UTILIZATION OF EXTRACTED DIETARY FIBER FROM CASSAVA PULP AND CASSAVA DISTILLER'S DRIED GRAINS AS FEED SUPPLEMENT FOR BROILER CHICKENS

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การใช้ประโยชน์ของใยอาหารที่สกัดได้จากกากมันสำปะหลังและกากมันเอทานอล เพื่อเป็นสารเสริมสำหรับไก่เนื้อ

นางสาวสุภัตรา โอกระโทก

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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สุภัตรา โอกระโทก : การใช้ประโยชน์ของใยอาหารที่สกัดได้จากกากมันสำปะหลัง และกากมันเอทานอลเพื่อเป็นสารเสริมสำหรับไก่เนื้อ (UTILIZATION OF EXTRACTED DIETARY FIBER FROM CASSAVA PULP AND CASSAVA DISTILLER'S DRIED GRAINS AS FEED SUPPLEMENT FOR BROILER CHICKENS) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.สุทิศา เข็มผะกา, 122 หน้า.

การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาสภาวะการสกัดใยอาหาร และการดัดแปลงใยอาหารจากกากมันสำปะหลัง และกากมันเอทานอลให้อยู่ในรูปแบบที่เหมาะสม และการประเมินผลของการเสริมใยอาหารดัดแปลงในอาหาร ต่อประสิทธิภาพการผลิต น้ำหนักอวัยวะย่อยอาหาร การย่อยได้ของสารอาหาร คลอเรสเตอรอลในเนื้อ ปริมาณจุลินทรีย์ในซีกัม ความเข้มข้นของกรดไขมันสายสั้น และกรดแลคติก การตอบสนองของระบบภูมิคุ้มกัน และการผลิตแอมโมเนียของไก่เนื้อ โดยมีรายละเอียด ดังนี้

ศึกษาสภาวะการสกัดใยอาหารจากกากมันสำปะหลัง และกากมันเอทานอลโดยใช้สารละลายโซเดียมไฮดรอกไซด์ (2 4 6 และ 8%) และประเมินองค์ประกอบของใยอาหารที่สกัดได้โดยการใช้ฟูเรียร์ทรานส์ฟอร์มอินฟราเรด (Fourier Transform Infrared; FTIR) ผลการศึกษาพบว่าสภาวะที่เหมาะสมสำหรับการสกัดใยอาหารจากกากมันสำปะหลัง และกากมันเอทานอล คือการใช้โซเดียมไฮดรอกไซด์ที่ระดับ 6% และ 4% ตามลำดับ โดยสามารถสกัดปริมาณใยอาหารทั้งหมดและใยอาหารที่ไม่ละลายน้ำได้ผลผลิตสูงสุด ซึ่งให้ผลที่สอดคล้องกับการวิเคราะห์ปริมาณใยอาหารด้วย FTIR และการวิเคราะห์องค์ประกอบหลัก (principle component analysis; PCA) ที่พบการกระจายของสเปกตรัมแยกจากกันอย่างชัดเจน

ศึกษาสภาวะที่เหมาะสมในการดัดแปลงใยอาหารที่ได้จากกากมันสำปะหลัง และกาก-มันเอทานอล โดยย่อยโพลิเมอร์สายยาวไปเป็นโอลิโกแซ็กคาไรด์โดยใช้เอนไซม์ เซลลูเลส : ไซลาเนส ที่ระดับ 0:0 9:3 36:12 และ 72:24 หน่วย/กรัมของสารตั้งต้น และทำการประเมินใยอาหารดัดแปลงโดยใช้วิธีการหมักในหลอดทดลอง พบว่าใยอาหารจากกากมันสำปะหลังมีประสิทธิภาพที่ดีกว่ากากมันเอทานอล อัตราส่วนเอนไซม์ที่เหมาะสมสำหรับปรับปรุงใยอาหารจากกากมันสำปะหลัง คือ 36:12 หน่วย/กรัมของสารตั้งต้น โดยสภาวะการย่อยดังกล่าวพบว่ามีปริมาณน้ำตาลรีดิวซ์ (D-กลูโคส) น้ำตาลทั้งหมด และน้ำตาลไม่รีดิวซ์ (D-ไซโลส) สูงที่สุด นอกจากนี้เมื่อทำการทดสอบในหลอดทดลอง พบว่าสามารถเพิ่มประชากรจุลินทรีย์ *Lactobacillus* และ *Bifidobacterium* และเพิ่มความเข้มข้นกรดไขมันสายสั้น และกรดแลคติก และลดค่าความเป็นกรด-ด่างหลังการบ่มที่ 24 ชั่วโมง ด้วยเชื้อจุลินทรีย์จากซีกัม

ศึกษาการตอบสนองของไก่เนื้อต่อใยอาหารดัดแปลงจากกากมันสำปะหลัง ใช้ไก่เนื้อเพศผู้ (Ross 308) อายุ 1 วัน จำนวน 336 ตัว แบ่งออกเป็น 4 กลุ่ม ๆ ละ 7 ซ้ำ ๆ ละ 12 ตัว อาหารทดลองมี 4 กลุ่ม ได้แก่ กลุ่มควบคุม และกลุ่มใยอาหารดัดแปลงจากกากมันสำปะหลัง 3 ระดับ คือ 0.5 1.0 และ 1.5% โดยพบว่าใยอาหารดัดแปลงจากกากมันสำปะหลังสามารถใช้เป็นแหล่งใยอาหารในอาหารไก่เนื้อได้ โดยไม่ส่งผลเสียต่อประสิทธิภาพการผลิต การเสริมใยอาหารดัดแปลงจากกากมัน-สำปะหลัง 1.0% ในอาหารไก่เนื้อสามารถส่งเสริมการทำงานของกึ๋น เพิ่มการย่อยได้ของสารอาหาร ลดไขมันในช่องท้อง และลดคอเลสเตอรอลในเนื้อไก่ เลือด และตับ นอกจากนี้ยังส่งผลดีในการเพิ่มจำนวนประชากร Lactic acid bacteria (LAB) และ *Bifidobacterium* spp. ในซีกัม เพิ่มความเข้มข้นของกรดไขมันสายสั้น และกรดแลคติค ลดการผลิตแอมโมเนีย อย่างไรก็ตามใยอาหารดัดแปลงจากกากมันสำปะหลังไม่มีผลต่อการทำงานของอิมมูโนโกลบูลิน และไลโซไซม์ในซีรัมของไก่เนื้อ

การศึกษาครั้งนี้สามารถสรุปได้ว่าการดัดแปลงใยอาหารจะมีประโยชน์สำหรับไก่เนื้อ ซึ่งผลแสดงอย่างชัดเจนว่าใยอาหารดัดแปลงจากกากมันสำปะหลังมีประโยชน์ต่อการพัฒนาของกึ๋นและจุลินทรีย์ในทางเดินอาหารของไก่เนื้อ

สาขาวิชาเทคโนโลยีและนวัตกรรมทางสัตว์ ลายมือชื่อนักศึกษา_____

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SUPATTRA OKRATHOK : UTILIZATION OF EXTRACTED DIETARY FIBER FROM CASSAVA PULP AND CASSAVA DISTILLER'S DRIED GRAINS AS FEED SUPPLEMENT FOR BROILER CHICKENS.
THESIS ADVISOR : ASST. PROF. SUTISA KHEMPAKA, Ph.D., 122 PP.

DIETARY FIBER/MODIFIED-DIETARY FIBER/CASSAVA PULP/CASSAVA DISTILLER'S DRIED GRAINS/BROILER

This study aimed to investigate the optimal conditions for extraction and modification of dietary fiber from dried cassava pulp (DCP) and cassava distiller's dried grains (CDG), and to evaluate the effects of modified-dietary fiber supplementation in diets on productive performance, weight of digestive organs, nutrient digestibility, meat cholesterol, cecal microbial population, short chain fatty acids (SCFA), and lactic acid concentration, immune response, and ammonia production of broilers.

The extraction conditions of dietary fiber from DCP and CDG treated with NaOH solution (2, 4, 6 and 8%) were studied. Fourier Transform Infrared (FTIR) was used to determine dietary fiber components. The results showed that the optimal condition for extracting fiber from DCP and CDG were under treated with 6% and 4% NaOH, respectively. These conditions yielded the highest contents of total dietary fiber (TDF) and insoluble dietary fiber (IDF). The results were associated with the semi-quantitative analysis of FTIR spectra integration and principal component analysis (PCA), with clearly separated spectral distribution.

Study on the optimal conditions of producing dietary fiber derived from DCP and CDG through hydrolysis of polymer chains into oligosaccharides with cellulase : xylanase at levels 0:0, 9:3, 36:12 and 72:24 U/g substrate, and assessment the modified-dietary fibers

using *in vitro* fermentation method. It revealed that dietary fiber from DCP was more efficient than CDG. The cellulase : xylanase ratio at 36:12 U/g substrate possessed an optimum level of modification dietary fiber from DCP. This condition generated the highest reducing sugar (D-glucose), total sugar and non-reducing sugar (D-xylose) contents. In addition, it also enhanced the *Lactobacillus* and *Bifidobacterium* populations, SCFA and lactic acid concentrations and reduced the pH value after 24 hours of incubation with cecal microbial.

The responses of broilers to M-DFCP were studied. A total of 336, one-day-old male chicks (Ross 308) were allocated to 4 groups in 7 replicate pens with 12 chicks each. Four dietary treatments composed of control and 3 M-DFCP inclusion levels : 0.5, 1.0 and 1.5%. It indicated that the M-DFCP can be used as dietary fiber in broiler diets without reducing productive performances. The inclusion of 1.0% M-DFCP in broiler diet possessed positive effects, enhancing gizzard function, improving nutrient digestibility, reducing abdominal fat, and cholesterol in chicken meat, blood, and liver. Moreover, the M-DFCP showed the potential effects, increasing cecal LAB and *Bifidobacterium* spp. populations, enhancing SCFA and lactic acid concentrations, and reducing ammonia production. However, the M-DFCP showed no effects on serum total immunoglobulin and lysozyme activity in broiler chickens.

From this study, it can be concluded that the modification of dietary fiber would useful for broilers, it is also apparent that there are potential beneficial effects of M-DFCP on gizzard development and gut microflora of broiler chickens.

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Supattra Okrathok

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF FIGURES (Continued)

Figure **Page**

LIST OF ABBREVIATIONS

ADG	=	Average daily gain
AMEn	=	Nitrogen corrected apparent metabolizable energy
ME	=	Metabolizable energy
ANOVA	=	Analysis of variance
BW	=	Body weight
BWG	=	Body weight gain
cal	=	Calorie
COS	=	Cello-oligosaccharides
CP	=	Crude protein
°C	=	Degree Celsius
CDG	=	Dried cassava distiller's dried grains
DCP	=	Dried cassava pulp
DF-CDG	=	Cassava distiller's dried grains extracted dietary fiber
DF-CP	=	Cassava pulp extracted dietary fiber
DM	=	Dry matter
EE	=	Ether extract
FCR	=	Feed conversion ratio
FI	=	Feed intake
FTIR	=	Fourier Transform Infrared
IDF	=	Insoluble dietary fiber
g	=	Gram

LIST OF ABBREVIATIONS (Continued)

GIT	=	gastrointestinal tract
kcal	=	Kilocalorie
kg	=	Kilogram
LAB	=	Lactic acid bacteria
M-DFCDG	=	Modified-dietary fiber from cassava distiller's dried grains
M-DFCP	=	Modified-dietary fiber from cassava pulp
NSPs	=	Non-starch polysarides
OM	=	Organic matter
PC	=	Principal component
PCA	=	Principal component analysis
PI	=	Productive index
SEM	=	Standard error of the mean
SCFA	=	Short chain fatty acids
SDF	=	Soluble dietary fiber
TDF	=	Total dietary fiber
Vs.	=	Versus
XOS	=	Xylo-oligosaccharides
%	=	Percentage

CHAPTER I

INTRODUCTION

1.1 Introduction

Broiler industry is one of the most important economic sectors in Thailand. This industry has grown significantly in the last 30 years and the future trend of production will also expand into larger annually. After a ban on the use of antibiotic as a growth promoter (AGPs) in livestock, formulating broiler diet has substantially strong focused on nutrients to improve gut health and manipulate the gastrointestinal tract (GIT) development to reach the crucial goals of production (Kheravii et al., 2018; Jacquier et al., 2019). Since the removal of AGPs from diets is associated with increased enteric disorders and reduced growth performance in broiler chickens, with serious economic consequences (Yang et al., 2009; Mateos et al., 2012; Kheravii et al., 2018). Therefore, several alternative ingredients function on gut health improvement such as probiotics, prebiotics, organic acids were investigated to replace antibiotics. Nowadays, dietary fiber has gained increasing interest in nutrition fields, and considered as a functional feed supplement.

Dietary fiber is defined as non-starch polysaccharides (NSPs) which is resistant to enzymatic digestion in the poultry GIT. These polysaccharides occur naturally in feedstuffs which include cellulose, non-cellulosic polysaccharides such as hemicellulose, pectic substances, gums, mucilage, and a non-carbohydrate component lignin (Dhingra et al., 2012; Knudsen, 2014; Choct, 2015). Dietary fiber is classified

into two groups as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF), which these components vary widely depending the type of feedstuffs. Dietary fiber plays an important role in the regulation of poultry bodies as its effect depends on structures and physicochemical properties (Michard, 2011). Previous studies have shown an important role of dietary fiber in GIT development, increasing gizzard activity, stimulating digestive enzyme production, improving nutrient digestibility, enhancing hindgut microbial fermentation as a consequence of improved animal health and performance (Sarikhan et al., 2010; Kalmendal et al., 2011; Mateos et al., 2012; Walugembe et al., 2015). In addition, dietary fiber can be fermented by the resident anaerobic microflora and resulted in improved intestinal balance and enhanced short-chain fatty acids (SCFA) production. It also has the positive functions on improving the microbial population balance and promoting the gut health and immune responses in broiler chickens (van der Wielen et al., 2000; Józefiak et al., 2004; Dunkley et al., 2007; Krás et al., 2013; Zduńczyk et al., 2015; Sabour et al., 2018). Previous studies also reported the positive effects of dietary fiber on lowering abdominal fat and meat cholesterol (Saki et al., 2011) and reducing ammonia production in excreta of poultry (Roberts et al., 2007). Currently, there is a trend towards the use of dietary fiber derived from natural plant sources as a feed supplement. In which, some previously neglected agro-industrial by-products can be considered as sources of natural dietary fiber for animal feed. Cassava by-products in the form of dried cassava pulp (DCP) and cassava distiller's dried grains (CDG) are annually generated in large amount from the cassava starch and bioethanol production process, respectively in Thailand. These waste by-products contain high in quantity of NSPs (29%), mainly cellulose and xylan approximately 20 and 4.2%, respectively

(Kosugi et al., 2009; Choct, 2015; Wan et al., 2015). Thus, the dietary fiber extracts from these DCP and CDG would useful for animals. Generally, the properties of dietary fiber in animal diets are depending on the fiber type, polymer chain composition, structure, and supplementation level. Choct (2015) demonstrated that NSPs are digested via fermentation and low molecular weight NSPs would change the activity of the gut microbiota. Ravn et al. (2017) reported that treating wheat brand with exogenous xylanase before in vitro fermentation assay with caecum originated microbiome, resulted in a decreased degree of polymerization (DP) of wheat bran, and in turn enhancing SCFA production by cecal bacteria fermentation.

Therefore, processing of cassava pulp and cassava distiller's dried grain fibers by enzymatic treatment might lead to the production of a dietary fiber feed supplement with beneficial effect on animal health and production .

1.2 Research objective

1.2.1 To investigate the optimal extraction conditions of dietary fiber derive from DCP and CDG.

1.2.2 To investigate the optimal conditions for modified-dietary fiber from DCP and CDG.

1.2.3 To evaluate the effects of modified-dietary fiber supplementation in diets on productive performance, weights of digestive organs, nutrient digestibility, meat cholesterol, cecal microbial population, SCFA and lactic acid concentration, immune response, and ammonia production of broilers.

1.3 Research hypothesis

1.3.1 The optimal extraction conditions of dietary fiber and modified-dietary fiber derived from DCP and CDG can possess the beneficial effects on broiler chickens.

1.3.2 The optimal levels of modified-dietary fiber inclusion in broiler diets can improve productive performance, increase gizzard activity, improve nutrient digestibility, improve the meat quality by reducing fat and cholesterol, improve microbial population, increase SCFA and lactic acid concentration, decrease excreta ammonia excretion, and improve immunity.

1.4 Scope of the study

This study aimed to investigate the optimal extraction and modification of dietary fiber from DCP and CDG for use as a feed supplement in broiler diets .The broilers aged 0-42 days were used to investigate the effects of modified-dietary fiber inclusion in broiler diets on productive performance, weight of digestive organ, nutrient digestibility, fat and meat cholesterol, cecal microbial population, SCFA and lactic acid, ammonia productions and immune response.

1.5 Expected results

1.5.1 Obtaining the knowledge of extracting dietary fiber from DCP and CDG for use as functional feed in animal diets .This knowledge will be applied to further extraction of specific dietary fiber sources.

1.5.2 Obtaining new alternative dietary fiber sources for use as functional feed in animal diets and enhancing the utilization of cassava by-products as well as reducing an environmental load of waste management.

1.5.3 The use of modified-dietary fiber derived from cassava by-products in broiler diets would help to improve health, production performance and improve quality meat to consumers.

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CHAPTER II

LITERATURE REVIEW

2.1 Dietary fiber

The term “dietary fiber” is principally composed of plant cell walls, which consists of non-starch polysaccharides (NPSs) and lignin. The major NSPs present in plant cell walls are mostly cellulose and a wide variety of non-cellulosic polysaccharides (Choct, 1997; Dhingra et al., 2012; Knudsen, 2014). An important aspect of dietary fiber is that their carbohydrates can resist to hydrolysis by animal digestive secretions (AACC, 2001; Józefiak et al., 2004).

2.1.1 Classification of dietary fiber

Several different classification systems have been suggested to classify the components of dietary fiber, however, the most widely accepted classification for dietary fiber has been differentiated dietary components on their solubility. Thus, the most appropriately dietary fiber is classified into two categories on the basis of water solubility as soluble dietary fiber (SDF) (ß-glucans, pectin, galactomannans, and gums), and insoluble dietary fiber (IDF) (cellulose, some hemicellulose, and lignin). In addition, each polysaccharide is specifically characterized, its monomeric residues and the bond linkage, which can be classified the dietary fiber compositions by its monomers residue and type of bond (Dhingra et al., 2012; Staffolo et al., 2012). The classification for dietary fiber by enzymatic-chemical extraction techniques is shown in Figure 2.1.

Most dietary fiber sources are found in several feedstuffs such as grains, legumes, including plant cell walls and yeast cell walls, etc. The feed ingredients contain several different types of carbohydrates, monosaccharides, disaccharides, oligosaccharides (degree of polymerization 3-12 units), and polysaccharides. Generally, the monogastric animals can digest monosaccharides and disaccharides (Choct, 2015), while most oligosaccharides and some polysaccharides are digested via fermentation by microorganisms in the lower gastrointestinal tract (GIT) (den Besten et al., 2013; Regassa and Nyachoti, 2018). The fiber sources and chemical structure of dietary fiber are shown in Table 2.1.

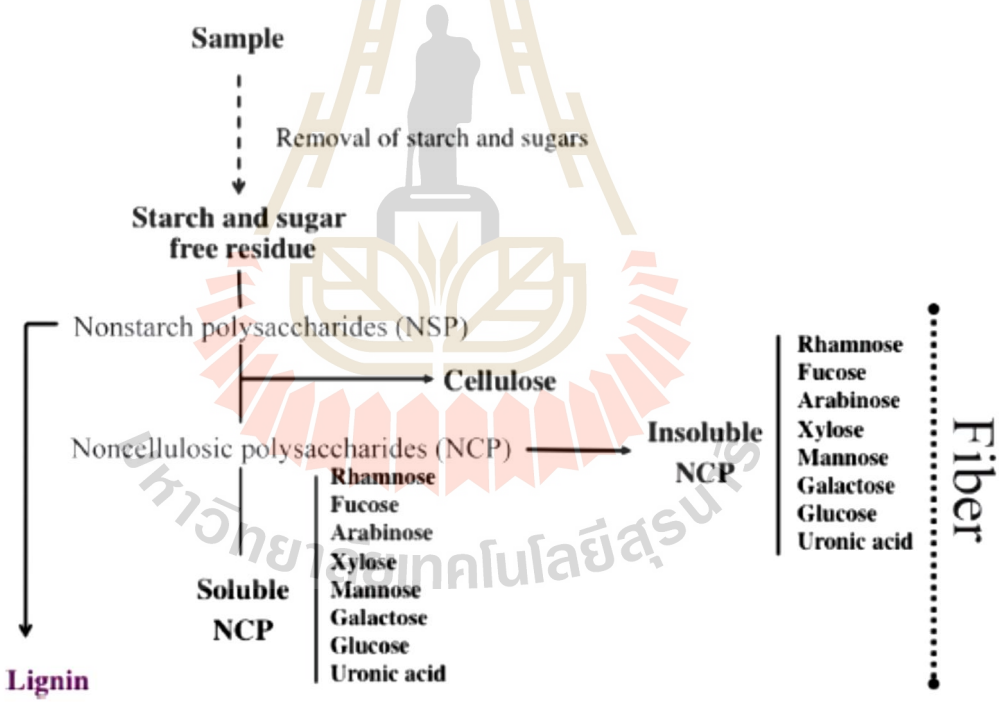

Figure 2.1 The classification of dietary fiber components by enzymatic-chemical procedure. NSP fall into three main groups, namely cellulose, insoluble non-cellulosic polymers, and soluble non-cellulosic polymers (pectic polysaccharides) (Choct, 1997; Knudsen, 2014).

Table 2.1 The fiber sources and chemical structure of dietary fiber.

Category	Monomeric residue	Linkage	Sources
Cellulose	Glucose	β-(1→4)	Most cereals, plant cell wall, legumes, sunflower seeds, oat hull, sugarcane bagasse
Arabinoxylans	Arabinose, xylose	β-(1→4)-linked xylose units	Wheat, rye, barley, oat, rice, sorghum, corn
Xylan	Xylose	β-(1→4)-xylose	Wheat, wheat brand
Mixed-linked β-glucans	Glucose	β-(1→3) and (1→4)	Barley, oat, rye, rice
β-glucans	Glucose	β-(1→3) and (1→6)	*S. cerevisiae*, *C. albicans*, seaweed
Mannans	Mannose	β-(1→4)	Coffee seed
Galactomannans	Galactose, mannans	β-(1→4)-linking mannan chains with α-(1→6)-linked galactosyl side groups	Locust bean gum, guar gum
Glucomannans	Glucose, mannans	β-(1→4)-linked mannan chain with interspersed glucose residues in the main chain	Sugar beet pulp, lilies, irises
Arabinans	Arabinose	α-(1→5)	Cereal co-products such as wheat bran
Galactans	Galactose	β-(1→4)	Sugar bean meal, sugar beet pulp
Pectin	D-galacturonic acid, L-rhamnose, L-arabinose, D-xylose, L-fucose	α-(1,4) D-galactomannans	Fruits, chicory, sugar beet pulp
Inulin	Fructose, glucose	β-(2-1)-D-fructosyl-fructose	Yam, rye, Jerusalem artichoke, chicory

Reference: modified from Choct (1997); Sinha et al. (2011); Staffolo et al. (2012); Lam and Cheung (2013).

