved normally and the hardness and color of feces was normal. No diarrhea, constipation, abdominal bulging or sunken was found. The hair on the rats was smooth and there was no appearance of skin laxation, wrinkle, redness or other phenomena. In conclusion, after oral administration of cassava DF diets, there were no deaths of the rats and no symptoms

of poisoning were seen by morphological observation throughout the experiments (Table 5.2). As there was no abnormal behavior or any deaths of the rats in this experiment, we can assume that DF from cassava pulp is safe for both rats and humans. Hong et al. (2012) studied acute toxicity and long-term feeding tests of DF from wheat bran which was used as a food additive to cookies. The results revealed that there were no deaths in any of the groups which received the doses which indicates that oral LD_{50} of wheat bran DF for male and female mice is more than 21.5 g/kg. The results suggest that DF cookies were non-toxic.

Table 5.2 Death rate of rats in control group and rats treated with diets containing 2.5% and 15.0% (w/w) of CDF for 14 days.

Group/Dose % (w/w)	**Death rate**[a]		**Side effect**
	Male	**Female**	
Control	0/5	0/5	No[b]
2.5	0/5	0/5	No
15.0	0/5	0/5	No

[a] Death rate = amount of rat deaths in this experiment/total rats in this experiment.
[b] No = No side effect during experiment.

Average daily consumption and body weight gain of rats are shown in Table 5.3. Control group has the highest food consumption which is significantly different (p>0.05) between diets containing 2.5% and 15.0% (w/w) of CDF in both sexes. This may be due to the high content of DF could cause hardness texture and changes in the diet's quality. The body weight gain of male rats is not different for either group after 7 and 14 days. Although the body weight gain of female rats was not different for

either group for 7 days, 15% of the group was lower than control group (p<0.05) for 14 days. These results support the previous reports of Gao et al. (2013).

Table 5.3 Average daily consumption and body weight gain of rats treated with diets containing 2.5% and 15.0% (w/w) of CDF for 14 days.

Sex	Group/Dose % (w/w)	Consumption (g/day)	Body weight gain (g)	
			Day 7	Day 14
Male	Control	21.04 ± 1.46[a]	21.33 ± 1.15[a]	20.33 ± 1.53[a]
	2.5	20.32 ± 1.50[b]*	21.20 ± 1.79[a]	18.20 ± 2.17[a]
	15.0	19.95 ± 1.65[b]*	18.60 ± 4.83[a]	15.40 ± 4.04[a]
Female	Control	17.85 ± 1.21[a]	12.80 ± 2.28[a]	12.40 ± 2.51[a]
	2.5	17.16 ± 1.43[b]*	13.80 ± 2.17[a]	11.00 ± 2.24[a]
	15.0	17.11 ± 1.34[b]*	12.00 ± 2.74[a]	8.60 ± 1.67[b]*

Values were means ± SD.

* p<0.05 vs. control group.

They found that the body weights of females fed with diets containing 5% (w/w) of rice hull fiber tend to be lower than the other group (0%, 2.5% and 3.75%). They suggest that dietary fiber can help in weight management. Because it does not contribute energy, it reduces the energy density of foods, consequently the weight gain is reduced. Overall, increasing of body weight after 14 days of male and female rats increased as usual when compared with the control group, which proves that CDF may not have significant effects on the growth of rats.

5.4.1.2 Hematology parameters

Compared with the control group, none of the haematology parameters

(Table 5.4) of both male and female rats in each dose group showed any significant differences (p>0.05) during the experiment. These results prove that CDF has no significant effects on the haematology parameters of rats.

5.4.1.3 Blood Chemistry

The serum biochemical parameters of male and female rats were also determined (Table 5.5). For male rats, serum total cholesterol in 2.5% group was significantly lower than that of the control group (p<0.05). While triglyceride in 15.0% group was also significantly lower than the control group (p<0.05). With regard to the female rats, blood urea nitrogen and serum triglyceride in 2.5% group was significantly lower than for the control group (p<0.05), while triglyceride in 15.0% group was highly significant lower than for the control group (p<0.01). As the results above indicate there is no toxic effect from CDF on rats. Moreover, CDF tends to decrease blood triglyceride, cholesterol and glucose concentration. We recommend that dietary fiber from cassava pulp can be used to reduce the risks of cardiovascular disease and diabetes in humans.

5.4.1.4 Organ weights

The gross morphological findings of the visceral organs of both male and female rats were normal (data not shown). The relative organ weights of male and female rats are shown in Table 5.6. The results show that the relative organ weight of male rats in both groups are not significantly different for the lungs, heart, liver, spleen, left adrenal gland and testis (p>0.05) while there was significant difference between the CDF diets and the control group for the right kidney (p<0.05). The relative organ weight for female rats in both groups were not significantly different for

the lungs, heart, liver, spleen, adrenal glands and ovaries (p>0.05). These results suggest that there is no toxic effect of CDF on any of the visceral organs of rats.

5.4.1.5 Histopathological examination

The livers of the rats were sliced into sections for histological examination (Figure 5.1 and 5.2). The internal structure of the liver cells of rats in each group for both males and female was clear and no expansion of cells or inflammatory cell infiltration was found. Furthermore, no degeneration or necrosis of the liver cells was observed. The structure of the hepatic lobular was normal and clear which means no expansion of the central vein or sinusoids was found.

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Table 5.4 Hematological parameters in rats treated with diets containing 2.5% and 15.0% (w/w) of CDF for 14 days.

Parameters	Relative organ weight (g/100 g)					
	Group/Dose (% w/w)					
	Male			Female		
	Control	2.5	15.0	Control	2.5	15.0
Hb (g/dL)	16.60 ± 1.60[a]	16.38 ± 0.79[a]	16.34 ± 0.79[a]	16.80 ± 1.32[a]	16.03 ± 1.32[a]	17.12 ± 0.86[a]
Hct (%)	49.87 ± 4.06[a]	49.30 ± 2.03[a]	49.18 ± 2.78[a]	48.90 ± 3.65[a]	47.28 ± 2.78[a]	50.10 ± 2.62[a]
WBC ($\times 10^3$ cell/mm^3)	6.91 ± 2.13[a]	6.26 ± 1.88[a]	6.31 ± 3.53[a]	5.84 ± 1.97[a]	6.15 ± 1.61[a]	8.72 ± 3.41[a]
PMNs (%)	22.67 ± 8.08[a]	18.00 ± 8.49[a]	17.60 ± 4.56[a]	22.80 ± 4.60[a]	23.50 ± 9.29[a]	16.80 ± 5.59[a]
Lymphocyte (%)	72.67 ± 7.02[a]	74.80 ± 6.42[a]	77.40 ± 2.41[a]	72.40 ± 5.18[a]	72.00 ± 9.93[a]	82.40 ± 5.18[a]
Monocyte (%)	4.00 ± 0[a]	5.00 ± 1.15[a]	3.80 ± 2.28[a]	4.00 ± 1.41[a]	4.50 ± 1.91[a]	3.60 ± 1.67[a]
Eosionphil (%)	1.33 ± 2.31[a]	2.40 ± 0.89[a]	0.80 ± 1.10[a]	1.60 ± 1.67[a]	1.50 ± 1.00[a]	1.20 ± 1.10[a]
MCV (fl.)	54.73 ± 2.40[a]	55.26 ± 2.60[a]	54.36 ± 2.39[a]	53.84 ± 0.74[a]	53.78 ± 1.52[a]	53.10 ± 1.15[a]
MCH (pg.)	18.23 ± 0.55[a]	18.34 ± 0.75[a]	18.04 ± 0.46[a]	18.48 ± 0.28[a]	18.20 ± 0.34[a]	18.14 ± 0.36[a]
MCHC (%)	33.23 ± 0.51[a]	33.22 ± 0.37[a]	33.26 ± 0.76[a]	34.36 ± 0.56[a]	33.88 ± 0.43[a]	34.16 ± 0.22[a]
Platelet count ($\times 10^3$ cell/mm^3)	832.67 ± 84.01[a]	847.20 ± 4.55[a]	778.20 ± 113.2[a]	804.04 ± 117.10[a]	742.50 ± 154.31[a]	962.40 ± 7.27[a]
RBC count	9.14 ± 1.15[a]	8.94 ± 0.65[a]	9.06 ± 0.60[a]	8.09 ± 2.71[a]	8.81 ± 0.75[a]	9.43 ± 0.35[a]

Values were means ± SD.
* $p<0.05$ vs. control group.

