PRODUCTION OF CRUDE EXTRACT AND MALTODEXTRIN FROM SWEET CASSAVA AND ITS EFFECT ON LONG ENDURANCE EXERCISE

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

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การผลิตสารสกัดหยาบและมอลโตเดกซ์ตรินจากมันสำปะหลังหวาน และผลต่อความทนทานในการออกกำลังกาย

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

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วัตถุประสงค์ของการศึกษานี้ เพื่อศึกษาผลของมอลโตเดกซ์ตรินและสารสกัดหยาบจากมันสำปะหลังชนิดหวานที่มีผลต่อความล้าของกล้ามเนื้อ และความทนทานในการออกกำลังกายในหนูขาวเพศผู้พันธุ์วิสตาร์ โดยทำการศึกษาการทดลองมีการบังคับให้หนูทดลองว่ายน้ำโดยใส่น้ำหนักที่หางซึ่งใช้ในการบ่งชี้ถึงความสามารถการทำงาน มีการวัดค่าความสัมพันธ์ของอวัยวะและน้ำหนักตัวของตับและกล้ามเนื้อลาย (soleus extensor digitorum longus (EDL) และ gastrocnemius) และปริมาณไกลโคเจนในตับและกล้ามเนื้อ gastrocnemius ของหนูทดลอง ในกระบวนการผลิตมอลโตเดกซ์ตรินด้วยการประยุกต์ใช้เอนไซม์ การหาปริมาณของเอนไซม์ที่เหมาะสมโดยใช้วิธีการพื้นผิวตอบสนอง ตัวแปรตามที่ใช้ในการพิจารณาคือ ร้อยละของมอลโตเดกซ์ตรินที่สกัดด้วยสารละลายที่เป็นกลาง จากผลการศึกษาพบว่า ปริมาณของมอลโตเดกซ์ตรินสูงสุดที่ได้ คือ ร้อยละ 17.63% และค่าสมมูลเดกซ์โทรส เท่ากับ 15 จากการใช้ปริมาณแอลฟา-อะไมเลส 0.2% (w/v) อุณหภูมิ 90 องศาเซลเซียส นาน 45 นาที

การศึกษาผลของมอลโตเดกซ์ตรินและสารสกัดหยาบที่มีต่อความทนทานในการออกกำลังกาย หนูทดลองถูกแบ่งออกเป็น 10 กลุ่ม กลุ่มละ 5 ตัว ประกอบด้วยกลุ่มควบคุม (double deionized distilled (DDD) water 1 ml/kg p.o.) กลุ่มมอลโตเดกซ์ตริน (250 500 mg/kg p.o.) และกลุ่มสารสกัดหยาบ (250 500 mg/kg p.o.) โดยห้ากลุ่มแรกได้รับสารได้รับการฝึกออกกำลังกาย โดยการว่ายน้ำที่ไม่ใส่น้ำหนักที่หาง (Ex) และห้ากลุ่มหลังไม่ได้รับการฝึกออกกำลังกาย (Non Ex) โดยให้สารดังกล่าววันละครั้งเป็นเวลา 16 วัน ในกลุ่มที่ได้รับการฝึกออกกำลังกายผลการเพิ่มความทนทานในการออกกำลังกายของมอลโตเดกซ์ตรินและสารสกัดหยาบ ได้แสดงให้เห็นจากการเพิ่มขึ้นอย่างมีนัยสำคัญของเวลาที่ใช้ในการว่ายน้ำจนเหนื่อย ในทั้งสองกลุ่ม เมื่อเปรียบเทียบกับกลุ่มควบคุม (Ex) ในกลุ่มที่ได้รับการฝึกออกกำลังกายและกลุ่มไม่ได้รับการฝึกการออกกำลังกาย ปริมาณไกลโคเจนในตับของกลุ่มมอลโตเดกซ์ตริน 500 mg/kg กลุ่มสารสกัดหยาบ 250 และ 500 mg/kg และปริมาณไกลโคเจนในกล้ามเนื้อ gastrocnemius ของทั้งสองกลุ่ม มีค่าสูงกว่ากลุ่มควบคุม (Ex) แสดงว่าการฝึกการออกกำลังกาย มีผลในการเพิ่มปริมาณไกลโคเจนในตับและกล้ามเนื้อ เมื่อนำตับและกล้ามเนื้อ gastrocnemius ในแต่ละกลุ่มไปทำการศึกษาการแสดงออกของยีนด้วยวิธี RT-PCR พบว่าการออกกำลังกายเหนี่ยวนำให้เกิดการแสดงออกของยีน AMPK α_1และ α_2 สูงกว่ากลุ่มควบคุม (Ex)