2.1.2 Roles of dietary fiber in poultry

Although dietary fiber has been considered for a nutrient diluent or as an anti-nutritional factor. However, there is a growing interest in the inclusion of dietary fiber in poultry diets due to their functional properties and health benefits. The moderate amounts of fiber might improve the development of organs, enzyme production, and nutrient digestibility in poultry (Mateos et al., 2012; Jha and Berrocoso, 2015). Moreover, Kheravii et al. (2018) have demonstrated that the manipulation of poultry diets, including the particles size and the dietary fiber composition, could improve gut health, nutrient utilization, and productive efficiency because their structural components could stimulate the activity in the gut.

Previous studies reported that the GIT, particularly the gizzard, it adapts rapidly in response to changes in diet composition. These showed a rapid enlargement in gizzard size when structural components such as dietary fibers (particularly IDF) are included in the diet. A well-developed and stronger gizzard potentially results in more frequent reverse peristalsis contractions that makes repeated reflux of digesta back into the proventriculus to re-expose digesta to HCl and pepsin, and increase the digesta retention time, this could be the reason for the improvement of nutrient digestibility (Svihus, 2011; Kheravii et al., 2018). In addition, diet is a key mediator of the composition and metabolic function of GIT microbiota. Also, the composition of dietary fiber could be responsible for large variations in their utilization, the physicochemical properties of dietary fiber sources may lead to changes in the gut environment, altering the growth of the gut microbial. Indeed, the maintenance of gut health and stability of the GIT ecosystem are a complex phenomenon, relying on a

delicate balance among the dietary components, the commensal microflora, and the tract mucosa (Montagne et al., 2003; Jha and Berrocoso, 2015).

The possible mechanisms underlying improved nutrient digestibility, performance, and gut health through manipulation of poultry diet by fiber or particle size are elucidated in Figure 2.2.

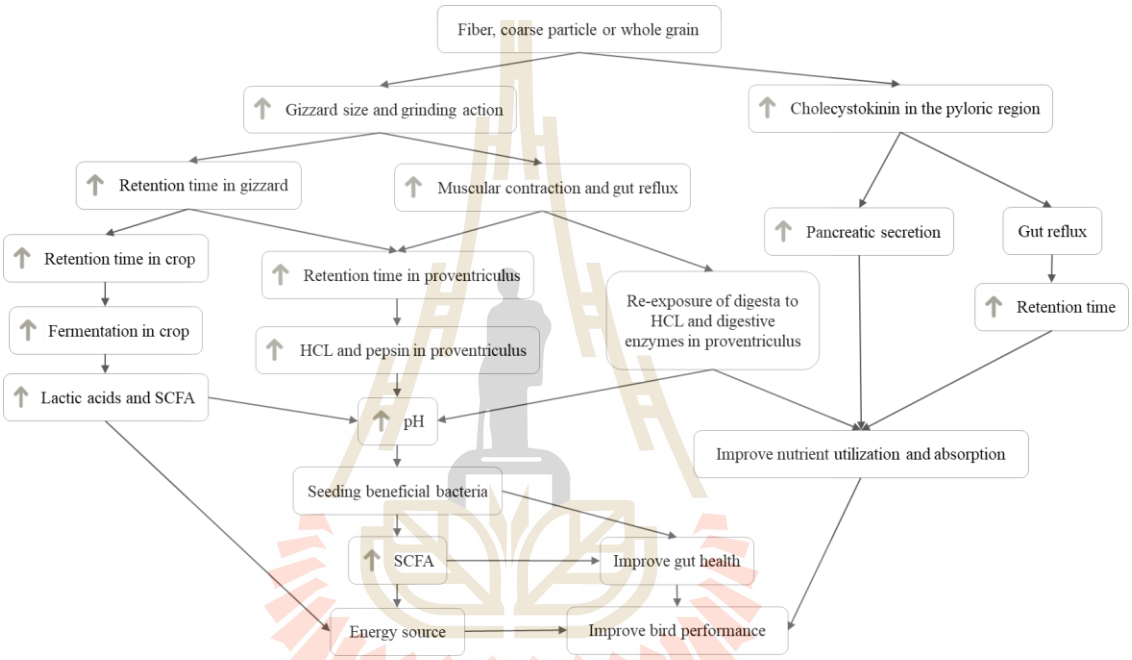

Figure 2.2 The possible mechanisms underlying improved nutrient digestibility, performance, and gut health through manipulation of poultry diet by fiber or large particle size (Kheravii et al., 2018).

2.2 Cassava by-products, sources of dietary fiber and interest for use as functional feed in animal diet

Cassava or tapioca (*Manihot esculenta Crantz*) is one of the most important economic crops in Thailand, it is also an important root plant in Asia. Cassava roots have a lot of applications in the food industries, bioethanol industries, and also in the

feed industries (cassava chips and cassava pellet). Currently, Cassava starch and bioethanol production are a huge and growing industry. Their processing also produce the yield dried cassava pulp (DCP) and cassava distiller's dried grains (CDG) as a by-product. Cassava by-products in the form of DCP and CDG are annually generated in a large amount (approximately 2.0-3.0 and 0.74-0.95 million tons per year, respectively) in Thailand.

The nutritional quality of DCP and CDG are variable. It depends on many factors including the extraction process, the kinds of cassava, and the cultivation of farmers (Chávez et al., 2005; Jorjong et al., 2013). The nutrient composition of DCP and CDG are presented in Table 2.2. Most of the NSPs in DCP were in insoluble forms (approximately 40-90 g kg^{-1}) whereas SDF was approximate from 13.93 to 16.21 (g kg^{-1}). The main sugars in the IDF were xylose, galactose, and mannose (Chauynarong et al., 2015).

Currently, using dietary fiber sources from agro-industrial by-products as an alternative has increased in animal feed. Thus, cassava by-products in both DCP and CDG may be useful for alternative fiber sources in Thailand. If the NSPs in cassava by-products could be extracted to dietary fiber and hydrolyzed to oligosaccharides, it would not only reduce the environmental pollution from cassava pulp disposal but also added more values for cassava crops.

Table 2.2 Chemical composition of cassava pulp and cassava distiller's dried grains.

Components (%)	**Cassava pulp**			**Cassava distiller's dried grains**	
	Kosugi et al. (2009)	Rattanachomsri et al. (2009)	Chauynarong et al. (2015)	Jorjong et al. (2013)	Wan et al. (2015)
Starch	60.60	61.19	-	-	15.79
Crude protein	2.50	-	1.87	7.94	3.70
Fat	-	-	0.33	1.29	-
Ash	-	-		10.22	24.08
Crude fiber	-	-	13.85	28.69	-
Non-starch polysaccharides	28.10	23.00	-	-	-
Glucan	19.10	-	-	-	-
Xylan	4.20	-	2.38	-	-
Arabinan	1.40	-	1.29	-	-
Galactan	0.50	-	4.06	-	-
Mannan	0.70	-	0.45	-	-
Cellulose	-	15.60	-	31.81	25.75
Hemicellulose	-	4.60	-	13.69	5.01
Lignin	2.20	2.80	-	11.30	8.69

2.3 Extraction of dietary fiber

Methods for extracting total dietary fiber include chemical, physical, enzymatic, and microbial methods, which have different effects on the composition and structure of the dietary fiber. The various extraction methods of dietary fiber are presented in Table 2.3. Moreover, further modifications of dietary fiber extracted can improve the quality of the dietary fiber. Dietary fiber modification methods are presented in Table 2.4.

Table 2.3 Extraction methods of dietary fiber.

Extraction methods	Examples
Chemistry methods	NaOH was used to extract dietary fiber from cereals.
	Alkalis was used to extract arabinoxylan from wheat bran and rice bran.
Physical methods	High temperature, high pressure, ultrasonic treatment increased the extraction rate of arabinoxylan.
	Micronization, ultrasonic, microwave, and extrusion cooking treatments had reduction effect on glucose breakdown rate and adsorption, and avoid increased in postprandial blood glucose level.
Enzymatic methods	Alcalase hydrolysis was used to extract dietary fiber from deoiled cumin.
	Dietary fiber extracted from defatted rice bran using thermal-stable α-amylase, and alcalase had glucose dialysis retardation index.
Microbial methods	Wheat bran soluble dietary fiber extracted by solid fermentation with Fungi.
Mixed methods	Dietary fiber extracted by shear emulsifying assisted enzymatic hydrolysis had excellent physicochemical and functional properties.
	Ultrasound-assisted solvent method was used to obtain dietary fiber from Corn Pericarp.

Reference: (Zhang et al., 2018).

Table 2.4 Modification methods of dietary fiber.

Modification methods	Effects
Micronization	Micronization processed dietary fiber enhanced the soluble dietary fiber content.
multidimensional swing high-energy nanoball-milling	Ultrafine bran dietary fiber increased the content of soluble dietary fiber and decreased the total phenolic content.
Enzyme	Cellulase and xylanase increased the extractable arabinoxylan and soluble dietary fiber content of dietary fiber.
Enzyme-micronization	Enzyme-micronization reduced the water and oil holding capacity, but increased the swelling capacity, cholesterol and sodium taurocholate absorption capacity of dietary fiber.
Chemical methods	Chemical reagents increased glucose adsorption capacity and α-amylase inhibitory activity of dietary fiber as a result improving the ability of insoluble fiber to reduce postprandial blood glucose.

Reference: (Zhang et al., 2018).

2.4 Fourier transform infrared

Fourier transform infrared (FTIR) spectroscopy is used for the study of polysaccharide, which provides information about the sample quantity, composition, structure and functional groups, and also the chemical bonds of the plant cell wall such

as sugars residue and complex carbohydrates. The application of FTIR spectroscopy has been studied the composition in food and feed such as the identify protein and carbohydrate molecular structure spectral features of dried distiller's grain soluble from wheat, quantitative analysis of tapioca starch, polysaccharide food additives, and studied the composition of dietary fiber from fruits (Cerna et al., 2003; Abeysekara et al., 2011; Sacithraa et al., 2013; Chylińska et al., 2016). This technique can detect very fast, without using chemical, non-destructive the sample, and only requires a small number of samples. FTIR method is the best for homogeneous samples or those composed of only a few materials (Lammers et al., 2008).

FTIR technique based on the selective absorption of mid-infrared radiation frequency, range between 4,000-400 cm^{-1}, of the chemical bonds of the sample. The functional of FTIR spectroscopy method, it consists of light source, interferometer, and detector. IR radiation is passed through a sample, then the sample absorbs some of the infrared radiation. The spectrum shows the molecular absorption and transmission, creating a molecular fingerprint for each sample. The IR absorption band of radiation occurs only when the frequency of incoming IR is the same frequency as the fundamental modes of vibration of the molecule, it contributed to the unique spectrum pattern, like a molecular fingerprint of the entire molecule. The functional diagram of FTIR spectroscopy method is shown in Figure 2.3.

The most intense of IR spectrum of these polysaccharides or dietary fiber was observed in the following regions: C–H stretching (3000-2800 cm^{-1}), C–H bending (1500-1300 cm^{-1}), C–O stretching and C–O–C glycoside (1260-1035 cm^{-1}), etc. Table 2.5 is the IR assignment of the main bands of dietary fiber in FTIR spectra.

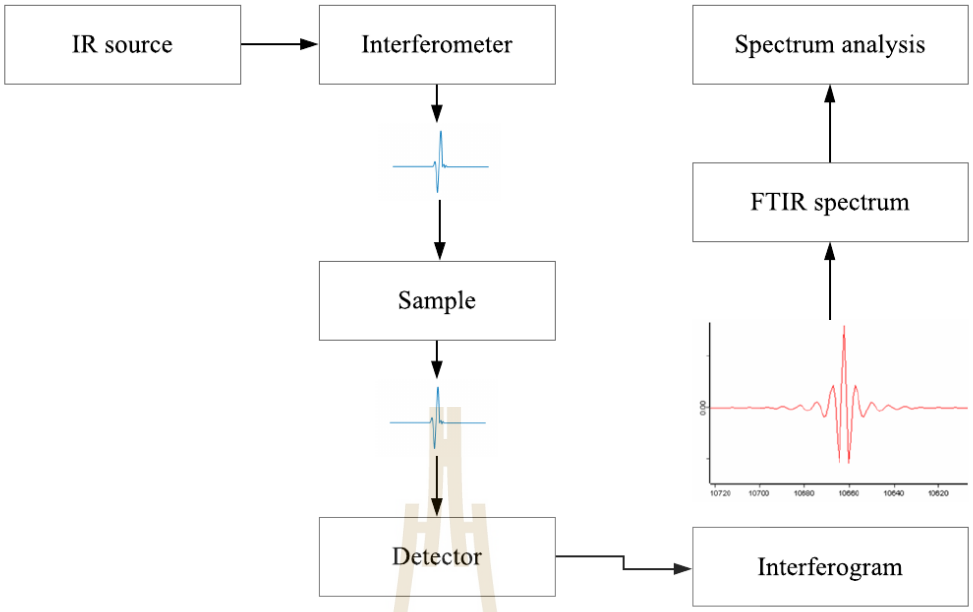

Figure 2.3 The functional diagram of FTIR Spectroscopy method; adapted from Sacithraa et al. (2013).

Table 2.5 IR assignment of the main bands of dietary fiber in FTIR spectra.

Wavenumber (cm^{-1})	**Assignments**	**Reference**
3000-2800	C–H stretching (aliphatic compounds)	Oh et al. (2005); Lammers et al. (2008); Abidi et al. (2014); Pouzet et al. (2017)
1680 - 1600	O–H bending of adsorbed water	Oh et al. (2005); Abidi et al. (2014); Pouzet et al. (2017)
1595, 1514	C–H deformation of lignin	Lammers et al. (2008)

Table 2.5 Continue.

Wavenumber (cm^{-1})	**Assignments**	**Reference**
1500-1300	C–H bending (crystalline versus amorphous structure of cellulose)	Oh et al. (2005); Marques et al. (2006); Abidi et al. (2014); Ying et al. (2017)
1260-1200	C–O stretching of hemicellulose	Corredor et al. (2009); Abidi et al. (2014); Cheikh Rouhou et al. (2018)
1160-1035	C–O–C glycoside in cellulose, C–O vibration of crystalline cellulose, C–O stretching and C–C stretching of cellulose	Corredor et al. (2009); Abidi et al. (2014); Ying et al. (2017); Cheikh Rouhou et al. (2018)
1000-980	C–O and ring stretching modes, C–O stretching of starch	Lammers et al. (2008); Abidi et al. (2014)
960-800	Vibration of the pyranose ring, glucose ring stretch	Lammers et al. (2008); Corredor et al. (2009)

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CHAPTER III

EXTRACTION CONDITION OF DIETARY FIBERS FROM CASSAVA PULP AND CASSAVA DISTILLER'S DRIED GRAINS AND ASSESSMENT THEIR COMPONENTS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY

3.1 Abstract

The aim of the present study was to investigate the extraction conditions of dietary fiber from dried cassava pulp (DCP) and cassava distiller's dried grains (CDG) under different NaOH concentrations, and the Fourier Transform Infrared (FTIR) was used to determine the dietary fiber components. The results showed that the optimal conditions for extracting dietary fiber from DCP and CDG were under treatment with 6% and 4% NaOH, respectively, as these conditions yielded the highest total dietary fiber (TDF) and insoluble dietary fiber (IDF) contents. These results were associated with the FTIR spectra integration for a semi-quantitative analysis, which obtained the highest cellulose content. The Principal Component Analysis (PCA) illustrated clear separation of spectral distribution in cassava pulp extracted dietary fiber (DF-CP) and cassava distiller's dried grains extracted dietary fiber (DF-CDG) when treated with 6% and 4% NaOH, respectively.

In conclusions, the optimal conditions for the extraction of dietary fiber from DCP and CDG were treated with 6% and 4% NaOH solution, respectively. In addition, FTIR spectroscopy proved itself to be a powerful tool for fiber identification.

Keyword: dietary fiber, FTIR spectroscopy, cassava pulp, cassava distiller's dried grains.

3.2 Introduction

Dietary fiber is found naturally in feedstuffs include non-starch polysaccharides (NSPs) such as cellulose, non-cellulosic polysaccharides (hemicellulose, pectic substances, gums, mucilage), and non-carbohydrate component lignin (Dhingra et al., 2012; Knudsen, 2014; Choct, 2015). It is classified into water soluble dietary fiber (SDF) and insoluble dietary fiber (IDF), the content and composition of SDF and IDF vary with feedstuff type. Dietary fiber plays an important role in the regulations of poultry bodies. Its effects depend on structures and physicochemical properties (Michard, 2011). In particular, IDF has been shown to improve gut health, litter and nutrient utilization, by increasing crop and gizzard activity, stimulating digestive enzyme production and enhancing bacterial fermentation in the hind gut (Mateos et al., 2012; Walugembe et al., 2015; de Vries, 2015). Therefore, the possibility of creating alternative dietary fiber sources derived from agro-industrial by-products could be a useful functional feed to improve animal health and provide the benefits of good waste management.

Cassava by-products in the form of dried cassava pulp (DCP) and cassava distiller's dried grains (CDG) are annually generated in large amounts (approximately 2.0-3.0 and 0.74-0.95 million tons per year) from the starch factory and the bioethanol

production process in Thailand, respectively. These by-products contain high NSPs which are mainly composed of cellulose, hemicellulose, and lignin (Kosugi et al., 2009; Wan et al., 2015). The fiber extracted from DCP and CDG can be further used in poultry diets as it provides health benefits and productive performance as well as good waste management. Previous studies reported several methods of extracting dietary fiber, unfortunately, there is little information available on the extraction of cassava by-products. A combination of enzymatic and solvent methods is usually used for dietary fiber extraction. For example, starch and protein are first removed by amylase and protease or a suitable solvents (such as a neutral, alkaline, acidic detergent). The different fiber extraction conditions affect the properties of dietary fiber in both composition and structure (Zhang et al., 2018). Daou and Zhang. (2013) reported that the extraction of dietary fiber from defatted rice bran, and the alkaline pretreatment with NaOH were the factors amongst others (e.g. concentration and soaking time) which significantly affected the purity of fiber fractions. Samanta et al. (2014) stated that the alkaline extraction (NaOH solution) can extract the yield of xylan in agricultural by-products. Crude fiber assay is still being used today as the legal measure of fiber in grains and finished feeds of non-ruminant animals. However, it is not a good indicator because some parts of the fiber can ferment in the large intestine or caecum. Thus, the true fiber contents have attracted more interest because of the potential improvements in the accuracy of future measurements (Choct, 2015). A power tool to identify the components in fiber could offer a rapid and reliable alternative technique.

Fourier Transform Infrared (FTIR) Spectroscopy has become an attractive alternative unlike traditional methods since it is a rapid analytical technique, which

uses non-destructive samples and minimizes hazardous chemical use. FTIR can provide information regarding the functional groups, chemical bonds, composition, structure, and quality of a product. IR spectra combined with chemometric techniques, such as PCA, can be used to obtain accurate dietary fiber components (Abeysekara et al., 2011; Chylińska et al., 2016; Pouzet et al., 2017). FTIR Spectroscopy has been used to study the relationship of feed intrinsic structures pertaining to protein molecular structures, carbohydrates, and starch matrices (Abeysekara et al., 2011), quantitative analysis of tapioca starch (Sacithraa et al., 2013), and polysaccharide food additives (Cerna et al., 2003) .It can be used to analyze the chemical composition of cell walls, the structure of natural fiber and fiber composition (Fan et al., 2012). The application of FTIR to identify the fractions of dietary fiber from DCP and CDG would be a useful tool for the assessment of dietary fiber components.

3.3 Objective

The objective of this study was to investigate the extraction conditions of dietary fiber from DCP and CDG by using different NaOH concentrations. In addition, FTIR was also used to determine the dietary fiber components combined with multivariate data analysis using PCA.

3.4 Materials and methods

3.4.1 Sample preparation

Fresh cassava pulp was obtained from the Korat Flour Industry Co., Ltd, Nakhon Ratchasima, Thailand. Fresh cassava distiller's dried grains was obtained from the Thai ethanol power Pub Co., Ltd, Khon Kaen, Thailand. There were dried in a hot

air oven at 55-60ºC, for 2 days and then were ground to pass through a 1.0 mm mesh sieve before being stored at 4ºC until further use. Prior to extraction, DCP and CDG were analyzed for dry matter, crude protein, crude ash, and ether extract according to the standard methods of AOAC (1990). The contents of total soluble and insoluble dietary fiber were determined using the total dietary fiber Kit (K-TDFR-100A, Megazyme International Ltd., Wicklow, Ireland). The chemical compositions of DCP and CDG are shown in Table 3.1.

Table 3.1 The chemical compositions of dried cassava by-products.

Compositions (%)	**Cassava pulp**	**Cassava distiller's dried grains**
Dry matter	93.79	92.26
Crude protein	2.30	10.14
Crude ash	1.87	16.58
Ether extract	0.22	1.02
Total dietary fiber	17.36	21.47
Soluble dietary fiber	2.29	3.09
Insoluble dietary fiber	15.07	18.38

3.4.2 Experimental design

The extraction conditions of dietary fiber from DCP and CDG were treated with various concentrations of NaOH at levels of 2 4 6 and 8% using a completely randomized design with 4 replications of each.

3.4.3 Dietary fiber extraction

The extraction procedure was slightly modified from Daou and Zhang (2013). A dried sample (1.0 g) was pretreated with 5.0 ml NaOH solution at different concentrations (2, 4, 6 and 8%), the mixture was soaked for 1 hour at room temperature, then centrifuged at 4,000 rpm for 15 minutes, and the residue was washed to pH 7.0 with distilled water. The sample was suspended in phosphate buffer (pH 6.0) ratio 1:30, and α-amylase (EC 3.2.1.1, Megazyme International Ltd., Wicklow, Ireland) was added, then the mixture was incubated at 95ºC in a boiling water bath for 1 hour. The sample was allowed to cool at room temperature. The dietary fiber was precipitated in 95% (v/v) ethanol at 60ºC for 1 hour, then was cooled to room temperature, and centrifuged at 4,000 rpm for 15 minutes. After centrifugation, the residue was washed with 78% (v/v) ethanol, 95% (v/v) ethanol, and acetone respectively, and finally dried at 55-60ºC overnight. A flow diagram of the dietary fiber extraction is shown in Figure 3.1.