Table 5.5 Serum biochemical parameters in rats treated with diets containing 2.5% and 15.0% (w/w) of CDF for 14 days.

Parameters	Relative organ weight (g/100 g)					
	Group/Dose (% w/w)					
	Male			Female		
	Control	2.5	15.0	Control	2.5	15.0
ALP (U/L)	95.00 ± 17.69[a]	120.00 ± 19.76[a]	114.60 ± 16.09[a]	65.60 ± 10.33[a]	74.00 ± 20.74[a]	60.40 ± 11.01[a]
ALT (U/L)	43.67 ± 24.99[a]	24.80 ± 4.55[a]	28.20 ± 2.28[a]	24.60 ± 3.97[a]	26.40 ± 7.44[a]	19.80 ± 3.83[a]
AST (U/L)	126.00 ± 15.00[a]	108.80 ± 17.57[a]	120.80 ± 47.92[a]	146.00 ± 44.82[a]	228.00 ± 96.29[a]	179.60 ± 70.22[a]
BUN (mg/dL)	20.33 ± 1.15[a]	20.00 ± 3.54[a]	18.80 ± 1.30[a]	27.40 ± 3.78[a]	21.20 ± 2.39[b]*	23.80 ± 5.07[a]
Creatinine (mg/dL)	0.54 ± 0.02[a]	0.50 ± 0.04[a]	0.51 ± 0.06[a]	0.72 ± 0.10[a]	0.62 ± 0.08[a]	0.71 ± 0.07[a]
TC (mg/dL)	82.33 ± 3.51[a]	69.60 ± 4.83[b]*	75.40 ± 7.99[a]	72.80 ± 9.83[a]	63.20 ± 10.08[a]	65.60 ± 10.43[a]
TG (mg/dL)	108.00 ± 34.22[a]	83.80 ± 12.77[a]	71.00 ± 13.00[b]*	116.60 ± 16.24[a]	82.40 ± 26.71[b]*	64.00 ± 7.31[c]**
Glucose (mg/dL)	326.00 ± 56.67[a]	275.20 ± 93.18[a]	305.20 ± 58.0[a]	185.20 ± 85.51[a]	160.40 ± 68.91[a]	131.80 ± 38.66[a]

Values were means ± SD.
* $p<0.05$ vs. control group.
** $p<0.01$ vs. control group.

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Table 5.6 Visceral organ weights of male and female rats treated with diets containing 2.5% and 15.0% (w/w) of CDF for 14 days.

Organs		Relative organ weight (g/100 g)					
		Group/Dose (% w/w)					
		Male			Female		
		Control	2.5	15.0	Control	2.5	15.0
Lung		0.43 ± 0.03[a]	0.51 ± 0.06[a]	0.46 ± 0.08[a]	0.59 ± 0.07[a]	0.54 ± 0.04[a]	0.62 ± 0.04[a]
Heart		0.33 ± 0.02[a]	0.33 ± 0.01[a]	0.34 ± 0.05[a]	0.40 ± 0.04[a]	0.37 ± 0.04[a]	0.38 ± 0.02[a]
Liver		2.96 ± 0.15[a]	3.04 ± 0.04[a]	3.04 ± 0.09[a]	2.98 ± 0.25[a]	3.26 ± 0.30[a]	3.09 ± 0.12[a]
Spleen		0.22 ± 0.03[a]	0.22 ± 0.03[a]	0.23 ± 0.06[a]	0.29 ± 0.02[a]	0.30 ± 0.02[a]	0.29 ± 0.03[a]
Kidney	L	0.32 ± 0.02[a]	0.33 ± 0.02[a]	0.33 ± 0.02[a]	0.35 ± 0.02[a]	0.35 ± 0.01[a]	0.34 ± 0.03[a]
	R	0.30 ± 0.00[a]	0.33 ± 0.03[b]*	0.33 ± 0.02[b]*	0.34 ± 0.01[a]	0.33 ± 0.02[a]	0.34 ± 0.03[a]
Adrenal gland	L	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]
	R	0.01 ± 0.00[a]	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.03 ± 0.00[a]	0.03 ± 0.00[a]	0.03 ± 0.01[a]
Testis[a]/Ovaries[b]	L	0.51 ± 0.09[a]	0.61 ± 0.06[a]	0.57 ± 0.04[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]
	R	0.50 ± 0.11[a]	0.60 ± 0.07[a]	0.58 ± 0.03[a]	0.03 ± 0.00[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]

Values were means ± SD.
[a] Testis for Male rats, [b] Ovaries for Female rats.
* $p<0.05$ vs. control group.