และกลุ่มที่ได้รับการฝึกการออกกำลังกายและไม่ได้รับการฝึกออกกำลังกาย ในกลุ่มสารสกัดหยาบมีการแสดงออกของยีน PGC 1α สูงกว่ากลุ่มควบคุม ซึ่งเกี่ยวข้องกับกลไกในไมโตคอนเดรีย ซึ่งสอดคล้องกับการศึกษาดูความเป็นพิษต่อเซลล์ มีการลดระดับลงของ MDA และ ROS และไม่มีความแตกต่างของระดับ SOD เมื่อเปรียบเทียบกับกลุ่มควบคุม ซึ่งเป็นไปได้ว่าองค์ประกอบของสารสกัดหยาบและมอลโตเดกซ์ตริน ประกอบด้วยคาร์โบไฮเดรต มีผลต่อการทำงานของตับและลดระดับปฏิกิริยาออกซิเจน จากผลการทดลองนี้แสดงให้เห็นถึงการเพิ่มความทนทานในการออกกำลังกายของมอลโตเดกซ์ตรินและสารสกัดหยาบ อย่างไรก็ตามยังคงต้องการการศึกษาเพิ่มเติมเพื่อทราบถึงกลไกการทำงานในการเพิ่มความทนทาน

สาขาวิชาเทคโนโลยีอาหาร
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ลายมือชื่อนักศึกษา ดอนางค์ โพนศรี
ลายมือชื่ออาจารย์ที่ปรึกษา

KAK ANANG POSRIDEE : PRODUCTION OF CRUDE EXTRACT AND MALTODEXTRIN FROM SWEET CASSAVA AND ITS EFFECT ON LONG ENDURANCE EXERCISE. THESIS ADVISOR : ASST. PROF. RATCHADAPORN OONSIVILAI, Ph.D., 139 PP.

SWEET CASSAVA/ENDURANCE EXERCISE/WISTAR RATS

The objectives of this study were to investigate the effect of maltodextrin and crude extract from sweet cassava (*Manihot esculenta* Crantz.) on exercise endurance capacity in male Wistar rats. The experiment was performed and the tail weight loaded forced swimming of the animal was employed as a criteria for physical work capacity. Relative organ weight (ROW) of liver and skeletal muscles (Soleus, extensor digitorum longus (EDL) and gastrocnemius), liver and gastrocnemius muscle glycogen content were determined. The extraction process required the maltodextrin by enzyme application. The enzyme reaction conditions for the solubilization were optimized via a response surface methodology (RSM). The selected dependent variable was a percentage yield. The highest yield (17.63%, Dextrose equivalent=15) of maltodextrin obtained from enzymatic digestion conditions was 0.2% of α-amylase (w/v), temperature 90°C and time 45 min.

To investigate the effect of maltodextrin and crude extract on exercise endurance capacity, rats were devided into 10 groups (n=5): control group (1 ml/kg DDD water), maltodextrin and sweet cassava extract at 250 mg/ml/kg for low dose group and 500 mg/ml/kg for the high dose group, respectively. Five groups had undergone exercise training (swimming without load, Ex) and five additional groups

had undergone non-exercise training (Non Ex). All treatments were given once daily for 16 days. Exercise endurance capacity of maltodextrin and crude extract were demonstrating significant increases in swimming time to exhaustion were found in two compounds, compared to the control (Ex) group. In exercise training and non-exercise training, liver glycogen contents in maltodextrin 500 mg/kg and crude extract 250, 500 mg/kg were significantly higher than the control group and gastrocnemius muscle glycogen content in all treated was significantly higher than the control group. Exercise training induced liver and gastrocnemius glycogen content. Moreover, the study of AMPK α1, 2 and PGC-1 α mRNA in liver and muscle were detected by RT-PCR. The result showed that the exercise training group increased expression of AMPK α1, and 2, compared with the control (Ex) group. In exercise and non-exercise training groups treated with crude extract showed increased expression of PGC 1α, compared with the control group. Related to the mechanism occouring in mitochondria including related to cells toxicity showed reduction of malondialdehyde (MDA) and reactive oxygen species (ROS) level but no change in superoxide dismutase (SOD) level when compared to the control group. Which is possible that compound of maltodextrin affected liver function and reduced reactive oxygen species.

In conclusion, the present results of exercise endurance capacity of maltodextrin and crude extract (mainly carbohydrate) may improve swimming time to exhaustion and exercise performance. However, further studies are needed to elucidate the mechanism underlying this action of crude extract and maltodextin.