3.4.4 Analysis of dietary fiber contents

The cassava pulp extracted dietary fiber (DF-CP) and cassava distiller's dried grains extracted dietary fiber (DF-CDG) were analyzed to determine the amounts of total, soluble and insoluble dietary fiber by using the Megazyme total dietary fiber kit (K-TDFR-100A, Megazyme International Ltd., Wicklow, Ireland), according to the manufacturer's instructions.

3.4.5 FTIR Spectroscopy

The infrared spectra were collected using Attenuated Total Reflectance (ATR)-FTIR spectroscopy with single reflection ATR sampling module and coupled

with a DTGS detector over the measurement range from 4,000-400 cm^{-1}. The measurements were performed with a spectral resolution of 4 cm^{-1} with 64 scans co-added

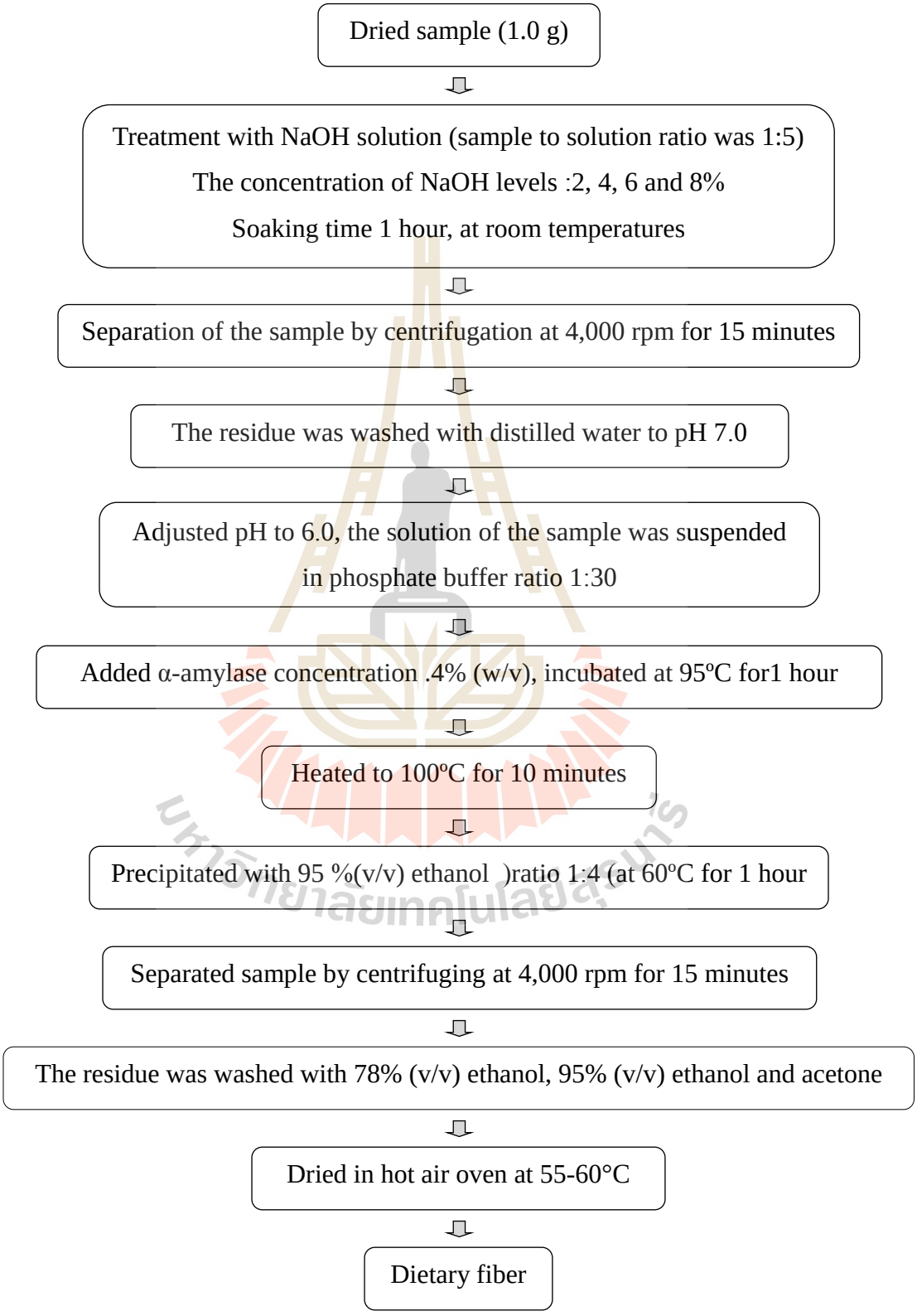

Figure 3.1 Flow diagram of dietary fiber extraction.

(Bruker Optics Ltd, Ettlingen, Germany). OPUS software was used for data acquisition and the spectra evaluation .The spectral changes of the functional groups were performed at the integral area of each peak such as cellulose, hemicellulose, lignin, and starch by using OPUS software.

3.4.6 PCA analysis

The FTIR spectra were exported to the Unscrambler X 10.5 (CAMO, Norway) for using PCA analysis. The spectral data were preprocessed by taking the 2^{nd} derivative with Savitzky-Golay method (3^{rd} polynomial, 9 smoothing points), normalization with Extended Multiplicative Signal Correction (EMSC) and PCA were performed for the determination of a significant variation between the spectra sets. In this study, PCA was used to compare the FTIR spectra of dietary fiber sources under treatment with different conditions of NaOH solution (2, 4, 6 and 8%). The output of PCA can be presented the sources of variability of data which were concentrated into the principal component (PC). The spectra were processed using the second derivative and vector normalized by the Savitzky-Golay method, and using the third polynomial and nine smoothing points setting (Jarvis and Goodacre, 2004).

3.4.7 Statistical analysis

Statistical analyses of dietary fiber contents (TDF, SDF, and IDF) and peak areas proportions from integrating FTIR spectra were performed using SPSS software version 18.0 (SPSS, Inc., 2010). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. A threshold level of $P < 0.05$ was used to determine significance.

3.4.8 Experimental location

The experiment were conducted at the Center for Scientific and Technological Equipment Building 10, Suranaree University of Technology and Beamline 4.1: Infrared Spectroscopy and Imaging, Synchrotron Light Research Institute (Public Organization), Thailand.

3.4.9 Experimental period

The experiment was done from May 2016 to February 2017.

3.5 Results

3.5.1 The components of dietary fiber extracted from dried cassava pulp and cassava distiller's dried grains

The DCP and CDG were treated with different concentrations of NaOH solution (2, 4, 6 and 8%) were performed to determine the optimal conditions of the dietary fiber extraction. The contents of TDF, SDF, and IDF after extraction are shown in Table 3.2. The DF-CP treated with NaOH at concentrations of 6 and 8% which yielded significantly greater amounts of TDF and IDF than the 2% NaOH ($P < 0.05$). In addition, DF-CDG treated with 4, 6 and 8% NaOH produced higher contents of TDF and IDF than the 2% NaOH ($P < 0.05$). The SDF content in both DF-CP and DF-CDG showed no significant differences ($P > 0.05$) between treatments.

3.5.2 FTIR spectra of dietary fiber extracted from dried cassava pulp and cassava distiller's dried grains

The spectral features of DF-CP and DF-CDG are shown in figure 3.2 and 3.3. In this study, FTIR spectra were used to detect the extracted dietary fiber treated with NaOH solution in the spectral region of 4,000 to 400 cm^{-1}. A total of 428 and 434 FTIR spectra of DF-CP and DF-CDG were analyzed. The fingerprint regions

of specific interest in this study were between 1,700 and 800 cm^{-1}, although many absorption bands associated with various NaOH solution from vibrational modes in the wavelength region are also present in DF-CP and DF-CDG. The FTIR spectra measurements were carried out to reveal the molecular characteristics of functional groups of dietary fiber such as cellulose, hemicellulose and lignin.

Table 3.2 The contents of total, soluble and insoluble dietary fiber of extracted dietary fiber.

Compositions (%)	**Concentration of NaOH levels**				**Pooled**
	2%	**4%**	**6%**	**8%**	**SEM**
Cassava pulp extracted dietary fiber					
Total dietary fiber	23.60[b]	24.29[b]	27.93[a]	27.60[a]	0.57
Soluble dietary fiber	3.65	2.57	3.19	2.84	0.19
Insoluble dietary fiber	19.95[b]	21.72[ab]	24.73[a]	24.76[a]	0.63
Cassava distiller's dried grains extracted dietary fiber					
Total dietary fiber	22.85[b]	25.12[a]	25.33[a]	25.35[a]	0.30
Soluble dietary fiber	2.38	2.59	2.33	2.32	0.06
Insoluble dietary fiber	20.46[b]	22.53[a]	23.00[a]	23.03[a]	0.31

[a, b] Means with different superscripts in a row are significantly different ($P < 0.05$)

The results of the semi-quantitative analysis of DF-CP and DF-CDG using FTIR spectra in term of proportions (%) of the functional groupies are prensented in Table 3.3. The results showed that DF-CP treated with 6% NaOH yielded the significant proportions of C–H bending of crystalline versus amorphous

structure of cellulose, C–O stretching of hemicellulose, and C–O–C glycoside, C–O and C–C stretching of cellulose contents compared to 2 and 4% NaOH ($P < 0.05$), but there was no significant difference compared to 8% NaOH treatment. While the proportions of the component in DF-CDG with peak area integration, treating CDG with 4% NaOH resulted in a significant yield of C–O–C glycoside, C–O and C–C stretching of cellulose compared to 2% NaOH ($P < 0.05$).

Table 3.3 The integral area from FTIR spectra of extracted dietary fiber.

Item	Concentration of NaOH levels				Pooled
	2%	4%	6%	8%	SEM
Cassava pulp extracted dietary fiber					
C–H stretching	5.33	6.22	4.52	5.08	0.38
O–H bending of adsorbed water	8.86	8.87	7.79	8.52	0.17
C–H deformation of lignin	0.66	0.79	0.75	0.73	0.04
C–H bending of crystalline cellulose	18.20^{bc}	17.06^{c}	19.54^{a}	18.57^{ab}	0.27
C–O stretching of hemicellulose	5.40^{a}	5.04^{b}	5.41^{a}	5.32^{a}	0.05
C–O–C glycoside, C–O and C–C stretching of cellulose	25.28^{b}	25.51^{b}	26.56^{a}	25.69^{ab}	0.17
C–O stretching of starch	17.96	18.21	18.75	19.11	0.24
Vibration of the pyranose ring	18.15	17.97	16.99	17.15	0.23
Cassava distiller's dried grains extracted dietary fiber					
C–H stretching	3.56^{ab}	3.89^{a}	2.43^{bc}	2.10^{c}	0.25
O–H bending of adsorbed water	7.52	6.84	6.98	6.41	0.18
C–H deformation of lignin	1.03	0.68	0.81	0.87	0.08
C–H bending of crystalline cellulose	29.03^{a}	27.30^{ab}	27.71^{ab}	26.38^{b}	0.37
C–O stretching of hemicellulose	5.60	6.02	5.80	5.34	0.12
C–O–C glycoside, C–O and C–C stretching of cellulose	20.57^{b}	22.58^{a}	21.95^{a}	22.28^{a}	0.255
C–O stretching of starch	22.67^{c}	23.23^{bc}	24.36^{ab}	25.64^{a}	0.353
Vibration of the pyranose ring	10.03^{b}	9.44^{b}	9.94^{b}	10.98^{a}	0.181

[a, b, c] Means with different superscripts in a row are significantly different ($P < 0.05$)

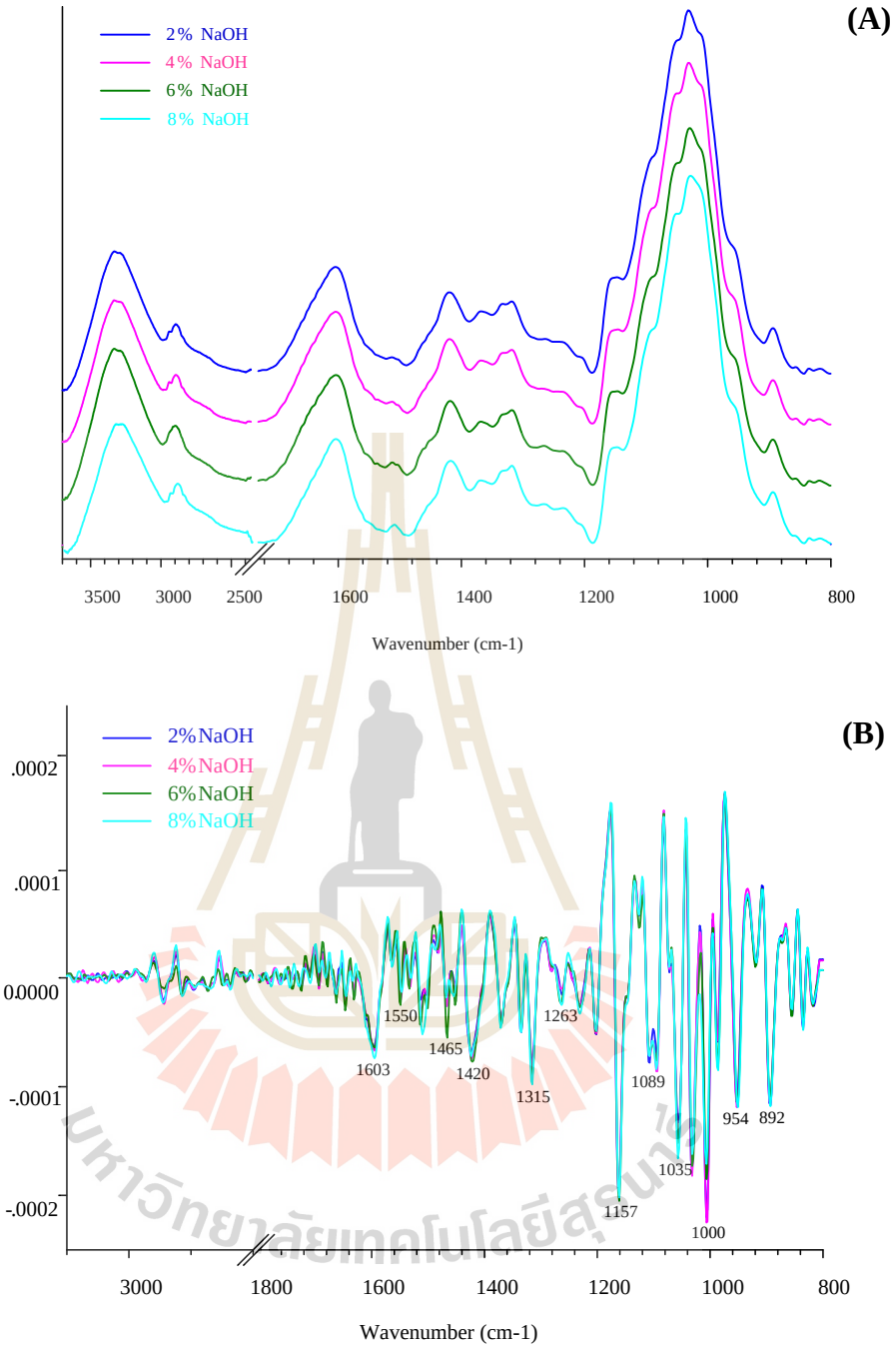

Fig 3.2 FTIR spectra of cassava pulp extracted dietary fiber (DF-CP) treated with NaOH solution at concentrations 2, 4, 6 and 8%, (A) Average original FTIR spectra of DF-CP, (B) 2nd derivative spectra of DF-CP.

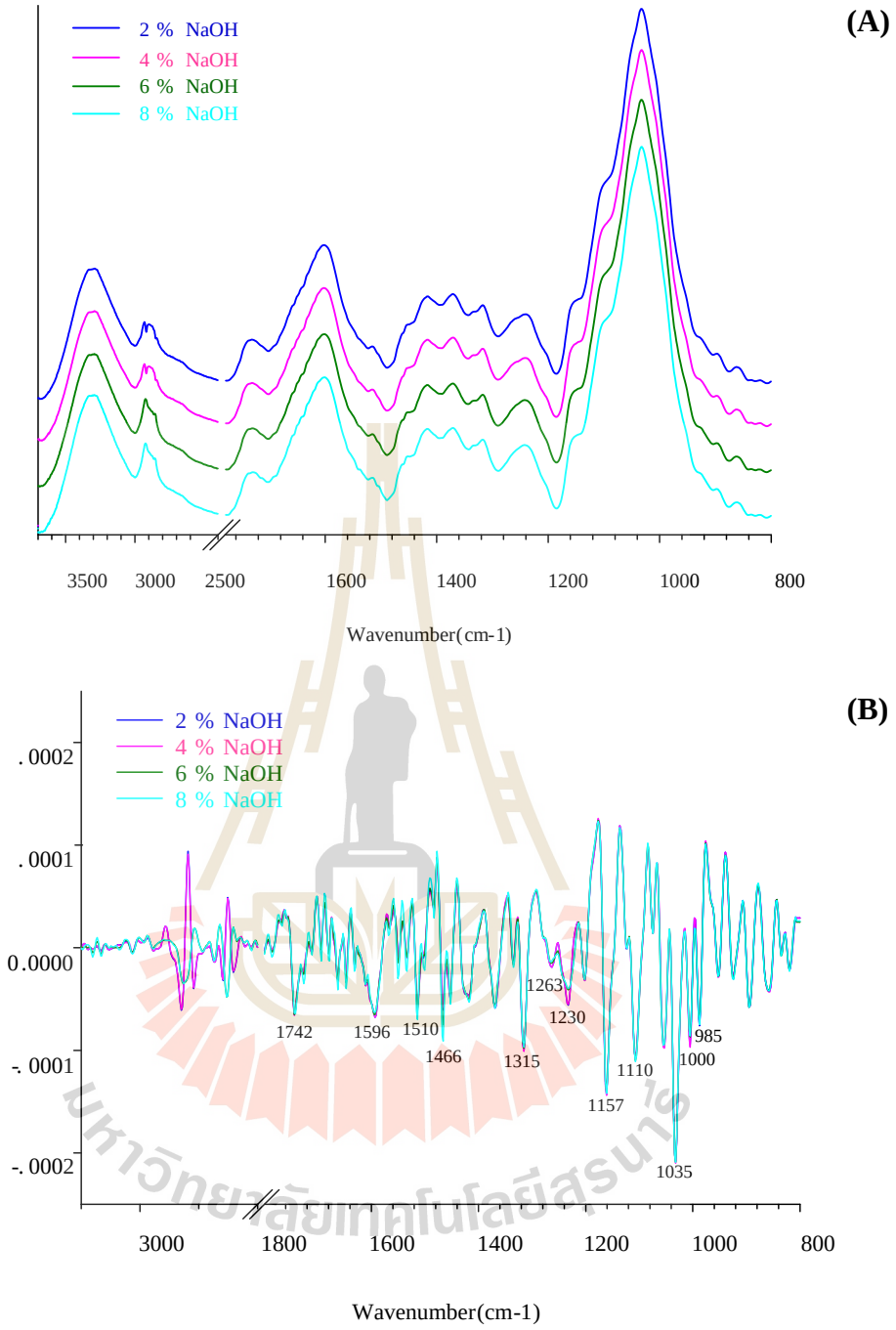

Fig 3.3 FTIR spectra of cassava distiller's dried grains extracted dietary fiber (DF-CDG) treated with NaOH solution at concentrations 2, 4, 6 and 8%, (A) Average original FTIR spectra of DF-CDG, (B) 2nd derivative spectra of DF-CDG.

3.5.3 PCA analysis, extracted dietary fiber components

In this study, the DF-CP and DF-CDG treated with NaOH solution at a concentration of 2, 4, 6 and 8% were identified by their spectral distribution by using PCA. The PCA scores were plotted to characterize the sample relationships between the spectra and the dietary fiber extraction treatments. The FTIR characterizes chemical structure by identifying the functional groups present in each sample.

The result of PCA scores from DF-CP are presented in Figure 3.4. The variability of PC-1 and PC-2 accounted for 58% and 13%, respectively. The scores plot of DF-CP treated with 6 and 8% NaOH appear clearly in the negative PC-1 score plot, while the scores plot of 2 and 4% NaOH treatments are clearly separated in the positive PC-1 score plot. The highest negative loading plot in PC-1 was observed in the C–O–C stretch of cellulose (centered at 1170 cm^{-1}), C–O vibrations of cellulose (centered at 1035 cm^{-1}), C–O and ring stretching (centered at 1000 and 985 cm^{-1}), which was oppositely correlated with the positive score plot in DF-CP treated with 2 and 4% NaOH group from second derivative spectrum (Figure 3.4b). While the treatment using 6% NaOH showed the scores plot differ from 8% NaOH and almost appear on the positive side of PC-2. The positive loading plot in PC-2 reveals O–H bending of adsorbed water (centered at 1603 cm^{-1}), C–H bending of crystalline cellulose (centered at 1407 cm^{-1}), C–O and C–O–C stretching of cellulose (centered at 1089 and 1016 cm^{-1}), and C–O stretching of starch (centered at 981 cm^{-1}) (Figure 3.4b).