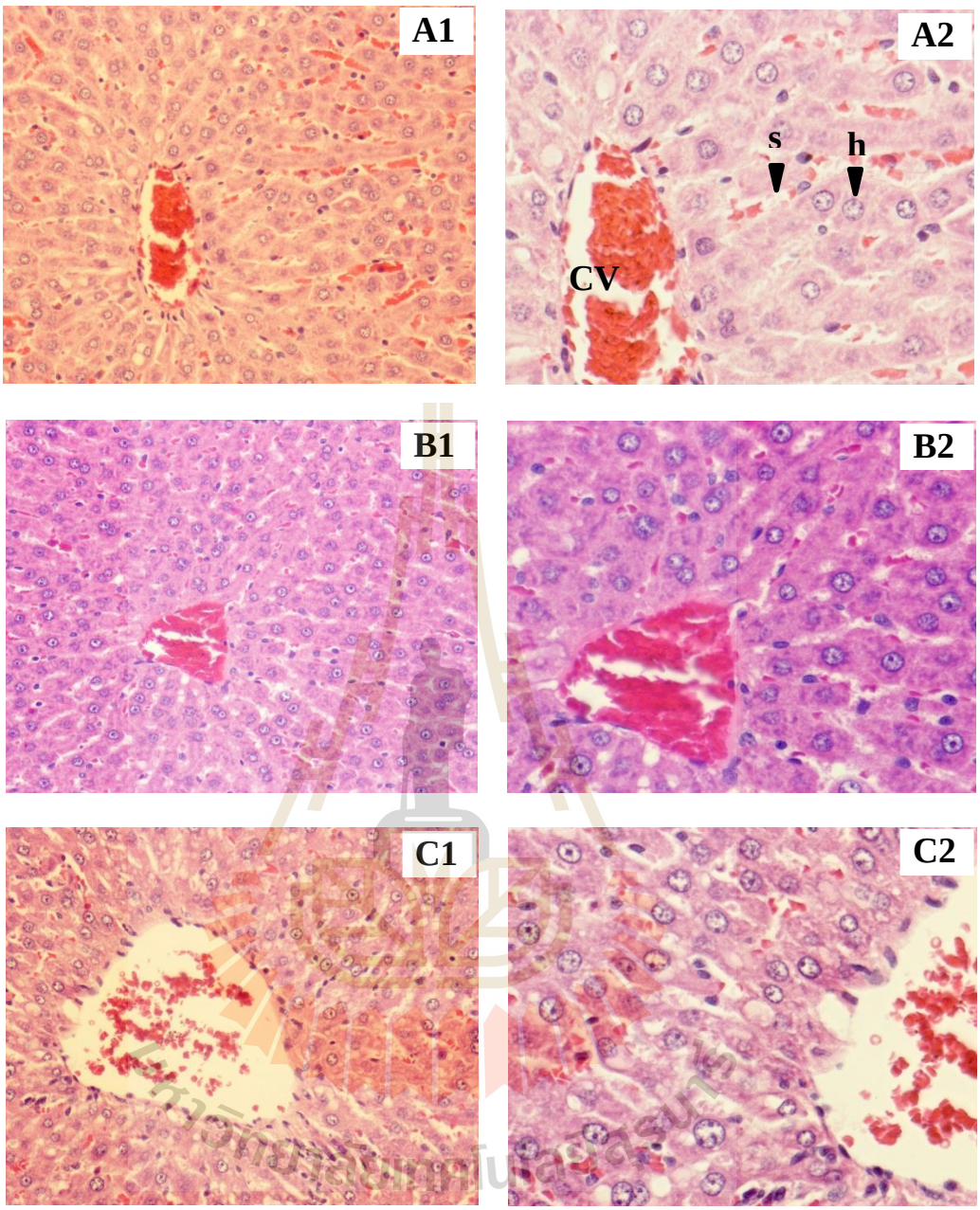

Figure 5.1 Tissue section of male liver rats; control (A1, B1), low dose (A2, B2), high dose (C1, C2); h = hepatocyte, cv = central vein, s = sinusoid. (Magnified × 200 for A1-C1 and × 400 for A2-C2).

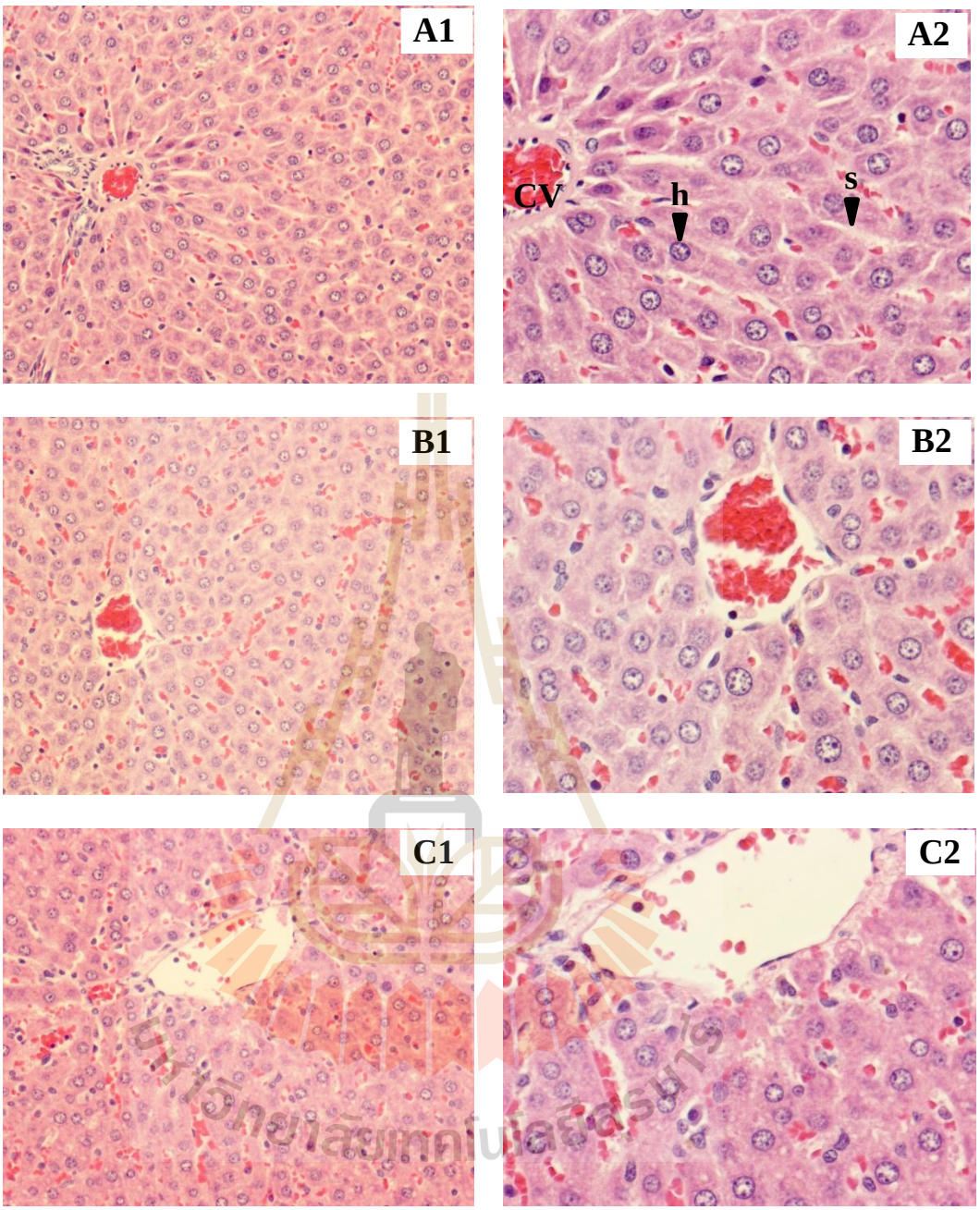

Figure 5.2 Tissue section of female liver rats; control (A1, B1), low dose (A2, B2), high dose (C1, C2); h = hepatocyte, cv = central vein, s = sinusoid. (Magnified×200 for A1-C1 and×400 for A2-C2).

5.4.2 Cholesterol-lowering properties of dietary fiber

5.4.2.1 Food intake, body weight gain and fresh fecal mass

Hyperlipidemia and hypercholesterolemia are the major risk factor that can progress of coronary artery diseases and development of atherosclerotic lesions. As increasing of lipid contents is a factor leading to the pathogenesis of atherosclerosis associated cardiac disorders (Shinde et al., 2013; Yoon et al., 2008). The induction of hyperlipidemia, principally hypercholesterolemia by feeding experimental animals with high cholesterol diets, have been suggested by many researches as a reliable model for atherosclerosis in humans (Shinde et al., 2013). Increased of lipid level especially hypercholesterolemia results because of extended absorption in the gut or endogenous synthesis (Hirunpanich et al., 2006). Simvastatin was used as positive control in this present study, due to it is potent hypolipidemic drug with mechanism of action and effects were inhibition of HMG-COA reductase, the rate limiting step in cholesterol biosynthesis, and resultant increase in LDL receptors (Shinde et al., 2013).