The score plot of the FTIR spectra of DF-CDG is presented in Figure 3.5. The variation of spectra in PC-1 and PC-2 accounted for 30% and 23%, respectively .The scores plot of DF-CDG treated with 2 and 4% NaOH appear in the

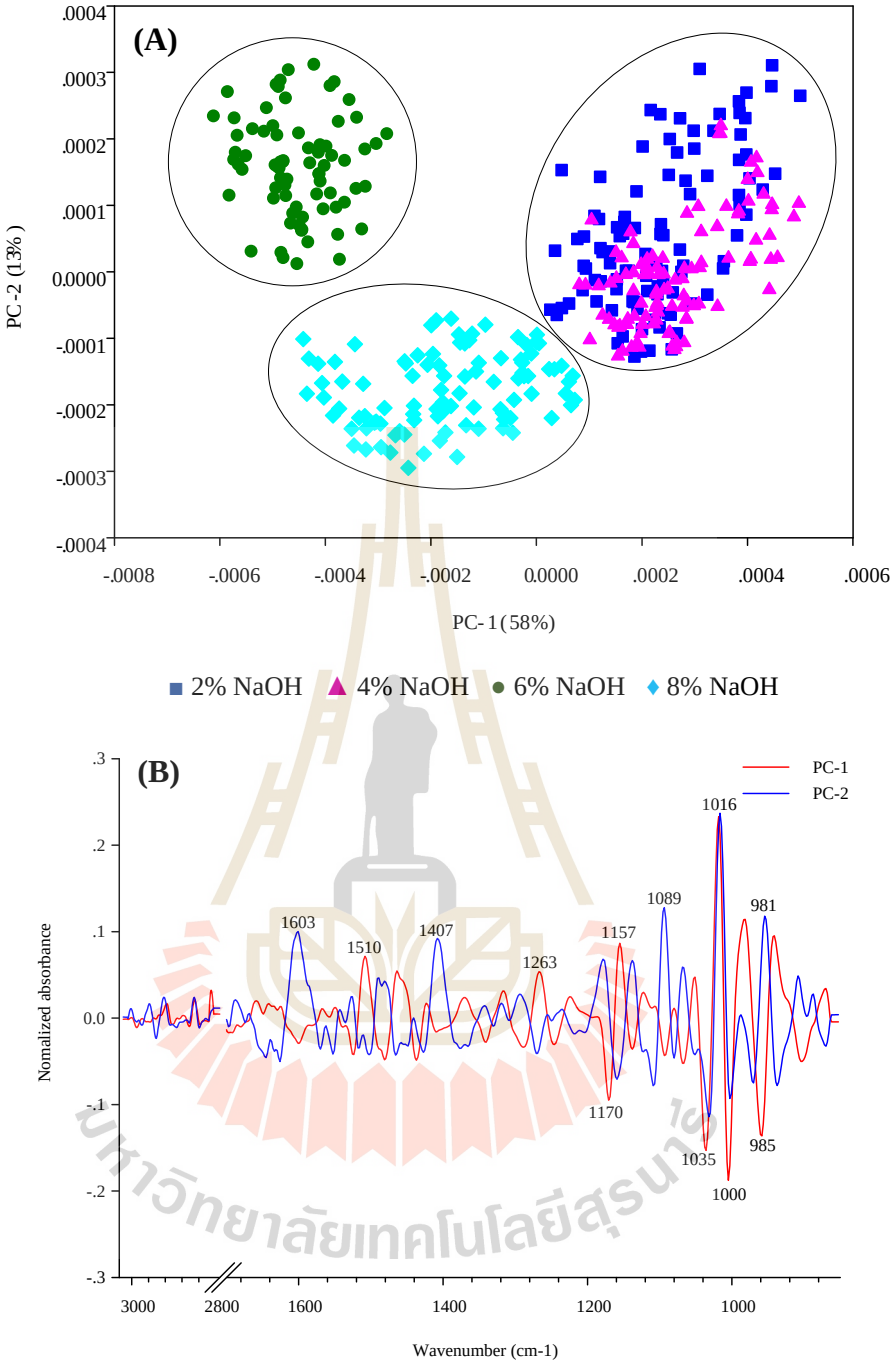

Fig 3.4 PCA scores scatter plot of FTIR spectra of cassava pulp extracted dietary fiber (DF-CP) treated with NaOH solution at concentrations 2, 4, 6 and 8%, (A) PCA scores plot, (B) PCA loading plot.

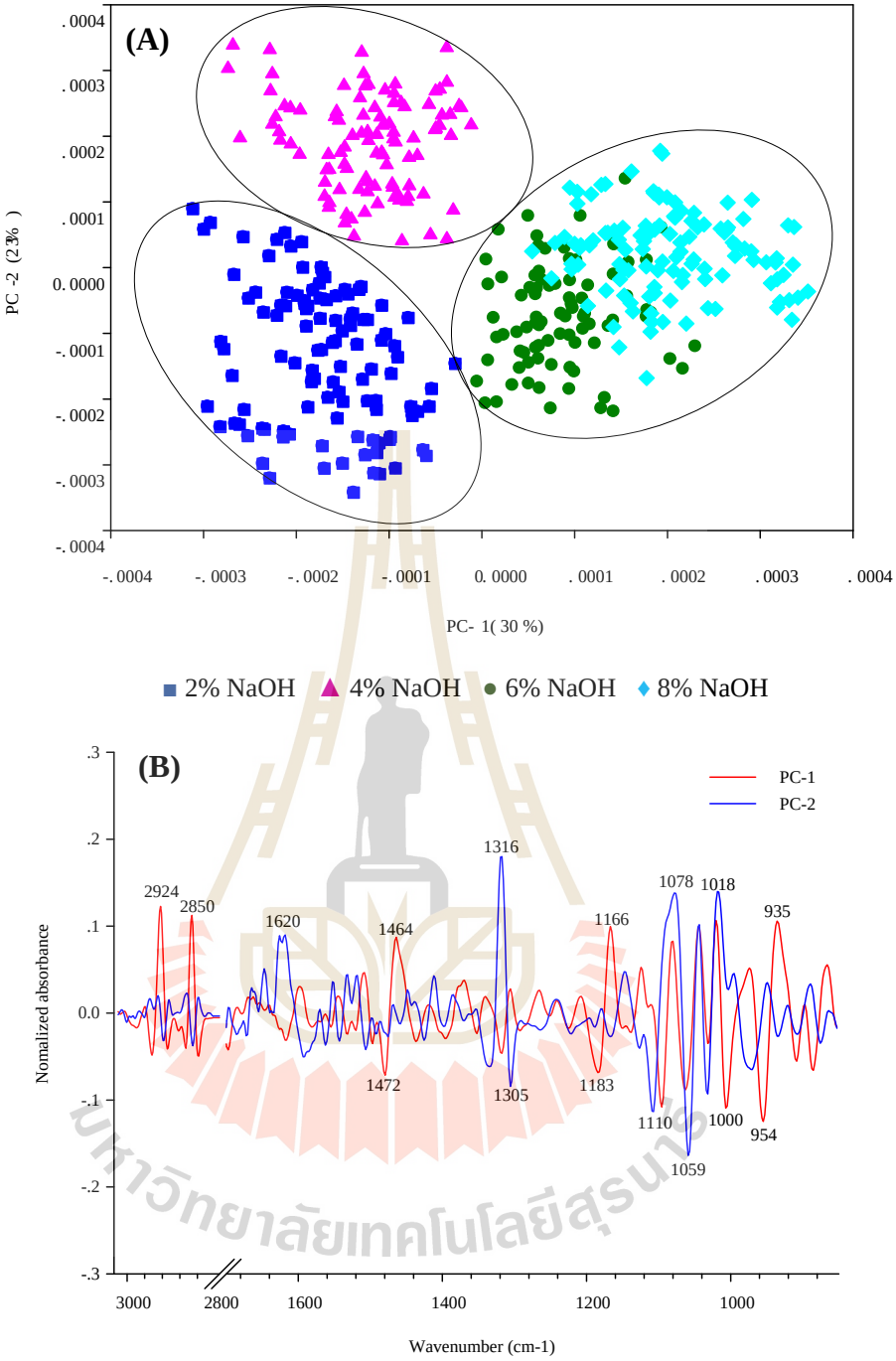

Fig 3.5 PCA scores scatter plot of the FTIR spectra of cassava distiller's dried grains extracted dietary fiber (DF-CDG) treated with NaOH solution at concentrations 2, 4, 6 and 8%, (A) PCA scores plot, (B) PCA loading plot.

negative PC-1 score plot, while the scores plot of 6 and 8% NaOH appears separately in the positive PC-1 score plot. The negative loading plot in PC-1 (Figure 3.5b) reveals C–H bending (centered at 1472 cm^{-1}), C–O and ring stretching (centered at 1000 cm^{-1}), and vibration of the pyranose ring, glucose ring stretch (centered at 954 cm^{-1}). The DF-CDG treated with 4% NaOH show the scores plot differed from that of 2% NaOH and almost appear in the positive PC-2 score plot. The positive loading plot in PC-2 (Figure 3.5b) reveal O–H bending of adsorbed water (centered at 1620 cm^{-1}), C–H bending of crystalline cellulose (centered at 1316 cm^{-1}), and C–O stretching and C–C stretching of cellulose (centered at 1078 and 1018 cm^{-1}).

3.6 Discussion

This study showed that the optimal conditions for extraction of dietary fiber from DCP and CDG were under treated with NaOH solution at 6-8% and 4-8%, respectively. The IDF represented a major component in both dietary fiber sources. In general, some hemicellulose such as ß-glucans, pectin, and gums are defined as SDF, whilst cellulose and lignin are defined as IDF (Dhingra et al., 2012; Choct, 2015). Cellulose is the main structural constituent in plant cell wall, and also in cassava by-products (Kosugi et al., 2009; Choct, 2015; Wan et al., 2015). Previous studies have shown that the defatted rice bran treated with NaOH solution can produce the maximum yield and purity of e TDF, SDF, and IDF (Daou and Zhang, 2013). Samanta et al. (2014) demonstrate that the extraction agricultural by-products with NaOH solution resulted in recovery of more than 90% of original xylan in plant materials. The results were similar to the highest xylan recovery from sugarcane bagasse

(Jayapal et al., 2012). Harun and Geok (2016) stated that rice straw treated with NaOH obtain the highest glucan and lower lignin composition.

FTIR technique can give information about the functional groups of C–O, C–O–C glycoside, and C–C from cellulose, hemicellulose, lignin, starch, and glucose in extracted dietary fiber (Lammers et al., 2008; Corredor et al., 2009; McKee et al., 2016). The semi-quantitative analysis of FTIR spectra was carried out by using OPUS software. The presented FTIR spectra were used to average the single spectra of each sample. The wavenumber of FTIR spectra was determined to be in the regions of 3500 -800 cm^{-1} for the peak area integration, and the total area of integrated peaks was defined as 100%. The peak area units were expressed as the relative proportions of the components in DF-CP and DF-CDG. These results were similar to the chemical composition of DF-CP treated with 6% NaOH and DF-CDG treated with 4% NaOH, which showed higher functional groups of cellulose.

The result of DF-CP and DF-CDG using different NaOH solution indicate that PCA analysis of FTIR spectroscopy reveals differences in DF-CP and DF-CDG treated with 6% and 4% NaOH solution respectively. These results were related to the semi-quantitative analysis by integral area obtained from the spectra. Also, the result showed mainly components of cellulose in both of dietary fiber sources. This result is consistent with Uthumporn et al. (2014), who found the predominant content of NSPs extracted from sago palm flour were cellulose, hemicellulose, pectin, and lignin by using FTIR. Chirinang et al. (2014) reported that the FTIR spectrum of dietary fiber from cassava pulp showing the band at 1031-1005 cm^{-1}. This band is the fingerprint of polysaccharides. These results show that FTIR spectroscopy can be used as a very reliable and quick tool for evaluating and monitoring dietary fiber.

3.7 Conclusions

It was demonstrated that DF-CP and DF-CDG treated with 6% and 4% NaOH solution respectively, obtained the highest TDF and IDF contents. Also, FTIR spectra integration for semi quantitative analysis of dietary fiber components showed that DF-CP and DF-CDG treated with 6% and 4% NaOH respectively, obtained the highest cellulose content. Their result was linked to IDF content in the chemical analysis. Moreover, the PCA analysis indicates the spectral distribution of dietary fiber components of DF-CP treated with 6% NaOH and DF-CDG treated with 4% NaOH have clearly separated the spectral distribution in PCA analysis. Finally, the optimal extraction condition of DF-CP and DF-CDG were treated with 6% and 4% NaOH solution, respectively. This study indicates that FTIR spectroscopy is a useful and rapid technique for fiber identification and semi-quantitative analysis.

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CHAPTER IV

OPTIMIZATION FOR MODIFIED-DIETARY FIBER FROM CASSAVA PULP AND CASSAVA DISTILLER'S DRIED GRAINS, AND ASSESSMENT THEIR EFFICACY FOR USE AS FUNCTIONAL FEED

4.1 Abstract

The objective of this study was to investigate the optimal ratio of cellulase : xylanase enzymes (4 levels 0:0, 9:3, 36:12 and 72:24 U/g substrate) in order to improve the dietary fiber extracted from cassava pulp (DCP) and cassava distiller's dried grain (CDG) and evaluate the modified-dietary fibers through in vitro fermentation method. The DCP and CDG were treated with NaOH at 6 and 4% NaOH respectively, following with enzymatic hydrolysis at various levels. The results showed that the ratio of cellulase : xylanase enzymes at levels 36:12 and 72:24 (U/g substrate) possessed an optimum level for the modification of dietary fiber from DCP. This hydrolysis condition generated the highest reducing sugar (D-glucose), total sugar and non-reducing sugar (D-xylose), and water holding capacity. While the dietary fiber from CDG under treated with cellulase and xylanase enzymes showed only the highest reducing sugar content of D-xylose. An assessment the efficacy of dietary fiber through in vitro fermentation method revealed that the dietary fiber extracted from DCP and CDG can increase Lactobacillus and Bifidobacterium

populations, SCFA and lactic acid productions and reduce pH values after 24 hours incubation. The dietary fiber from DCP was more efficient than the CDG. In addition, the modified-dietary fiber from cassava pulp (M-DFCP) with enzymes showed better efficacies than the cassava pulp extracted dietary fiber (DF-CP). Unfortunately, there was no significant difference between modified-dietary fiber from cassava distiller's dried grains (M-DFCDG) and cassava distiller's dried grains extracted dietary fiber (DF-CDG). In conclusion, it is suggested that the ratio of cellulose : xylanase at 36:12 U/g substrate could be an appropriate level for modifying dietary fiber from DCP.

Key words: modified-dietary fiber, cassava pulp, cassava distiller's dried grain, in vitro fermentation

4.2 Introduction

Poultry diets must be formulated to provide all of the birds' nutrient requirements. In addition, the formulation should also consider to provide an alternative approach to improve health, reduce the risk of pathogenic infection or reduce the inflammation in the gastrointestinal tract (GIT) (Choct, 2009; Gaggìa et al., 2010). Recently, the use of dietary fiber as an alternative feed additive in poultry diets has gain interesting due to it exists several beneficial functions on animal health such as enhancing the immune system, preventing and treating diseases (Vermeulen et al., 2017). Moreover, dietary fiber can be fermented by cecal microorganisms and produced short chain fatty acids (SCFA). As a result, this end product could reduce pH and inhibit the growth of pathogenic bacteria in cecal (Józefiak et al., 2004; Zduńczyk et al., 2015). However, dietary fiber has variable properties depending on

source, extraction process, composition and chemical structure of fiber with suffer different impacts in poultry.

Currently, there is a trend towards the use of dietary fiber from natural plant sources as feed supplement. Indeed, some agro-industrial by-products can be considered as sources of natural dietary fiber. Cassava by-products in both of dried cassava pulp (DCP) and cassava distiller's dried grains (CDG) are an interesting alternative fiber sources. Due to cassava by-products contain high non-starch polysaccharides (NSPs) contents, approximately 29%, all of which can be regarded as significant sources of dietary fiber. NSPs contents in cassava is almost in the form of insoluble fiber, in which the cecal microorganisms may not be digested or not fully utilized of this NSPs. However, the extraction and modification of dietary fiber by hydrolyzing NSPs bond with enzymes into the form of oligosaccharides may be increased their efficacies and properties for use as feed supplement. Previous studies have shown the possibility of using exogenous enzymes to hydrolyze the NSPs bond in animal feedstuffs. For instance, Ravn et al. (2017) reported that treating wheat bran with exogenous xylanase can decrease the average degree of polymerization, as this enzyme degraded wheat bran arabinoxylan into arabinoxylo-oligosaccharides. In addition, this oligosaccharides also promote microbial population growth and enhance SCFA production via in vitro fermentation assay inoculated with cecal microbial. Previous studies also reported the multicomponent enzymes can decrease degree of polymerization of wheat xylan, and in turn enhancing acetic acid and butyric acid after in vitro fermentation assay (Yacoubi et al., 2016).

4.3 Objective

This study aimed to investigate the optimal ratio of cellulose:xylanase enzymes at levels 0:0, 9:3, 36:12, and 72:24 U/g substrate in order to improve the dietary fiber derived from DCP and CDG and to evaluate the modified-dietary fiber efficacies by using *in vitro* fermentation method inoculated with cecal microbial.

4.4 Materials and methods

4.4.1 Experimental design

The ratios of cellulase : xylanase (3:1) enzymes 4 levels; 0:0, 9:3, 36:12 and 72:24 (U/g substrate (were used to hydrolyze dietary fiber from DCP and CDG, and evaluate the modified-dietary fibers using *in vitro* fermentation assay .The trial was conducted under a completely randomized design with 4 replications of each group .The *in vitro* fermentation assay were compares with cecal inoculum as a control (Table 4.1).

Table 4.1 Dietary fiber and enzyme ratios of the sample in the *in vitro* fermentation.

Treatment	**Dietary fiber source**	
	DCP[1]	**CDG**[2]
cellulase : xylanase (U/g substrate)	0:0	0:0
	9:3	9:3
	36:12	36:12
	72:24	72:24
Control (cecal inoculum)	-	-

[1] Cassava pulp

[2] Cassava distiller's dried grain

4.4.2 Extraction and modification of dietary fiber from cassava pulp and cassava distiller's dried grain

Dried cassava pulp and cassava distiller's dried grains were prepared samples, as in chapter III, section 3.4.1.

The procedure of dietary fiber extraction was modified from Daou and Zhang (2013). Dried samples were pretreated with 6% (w/v) NaOH solution for DCP and 4% (w/v) NaOH solution for CDG (sample to solution volume ratio was 1:5), the mixture was soaked for 1 hour at room temperature, and then washed with distilled water to reach pH 7.0. The sample was suspended in phosphate buffer (pH 6.0) at a ratio of 1:30, and 0.4% (w/v) α-amylase (EC 3.2.1.1, Megazyme International Ltd., Wicklow, Ireland) was added. The mixed sample was incubated in a water bath at 95°C for 1 hour. The dietary fiber extraction was precipitated in 95% (v/v) ethanol at 60°C for 1 hour, and cooled to room temperature. The sample was then filtered and the residue was washed with 78% (v/v) ethanol, followed with 95% (v/v) ethanol, and acetone, respectively, then dried the extracted dietary fiber in a hot air oven at 55-60°C overnight. The procedure of modified-dietary fiber after the sample incubated with α-amylase, the mixed sample was cooled to 60°C, and adjusted to pH 4.5 with 1.0 N HCl solution. The cellulase (EC 3.2.1.4, Megazyme International Ltd., Wicklow, Ireland) and xylanase (EC 3.2.1.8, Megazyme International Ltd., Wicklow, Ireland) enzymes were added at ratio 0:0, 9:3, 36:12, and 72:24 (U/g substrate). The sample was then incubated in a water bath at 60°C for 10 hours. At the end of the incubation time, the sample was heated at 100°C for 10 minutes to inactivate the enzymes, then cooled to room temperature. The modified-dietary fiber was precipitated in 95% (v/v) ethanol in a water bath at 60°C for 1 hour, and cooled to room temperature. The

sample was then filtered and the residue was washed with 78% (v/v) ethanol, 95% (v/v) ethanol, and acetone, respectively, and then dried modified-dietary fiber in a hot air oven at 55-60°C overnight. A flow diagram of dietary fiber extraction and modification is shown in Figure 4.1.

4.4.2.1 Chemical analysis

The cassava pulp extracted dietary fiber (DF-CP), cassava distiller's dried grains extracted dietary fiber (DF-CDG), modified-dietary fiber from cassava pulp (M-DFCP), and modified-dietary fiber from cassava distiller's dried grains (M-DFCDG) were analyzed for total sugar and reducing sugar according to the phenol sulfuric acid method (Dubois et al., 1956) and dinitrosalicylic acid (DNS) method (Miller, 1995), respectively. The assay were calibrated with D-glucose and D-xylose standards (Sigma-Aldrich, St Louis, MO, USA). In addition, non-reducing sugar content was calculated as follows:

Non-reducing sugar = total sugar – reducing sugar

The contents of TDF, SDF and IDF were determined using the Megazyme total dietary fiber Kit (K-TDFR-100A, Megazyme International Ltd., Wicklow, Ireland), according to manufacturer instructions. The functional properties were measured included the water holding capacity (WHC) and oil holding capacity (OHC), according to the method of Yaich et al. (2015). WHC and OHC were estimated with the following formula:

$$\text{WHC (g/g)} = \frac{\text{weights of water adsorbed (g)}}{\text{weights of sample (g)}}$$

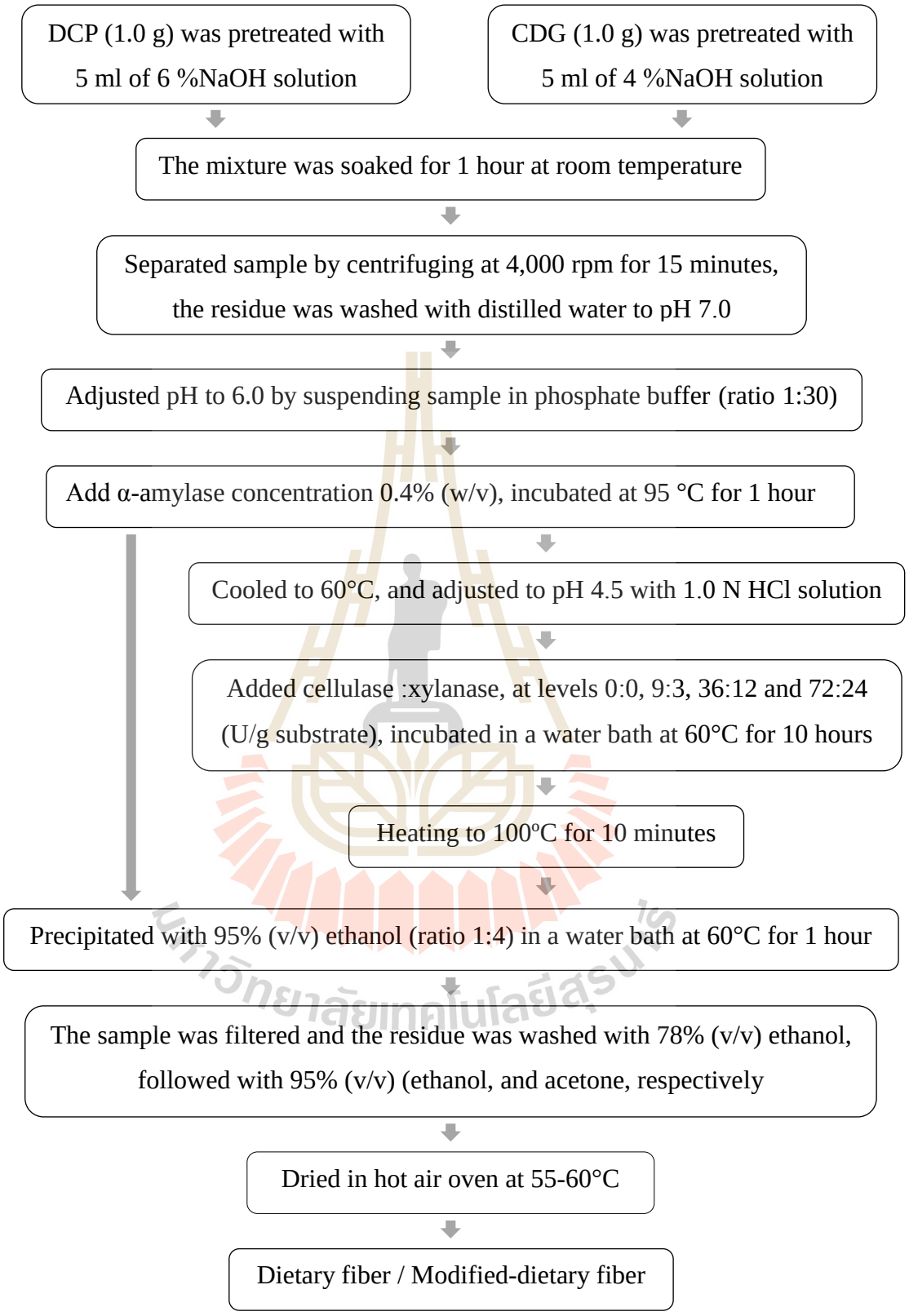

Figure 4.1 Flow diagram of dietary fiber extraction and modification.

$$\text{OHC (g/g)} = \frac{\text{weights of oil adsorbed (g)}}{\text{weights of sample (g)}}$$

4.4.3 *In vitro* incubation procedure

The evaluation of modified-dietary fibers under the in vitro fermentation method inoculated with cecal microbial, the procedure modified from Dunkley et al. (2007) and Donalson et al. (2008). The cecal digesta were obtained from commercial layers (Isa Brown) at 50-60 weeks of age. Cecal digesta were collected into an empty sterile 50 ml conical tube (the samples were used within 15 minutes of slaughter), and mixed thoroughly to obtain a uniform pooled digesta. The cecal contents were diluted to a 1:3,000 concentration (w/v) with anaerobic phosphate buffer to formulate the inoculum, Carbon dioxide (CO_2) was flushed over the cecal inoculum during the preparation.