In this experiment, we compared the results between the normal group (rat fed basal diet) and the group of rats fed with high fat diets to induce hypercholesterolemia. This diet was composed of: fiber free diet (control), fiber free diets together with simvastatin (10 mg/kg/day) which is a commercial medicine used to lower cholesterol levels, 5% of cassava dietary fiber (CDF) containing diet and 5% of cellulose containing diet. Food intake, body weight gain and fresh fecal mass of the rats are shown in Table 5.7. On the basis of daily observations, the rats behaved actively and healthily throughout the experiment. After 30 days of feeding, the normal group had consumed the highest amount but there was no difference between the CDF

and cellulose groups (p>0.05). On the other hand, the control and simvastatin groups had consumed significantly lower amounts of food than the other groups (p<0.05). The results show that the experiment diets with or without fiber have similar results for food intake. The simvastatin group showed significantly the lowest body weight gained when compared with the other groups (p<0.05). This might be due to the side effects of simvastatin and the daily gavage which could have led to stomach irritation in the rats' eating behavior. Rat fecal mass in the group fed on a high fat diet was significantly lower than for the normal group (p<0.05). These results suggest that the high fat diet might disrupt the rats' excretory system.

Table 5.7 Food intake (Average daily consumption), body weight gain and fresh fecal mass (g) of male rats fed various diets for 30 days.

Parameter	**Group (mean ± S.D.)**				
		Hyperlipidemia rats			
	Normal	**Control**	**Simvastatin**	**CDF**	**Cellulose**
Food intake (g/day)	20.26 ± 2.71^{a}	18.45 ± 4.00^{b}	16.48 ± 3.13^{c}	19.60 ± 3.90^{a}	19.72 ± 3.22^{a}
Body weight gain (g)	97.50 ± 7.58^{a}	99.17 ± 18.0^{a}	65.83 ± 13.57^{b}	96.67 ± 13.66^{a}	100 ± 7.89^{a}
Fecal mass (g/day)	4.68 ± 1.38^{a}	2.35 ± 2.12^{b}	2.47 ± 2.53^{b}	2.76 ± 1.27^{b}	3.07 ± 1.28^{b}

Mean with different superscripted letters in the same row differ significantly (p<0.05).

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5.4.2.2 Biochemical assays of serum cholesterol, triglyceride and glucose

As shown in Table 5.8, the concentrations of serum total cholesterol, serum triglyceride, serum HDL cholesterol, serum LDL cholesterol and glucose of rats were investigated. The initial serum total cholesterol was 68.33-84 mg/dL, serum triglyceride was 69.50-89.83 mg/dL, HDL cholesterol was 46.17-55.0 mg/dL and glucose was 98.50-115.17 mg/dL. LDL cholesterol content could not be detected, which may be due to the limitation of equipment. At the end of feeding (30 days), the results showed a significant (p<0.05) difference in the serum total cholesterol and HDL cholesterol levels of the normal and high fat diet groups. When compared with the control group, the consumption of CDF and the simvastatin group significantly (p<0.05) lowered the serum total cholesterol level by 9.18% and 11.0%, respectively (Table 5.8), while the cellulose group showed no significant difference in the total cholesterol level. These results reveal that the addition of CDF in diets can effectively decrease the serum total cholesterol level (including the hypocholesterolemic effect of CDF being much greater than the cellulose effect, but most similar to simvastatin). The dietary fiber derived from other agricultural products (such as sugar beet pulp, apple pomace, peel of sweet orange, and carrot pomace etc.) may also have hypocholesterolemic and hypolipidemic properties. Moreover, different sources of fibers may have significantly different effects on plasma lipids. Some research attempts have also indicated that the hypolipidemic and hypocholesterolemic effects of fibers might depend on their types, qualities, sources, and physicochemical properties (Chau and Cheung, 1999; Chau, Huang and Lin, 2004; Hsu et al., 2006; Leontowicz et al., 2001; Martín-Carrón et al., 1999; Shorey et al., 1985). According to

Furda (1990), the fibers with higher in cation-exchange capacity could entrap, destabilize and break down the emulsion of lipid, subsequently decreasing the dispersion and absorption of cholesterol and lipids in the small intestine. Thus, the absorption and utilization of lipid and cholesterol in the intestinal system could not be effective, consequently the hypocholersterolemic effect of fibers. The higher cation-exchange capacity of CDF relative to the cellulose (Table 3.7) might therefore partially explain their cholesterol-lowering effects (Tables 5.8). In future study, it is worth investigating in depth the effects of cassava pulp fiber on lipoprotein cholesterol metabolism.

When compared to the control group, the simvastatin, CDF, and cellulose groups show a significant decrease ($p<0.05$) in the level of serum triglyceride with 49.0%, 51.3%, and 49.0%, respectively. Davignon and Cohn (1996) indicated that decreasing plasma triglyceride levels are associated with a lower risk of coronary heart disease. Moreover, dietary fiber reduced the triglyceride level which may be due to the direct interference of triglyceride absorption as well as the increase of triglyceride excretion via fecal fat (Miettinen, 1987).

The consumption of the CDF group maintained HDL cholesterol levels at the same level as the simvastatin and cellulose consumption groups ($p<0.05$), while showing higher content of HDL cholesterol than the control group ($p>0.05$). A previous study by Uberoi, Vadhera and Soni (1992) illustrated that the lowering of the total amount of cholesterol might be generally related to LDL and VLDL cholesterol fractions. In this study, the results show a 51.3% reduction in the serum triglyceride level (Table 5.8) which is possibly due to the reduced adsorption of VLDL and LDL, leading to lower levels of circulating forms of triglyceride.

As shown in Table 5.8, there was no significant (p>0.05) difference in the glucose content of the CDF, normal, control, and cellulose groups. In contrast, the simvastatin group had a lower glucose level than the other groups (p<0.05).

5.4.2.3 Total lipids content in liver and feces

A comparison between the normal and hyperlipidemic groups for liver and fecal total lipids is shown in Figure 5.3. The results indicate that there is a significant (p<0.05) difference between the control and CDF groups in the total amount of lipids (both groups had shown high content of total lipids in rats that were fed high fat diets for 30 days). Thus, the high fat diets induce an accumulation of fat in rats' livers. The level of liver total lipids obtained from the CDF and simvastatin groups is significantly (p<0.05) lower than the total lipid levels of the control and cellulose groups. Therefore, CDF prevents the accumulation of lipids in the livers of animals consuming a high-fat diet; this demonstrates a hepatoprotective effect. With regard to the feces, no significant differences were observed between the CDF and simvastatin groups, which had higher total lipid levels than the other groups. The results indicated that CDF causes an increase in the excretion of lipids via feces.

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Table 5.8 The concentration of serum total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol and glucose of male rats at initial day and day 31th.

Parameter (mg/dL)	Group (mean ± S.D.)				
	Normal	Hyperlipidemia rats			
		Control	Simvastatin	CDF	Cellulose
			At day 0th		
Total cholesterol	84.0 ± 3.46^{a}	80.67 ± 6.86^{a}	82.17 ± 12.25^{a}	73.83 ± 9.15^{ab}	68.33 ± 8.7^{b}
Triglyceride	82.50 ± 12.50^{ab}	69.50 ± 5.09^{c}	84.17 ± 13.21^{a}	89.83 ± 5.15^{a}	88.33 ± 7.06^{a}
HDL cholesterol	55.0 ± 6.0^{a}	52.67 ± 3.14^{ab}	54.83 ± 7.55^{a}	49.33 ± 6.31^{ab}	46.17 ± 5.71^{b}
LDL cholesterol	< 30	< 30	< 30	< 30	< 30
Glucose	115.17 ± 4.75^{a}	99.83 ± 5.98^{bc}	98.50 ± 12.66^{c}	110.33 ± 10.01^{ab}	1 00.67 ± 8.12^{bc}
			At day 31th		
Total cholesterol	77.67 ± 6.59^{c}	147.0 ± 12.44^{a}	130.83 ± 15.28^{b}	133.50 ± 4.93^{b}	141.17 ± 9.20^{ab}
Triglyceride	97.0 ± 12.38^{b}	187.17 ± 11.32^{a}	95.67 ± 9.33^{b}	91.17 ± 8.28^{b}	95.50 ± 7.20^{b}
HDL cholesterol	43.33 ± 7.66^{a}	28.17 ± 6.91^{c}	36.67 ± 3.67^{b}	35.17 ± 4.22^{b}	32.83 ± 4.79^{bc}
LDL cholesterol	< 30	< 30	< 30	< 30	< 30
Glucose	189.50 ± 19.59^{b}	212.17 ± 13.36^{a}	171.33 ± 8.19^{c}	203.83 ± 4.96^{ab}	202.33 ± 10.33^{ab}

Mean with different superscripted letters in the same row differ significantly (p<0.05)

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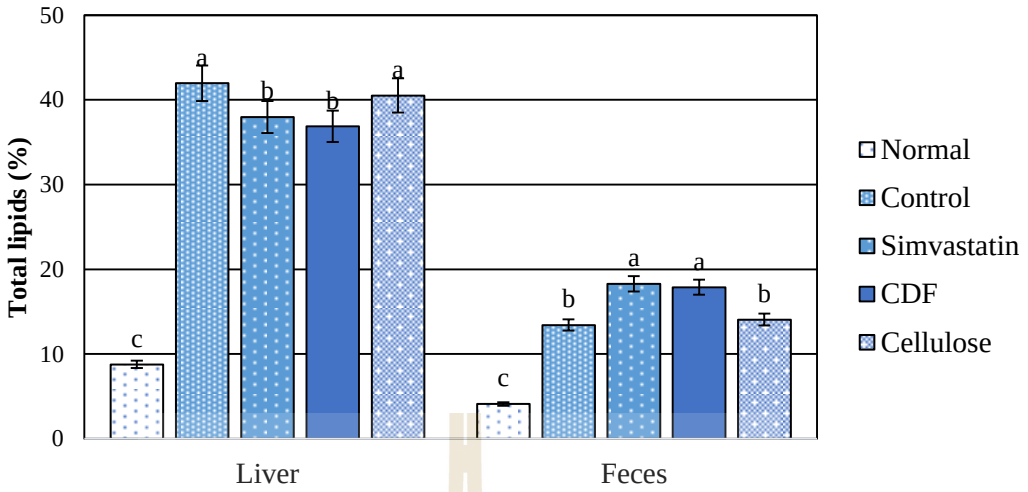

Figure 5.3 Total lipids content (%) in liver and feces of male rats fed various diets for 30 days.

5.4.2.4 Cholesterol content in liver and feces

The results of cholesterol content were also the same as for total lipids. A comparison between normal and hyperlipidemic groups for liver and fecal cholesterols is shown in Figure 5.4. The results indicate that the normal group had significantly (p<0.05) lower cholesterol content than the group of rats fed with a high fat diet. The level of liver cholesterol obtained from the CDF and simvastatin groups is significantly (p<0.05) lower than that for the control and cellulose groups. With regard to the cholesterol content in feces, no significant difference was observed between the CDF and simvastatin groups. In addition, both the control and cellulose groups had a lower cholesterol level in feces than the CDF and simvastatin groups. When compared to control, the results indicate that CDF decreases serum triglyceride and total cholesterol (Table 5.8) which is similar to total liver lipids and cholesterol content (Figure 5.3 and 5.4). These results may be due to the promoted excretion of

cholesterol and lipids in stools as the data shows a higher cholesterol content in the feces.

According to the chemical composition of CDF (Table 3.5), it contains high amount of NDF which mean cellulose, hemicellulose and lignin. Hemicellulose has an important physical property is cation exchange capacity. Hemicelluloses could prevent cholesterol absorption by directly bind cholesterol in the intestine. Moreover, microflora in the gut can digest hemicelluloses increasing the number of beneficial bacteria and creating short-chain fatty acids which colon cells use as fuel and decrease cholesterol (Jalili, Medeiros and Wildman, 2007; Mudgil and Barak, 2013; Mudgil, Barak and Khatkar, 2012).

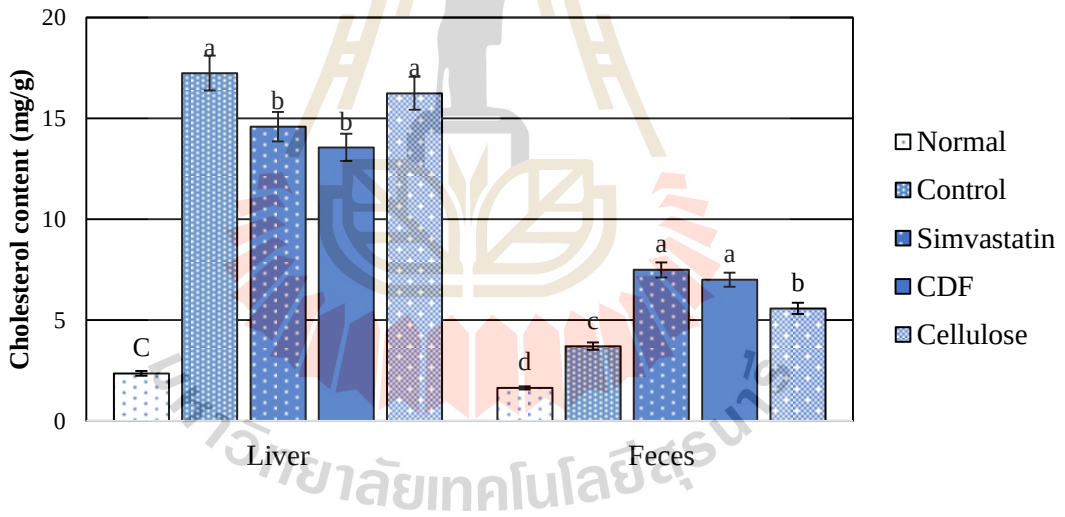

Figure 5.4 Cholesterol content (mg/g) in liver and feces of male rats fed various diets for 30 days.

5.4.2.5 Gross morphological finding of visceral organ

The gross morphological finding of the visceral organ was normal except for the livers (data not shown). The visceral organ weights of rats are shown in Table 5.9. The results show that the relative organ weight of rats is not significantly

different for lungs, heart, spleen, kidney, left and right adrenal gland and left and right testis (p>0.05) while there is a significant difference between the normal groups and the rats fed on a high-fat diet for liver (p<0.05).

Table 5.9 Visceral organ weights of male rats fed various diets for 30 days.