Approximately 250 mg of each sample was added to sterilized 10 ml vial, with 5 ml of cecal inoculum was added to the vial under anaerobic conditions. In this study have nine treatment groups with 4 replications (Table 4.1), a vial with only cecal inoculum was used as the control. The samples for analysis were conducted at 2 time periods (0 and 24 hours). All 24 hours samples were flushed with CO_2 (anaerobic conditions) and were capped with rubber stoppers and crimp aluminum seals. The in vitro fermentation study was conducted at 37°C in an incubator (anaerobic conditions). For each time point (0 and 24 hours), a 1.0 ml of each samples were centrifuged at 10,000×g for 10 minutes, 4°C. The supernatants were collected, and stored at -20°C for SCFA and lactic acid analysis. Samples (1.0 ml) for the enumeration of bacteria (*Lactobacillus* spp., *Bifidobacterium* spp., and *E.coli*) were taken from the each

fermented samples at after 24 hours. The bacteria analysis were determined using dilution plate count technique on selective medium. The pH was measured in each samples at after 24 hours fermentation.

4.4.3.1 SCFA and lactic acid analysis

The concentration of SCFA (acetic acid, propionic acid, and butyric acid) and lactic acid were analyzed based on Mookiah et al. (2014). A 2 mM 4-methylvaleric acid (Alfa Aesar, United Kingdom) and a 2 mM fumaric acid (Alfa Aesar, United Kingdom) was added into the sample as an internal standard for SCFA and lactic acid measurement, respectively. The analysis was conducted with a gas chromatograph (Agilent 7890B; Agilent Technologies, USA), with flame ionisation detection (FID) and nitrogen as carrier gas. The fused silica capillary column (0.32 mm × 25 m; CP-Sil 5 CB column, Agilent J&W GC Column, USA).

The SCFA and lactic acid concentrations were calculated according to the method of Donalson et al. (2008). The net SCFA and lactic acid concentrations which produced during the 24 hours fermentation were calculated as follows:

Net short chain fatty acid production = S (t 24) – S (t 0)

Net lactic acid production = L (t 24) – L (t 0)

where; S are the SCFA concentrations (mM/ml) at 0 hour and after 24 hours.

L are the lactic acid concentrations (mM/ml) at 0 hour and after 24 hours.

4.4.4 Statistical analysis

Data were subjected to a one-way ANOVA analyzed as a CRD using SPSS version 18.0 (SPSS, Inc., 2010). Significant differences among treatments were

assessed by Tukey's post hoc test. A threshold level of $P < 0.05$ was used to determine significance.

In the study of dietary fiber compositions, orthogonal contrasts were used to compare the effects of :1 (dietary fiber from DCP vs .dietary fiber from CDG, 2 (DF-CP vs. M-DFCP treated with cellulase : xylanase, and 3 (DF-CDG vs. M-DFCDG treated with cellulase : xylanase.

In the study of in vitro fermentation method, orthogonal contrasts were used to compare the effects of: 1) control (cecal inocula) vs. dietary fiber form DCP and CDG, 2) dietary fiber form DCP vs. dietary fiber form CDG, 3) DF-CP vs. M-DFCP treated with cellulase : xylanase, and 4) DF-CDG vs. M-DFCDG treated with cellulase : xylanase.

4.4.5 Experimental location

The experiment was conducted at the Center for Scientific and Technological Equipment Building 14, Suranaree University of Technology.

4.4.6 Experimental period

The experiment was done from March 2017 to November 2017.

4.5 Results

4.5.1 Chemical composition and functional properties of dietary fiber extracted from cassava pulp and cassava distiller's dried grains

The chemical composition and functional properties of dietary fiber extracted from DCP and CDG treated with cellulase : xylanase enzymes at level; 0:0, 9:3, 36:12 and 72:24 (U/g substrate) are presented in Table 4.2.

The reducing sugar, total sugar, and non-reducing sugar of dietary fiber extracted from DCP and CDG were significantly different ($P < 0.05$) among treatments. Furthermore, orthogonal contrasts revealed the values of reducing sugar, total sugar, and non-reducing sugar (both in D-glucose and in D-xylose) which M-DFCP treated with cellulase : xylanase enzymes were higher than the DF-CP group ($P < 0.05$). While M-DFCDG treated with cellulase : xylanase enzymes showed no significant differences in reducing sugar, total sugar, and non-reducing sugar values ($P > 0.05$), excepted in reducing sugar content of D-xylose was significantly influenced by enzymes (orthogonal contrasts, $P < 0.05$). Moreover, the ratios of cellulase : xylanase enzymes at 36:12 and 72:24 (U/g substrate) showed the higher contents of reducing sugar (D-glucose), total sugar and non-reducing sugar (D-xylose) ($P < 0.05$) compared to other ratios (0:0 and 9:3 U/g substrate).

The SDF, IDF, and TDF contents of dietary fiber extracted from DCP were higher than the dietary fiber extracted from CDG ($P < 0.05$). However, there were no significant effects of M-DFCP treated with enzymes on the SDF, IDF, and TDF compositions ($P > 0.05$). While the M-DFCDG treated with cellulase : xylanase enzymes has been shown to increase the IDF and TDF ($P < 0.05$).

The present study showed that the M-DFCP had higher WHC (g of water per g of sample) than the M-DFCDG ($P > 0.05$). However, the OHC (g of oil per g of sample) in both dietary sources did not show any significant differences ($P > 0.05$).

Table 4.2 Chemical composition and functional properties of dietary fiber extracted from cassava pulp and cassava distiller's dried grains treated with cellulase and xylanase enzymes.

Item	Dietary fiber from DCP				Dietary fiber from CDG				Pooled SEM	Orthogonal contrasts[2]		
	DF-CP	M-DFCP[1]			DF-CDG	M-DFCDG[1]						
	0:0	9:3	36:12	72:24	0:0	9:3	36:12	72:24		1	2	3
Glucose standard (mg/g)												
Reducing sugar	6.46^{b}	6.51^{b}	7.32^{a}	7.92^{a}	4.61^{c}	4.65^{c}	4.67^{c}	4.79^{c}	0.234	<0.01	<0.01	0.61
Total sugar	19.88^{c}	22.90^{c}	26.58^{b}	32.57^{a}	8.32^{d}	8.54^{d}	8.65^{d}	8.93^{d}	1.674	<0.01	<0.01	0.69
Non-reducing sugar [3]	13.42^{c}	16.40^{bc}	19.26^{b}	24.65^{a}	3.71^{d}	3.89^{d}	3.98^{d}	4.14^{d}	0.169	<0.01	<0.01	0.70
Xylose standard (mg/g)												
Reducing sugar	3.99^{c}	4.23^{c}	4.89^{b}	5.50^{a}	2.24^{d}	2.55^{d}	2.58^{d}	2.66^{d}	0.213	<0.01	<0.01	0.03
Total sugar	11.77^{b}	13.27^{b}	17.09^{a}	18.83^{a}	5.06^{c}	5.32^{c}	5.11^{c}	5.48^{c}	0.998	<0.01	<0.01	0.72
Non-reducing sugar [3]	7.78^{b}	9.05^{b}	12.20^{a}	13.33^{a}	2.82^{c}	2.77^{c}	2.53^{c}	2.82^{c}	0.116	<0.01	<0.01	0.11
Soluble dietary fiber (%)	3.23^{a}	3.20^{a}	3.25^{a}	3.51^{a}	2.18^{b}	2.25^{b}	2.27^{b}	2.28^{b}	0.109	<0.01	0.65	0.66
Insoluble dietary fiber (%)	24.96^{a}	25.15^{a}	25.72^{a}	25.64^{a}	22.22^{b}	22.77^{b}	23.20^{b}	23.16^{b}	0.256	<0.01	0.15	0.03
Total dietary fiber (%)	28.19^{a}	28.35^{a}	28.97^{a}	29.15^{a}	24.40^{b}	25.02^{b}	25.47^{b}	25.44^{b}	0.344	<0.01	0.09	0.02
Water holding capacity (gw/gs)	2.44^{bc}	2.39^{cd}	2.58^{ab}	2.62^{a}	2.20^{e}	2.28^{de}	2.27^{de}	2.37^{cd}	0.027	<0.01	0.02	0.01
Oil holding capacity (go/gs)	4.12	4.29	4.32	4.33	4.11	4.21	4.2	4.27	0.022	0.09	<0.01	0.09

[a, b, c, d, e] Means with different superscripts in a row are significantly different ($P < 0.05$).

[1] Cellulase and xylanase ratios, 0:0, 9:3, 36:12, and 72:24 U/g substrate.

[2] Orthogonal contrasts: 1) dietary fiber from DCP vs. dietary fiber from CDG, 2) DF-CP vs. M-DFCP treated with cellulase: xylanase, and 3) DF-CDG vs. M-DFCDG treated with cellulase: xylanase.

[3] Non-reducing sugar = total sugar - reducing sugar.

4.5.2 Effects of modified-dietary fibers from cassava pulp and cassava distiller's dried grains on microbial populations using *in vitro* fermentation method.

The results of modified-dietary fibers with cellulase : xylanase enzymes (M-DFCP and M-DFCDG) on microbial populations after in vitro fermentation is presented in Table 4.3. The orthogonal contrasts revealed that dietary fiber extracted from DCP and CDG can increase *Lactobacillus* and *Bifidobacterium* populations compared to the control (cecal inoculum) ($P > 0.05$), but there showed no significant changes in the *E. coli* populations ($P > 0.05$). The dietary fiber from DCP (DF-CP and M-DFCP) can increase *Bifidobacterium* spp. than the dietary fiber from CDG (DF-CDG and M-DFCDG) ($P < 0.05$). However, in both extracted dietary fiber (DF-CP and DF-CDG) and modified-dietary fiber with cellulase and xylanase enzymes (M-DFCP and M-DFCDG) showed no significant changes in *E. coli* populations ($P > 0.05$).

4.5.3 Effects of modified-dietary fibers from cassava pulp and cassava distiller's dried grains on SCFA and lactic acid and pH value after *in vitro* fermentation method.

The results of modified-dietary fiber with cellulase : xylanase enzymes (M-DFCP and M-DFCDG) on SCFA and lactic acid concentrations and pH values after the in vitro fermentation are presented in Table 4.4. The orthogonal contrasts showed that dietary fiber extracted from DCP (DF-CP and M-DFCP) and dietary fiber extracted from CDG (DF-CDG and M-DFCDG) have shown to reduce pH and increase SCFA (acetic, propionic, and butyric acid) and lactic acid concentrations compared to the control (cecal inoculum) ($P < 0.05$). The dietary fiber extracted from DCP (DF-CP and M-DFCP) showed better results on pH value, and SCFA and lactic

acid production than the dietary fiber extracted from CDG (DF-CDG and M-DFCDG) ($P < 0.05$). Moreover, the M-DFCP with cellulase and xylanase enzymes can increase SCFA and lactic acid production after 24 hours fermentation compared to the DF-CP ($P < 0.05$). Unfortunately, there showed no significant difference ($P > 0.05$) between DF-CDG and M-DFCDG.

The use of different ratios of cellulase and xylanase enzymes to improve dietary fiber revealed that the M-DFCP treated with cellulase : xylanase at ratios of 36:12 and 72:24 (U/g substrate) produced higher SCFA (acetic, propionic, and butyric acid) concentrations ($P < 0.05$) than the DF-CP and control group. The lactic acid concentration was significantly increased, and in turn, pH value was reduced ($P < 0.05$) with increasing enzyme levels. Additionally, treating M-DFCDG with cellulase : xylanase at levels 36:12 and 72:24 U/g substrate has shown to increase propionic acid concentration compared to the DF-CDG and control groups ($P < 0.05$). However, there was no significant difference in other parameters ($P > 0.05$).

4.6 Discussion

This optimal conditions of producing dietary fiber derived from DCP through hydrolysis of the polymer chains into oligosaccharides with cellulase and xylanase enzymes at ratio 36:12 U/g substrate. This hydrolysis condition generated the highest reducing sugar (D-glucose), total sugar and non-reducing sugar (D-xylose) contents, and water holding capacity in M-DFCP. Therefore, it indicates that the structure of dietary in DCP includes less complexity molecules and can be easily digested into oligosaccharides. The results from FTIR indicated that the main component of fiber in DCP composed has a simple primary structure of cellulose, a linear chain of glycosidic C–O–C linkages. While

Table 4.3 Microbial populations (log CFU/ml) after the in vitro fermentation of dietary fiber with chicken cecal inoculum.

Item	Dietary fiber from DCP				Dietary fiber from CDG				Control[2]	Pooled SEM	Orthogonal contrasts[3]			
	DF-CP	M-DFCP[1]			DF-CDG	M-DFCDG[1]								
	0:0	9:3	36:12	72:24	0:0	9:3	36:12	72:24			1	2	3	4
Lactobacillus spp.	6.30[a]	6.21[a]	6.21[a]	6.29[a]	6.38[a]	6.42[a]	6.21[a]	6.00[ab]	5.53[b]	0.054	<0.01	0.98	0.64	0.20
Bifidobacterium spp.	6.14[a]	6.16[a]	6.10[a]	6.08[ab]	5.96[ab]	5.91[ab]	5.93[ab]	5.80[ab]	5.70[b]	0.035	<0.01	<0.01	0.78	0.42
E .coli	4.96	4.92	4.73	4.97	4.94	4.79	4.76	4.81	4.63	0.038	0.06	0.40	0.50	0.26

[a, b] Means with different superscripts in a row are significantly different ($P < 0.05$).

[1] Cellulase and xylanase ratios, 0:0, 9:3, 36:12, and 72:24 U/g substrate.

[2] Control = cecal inoculum.

[3] Orthogonal contrasts: 1) Control vs. dietary fiber from DCP and CDG, 2) dietary fiber from DCP vs. dietary fiber from CDG, 3) DF-CP vs. M-DFCP treated with cellulase: xylanase, and 4) DF-CDG vs. M-DFCDG treated with cellulase: xylanase.

Table 4.4 Concentrations of short chain fatty acids, lactic acid and pH after the *in vitro* fermentation of dietary fiber with chicken cecal inoculum.

Item	Dietary fiber from DCP				Dietary fiber from CDG				Control[2]	Pooled SEM	Orthogonal contrasts[3]			
	DF-CP	M-DFCP[1]			DF-CDG	M-DFCDG[1]								
	0:0	9:3	36:12	72:24	0:0	9:3	36:12	72:24			1	2	3	4
Short-chain fatty acid4 (mM/ml)														
Acetic	35.58^{cd}	57.72^{abc}	69.23^{ab}	78.83^{a}	34.76^{d}	35.32^{cd}	52.28^{bcd}	53.02^{bcd}	34.69^{d}	3.049	<0.01	<0.01	<0.01	0.05
Propionic	8.24^{cd}	16.61^{ab}	18.76^{ab}	20.57^{a}	8.14^{cd}	13.72^{bc}	15.24^{ab}	15.31^{ab}	7.18^{d}	0.870	<0.01	<0.01	<0.01	<0.01
Butyric	2.27^{b}	3.52^{a}	3.63^{a}	3.74^{a}	2.24^{b}	3.05^{ab}	3.07^{ab}	3.01^{ab}	1.11^{c}	0.157	<0.01	0.01	<0.01	<0.01
Lactic acid4 (mM/ml)														
	7.77^{d}	14.54^{c}	20.97^{b}	39.52^{a}	6.68^{d}	6.54^{d}	7.10^{d}	7.50^{d}	5.82^{d}	1.130	<0.01	<0.01	<0.01	0.47
pH after 24 hours the *in vitro* fermentation														
	6.80^{c}	6.73^{d}	6.72^{d}	6.66^{e}	6.85^{b}	6.84^{bc}	6.85^{b}	6.85^{b}	7.03^{a}	0.017	<0.01	<0.01	<0.01	0.78

[a, b, c, d, e] Means with different superscripts in a row are significantly different ($P < 0.05$).

[1] Cellulase and xylanase ratios, 0:0, 9:3, 36:12, and 72:24 U/g substrate.

[2] Control =Cecal inoculum.

[3] Orthogonal contrasts: 1) Control vs. dietary fiber from DCP and CDG, 2) dietary fiber from DCP vs. dietary fiber from CDG, 3) DF-CP vs. M-DFCP treated with cellulase: xylanase, and 4) DF-CDG vs. M-DFCDG treated with cellulase: xylanase.

[4] Net production of short chain fatty acids and lactic acid were subtracted the baseline (time 0) from 24 hours samples.

the parameters mentioned above of dietary fiber extracted from CDG were unaffected by enzymatic hydrolysis, except for reducing sugar when measurement based on the xylose standard. The dietary fiber extracted from CDG was observed very negligible amounts of reducing sugars and total sugar. The CDG might be unavailable for digestion by cellulase and xylanase enzymes. Evidence has shown that the fiber in CDG contained high lignin content, approximately 8.69% (Wan et al., 2015), was most likely difficult to degradation. In addition, the FTIR analysis revealed the major component of cellulose in CDG almost represent in the form of crystalline cellulose. With this structure, cellulose chains are packed into partially crystalline fibers, the chains are arranged in sheets, with hydrogen bonding between chains and between monomers in each chain. The crystalline cellulose is exceptionally stiff and strong (Altaner et al., 2014). Previous studies reported that the production of xylo-oligosaccharides from sugarcane bagasse was affected by temperature, dosage of enzyme and incubation time. These factors influenced the differences in reducing sugars concentrations (Jayapal et al., 2013). Moreover, the WHC (gw/gs) is defined as the amount of water that is retained by 1.0 g of dry fiber that revealed a significant difference in M-DFCP of the cellulase : xylanase enzymes at ratio of 72:24 (U/g substrate), it demonstrated that the WHC of M-DFCP was 2.62 (gw/gs) which is similar the WHC of oat hull (2.13 gw/gs), and rice hull (2.58 gw/gs) (Jacometti et al., 2015). The WHC indicates the physical characteristics of fiber is an important determinant of hydratability, there have important physiological effects on the GIT. However, it was difficult to compare the values of WHC, it due to depended on fiber source, conditions (such as temperature, pH, time, and centrifugation) and methods of measurement (Yaich et al., 2015).

This study showed the dietary fiber extracted from DCP and CDG can increase Lactobacillus and *Bifidobacterium* populations, increase SCFA (acetic acid,

propionic acid, and butyric acid) and lactic acid productions, and reduce pH value after the in vitro fermentation. This phenomenon probably due to the beneficial microbial in cecal inocula of chickens, almost can use this dietary fiber as a nutrients source and produce the SCFA and lactic acid are the end products. These end products generated the low-pH environment, thus making a more favorable environments for development of the lactic acid bacteria (such as; *Lactobacillus* spp. and *Bifidobacterium* spp.). This is in agreement with Donalson et al. (2008) who reported that the cecal microbial can ferment cellulose and thus promote the acetic acid production. Additionally, the in vitro fermentation of soybean meal, soybean hull, and alfalfa (their fiber classified as IDF), it revealed that after the fermentation process, all substrates can increase the beneficial bacteria and enhance acetic acid, propionic acid, butyric acid and isobutyric acid (Dunkley et al., 2007; Zduńczyk et al., 2015). Meimandipour et al. (2009) reported that the increases number of lactic acid bacteria, such as *Lactobacillus* spp. and *Bifidobacterium* spp., were correlated with the increases butyric acid concentrations. Therefore, dietary fiber is considered to play an important role in changing the microbial populations in the GIT. In this study, the modification of dietary fiber through enzymatic hydrolysis can improve the functional properties of fiber in DCP, this is because of the cellulase and xylanase enzymes have capability to degrade the NSPs bond of DCP into oligosaccharides, results in better fermentation of microbial. This results are in accordance with the in vitro study observed by Ravn et al. (2017), the xylanase can hydrolyze the the xylan-backbone into arabinoxylo-oligosaccharides in wheat bran. After testing this oligosaccharides using in vitro fermentation with cecal inocula of chickens, a significant increase in butyrate-producing bacteria. In general, the degrees of polymerization influence their fermentation properties and products. The low molecular weight of the degree of polymerization is associated with the high concentrations of

acetic acid and butyric acid (Yacoubi et al., 2016). In the present study, the use of cellulase and xylanase enzymes at levels of 36:12 and 72:24 U/g substrate can modify the dietary fiber structure, resulting in increased acetic acid, propionic acid, and butyric acid productions. The SCFA play an important role on the modulate the microbial populations, this may inhibit some pathogenic bacteria in GIT, whilst providing some energy-yielding substrates to GIT epithelium membrane, and also promote the animal health (Józefiak et al., 2004; Mookiah et al., 2014). Taking into consideration the beneficial effects of dietary fiber in chicken diets to produce a minimum production cost, the enzyme ratio of 36:12 U/g substrate would be an appropriate level for modification the dietary fiber from DCP.

Unfortunately, there was no significant difference between M-DFCDG and DF-CDG, there showed no effects on SCFA, lactic acid, pH value and number of the microbial population. This is because the differences of fiber type and polymer chain composition in CDP and CDG that mentioned above. In the present study, the ratio of cellulase and xylanase cannot degrade the complex structure of crystalline cellulose and lignin in CDG, resulting in cecal microbial less fermented and a consequence of lowering SCFA and lactic acid productions. Additional processes prior extraction and modification using the pretreatment methods such as heat and high pressure, ultrasonic treatment, and microbial method may aid in improving dietary fiber utilization (Zhang et al., 2018).

4.7 Conclusion

The dietary fiber extracted from DCP was more efficient than the CDG. The ratio of cellulose:xylanase at 36:12 U/g substrate possessed an optimum level for the

modification of dietary fiber from DCP . This hydrolysis condition generated the highest reducing sugar, total sugar and non-reducing sugar contents, and water holding capacity.

In addition, the modified-dietary fiber from the cassava pulp (M-DFCP) also enhanced the *Lactobacillus* and *Bifidobacterium* populations, SCFA and lactic acid concentrations and reduced pH value after 24 hours incubation through in vitro fermentation with cecal microbial.

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CHAPTER V

EFFECT OF MODIFIED-DIETARY FIBER FROM CASSAVA PULP IN DIETS ON PRODUCTIVE PERFORMANCE, NUTRIENT DIGESTIBILITY, WEIGHT OF DIGESTIVE ORGAN, ABDOMINAL FAT, AND CHOLESTEROL IN MEAT OF BROILER CHICKENS

5.1 Abstract

This study aimed to investigate the effects of modified-dietary fiber from cassava pulp (M-DFCP), mostly classified as insoluble dietary fiber (IDF), as feed supplement on productive performance, nutrient digestibility, weight of digestive organ, abdominal fat storage, and cholesterol in meat and blood of broiler chickens. A total of 336 one-day-old male broiler chickens (Ross 308) were allocated to 4 groups in 7 replicate pens with 12 chicks each, based on a completely randomized design. Four dietary treatments composed of control and 3 M-DFCP inclusion levels: 0.5, 1.0 and 1.5%. The results showed that M-DFCP showed no negative effects on growth performance in broiler chickens. The inclusion of M-DFCP in diets at 1.0-1.5% had positive effects on increased gizzard weight, reduced gizzard pH, and reduced abdominal fat. The M-DFCP at 1.0% can also increase nutrient digestibility (dry matter, organic matter, and ether extract). In addition, the supplementation of M-DFCP

at 1.0-1.5% in diets represented lower cholesterol in serum, breast and thigh meats, and liver of broiler chickens. In conclusion, these results indicate that M-DFCP can be used as IDF source in broiler diets. The inclusion of 1.0% M-DFCP in broiler diet has positive effects on enhancing gizzard function, improving nutrient digestibility, reducing abdominal fat and cholesterol in chicken meat, blood, and liver.

Key words: modified-dietary fiber from cassava pulp, gizzard, digestibility, abdominal fat, cholesterol

5.2 Introduction

In addition to produce optimized growth performances, formulating poultry diet has recently focused on nutrient improving gastrointestinal tract health and development (Kheravii et al., 2018a). The ban on the use of antibiotics as growth promoters in diets contributed to increased enteric disorders and reduced growth performance in broiler chickens, with serious economic consequences (Yang et al., 2009; Mateos et al., 2012; Kheravii et al., 2018a). Therefore, several alternative ingredients acting on gut health improvement such as probiotics, prebiotics, organic acids, etc. were investigated to replace antibiotics. Nowadays, dietary fibers have gained increasing interest in feed formulation, and are considered as a functional feed supplement. Previous studies have shown an important role of dietary fiber on gastrointestinal development, digestive enzyme activities, and gizzard function, producing a positive effect on animal health, nutrient digestibility and production performances (Sarikhan et al., 2010; Kalmendal et al., 2011; Mateos et al., 2012; Walugembe et al., 2015). In addition, dietary fiber has also been reported to lower fat and cholesterol contents in chicken meat (Saki et al., 2011).

Currently, there is a trend towards the use of modified-fibers derived from natural plant sources as feed supplements. Indeed, some previously neglected agro-industrial by-products can be considered as sources of natural dietary fibers for animal feed. Cassava pulp is a by-product of starch manufacturing containing high quantity of non-starch polysaccharides (NSPs) (29%), mainly cellulose and xylan, approximately 20 and 4.2%, respectively (Kosugi et al., 2009; Choct, 2015). Dried cassava pulp (DCP) can be used as an energy source in broiler and laying hen diets, but the inclusion level should not exceed than 10 and 20%, respectively (Khempaka et al., 2009; 2016). The negative effects of higher proportions are due to its fiber content. However, added at the optimal level, DCP can also reduce abdominal fat in broilers and cholesterol in egg yolk from laying hens. Thus, the dietary fiber extracts from cassava pulp might be useful for animal health and production performances. Generally, the properties of dietary fiber in animal diets are depending on the fiber type, polymer chain composition, structure, and supplementation level. A pre-treatment of crude dietary fibers by enzymatic hydrolysis in vitro may change their structure and in turn, their digestive properties. For instance, Ravn et al. (2017) reported that treating wheat bran with exogenous xylanase before in vitro fermentation assay with caecum originated microbiome, resulted in increased prebiotic oligosaccharide production, as this enzyme degraded wheat bran arabinoxylan into arabinoxylo-oligosaccharides, and in turn enhancing short chain fatty acids (SCFA) production by cecal bacteria. Therefore, processing of cassava pulp fibers by enzymatic treatment might lead to the production of a dietary fiber feed supplement with beneficial effect on animal health and production.

5.3 Objective

In this study, the modified-dietary fibers were produced from DCP by NaOH extraction and subsequent hydrolysis of the polymer chains into oligosaccharides with cellulase and xylanase enzymes. Then the modified-dietary fiber from DCP was supplemented in broiler diets and investigated its effects on productive performance, nutrient digestibility, weight of digestive organ, and abdominal fat storage and cholesterol in meat of broilers.

5.4 Materials and Methods

All experiments were conducted according to the principles and guidelines approved by the Animal Care and Use Committee of Suranaree University of Technology.

5.4.1 Extraction and modification of dietary fiber from cassava pulp

Fresh cassava pulp was obtained from the Korat Flour Industry Co., Ltd, Nakhon Ratchasima, Thailand. The preparation process of cassava pulp was similar as explained in chapter III, section 3.4.1. In addition, the modified-dietary fiber from cassava pulp was also similar as in chapter IV, section 4.4.2, (Okrathok et al., 2019). In briefly, DCP was pretreated with 6% (w/v) NaOH solution (sample to solution volume ratio was 1:5), the mixture was soaked for 1 hour at room temperature, and then washed with water to reach pH 7.0. The sample was suspended in phosphate buffer (pH 6.0) at a ratio of 1:30, and 0.4% (w/v) α-amylase (from *Bacillus subtilis*, SinoBios, China) was added. The mixed sample was incubated in a water bath at 95°C for 1 hour, then cooled to 60°C, and adjusted to pH 4.5 with 1.0 N HCl solution. The cellulase (from *Trichoderma reesei*, SinoBios, China) and xylanase (from

Trichoderma reesei, SinoBios, China) enzymes were added at levels of 36 and 12 U/g substrate, respectively. The sample was then incubated in a water bath at 60°C for 10 hours. At the end of the incubation time, the sample was heated at 100°C for 10 minutes to inactivate the enzymes, then cooled to room temperature. The modified-dietary fiber was precipitated in 95% (v/v) ethanol in a water bath at 60°C for 1 hour, and cooled to room temperature. The sample was then filtered and the residue was washed with 78% (v/v) ethanol, followed with 95% (v/v) ethanol, and pure acetone, successively, and then dried in a hot air oven at 55-60°C overnight. The dried M-DFCP was ground to pass through a 1.0 mm mesh sieve and stored at 4°C until used.

5.4.1.1 Chemical analysis of modified-dietary fiber from cassava pulp

The M-DFCP was analyzed for dry matter (DM) and ether extract (EE) according to the standard methods of AOAC (1990). Total nitrogen was determined by the Dumas combustion technique (AOAC, 2006) using a rapid MAX N exceed, N/protein analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) with L-aspartic acid (Sigma-Aldrich, St Louis, MO, USA) as a calibration standard. The nitrogen content was used to calculate the crude protein (CP) concentration according to the formula: N (%) × 6.25 g of protein per g of sample.

The contents of total, soluble, and insoluble dietary fiber of M-DFCP were determined using the Megazyme total dietary fiber Kit (K-TDFR-100A, Megazyme International Ltd., Wicklow, Ireland), according to manufacturer instructions. The cello-oligosaccharides (COS) and xylo-oligosaccharides (XOS) compositions of the M-DFCP were performed with an ion chromatography instrument (Dionex ICS-5000, Thermo Scientific, Waltham, USA) equipped with a CarboPac

PA200 column (Thermo Scientific, each 3 mm × 250 mm) by a procedure modified from Yong et al. (2013).

The COS (DP between 2 and 5) and XOS (DP between 2 and 6) standards were purchased from Sigma-Aldrich (St Louis, MO, USA), and Megazyme (Megazyme International Ltd., Wicklow, Ireland) respectively.

Table 5.1 Chemical composition of modified-dietary fiber from cassava pulp (as-fed basis).

Composition(%)	**M-DFCP**[1]
Dry matter	94.14
Crude protein	1.70
Ether extract	0.19
Total dietary fiber	28.07
Soluble dietary fiber	2.22
Insoluble dietary fiber	25.85
Cello-oligosaccharides	2.47
Xylo-oligosaccharides	16.26

[1] Modified-dietary fiber from cassava pulp.

5.4.2 Birds and housing

A total of 336, one-day-old male chicks (Ross 308) were purchased from a local commercial hatchery. The chicks were weighed (40.0 ± 0.22 g of initial body weight) and randomly divided into 4 groups of 84 birds, each group including 7 replicates of 12 birds. The chicks were placed in floor pens (1.0 × 2.0 m) on rice husk,

equipped with one tray feeder and 2 nipple drinkers. The housing temperature was maintained at 33°C from day 1 to day 7 and then the temperature was gradually reduced and kept at 24°C until the end of the trial. The chicks received almost continuous light of 23 hours/day for 0-10 days of age, and this was reduced to 18 hours/day from day 11 onwards. The birds were vaccinated for Newcastle disease and Infectious Bronchitis vaccines on day 7 and for Infectious Bursal Disease vaccine on day 14.

5.4.3 Experimental diets and design

Four dietary treatments were as follows: control and M-DFCP at 0.5, 1.0, and 1.5%. All diets were formulated to reach similar levels of calculated metabolizable energy (ME) and CP for each period. The diets were formulated to meet or to exceed the minimum nutrient requirements of broiler chickens as recommended by NRC (1994) and Ross 308: broiler nutrition specification (2014) for starter (0-10 days), grower (11-21 days), and finisher (22-42 days) period. Feed in mash form and water were provided ad libitum throughout the experimental period. Calculated and analyzed values for the experimental diets are presented in Table 5.2 and 5.3. The experiment was conducted under a completely randomized design.

5.4.4 Experimental procedures and samples analyses

The body weight (BW) and feed intake (FI) were measured at 21 and 42 days of age and were used to calculate body weight gain (BWG), average daily gain (ADG), feed conversion ratio (FCR), and productive index (PI).

On day 21 and 42, two birds per pen (14 birds per treatment) were randomly selected, weighed, and used for blood and digestive collection. Blood samples were collected from wing vein and allowed to clot in polypropylene tubes at

room temperature for triglycerides and cholesterol analyses. Subsequently, all chickens were stunned and killed by cervical dislocation. The birds were then eviscerated, and the proventriculus, gizzard, small intestine, and liver were collected. The pH of the gizzard contents was immediately measured following the method of Morgan et al. (2014). The weights of liver, proventriculus, gizzard (empty), duodenum, jejunum, ileum, and abdominal fat were measured (expressed as g/100 g of live BW). The length of duodenum, jejunum, and ileum were also measured (expressed as cm/100 g of live BW). In addition, liver (on day 21 and 42), breast and thigh meats (on day 42) were collected and kept in a clean plastic bag, then stored at -20°C for later cholesterol analysis according to the method of Rowe et al. (1999), using gas chromatography (Agilent 7890B; Agilent Technologies, USA) with cholesterol (Sigma-Aldrich, St Louis, MO, USA) as an external standard and 5-α-cholestane as an internal standard (Sigma-Aldrich, St Louis, MO, USA).

5.4.5 Digestibility study

On Day 21, three birds with BW similar to the average weight of the group were selected from each pen and placed in the metabolic cages (3 birds per cage) for nutrient digestibility measurement using the index method. The experimental diets contained 0.3% titanium dioxide (TiO_2) (Sigma Sigma- Aldrich, St Louis, MO, USA) as an indigestible marker. Feed and water were provided ad libitum. The trial lasted 7 days (21 to 28 days of age), 4 days for adaptation of birds to metabolic cages and last 3 days for excreta collection. About 200 g of excreta were collected on days 26-28 in a clean plastic bag on ice. Excreta were stored at -20°C immediately. These excreta samples were oven dried at 60°C for 3 days, and ground to pass through a 1.0 mm mesh sieve for later analyses.

Diets and excreta were analyzed for DM, organic matter (OM), and EE according to the standard methods of AOAC (1990), and nitrogen content was determined by Dumas combustion technique according to the AOAC (2006), using a rapid MAX N exceed, N/protein analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) with L-aspartic acid (Sigma - Aldrich, St Louis, MO, USA) as a calibration standard. Titanium dioxide concentrations in both the diets and excreta samples were analyzed by the method of Short et al. (1996). The concentration of TiO2 marker and nutrients in the diets and excreta were used to calculate the digestibility and retention. The nutrient digestibility in the assay diets were calculated according to the method of Kong and Adeola (2014). With digestibility was calculated as follows:

$$\text{Digestibility (\%)} = 100 - \left(\left(\frac{\text{CI input x CC output}}{\text{CI output x CC input}} \right) \right) \text{ x100}$$

where; CI input and CI output are the concentration of index marker compound (%) in feed and excreta, respectively.

CC input and CC output are the concentration of component (%) in feed and excreta, respectively.

5.4.6 Statistical Analysis

Data were analyzed using one-way ANOVA of SPSS version 18.0 (SPSS, Inc., 2010). Significant differences among treatments were assessed by Tukey's post hoc test. The effects of M-DFCP inclusion in diets were determined using orthogonal polynomials for linear effects. A threshold level of $P < 0.05$ was used to determine significance.

Table 5.2 Nutrient composition of the experimental diets (as-fed basis).

Ingredients	Starter diets				Grower diets				Finisher diets			
	Control	M-DFCP[1]			Control	M-DFCP[1]			Control	M-DFCP[1]		
		0.5%	1.0%	1.5%		0.5%	1.0%	1.5%		0.5%	1.0%	1.5%
Corn	51.83	51.83	51.83	51.83	52.42	52.42	52.42	52.42	54.76	54.76	54.76	54.76
Soybean meal, 44% CP	31.55	31.55	31.55	31.55	28.05	28.05	28.05	28.05	24.82	24.82	24.82	24.82
Full-fat soybean	5.00	5.00	5.00	5.00	7.00	7.00	7.00	7.00	8.50	8.50	8.50	8.50
Meat meal, 58% CP	3.13	3.16	3.20	3.23	3.12	3.16	3.20	3.23	1.05	1.08	1.12	1.16
Cassava starch	2.53	1.69	0.84	0.00	2.53	1.68	0.84	0.00	2.55	1.7	0.85	0.00
Rice bran oil	2.64	2.95	3.26	3.57	3.60	3.91	4.21	4.52	4.82	5.14	5.45	5.76
M-CPDF[1]	0.00	0.50	1.00	1.50	0.00	0.50	1.00	1.50	0.00	0.50	1.00	1.50
Calcium carbonate	0.95	0.95	0.95	0.95	0.89	0.89	0.89	0.89	1.06	1.06	1.06	1.06
Monocalcium phosphate	1.02	1.02	1.02	1.02	0.98	0.98	0.98	0.98	1.12	1.12	1.12	1.12
Sodium chloride	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.51	0.44	0.44	0.44	0.44
L -lysine	0.05	0.05	0.05	0.05	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08
DL -methionine	0.27	0.27	0.27	0.27	0.28	0.28	0.28	0.28	0.26	0.26	0.26	0.26

Table 5.2 Continue.

Ingredients	Starter diets				Grower diets				Finisher diets			
	Control	M-DFCP[1]			Control	M-DFCP[1]			Control	M-DFCP[1]		
		0.5%	1.0%	1.5%		0.5%	1.0%	1.5%		0.5%	1.0%	1.5%
L -threonine	0.02	0.02	0.02	0.02	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.04
Premix[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calculated composition												
ME (kcal/kg)	3,010	3,010	3,010	3,010	3,100	3,100	3,100	3,100	3,200	3,200	3,200	3,200
Calcium (%)	0.90	0.90	0.90	0.90	0.87	0.87	0.87	0.87	0.79	0.79	0.79	0.79
Available phosphorus (%)	0.46	0.46	0.46	0.46	0.45	0.45	0.45	0.45	0.39	0.39	0.39	0.39
Digestible lysine (%)	1.18	1.18	1.18	1.18	1.15	1.15	1.15	1.15	1.03	1.03	1.03	1.03
Digestible methionine (%)	0.57	0.57	0.57	0.57	0.58	0.58	0.58	0.58	0.53	0.53	0.53	0.53
Digestible methionine + cysteine (%)	0.88	0.88	0.88	0.88	0.87	0.87	0.87	0.87	0.80	0.80	0.80	0.80
Digestible threonine (%)	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.77	0.69	0.69	0.69	0.69

[1] Modified-dietary fiber from cassava pulp.

[2] Premix (0.5%) provided the following (per kg of diet): vitamin A, 15,000 IU; vitamin D3, 3,000 IU; vitamin E, 25 IU; vitamin K3, 5 mg; vitamin B1, 2 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15 µg; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

Table 5.3 Chemical composition of the experimental diets (as-fed basis).

Composition(%)	Starter diets				Grower diets				Finisher diets			
	Control	M-DFCP[1]			Control	M-DFCP[1]			Control	M-DFCP[1]		
		0.5%	1.0%	1.5%		0.5%	1.0%	1.5%		0.5%	1.0%	1.5%
Dry matter	90.23	90.41	90.71	90.89	89.98	90.01	89.87	89.98	91.27	90.50	90.93	90.90
Crude protein	23.39	22.86	22.25	22.27	21.45	21.79	21.36	22.23	19.88	19.90	19.44	19.90
Ether extract	4.75	5.38	4.84	5.89	7.27	7.97	8.42	8.15	9.05	9.30	9.45	9.56
Ash	6.40	6.45	6.55	5.91	5.86	5.97	6.01	5.96	5.63	5.93	5.89	5.83
Crude fiber	2.64	3.42	3.78	4.17	3.11	3.15	3.76	4.06	2.81	3.26	3.51	4.23
Total dietary fiber	11.23	11.52	11.81	11.86	10.47	10.52	10.57	10.66	10.28	10.33	10.72	10.94
Soluble dietary fiber	2.60	2.62	2.64	2.66	2.49	2.58	2.56	2.49	2.36	2.34	2.41	2.43
Insoluble dietary fiber	8.63	8.90	9.17	9.20	7.98	7.94	8.01	8.17	7.92	7.99	8.31	8.51
IDF : SDF2	3.32	3.40	3.47	3.46	3.21	3.08	3.13	3.28	3.36	3.41	3.45	3.50

[1] Modified-dietary fiber from cassava pulp.

[2] Ratios of insoluble and soluble dietary fiber

5.4.7 Experimental location

The experiment was conducted at Suranaree University of Technology's poultry farm, the Center for Scientific and Technological Equipment Building 14, Suranaree University of Technology.

5.4.8 Experimental period

The experiment was done from December 2017 to June 2018.

5.5 Results

5.5.1 Productive Performance

The effect of M-DFCP in broiler diets on productive performance is shown in Table 5.4. The M-DFCP revealed no negative effects on BW, FI, FCR and ADG of broilers in all periods (0 to 21, 21 to 42, and 0 to 42 days of age) ($P > 0.05$). The PI of broilers fed the diets containing M-DFCP was also not significantly different compared to the control diet ($P > 0.05$).

5.5.2 Gizzard pH, Digestive Organ Weight and Abdominal Fat

The results of the inclusion of M-DFCP in diets on gizzard pH, digestive organ weight, small intestine length and abdominal fat in broilers aged 21 and 42 days are presented in Table 5.5. A significant reduction in gizzard pH of broilers fed 1.5% M-DFCP diet was observed as compared to the control diet ($P < 0.05$) and this value was linearly decreased with increasing M-DFCP levels ($P < 0.01$). The relative gizzard weight also linearly increased with increasing M-DFCP in diet. Indeed, a significant increase in gizzard weight was found in broiler groups fed diets containing M-DFCP at levels of 1.0-1.5% compared to control ($P < 0.05$). The M-DFCP did not show any significant effects on the relative weights of proventriculus, small intestine and liver ($P > 0.05$). Similarly, the

relative length of the duodenum, jejunum and ileum was not significantly affected by the treatments.

Table 5.4 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in the diet on growth performance of broiler chickens.

Item	Control	M-DFCP[1]			Pooled SEM
		0.5%	1.0%	1.5%	
Body weight (BW), g/chick					
1 - 21 days	826.5	821.7	830.2	816.7	2.73
21 - 42 days	2577.2	2569.8	2581.7	2587.0	18.30
1 - 42 days	2577.2	2569.8	2581.7	2587.0	18.30
Feed intake (FI), g/chick					
1 - 21 days	990.4	998.6	992.3	976.7	3.35
21 - 42 days	3009.0	2970.4	2923.5	3005.2	23.53
1 - 42 days	3999.5	3968.9	3936.7	3981.9	25.42
Feed conversion ratio (FCR)					
1 - 21 days	1.26	1.27	1.26	1.26	0.00
21 - 42 days	1.71	1.70	1.67	1.68	0.01
1 - 42 days	1.58	1.57	1.55	1.56	0.01
Average Daily Gain (ADG), g/d					
1 - 21 days	37.46	37.22	37.63	36.98	0.13
21 - 42 days	82.81	83.24	83.40	84.33	0.74
1 - 42 days	60.41	60.23	60.52	61.22	0.44
Productive index (PI)					
1 - 21 days	296.38	291.40	299.75	294.09	1.40
21 - 42 days	249.24	250.33	249.99	249.27	2.67
1 - 42 days	389.29	388.07	396.82	388.82	3.09

[1] Modified-dietary fiber from cassava pulp.

Table 5.5 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in broiler diets on gizzard pH, the weight of digestive organs, intestine and abdominal fat.