Organs		Relative organ weight (g/100 g)				
		Normal	Hyperlipidemia rats			
			Control	Simvastatin	DF	Cellulose
Lung		0.41 ± 0.04^{a}	0.47 ± 0.07^{a}	0.51 ± 0.12^{a}	0.45 ± 0.06^{a}	0.49 ± 0.12^{a}
Heart		0.34 ± 0.05^{a}	0.34 ± 0.04^{a}	0.36 ± 0.03^{a}	0.33 ± 0.03^{a}	0.32 ± 0.01^{a}
Liver		3.31 ± 0.20^{b}	5.06 ± 0.29^{a}	4.80 ± 0.30^{a}	5.17 ± 0.40^{a}	5.05 ± 0.39^{a}
Spleen		0.21 ± 0.01^{b}	0.36 ± 0.10^{a}	0.27 ± 0.06^{ab}	0.33 ± 0.12^{a}	0.31 ± 0.08^{ab}
Kidney	L	0.31 ± 0.02^{a}	0.28 ± 0.03^{b}	0.30 ± 0.03^{ab}	0.29 ± 0.01^{ab}	0.29 ± 0.02^{b}
	R	0.32 ± 0.01^{a}	0.28 ± 0.01^{b}	0.30 ± 0.01^{ab}	0.28 ± 0.01^{b}	0.29 ± 0.03^{b}
Adrenal gland	L	0.01 ± 0^{a}	0.02 ± 0^{a}	0.02 ± 0.01^{a}	0.02 ± 0^{a}	0.02 ± 0^{a}
	R	0.01 ± 0^{a}	0.02 ± 0^{a}	0.02 ± 0.01^{a}	0.01 ± 0^{a}	0.02 ± 0^{a}
Testis	L	0.50 ± 0.03^{b}	0.53 ± 0.03^{ab}	0.54 ± 0.03^{a}	0.49 ± 0.03^{b}	0.49 ± 0.04^{b}
	R	0.50 ± 0.03^{b}	0.52 ± 0.02^{ab}	0.55 ± 0.03^{a}	0.49 ± 0.03^{b}	0.49 ± 0.05^{b}

Mean with different superscripted letters in the same row differ significantly (p<0.05)

The results could be suggested that there is no toxic effect of dietary fiber on the visceral organs of rats. The liver of rats fed on a high fat diet are significantly larger than the normal group. This may be due to the effect of the formula of high fat diet from which fat accumulates in liver as shown by the previous

results (Figure 5.3, 5.4). There is no significant differences in the liver weights among the rats fed on high fat diets (Table 5.9). Variations in the appearance and weight of the livers are generally associated to the amount of cholesterol and oil consolidated in the experimental diets (Beynen et al., 1986).

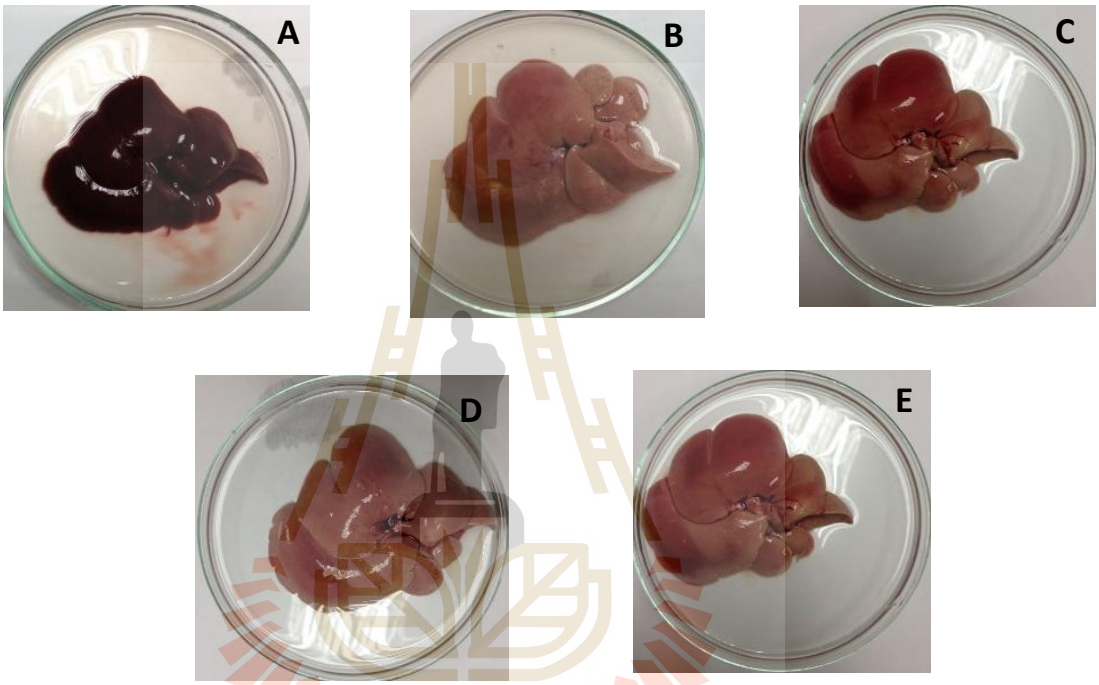

Figure 5.5 Liver from male rats fed on various diets for 30 days. (A = Normal; B = Control; C= Simvastatin; D = Cassava dietary fiber; E = Cellulose).

The gross morphology of liver rats are shown in Figure 5.5. The liver color of the normal and high fat diet group exhibited clear differences (by sight). For control, simvastatin, CDF and cellulose were paler than for the normal group. Moreover, the size of the high fat diet was larger than for the normal group as well which correlates with the result in Table 5.9. The abnormal liver color in rats fed on a high-fat diet group might be from the effect of fat accumulated in the liver. Thus, high-fat diet in

this experiment cause an increase of serum total cholesterol, serum triglyceride, liver total lipids and liver cholesterol which correlate with the results in Table 5.8, and Figure 5.3 and 5.4.

5.4.2.6 Hematogram of liver

A hematogram (hematoxylin and eosin, 20x and 40x) of a liver biopsy is exhibited in Figure 5.6. For the normal group, the liver cell was clear and no expansion of cells and no degeneration of cells were observed. The structure of hepatocyte (h) was normal and no expansion of the central veins and sinusoids (s) were found. For the group of rats fed on the high-fat diet, there was no expansion of the central veins, but they showed marked fatty degeneration of cells and the accumulation of fat in the livers could clearly be seen, especially in the control group. Moreover, some liver cells disappeared and there was an expansion of the cells. From this study, CDF revealed better liver hematograms than the other groups when compared with the normal group. Therefore, CDF may protect liver cells from the effect of fat in diets.

5.4.2.7 Short-chain fatty acids in feces

Colonic microflora normally use carbohydrates as a substrate for fermentation and the main fermentation products are short-chain fatty acids (SCFAs; acetic acid, propionic acid and butyric acid) and gases (Co_2, CH_4 and H_2). These end products are excreted in the stool or absorbed from the colon (Henningsson, Björck and Nyman, 2001). As expected, acetic acid was the predominant fatty acid in the feces of all the animals (Sembries et al., 2006; Wong et al., 2006). Acetic acid is also the most frequently found chemical in this study (Table 5.10). The CDF group had the highest content of acetic acid, propionic acid, and butyric acid. Although, the main