Item	Control	M-DFCP[1]			Pooled	Linear
		0.5%	1.0%	1.5%	SEM	trend
21 days of age						
Digesta pH of the gizzard	2.05[a]	2.04[ab]	2.01[ab]	1.91[b]	0.02	< 0.01
Relative weight, g/100 g of live BW						
Proventriculus	0.55	0.55	0.56	0.56	0.01	NS[2]
Gizzard	1.62[c]	1.65[bc]	1.74[ab]	1.77[a]	0.02	< 0.01
Liver	2.20	2.23	2.26	2.39	0.04	NS
Duodenum	1.11	1.10	1.11	1.12	0.02	NS
Jejunum	1.44	1.45	1.45	1.47	0.02	NS
Ileum	1.38	1.36	1.38	1.38	0.03	NS
Abdominal fat	1.26[a]	1.16[ab]	1.10[b]	1.08[b]	0.02	< 0.01
Small intestinal length, cm/100 g of live BW						
Duodenum	3.49	3.38	3.55	3.47	0.04	NS
Jejunum	5.76	5.96	6.16	6.44	0.10	NS
Ileum	6.17	6.20	6.14	6.21	0.08	NS
42 days of age						
Digesta pH of the gizzard	2.22[a]	2.21[a]	2.13[ab]	2.04[b]	0.02	< 0.01

Table 5.5 Continue.

Item	Control	M-DFCP[1]			Pooled	Linear
		0.5%	1.0%	1.5%	SEM	trend
Relative weight, g/100 g of live BW						
Proventriculus	0.37	0.38	0.35	0.35	0.01	NS
Gizzard	1.22[b]	1.27[ab]	1.35[a]	1.35[a]	0.02	< 0.01
Liver	12.64	12.86	13.68	13.66	0.27	NS
Duodenum	0.67	0.68	0.69	0.69	0.01	NS
Jejunum	1.15	1.15	1.14	1.16	0.01	NS
Ileum	0.96	0.93	0.93	0.90	0.02	NS
Abdominal fat	11.65[a]	9.55[b]	9.14[b]	9.13[b]	0.30	< 0.01
Small intestinal length, cm/100 g of live BW						
Duodenum	1.27	1.22	1.23	1.23	0.02	NS
Jejunum	2.46	2.49	2.52	2.51	0.03	NS
Ileum	2.49	2.54	2.57	2.57	0.05	NS

[a, b, c] Means with different superscripts in a row are significantly different ($P < 0.05$).

[1] Modified-dietary fiber from cassava pulp.

[2] Not significant.

Interestingly, the M-DFCP showed a beneficial effect on abdominal fat storage. Indeed, increasing M-DFCP in diets linearly decreased the abdominal fat deposition in both periods (21 and 42 days of age). A significant reduction in abdominal fat was found in broiler group fed M-DFCP at levels of 1.0-1.5% (21 days of age) and 0.5-1.5% (42 days of age) ($P < 0.05$).

5.5.3 Nutrient Digestibility and Utilization

The effect of inclusion of M-DFCP in broiler diets on nutrient digestibility and retention are presented in Table 5.6. An improvement of DM, OM, and EE digestibilities was found in broilers fed 1.0% M-DFCP diet compared to other treatments ($P < 0.05$). However, no significant effect was found considering N retention ($P > 0.05$).

Table 5.6 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in broiler diets on nutrient digestibility and retention.

Item	Control	M-DFCP[1]			Pooled	Linear
		0.5%	1.0%	1.5%	SEM	trend
Digestibility, %						
Dry matter	66.91[b]	67.06[b]	68.76[a]	66.88[b]	0.23	NS[2]
Organic matter	72.72[b]	72.77[b]	74.06[a]	72.52[b]	0.18	NS
Ether extract	76.97[b]	76.24[b]	78.98[a]	78.01[ab]	0.32	< 0.01
Nitrogen retention, %	68.14	67.05	68.65	68.12	0.35	NS

[a, b] Means with different superscripts in a row are significantly different ($P < 0.05$).

[1] Modified-dietary fiber from cassava pulp.

[2] Not significant.

5.5.4 Serum Total Cholesterol and Triglycerides

The effects of M-DFCP inclusion in broiler diets on blood serum total cholesterol and triglycerides are shown in Table 5.7. The concentrations of serum total cholesterol and triglycerides were linearly decreased with increasing M-DFCP levels in both periods (21 and 42 days of age), except in broilers aged 42 days, there was no statistically significant difference in triglyceride content ($P > 0.05$).

Table 5.7 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in the diet on blood serum total cholesterol and triglycerides of broiler chickens.

Item	Control	M-DFCP[1]			Pooled	Linear
		0.5%	1.0%	1.5%	SEM	trend
Total cholesterol (mg/dl)						
1 - 21 days	135.42[a]	123.57[b]	121.58[b]	122.14[b]	1.57	< 0.01
1 - 42 days	120.00[a]	112.42[ab]	108.50[b]	108.10[b]	1.58	< 0.01
Triglycerides (mg/dl)						
1 - 21 days	85.50[a]	61.50[b]	61.17[b]	62.67[b]	3.33	< 0.01
1 - 42 days	75.42	64.00	62.50	63.70	2.69	NS[2]

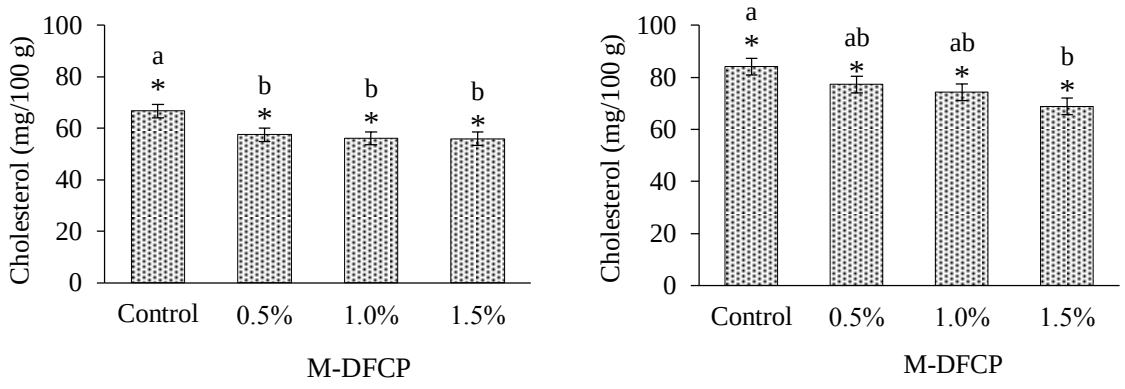
[a, b] Means with different superscripts in a row are significantly different (P < 0.05).

[1] Modified-dietary fiber from cassava pulp.

[2] Not significant.

5.5.5 Cholesterol Content in Meat and Liver

The effect of M-DFCP inclusion in broiler diets on cholesterol content in meat and liver are shown in Figures 5.1 and 5.2. The cholesterol contents in breast and thigh meats were linearly decreased as M-DFCP increased (P < 0.01), the addition of M-DFCP in diets at levels of 0.5-1.5% and 1.5% represented lower cholesterol in breast and thigh meats compared to the control (P < 0.05). Similarly, a significant reduction in liver cholesterol was found in broiler groups fed 1.0-1.5% M-DFCP in both periods (P < 0.05), while only a tendency was observed at 0.5%.

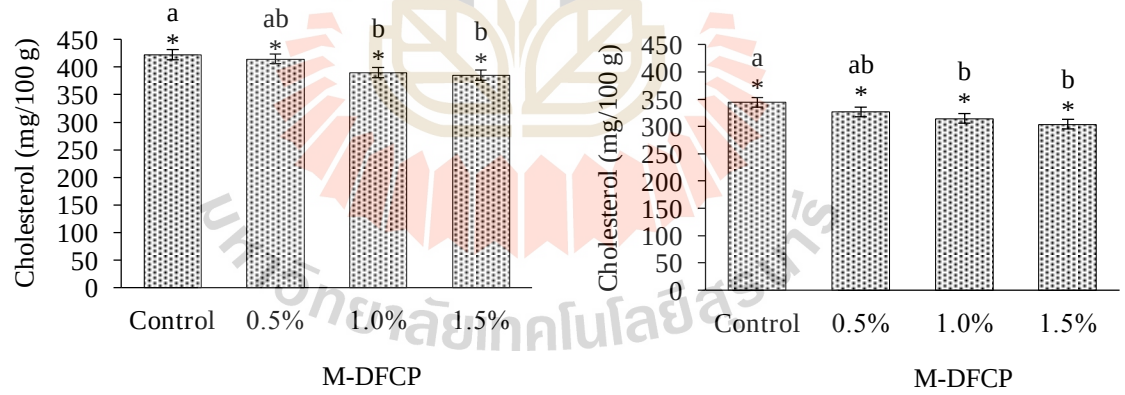


a) Cholesterol in Breast b) Cholesterol in Thigh

Figure 5.1 Figure 5.1 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in the diet on cholesterol in chicken meats (mg/100 g) at 42 days of age.

[a, b] Means with no common superscripts are significantly different ($P < 0.05$).

*Significant linear effect ($P < 0.05$).



a) Cholesterol in Liver (21 days of age) b) Cholesterol in Liver (42 days of age)

Figure 5.2 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in the diet on cholesterol in liver (mg/100 g) at 21 and 42 days of age.

[a, b] Means with no common superscripts are significantly different ($P < 0.05$).

*Significant linear effect ($P < 0.05$).

5.6 Discussion

This study showed for the first time that the inclusion of M-DFCP at the level of 1.0% in broiler diets can improve nutrient digestibility (DM, OM, and EE) without impacting production parameters (BW, FCR, ADG, and PI). The M-DFCP has also been shown to improve gizzard development and lower abdominal fat, and cholesterol in serum, liver and meat of broilers. Several previous studies reported the positive effects of fibers from oat hull, soybean hull, rice hull, barley hull, and sugarcane bagasse on growth performance of broilers (Jiménez-Moreno et al., 2011; Jiménez-Moreno et al., 2013; Incharoen, 2013; Adibmoradi et al., 2016; Kheravii et al., 2017). Moreover, Jung et al. (2019) also indicated the supplementation of modified-insoluble dietary fiber from oak (Quercus mongolica) at 1.0% can promote body weight of broilers, without affecting FI and FCR. In contrast, Kimiaeitalab et al. (2017) showed that no significant effects of dietary fiber on growth performance were found when adding 3.0% sunflower hulls in broiler diets. Similarly, Sabour et al. (2018) reported that dietary fiber from sugar beet pulp (mainly as SDF) and rice hull (mainly as IDF) had no significant effect on growth performance of broilers. Kheravii et al. (2017) has also reported the addition of 2.0% lignocellulose in diet showed no significant effect on broilers performance. Although there have not been consistent reported on the impact of dietary fiber on growth performance in broiler chickens. The existence of these contradictory results may depend on the dietary fiber type, structure and properties, source, and level of usage (Mateos et al., 2012; Adibmoradi et al., 2016; Sabour et al., 2018)

No research studies have been conducted so far to study the effects of M-DFCP in broilers. However, some previous studies revealed that unmodified dried cassava pulp supplied in adequate amount in broiler diets, can lower abdominal fat (Khempaka et al., 2009) and cholesterol in egg yolk (Khempaka et al., 2016). Morgan and Choct (2016)

also revealed the beneficial effect of cassava on abdominal fat pad reduction when using in broiler diets under pellet form. In our study, the M-DFCP has been shown to improve gizzard development of broilers. Significant differences in relative gizzard weight enhancement and in pH reduction in gizzard were found in bird groups fed diets containing M-DFCP at any level (1.0 -1.5% and 1.5%, respectively). This resulted in the improvement of the digestibility of nutrients as mentioned above. In general, gizzard has an important additional function of grinding feed material in poultry (Classen et al., 2016). The M-DFCP showed positive impact on gizzard weight, this probably due to the action of insoluble fraction, because of its hardness would retain for a longer time, inducing thickening of the gizzard muscular wall. Indeed, the M-DFCP used in this study was composed of IDF 25.85%, that could account for this effect on gizzard development. This finding is consistent with previous studies that have revealed the supplementation of IDF in broiler diets can increase gizzard weight and stimulate gizzard function (Kheravii et al., 2018a; Jung et al., 2019), prolong retention time of diets from crop into gizzard, increase gizzard activity and control of feed reflux into gut (Mateos et al., 2012; Guzmán et al., 2016; Yokhana et al., 2016; Kheravii et al., 2018b; Makivic et al., 2019).

Feeding broilers with M-DFCP resulted in lowering gizzard pH, this phenomenon probably due to several reasons, such as the dietary fiber could increase bacterial fermentation in crop through enhancing lactic acid and SCFA production (Classen et al., 2016). On other hand, a reduction of gizzard pH could be the result of increased HCl production and pepsin activity in the proventriculus (Kheravii et al., 2018b; Makivic et al., 2019). Moreover, it has been reported that dietary fiber could stimulate bile salt and endogenous enzyme secretions, and synchronize the digestion and absorption of nutrients (Mateos et al., 2012). All these activities might explain the enhancement of DM, OM, and EE digestibilities when M-DFCP was supplemented at 1.0% in diet. Similar to

previous studies, Adibmoradi et al. (2016) revealed that the supplementation of 1.5% barley hulls and 0.75% rice hulls, both these fiber sources defined as IDF, can improve the digestibility of DM and ash in broilers. The addition of 3.0% sunflower hulls in diets also increased the AMEn at day 9 and day 21, but did not affect the digestibility of nutrients (DM, OM and N) in broilers (Kimiaeitalab et al., 2017). In this respect, the positive effect of IDF on nutrient digestibility is consistent with a raise in gizzard development and digestive enzyme secretion (Mateos et al., 2012).

A reduction of abdominal fat, serum triglyceride and cholesterol, and meat and liver cholesterol were observed in broiler groups fed diets containing M-DFCP. The lowering in cholesterol concentration and fat pad could be due to the reduced lipid and cholesterol emulsification (Hemati Matin et al., 2016), or to the inhibition of bile acid reabsorption (Safaa et al., 2014). Both factors contribute to increase bile acids synthesis by conversion esterification of cholesterol (Hemati Matin et al., 2016) The fermentation of dietary fiber in distal intestinal tract could induce an increased SCFA production, able to interfere with liver metabolism and cholesterol synthesis (Chen, 2003; Safaa et al., 2014). Altogether, this suggests that the beneficial effects of M-DFCP in broiler diets result in the production of low cholesterol meat.

5.7 Conclusion

This study demonstrates that M-DFCP can be used as IDF source in broiler diets without reducing productive performances. In addition, the inclusion of 1.0% M-DFCP has positive effects, enhancing gizzard function, improving nutrient digestibility, and decreasing lipid and cholesterol absorption, resulting in lower abdominal fat and cholesterol in chicken meat and liver.

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CHAPTER VI

EFFECT OF MODIFIED-DIETARY FIBER FROM CASSAVA PULP IN DIETS ON CECAL MICROBIAL POPULATION, SHORT CHAIN FATTY ACID AND AMMONIA PRODUCTION IN BROILER CHICKENS

6.1 Abstract

The objective of this study was to investigate the effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in broiler diets on cecal microbial populations, short chain fatty acid (SCFA), lactic acid, ammonia production and immune responses. Chicks were fed the control and 3 levels of M-DFCP (0.5, 1.0, and 1.5%). The results revealed that the inclusion M-DFCP at 1.0 and 1.5% in broiler diets showed positive effects on stimulating the growth of lactic acid bacteria (LAB) and *Bifidobacterium* population, enhancing SCFA (acetic, propionic, butyric acid and branched SCFA) and lactic acid concentrations in growing periods. In addition, the M-DFCP also reduced cecal and excreta ammonia production of broilers in both periods (0-21 and 22-42 days of age). However, the M-DFCP showed no effects on serum total immunoglobulin (Ig) and lysozyme activity. In conclusion, it indicates that M-DFCP can be used as dietary fiber in broiler diets, with the optimum inclusion level approximately 1.0%.

Key words :modified-dietary fiber, cassava pulp, microbial population, short chain fatty acid, ammonia.

6.2 Introduction

Managing nutrition is one of the crucial goals to improve gut health and production in poultry afterwards the use of antibiotics as growth promoters (AGPs) were banned in feed. Since an improvement of gut health is a key aspect to ensure animal well-being as it links to balanced microbiome and improved immune system (Huyghebaert et al., 2011; Jacquier et al., 2019). Therefore, several alternative ingredients to AGPs are mainly responsibility to preserve the gut microbiota balance in chicken such as probiotics, prebiotics, organic acids, enzymes, etc. Nowadays, dietary fiber has gained renewed interest in poultry nutrition. Previous research has shown that dietary fiber plays an important role in modulating gastrointestinal tract (GIT) microbial community of host, and consequently possess beneficial actions on gut health (Adams et al., 2018; Yadav and Jha, 2019). Thus, developing the understanding of dietary fiber in poultry nutrition as functional feed may process contributed to improving animal health.

Dietary fiber is resistant to digestion by endogenous enzymes in small intestine of chickens, thereby it passes to the lower GIT and in being substrates for fermentation by microorganisms. As a result, SCFA such as acetic, propionic, and butyric acids and other metabolites (lactic and succinic acid) are the main end products of dietary fiber fermentation. (Dunkley et al., 2007; den Besten et al., 2013; Regassa and Nyachoti, 2018). The SCFA formation reduces the pH in cecal environment, which creates unfavorable conditions for pathogenic bacteria proliferation, while this environment

favours the growth of beneficial bacteria, results in improved the microbial population balance and promoted the gut health of monogastric animals (Krás et al., 2013; Rinttilä and Apajalahti, 2013; Zduńczyk et al., 2015; Kheravii et al., 2018a). As well as, reduction of ammonia concentration in poultry excreta (Roberts et al., 2007). Moreover, the SCFA also appeared to have immunity related functions in broiler chickens (Sabour et al., 2018).

Currently, there is an interesting trend of using modified-dietary fiber derived from natural plant sources, such as agro-industrial by-products, as feed supplement in poultry diets. Cassava pulp is a by-product from cassava starch manufacturing, this pulp contains high quantity of non-starch polysaccharides (NSPs), approximately 29%, which mainly comprises of cellulose and xylan approximately 20 and 4.2%, respectively (Kosugi et al., 2009; Choct, 2015). The IDF (lignocellulose) has been shown to modulate gut microflora and SCFA production (Boguslawska-Tryk et al., 2015; Kheravii et al., 2017). It's therefore important to consider nutritional strategies to enhance SCFA when formulating feed for poultry in the future (Kheravii et al., 2018b). Ravn et al. (2017) reported that treating wheat brand with exogenous xylanase prior assessment its effect through in the in vitro cecal fermentation assay, resulting in decreased degree of polymerization of wheat bran arabinoxylan into arabinoxylo-oligosaccharides, and in turn enhancing SCFA production by cecal bacteria fermentation. Courtin et al. (2008), suggest that low-molecular-weight of arabinoxylan act as prebiotics. Ideally, the dietary fiber extracts from cassava pulp and processing of crude dietary fibers by enzymatic treatment may change their structure or degree of polymerization. Therefore, the efficiency properties of dietary fiber in animal diets on gut microflora were related to fiber type, polymer chain composition,

structure, and supplementation level, and also dietary fiber level may influence the SCFA profile.

6.3 Objective

The objective of this study was to investigate the effects of modified-dietary fiber from cassava pulp inclusion in the diets on cecal microbial population, short chain fatty acid, lactic acid, ammonia production and immune responses.

6.4 Materials and Methods

All experiments were conducted according to the principles and guidelines approved by the Animal Care and Use Committee of Suranaree University of Technology.

6.4.1 Preparation of Modified-dietary fiber from cassava pulp

Fresh cassava pulp was obtained from the Korat Flour Industry Co., Ltd, Nakhon Ratchasima, Thailand. There were prepared the DCP similar as described in chapter III, section 3.4.1.

The modification of dietary fiber from cassava pulp was similar as in chapter V, section 5.4.1

6.4.2 Birds and housing

The birds used in these experiment were the same group as in chapter V.

6.4.3 Experimental diets and design

Four experimental diets were used :control and M-DFCP at 0.5, 1.0 and 1.5%, as in chapter V, section 5.4.3. The experiment was conducted under a completely randomized design. The calculated and analyzed values for the experimental diets are presented in chapter V, Table 5.2 and 5.3.

6.4.4 Sampling procedures

Day 26 to 28, the fresh excreta were collected, as described earlier in chapter V, section 5.4.5, the excreta were stored at -20°C for ammonia analysis.

On 21 and 42 days of age, two birds per replicate (14 birds per treatment) were randomly selected, weighed individually (as in chapter V, section 5.4.4), and used for blood and cecal digesta collection. About 3.0 ml blood samples from these chicks were collected in polypropylene tubes. Blood serum were separated by centrifugation at 1,000×g, for 15 min, then pure serum samples were aspirated by a pipette, transferred into 1.5 ml sterilized tubes, and stored at -20°C for further analysis. Subsequently, all chickens were euthanized and the carcasses were opened immediately. Both cecal were removed, the digesta of cecal were collected aseptically from each bird into an empty sterile 15 ml conical tube (right side for microbial analysis and left side for SCFA, lactic acid and ammonia production) on ice, and then were kept frozen at -20°C until analysis.

6.4.4.1 Cecal microbial populations analysis by quantitative real-time PCR

The contents of cecal digesta were used for quantification of LAB, *Bifidobacterium* spp., *Enterobacter*, and *E. coli*. Bacterial DNA was isolated using the QIAamp Fast DNA Stool kit (Qiagen Inc., Hilden, Germany) according to the instructions of the manufacturer. The extracted DNA was quantified with a NanoVue Plus Nano Drop spectrophotometer (GE Healthcare, UAS) to evaluate the purity and concentration, and then was stored in -80°C for further analysis.

The populations of cecal microbial were analyzed by quantitative real-time PCR (qPCR). The extracted DNA was used as DNA templates for PCR amplification. The primer sequences in this study was determine according to Rezaei et al. (2015). The following primers were used to quantify different bacteria populations: for LAB, F-5'-CATCCAGTGCAAACCTAAGAG-3' and R-5'-GATCCG CTTGCCTTCGCA-3'; for *Bifidobacterium* spp., F-5'-GGGTGGTAATGCCGGA TG-3' and R-5'-TAAGCCATG GACTTTCACACC-3'; for *Enterobacter*, F-5'-CATTGACGTTAC CCGCAGAAGAAGC -3' and R-5'-CTCTACGAGACTCAAGCTTGC-3'; and for *E. coli*, F-5'-GTGTGATATC TACCCGCTTCGC-3' and R-5'-AGAACGCTTTGTGGTTAATC AGGA-3'.