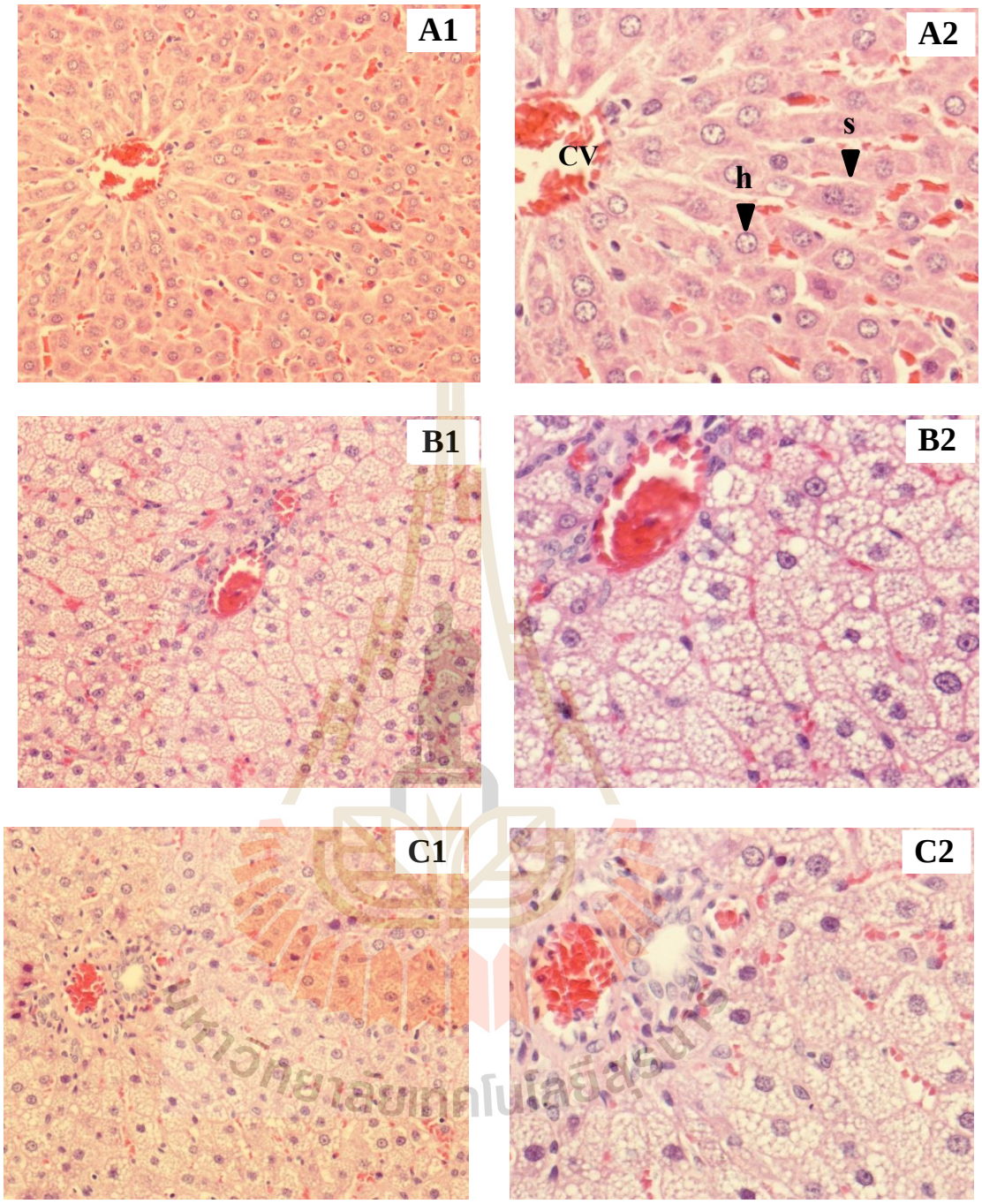

Figure 5.6 Hematogram of liver from male rats fed various diets for 30 days (A = Normal; B = Control; C= Simvastatin; D = Cassava dietary fiber; E = Cellulose; h = hepatocyte, cv = central vein, s = sinusoid). Hematoxylin and eosin staining, magnified×200 for A1-E1 and×400 for A2-E2.

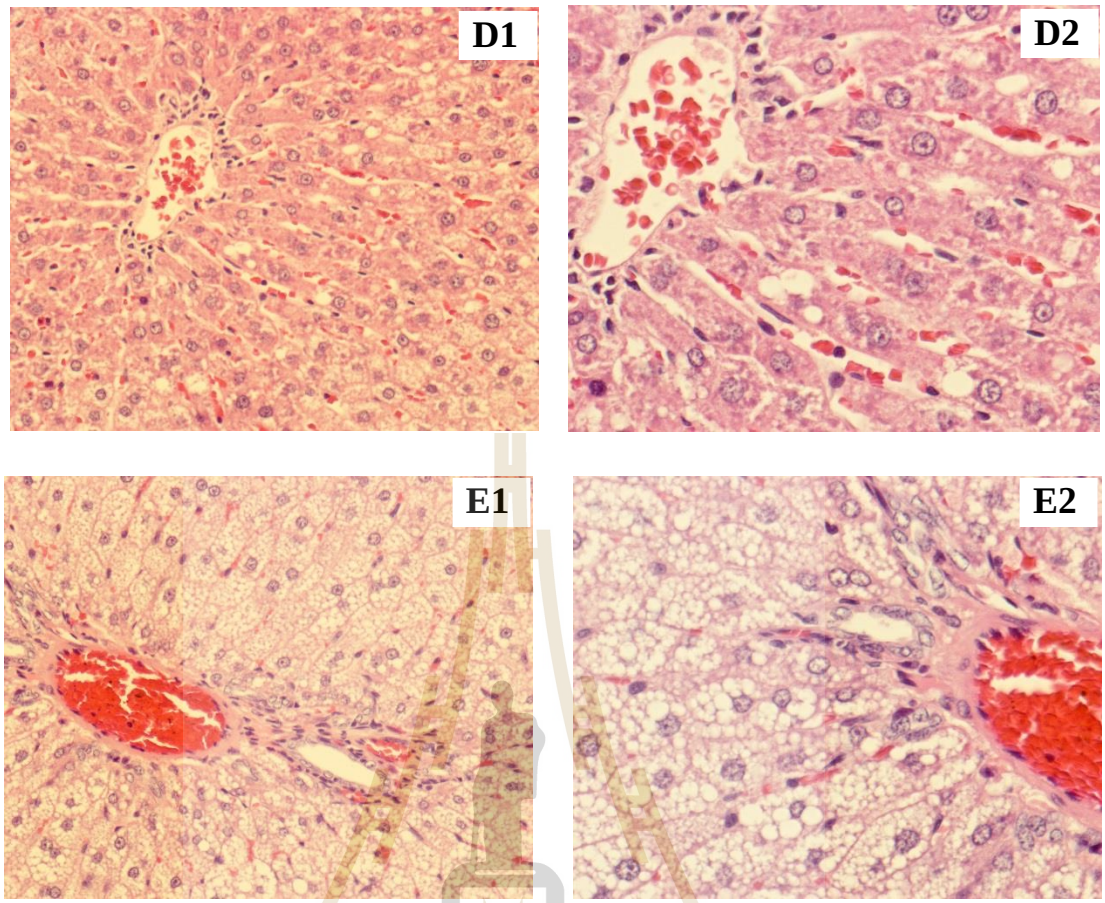

Figure 5.6 Hematogram of liver from male rats fed various diets for 30 days (A = Normal; B = Control; C= Simvastatin; D = Cassava dietary fiber; E = Cellulose; h = hepatocyte, cv = central vein, s = sinusoid). Hematoxylin and eosin staining, magnified×200 for A1-E1 and×400 for A2-E2 .

composition of CDF is in the insoluble form of dietary fiber (Table 3.5), it is often believed incorrectly that the insoluble part is not fermented by bacteria in the large intestine. Some reports reveal that the fermentability of oat bran which has high insoluble fiber (about 80% of IDF), can be fermented in the large bowel (Mongeau and Brooks, 2016) However, there were no significant differences between the normal, control, and simvastatin groups in this study. The cellulose group, on the

other hand, had (p<0.05) the lowest short-chain fatty acids content at a significant level. This may be due to cellulose being an insoluble dietary fiber which has low fermentability and low prebiotic properties. The fermentability is a unique property to each dietary fiber. This property depends on the nature and arrangement of the structure of fiber and also on its physical characteristics, for example, particle size (Mongeau and Brooks, 2016). From the results for the short-chain fatty acids of the CDF group (Table 5.10), we can conclude that the CDF group might have a fermentative capacity. The production of short-chain fatty acids in the intestine has been correlated with a reduction risk of some diseases including irritable bowel syndrome, inflammatory bowel diseases, cardiovascular disease, and cancer (Jenkins, Kendall and Vuksan, 1999; Wong et al., 2006). Moreover, it has been proposed that propionic acid may lower plasma cholesterol concentrations by inhibiting hepatic cholesterogenesis (Chen, Anderson and Jennings, 1984).