The qPCR assay was performed with LightCycler® 480 Instument II (Roche Diagnostics, GmbH, Mannheim, Germany). The PCR reaction was performed in white LightCycler® 480 Multiwell Plate 96 plates (Roche, Mannheim, Germany) in a final volume of 10 µL using the QuantiFast® SYBR® Green PCR kit (Qiagen Inc., Valencia, USA). Each reaction included 5.0 µl of 2×SYBR Green Master Mix, 0.4 µl of 10 µM forward primer, 0.4 µl of 10 µM reverse primer, 2.0 µl of DNA samples and 2.2 µl of nuclease-free water. Each sample was analyzed with triplicate reactions. The reaction conditions for amplification of DNA were initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 20 seconds, primer annealing at 50°C for *E.coli*, 58°C for LAB, 60°C for *Bifidobacterium* spp. and *Enterobacter* for 30 seconds respectively, and extension at 72°C for 20 seconds (Sharmila et al., 2015). To confirm the specificity of amplification, melting curve analysis was carried out after the last cycle of each amplification.

Absolute quantification of cecal microbial population was achieved by using standard curves constructed by amplification of known amount of target bacteria DNA. Standard bacterial strains used in this study including *Lactobacillus acidophilus* (ATCC 4356), *Bifidobacterium bifidum* (ATCC 29521), *Enterobacter cloacae* (ATCC 13047), and *Escherichia coli* (ATCC 25922) were obtained from the American Type Culture Collection (ATCC; Manassas, USA.).

6.4.4.2 SCFA, lactic acid and ammonia analysis

The cecal digesta was treated with 24% meta-phosphoric acid in 1.5 M H_2SO_4 (sample to solution ratio was 1:1) and vortex to mix. The samples was left overnight at room temperature, and then centrifuged at 10,000×g, 4°C, for 20 min, and the supernatant was used for analysis. The concentration of SCFA (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acid) and lactic acid were analyzed, as in chapter IV, section 4.4.3.1.

The content of ammonia production in cecal digesta and excreta were determined by a procedure modified from Willis et al. (1996). Add 250 mg of sample to the tube and 50 ml of 5% Li_2CO_3 was added, vortex to mix and centrifuged at 10,000×g, 4°C, for 15 min. Transfer up to 500 µl of supernatant to a 15 ml tube, add 4 ml of salicylate reagent followed by 1 ml of hypochlorite reagent and vortex briefly. The mixture was incubated at room temperatures for 30 min and measured the absorbance at 685 nm. Finally, comparison with a calibration curve of ammonium standard.

6.4.4.3 Serum total immunoglobulin and lysozyme analysis

Serum samples were used for total immunoglobulin (Ig) analysis by a total protein kit, (Micro Lowry, Peterson's Modification, Sigma, Product

Codes: TP0300-1KT). Serum lysozyme activity was determined by using the method described by Kreukniet et al. (1995), using Micrococcus lysodeikticus cells as substrate.

6.4.5 Statistical Analysis

All data were analyzed as a completely randomized design with 4 treatments using one-way ANOVA of SPSS version 18.0 (SPSS, Inc., USA, 2010). Significant differences between means were separated by Tukey's post hoc test. Adequate contrasts were performed to study the linear responses of the different variables to increase levels of the M-DFCP inclusion in the diets. A threshold level of $P < 0.05$ was used to determine significance.

6.4.6 Experimental location

The experiment was conducted at Suranaree University of Technology's poultry farm, the Center for Scientific and Technological Equipment Building 11 and Building 14, Suranaree University of Technology.

6.4.7 Experimental period

The experiment was done from December 2017 to September 2018.

6.5 Results

6.5.1 Cecal microbial populations

The results of microbial populations in cecal contents of broiler fed M-DFCP is shown in Figures 6.1. The M-DFCP can change the cecal LAB of broilers in both periods (21 and 42 days of age). The populations of LAB were linearly increased with increasing M-DFCP levels in diets ($P < 0.01$), as its number were

significantly higher in broilers fed 1.0 and 1.5% compared to the control group (P < 0.05). At 21 days of age, the *Bifidobacterium* spp. population increased linearly with

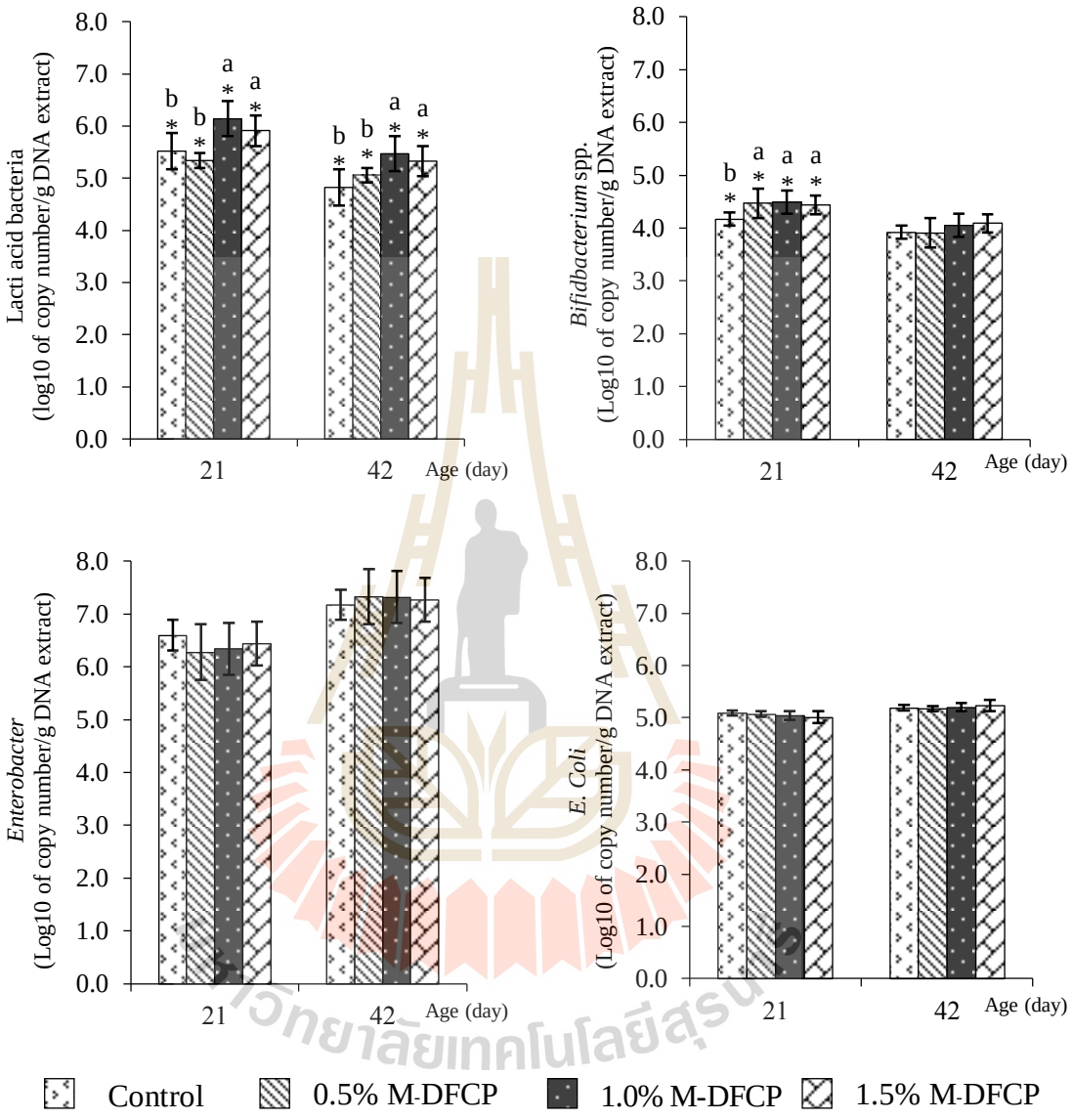

Figure 6.1 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in the diet on cecal microbial population of broilers (log10 of copy number/g DNA extract).

[a, b] Means with no common superscripts are significantly different (P < 0.05).

[*] Significant linear effect (P < 0.05).

increasing M-DFCP levels in diets, a significant enhancement was observed in all M-DFCP diets (0.5-1.5%) as compared to the control. However, no such significant difference was observed in broilers aged 42 days ($P > 0.05$). In addition, feeding broilers with all M-DFCP diet groups also showed no significant differences in the populations of *Enterobacte*r and *E. coli* ($P > 0.05$) as compared to the control group.

6.5.2 SCFA and lactic acid concentrations

The effects of M-DFCP inclusion in broiler diets on concentrations of cecal SCFA and lactic acid are shown in Table 6.1. At 21 days of age, cecal SCFA contents were linearly increased with increasing M-DFCP levels in broiler diets ($P < 0.01$). The broiler groups fed diets containing 1.0% M-DFCP produced significantly higher acetic, propionic, butyric acid and branched SCFA than the control group ($P < 0.05$). Indeed, the inclusion of M-DFCP (0.5 to 1.5%) in diets can enhance the production of butyric acid ($P < 0.05$) as compared to the control diet. While the highest valeric acid concentration was found in 0.5% and control diet ($P < 0.05$). In contrast, at 42 days of age, cecal SCFA (acetic, propionic, butyric, valeric acid and branched SCFA) was not significantly affected by the treatments ($P > 0.05$).

A significant increase in cecal lactic acid production of birds was found in both periods (21 and 42 days of age). The lactic acid concentration was linearly increased with increasing M-DFCP levels in diets ($P < 0.01$), the significant differences was found in broilers fed 1.0 and 1.5% M-DFCP than the control group.

Table 6.1 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in the diet on cecal SCFA and lactic acid concentrations of broiler chickens at 21 and 42 days of age.

Items	Control	M-DFCP[1]			Pooled SEM	Linear trend
		0.5%	1.0%	1.5%		
21 days of age						
Short-chain fatty acids (% of total SCFA)						
Acetic acid	48.77^{b}	51.44^{b}	63.20^{a}	63.19^{a}	1.83	< 0.01
Propionic acid	2.94^{b}	3.98^{ab}	5.00^{a}	4.78^{ab}	0.28	< 0.01
Butyric acid	2.07^{b}	3.67^{a}	4.13^{a}	4.17^{a}	0.23	< 0.01
Branched SCFA[2]	0.28^{c}	0.46^{bc}	0.74^{ab}	0.95^{a}	0.07	< 0.01
Valeric acid	45.94^{a}	40.45^{a}	26.93^{b}	26.92^{b}	2.17	< 0.01
Lactic acid (umol/g of digesta)						
	15.58^{c}	16.26^{c}	19.46^{b}	24.98^{a}	0.84	< 0.01
42 days of age						
Short-chain fatty acids (% of total SCFA)						
Acetic acid	77.07	77.60	77.15	78.77	0.46	NS[3]
Propionic acid	5.44	5.95	5.87	6.22	0.18	NS
Butyric acid	4.17	4.40	4.44	4.44	0.16	NS
Branched SCFA[2]	0.93	0.92	1.01	1.10	0.06	NS
Valeric acid	12.39	11.13	11.53	9.47	0.60	NS
Lactic acid (umol/g of digesta)						
	23.65^{b}	24.29^{b}	32.53^{a}	32.50^{a}	0.92	< 0.01

$^{a, b, c}$ Means with different superscripts in a row are significantly different ($P < 0.05$).

[1] Modified-dietary fiber from cassava pulp.

[2] Branched SCFA = isobutyric acid + isovaleric acid.

[3] Not significant.

6.5.3 Ammonia production in cecal digesta and excreta

The effects of the inclusion of M-DFCP in broiler diets on ammonia production are shown in Table 6.2. The ammonia concentration in cecal and digesta decreased linearly as increasing M-DFCP in broiler diets ($P < 0.01$). A significant reduction in ammonia concentration was found in in broiler fed 0.5 to 1.5% M-DFCP ($P < 0.05$) in both periods (21 and 42 days of age).

Table 6.2 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in broiler diets on ammonia production of cecal digesta and excreta.

Items	**Control**	**M-DFCP[1]**			**Pooled**	**Linear**
		0.5%	**1.0%**	**1.5%**	**SEM**	**trend**
Ammonia production (mg/g of cecal digesta)						
21 days of age	0.90[a]	0.73[b]	0.72[b]	0.72[b]	0.02	< 0.01
42 days of age	1.11[a]	0.95[b]	0.89[bc]	0.82[c]	0.03	< 0.01
Ammonia production (mg/g of excreta)						
	1.96[a]	1.68[b]	1.63[b]	1.64[b]	0.04	< 0.01

[a, b, c] Means with different superscripts in a row are significantly different ($P < 0.05$).

[1] Modified-dietary fiber from cassava pulp.

6.5.4 Serum total immunoglobulin and lysozyme concentration

The effects of M-DFCP inclusion in broiler diets on serum total Ig and lysozyme activity are shown in Table 6.3. The M-DFCP inclusion in diets did not

show any significant effects on total Ig and lysozyme activity in both periods (21 and 42 days of age) of broilers (P > 0.05).

Table 6.33 Effects of the inclusion of modified-dietary fiber from cassava pulp (M-DFCP) in the diet on total immunoglobulin and lysozyme activity in blood serum of broiler chickens.

Items	Control	M-DFCP[1]			Pooled SEM	Linear trend
		0.5%	1.0%	1.5%		
Total Ig (mg/ml)						
21 days of age	18.35	18.41	18.45	18.42	0.21	NS[2]
42 days of age	18.21	18.43	18.52	18.53	0.22	NS
Lysozyme (μg/ml)						
21 days of age	4.21	4.20	4.25	4.26	0.02	NS
42 days of age	4.21	4.22	4.23	4.26	0.02	NS

[1] Modified-dietary fiber from cassava pulp.

[2] Not significant.

6.6 Discussion

It is generally accepted the dietary fiber plays an important role in the shift of gut microbial populations and their metabolites (SCFA and lactic acid) (Rinttilä and Apajalahti, 2013; Regassa and Nyachoti, 2018). In the current study, the inclusion of M-DFCP in broiler diets can promote the growth of LAB and Bifidobacterium spp. populations and consequently enhance the SCFA and lactic acid productions. In addition, the M-DFCP also has shown to reduce the ammonia concentration in both

cecal digesta and excreta. The optimum supplementation level of M-DFCP for broilers should be 1.0% in diets.

The M-DFCP can modulate the number of LAB and *Bifidobacterium* spp., this probably due to the fiber content in M-DFCP is easily fermented in the caecum. The M-DFCP used in this study is the degradation products principally contain the major portions of cellulose and xylan derived from DCP through cellulase and xylanase enzymatic hydrolysis. This M-DFCP is classified as insoluble fiber and composed of COS and XOS approximately 2.47 and 16.26%, respectively. Generally, the COS and XOS are mixtures of oligosaccharides formed by glucose, xylose, and arabinose, a structure to function relationship in term of prebiotics. Thus, the M-DFCP has been shown to provide as an energy source for the beneficial bacteria (LAB and *Bifidobacterium* spp.). As a consequence of enhancing beneficial cecal fermentation products such as acetic, propionic, butyric, and lactic acid in broiler chickens. This is in agreement with the previous studies revealed the bacteria strains such as *Lactobacillus* spp. and *Bifidobacterium* spp. were able to utilize COS and XOS (Moura et al., 2007; Hasunuma et al., 2011). These results were in agreement with Walugembe et al. (2015) who indicated that caecal microbiota is changed by modification in diet, the increase in fermentable dietary fiber concentration in diets results in increased caecal SCFA concentrations. De Maesschalck et al. (2015) reported that supplementing XOS in broiler diets resulted in increased caecal *Lactobacillus* and butyrate-producing bacteria populations. They also reported that the in vitro fermentation assay of XOS revealed cross-feeding between *Lactobacillus crispatus* and *Anaerostipes butyraticus*, which lactic acid and butyric acid produced by *L. crispatus* and *A. butyraticus* during fermentation. These data show the beneficial

effects of XOS on broiler GIT function, which potentially can be explained by stimulation of butyrate-producing bacteria.

As well as, the supplementation of COS in diets can ameliorate heat stress in broilers through improving intestinal microflora, morphology and barrier integrity. Song et al. (2013) reported that broilers on the COS-supplemented diets had higher cecal *Lactobacillus* and lower *E. coli* populations, because of COS can be metabolized by microbes which generate SCFA such as butyric acid. Moreover, in broilers fed 0.5% lignocellulose had better SCFA production (Boguslawska-Tryk et al., 2015). Similarly, the findings of this study revealed the positive effects of COS and XOS in M-DFCP on proving the suitable environment for beneficial microbes and stimulating the production of SCFA (acetic, propionic and butyric acid) and lactic acid in cecal. In general, high SCFA and lactic acid concentrations create a lower pH cecal environment, resulting in the inhibition viability of pathogenic bacteria in GIT (Mookiah et al., 2014; Kheravii et al., 2018b). However, the M-DFCP did not influence the cecal *Enterobacter* and *E. coli* populations of broilers. Previous studies have documented the changes in microflora during the period, caecal contain the largest number of bacteria in the chicken GIT, and it can be divided into 3 populations: dominating, sub-dominating, and temporary. These microbial populations are influenced by many factors such as diet, health status, and age (Józefiak et al., 2004; Amit-Romach et al., 2004).

In poultry housing, ammonia is a major issue of aerial pollution, it can adversely affect the health, productive performance, and welfare of animals (Rothrock et al., 2008). In this study demonstrated that the feeding broilers with M-DFCP resulted in a significant reduction in cecal and excreta ammonia concentrations of

broilers, this probably due to the M-DFCP has capable of activating the metabolism and growth of beneficial bacteria and producing SCFA, as a consequence of lowering pH in cecal. A reduction in cecal pH has been reported to associate with the lower ammonia emission, due to low pH value may inhibit bacterial enzymes that involved in the breakdown of uric acid to ammonia (Roberts et al., 2007). Indeed, the concentration of ammonia is proportional to uric acid excretion which the conversion of uric acid to ammonia requires urease (McCrory and Hobbs, 2001). Additionally, Liu et al. (2007) has also reported that feeding *Lactobacillus reuteri* supplemented diet can noticeably reduce the concentration of ammonia in the excreta of broiler because of a reduction of excreta urease activity produced by *Lactobacillus reuteri*.

Although the inclusion of M-DFCP in broiler diets showed the positive impacts on the growth of LAB and *Bifidobacterium* spp. SCFAs, there no significant effects on serum total Ig and lysozyme activity. In our study, total Ig and lysozyme were determined in blood serum, if we are analyzed total Ig and lysozyme activity in mucosa from gut may find positive effects. Normally, SFCAs, mainly acetic, propionic and butyric acid provide energy sources for epithelial cells in gut and have profound effects on immunomodulation, butyric acid has also been shown stimulated of host immune system (Pourabedin and Zhao, 2015; De Maesschalck et al., 2015).

6.7 Conclusions

The M-DFCP contains the major portions of COS and XOS. The optimum inclusion of 1.0% M-DFCP in broiler diets showed the positive effects on improving cecal LAB and *Bifidobacterium* populations, enhancing SCFA and lactic acid

production, resulting in lower ammonia concentration. However, the M-DFCP showed no effects on total Ig and lysozyme in serum of broiler chickens.

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CHAPTER VII

OVERALL CONCLUSION AND IMPLICATION

7.1 Conclusion

Cassava by-products in the form of DCP and CDG are annually generated in large amount from the cassava manufactures in Thailand. These waste by-products contain high in quantity of non-starch polysaccharides, mainly cellulose (20%) and xylan (4.2%). Thus, the dietary fiber extracts from these DCP and CDG would useful for animals. In this study, modified-dietary fibers were produced from cassava by-products through NaOH extraction and subsequent hydrolysis of the polymer chains into oligosaccharides with cellulase and xylanase enzymes. They were tested in broiler diets in order to investigate their potential effects for use as functional feed in broilers. The main results are summarized as follows:

7.1.1 The optimal conditions for extracting dietary fiber from DCP and CDG were under treated with 6% and 4% NaOH solution, respectively. These conditions yielded the highest total and insoluble dietary fiber contents. These results were associated with the FTIR spectrum integration for semi-quantitative analysis, which obtained the highest cellulose content and clearly separated the spectral distribution in PCA analysis.

7.1.2 The optimal conditions of producing dietary fiber derived from DCP and CDG through hydrolysis of the polymer chains into oligosaccharides with cellulase and xylanase enzymes. It revealed the dietary fiber source from DCP was

more efficient than the CDG. The ratio of cellulase : xylanase at 36:12 U/g substrate possessed an optimum level for the modification of dietary fiber from DCP. This hydrolysis condition generated the highest reducing sugar (D-glucose), total sugar and non-reducing sugar (D-xylose) contents, and water holding capacity. In addition, the M-DFCP also enhanced the Lactobacillus and Bifidobacterium populations, SCFA and lactic acid concentrations and reduced pH value after 24 hours incubation through in vitro fermentation with cecal microbial.

7.1.3 The M-DFCP can be used as functional feed in broiler diets without reducing productive performances. The optimum inclusion level of 1.0% M-DFCP possessed positive effects, enhancing gizzard function, improving nutrient digestibility, and decreasing abdominal fat and cholesterol in chicken meat and liver. In addition, the M-DFCP showed the potential effects, improving cecal LAB and Bifidobacterium populations, enhancing SCFA and lactic acid productions, and reducing ammonia concentration. However, the M-DFCP showed no effects on total immunoglobulin and lysozyme activity in serum of broiler chickens.

7.2 Implication

This present study demonstrated that the modification dietary fiber from DCP can be used as a functional feed for broilers. The M-DFCP has the valuable prebiotic properties since it is composed of cello-oligosaccharides and xylo-oligosaccharides (approximately 2.46 and 16.26%, respectively). Future development of quality extraction and modification of dietary fiber could therefore influence more benefits in broilers. However, it is interesting to note that the functional properties of dietary fiber from CDG were little changed under treated with cellulase and xylanase enzymes.

This is most likely due to the complexity of dietary fiber structures in CDG such as crystalline of cellulose and lignin contents. These complex polysaccharides are difficult to degrade by enzymes. Additional processes prior extraction and modification using the pretreatment methods such as heat and high pressure, ultrasonic treatment, and microbial method may aid in improving dietary fiber utilization.

BIOGRAPHY

Supattra Okrathok was born on 26th September 1987 in Nakhon Ratchasima, Thailand. In 2010, she obtained her Bachelor of Science in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology. In 2013, she received her Master of Science in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology. In 2013, she was awarded got a scholarship by the Royal Golden Jubilee Ph.D. (RGJ-PHD) Program (Grant number PHD/0054/2556) for her Doctor of Philosophy (Ph.D. degree) study in Animal Production Technology at the School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima. During her doctoral study, she had an opportunity to go abroad for training in the nutrition section and microbiology section at the Department of Animal Nutrition, Faculty of Animal Sciences, Wageningen University & Research, Wageningen, The Netherlands, for 6 months (from 1st Sep 2018 to 28th Feb 2019). During her Ph.D. study, she has published one article "**Okrathok, S.,** Sirisopapong, M., Molee, W., and Khempaka. S. (2019). *In vitro* fermentation of extracted dietary fiber from cassava pulp and cassava distiller' s dried grain and their effects on microbial populations , short chain fatty acids and lactic acid production. **Khon Kaen Agric. J.** 47:271-280."