Finally, consumption of fibers could promote hypolipidemic and hypocholesterolemic actions by a combination of the physiological effects including decreased transit time, reduced rate of lipids and cholesterol absorption, higher bile acid adsorption, increased cholesterol catabolism to bile acids, and retarded cholesterol biosynthesis (Hughes, 1991; Lairon, 2008; Marlett, Cho and Dreher, 2001; Miettinen, 1987; Uberoi, Vadhera and Soni, 1992). The above-mentioned beneficial effects of insoluble dietary fiber fraction might occur when it is added at a level of not less than 5% in a hypercholesterolemic diet containing cholesterol and soybean oil at 1% and 4% levels, respectively (Chau, Huang and Lin, 2004; Hsu et al., 2006).

Table 5.10 Concentration of short chain fatty acids (SCFAs) in feces (mg/g dried feces).

Parameter (mg/g)	Group (mean ± S.D.)				
	Normal	Hyperlipidemia rats			
		Control	Simvastatin	CDF	Cellulose
Total SCFA	3.14 ± 0.58[a]	2.71 ± 1.02[ab]	2.37 ± 0.72[ab]	4.03 ± 0.76[a]	0.85 ± 0.58[b]
Acetic acid	1.88 ± 1.0[a]	1.28 ± 1.08[ab]	0.92 ± 0.65[b]	1.95 ± 1.03[a]	0.44 ± 0.54[b]
Propionic acid	0.72 ± 0.17[ab]	0.82 ± 0.67[ab]	0.78 ± 0.47[ab]	1.42 ± 0.57[a]	0.24 ± 0.38[b]
Butyric acid	0.54 ± 0.12[ab]	0.61 ± 0.33[a]	0.67 ± 0.39[a]	0.66 ± 0.09[a]	0.18 ± 0.28[b]

Mean with different superscripted letters in the same row differ significantly (p<0.05)

5.5 Conclusions

There was no deaths or abnormal behaviors of rats fed on 15% (w/w) of CDF diets in male and female rats. The CDF diet exhibited no toxic or harmful actions on the rat model. Also, the CDF diet was able to decrease the concentration of TC and TG in serum. The decreasing of TC and TG in the rat model should be an interesting topic for the future study. Based on this experiment, the no-observed-adverse-effect-level (NOAEL) for cassava pulp dietary fiber was 15% for both genders equivalent to 10.01 g/kg body weight/day for males and 11.21 g/kg body weight/day for females. The equivalent human doses would be 1.60 and 1.79 g/kg body weight/day, or 96.10 and 107.62 g/day, respectively, based on the conversion factor provided by Gao et al. (2013). Moreover, long term feeding test of CDF should be investigated. Finally, no toxic effect of cassava dietary fiber on rats, suggests that CDF could be used in human food.

The present study demonstrates that cassava dietary fiber has hypolipidemic and hypocholesterolemic effects when compared to simvastatin and cellulose. The consumption of CDF significantly (p<0.05) decreased the levels of serum triglyceride, serum total cholesterol, liver total lipids, and liver cholesterol, while it also significantly (p<0.05) increased the levels of fecal total lipids and cholesterol when compared with the control group. Moreover, a comparison between modern medicine (simvastatin) and CDF found that there were no significant (p>0.05) differences in terms of serum triglyceride, serum total cholesterol, liver total lipids, and liver cholesterol. The hypocholesterolemic and hypolipidemic effects of CDF might be correlated with the enhanced excretion of lipids and cholesterol via stools. Thus, CDF could be a potential cholesterol-lowering ingredient in food products.

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CHAPTER VI

SUMMARY

Cassava pulp could be a new source of dietary fiber production by enzymatic extraction at the optimal conditions of 0.1% w/v, 1% v/v and 0.1% v/v for concentration of α-amylase, neutrase and amyloglucosidase enzymes, respectively. In addition, dietary fiber prepared from cassava pulp (CDF) contained 79.03% neutral detergent fiber (NDF: Cellulose, Hemicellulose, Lignin) and a high content of cellulose. The hydration properties of CDF such as swelling, water retention capacity and water holding capacity showed values of 4.82 mL/g, 8.36 g/g dry weight and 8.17g/g, respectively. Finally, there was no starch granule embedded inside the fiber matrix that suggests a high porosity of the fiber structure. According to *in vitro* study, CDF had potential to bind lard, cholesterol and bile acid which correlated to cholesterol-lowering property. Moreover, assessment of prebiotic potential for some *Lactobacillus* strains showed CDF has prebiotic activity for *Lactobacillus plantarum* TISTR 1465 and might be act as prebiotic substance.

There was no deaths or abnormal behaviors of rats fed with 15% (w/w) of CDF diets in male and female rats. The CDF diet exhibited no toxic or harmful actions on the rat model. Also, the CDF diet was able to decrease the concentration of tatal cholesterol and triglyceride in serum. Based on this experiment, the no-observed-adverse-effect-level (NOAEL) for CDF was 15% for both genders equivalent to 10.01 g/kg body weight/day for males and 11.21 g/kg body weight/day for females. The

equivalent human doses would be 1.60 and 1.79 g/kg BW/d, or 96.10 and 107.62 g/day, respectively, based on the conversion factor provided by Gao et al. (2013). Finally, no toxic effect of CDF on rats, suggests that it could be used in human food. The present study demonstrates that CDF has hypolipidemic and hypocholesterolemic effects when compared to simvastatin and cellulose. The consumption of CDF significantly (p<0.05) decreased the levels of serum triglyceride, serum total cholesterol, liver total lipids and liver cholesterol, while it also significantly (p<0.05) increased the levels of fecal total lipids and cholesterol when compared with the control group. Moreover, a comparison between modern medicine (simvastatin) and CDF found that there were no significant (p>0.05) differences in terms of serum triglyceride, serum total cholesterol, liver total lipids, and liver cholesterol. The hypocholesterolemic and hypolipidemic effects of CDF might be correlated with the enhanced excretion of lipids and cholesterol via stools. Thus, CDF could be a potential cholesterol-lowering ingredient in food products.

มหาวิทยาลัยเทคโนโลยีสุรนารี

BIOGRAPHY

Ms. Pornariya Chirinang was born in May 27, 1979 in Roi-et Province, Thailand. She earned Bachelor's Degree in B.Sc. (Food Technology) from School of Food Technology, Institute of Agricultural Technology, Suranaree University of Technology in 2001. Then, she started for her Master degree (Food Technology) in 2002 and graduated in 2006 at Suranaree University of Technology. Her research was Acid, alkaline and enzymatic hydrolysis of mushroom for flavored sauce production. The results from this research have been published; Chirinang, P. and Intarapichet, K-O. (2009). Amino acids and antioxidant properties of the oyster mushrooms, *Pleurotus ostreatus* and *Pleurotus sajor-caju*. ScienceAsia. 35, 326-333. In 2010, she attended Ph.D. Program at School of Food Technology, Suranaree University of Technology, Thailand. During her graduate study, she obtained opportunities to present her research work at the 5th International Conference on Natural Products for Health and Beauty (Phuket, Thailand, 6-8th May, 2014) and at 2016 International Conference on Food Properties (Bangkok, Thailand, May 31th-June 2nd, 2016).