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Kakanang Posridee

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LIST OF ABBREVIATIONS

AMPK	=	5′-AMP-activated protein kinase
ALT	=	Alanine aminotransferase
AST	=	Aspartate aminotransferase
ATP	=	Adenosine triphosphate
BUN	=	Blood urea nitrogen
°C	=	Degree Celsius
dL	=	Decilitre
DE	=	Dextrose equivalent
EDL	=	Extensor digitorum longus
Ex	=	Exercise training
g	=	Gram
kg	=	Kilogram
L	=	Litre
LDH	=	lactate dehydrogenase
MDA	=	malondialdehyde
mg	=	Milligram
mL	=	Millilitre
mM	=	Millimolar
mm^3	=	Cubic millimeter
nm	=	Nanomater
Non Ex	=	non exercise training

LIST OF ABBREVIATIONS (Continued)

ppm	=	Parts per million
PGC	=	Receptor γ coactivator
ROS	=	Reactive oxygen species
(RT)-PCR	=	Reverse transcription polymerase chain reaction
SOD	=	Superoxide dismutase
U	=	Units
µg	=	Microgram
µL	=	Microlitre
µM	=	Micromolar

CHAPTER I

INTRODUCTION

1.1 Background and significance of the study

Food is the key to good health. Good nutritional and necessary consume is the importance of good health. So, the consumption-based on nutrition facts will be to the development of health and physical fitness. The athletes must diet attention from the beginning practice, competition and after that because it is difficult to get adequate nutrition to the body in a short time. Each sport and age category will have unequal energy. More than half of the athletes will consume the dietary supplement, help long exercise for endurance sports example marathon, triathlon, cycling, etc. When before and during a competition or activities can take some fat and protein all of the day, but the carbohydrate can easily release energy to use. So, carbohydrate supplement is high value and rare item.

Cassava (*Manihot esculenta* Crantz.) is the third most important crop in Thailand, rice, and maize. The root crop is known by many names in Thailand, such as cassava or tapioca. Total cassava root crops of Thailand in 2011 are 21,060,903 tones. The main production of the crop is now found in the northeast of Thailand, especially in Nakhon Ratchasima which can produce about 5,003,878 tones. (Thailand Tapioca starch, 2011)

The proximate composition, mineral content and cyanogenic potential were determined using standard methods. The cassava had moisture content (33.14-45.86%),

protein (1.17-3.48%), ash (1.71-2.34%), crude fiber (1.38-3.20%), fat (0.74-1.49%) and carbohydrate (83.42-87.35%). Mineral contents were 0.60-1.60, 1.35-1.58 and 1.06-2.13 mg/100g for Ca, Mg and P respectively, and 0.16-0.24, 0.021-0.030, 0.04-0.13, 0.25-0.36 and 0.25-0.37 mg/100g for Fe, Mn, Zn, K and Na respectively. (Emmanuel et al., 2012)

Yen et al. (2013) reported the effects of sweet cassava on endurance exercise in rats. The result showed the running time to exhaustion of the supplement group (took cassava supplement) was significantly longer than that of the normal group by 49% (enhance 64 minutes from 43 minutes).

Sun and Wang (2010) reported the effects of *Astragalus membranaceus* polysaccharides on endurance exercise. *Astragalus membranaceus* is a famous Chinese medicinal herb. Polysaccharides are the important functional constituent of this herb. The result showed that the supplement could prolong the swimming time of mice, and enhance exercise endurance capacity. Process the ability to retard and lower the production of blood lactate, and prevent the increase of serum blood urea nitrogen (BUN) after exercise.

Nui et al. (2008) reported that the modulating effect of *Lycium barbarum* polysaccharides (LBP) on the oxidative stress induced by an exhaustive exercise in *vivo*. The result showed LBP administration increases glycogen level and antioxidant enzyme activities, and decreases malondialdehyde (MDA) level and creatinine kinase activities. So, LBP administration can decrease the oxidative stress induced by the exhaustive exercise.

Coyle et al. (2001) reported that a low-fat diet reduces intramuscular triglyceride (IMTG) concentration, body lipolysis, total fat oxidation, and nonplasma fatty acid

oxidation during exercise. The result showed a low fat (2% of energy) and a high-carbohydrate diet lowers whole-body lipolysis, total fat oxidation, and nonplasma fatty acid oxidation during exercise in the fasted state in association with a reduced concentration of intramuscular triglyceride.

Coyle and Coggan (1984) reported the effect of carbohydrate feeding in delaying fatigue during prolonged exercise in human. Carbohydrate feedings prevent hypoglycemia, release for energy during mild exercise, and improve endurance during low-intensity exercise. The carbohydrate feedings delay fatigue by apparently slowed the depletion of muscle glycogen.

The aim of this work is to study chemical composition of crude extract and maltodextrin from sweet cassava to prepare supplement for long endurance athletes.

1.2 Research objectives

1. To study chemical properties of crude extract and maltodextrin from sweet cassava.
2. To evaluate the durability properties of crude extract and maltodextrin from sweet cassava in rats
3. To study changes in gene expression (AMPK α1, AMPK α2 and PGC 1α) of rats, that result from nutrient carbohydrate of sweet cassava with exercise.
4. To study the functional properties of crude extract and maltodextrin from sweet cassava for application in dietary supplement for athletes.

1.3 Research hypothesis

Carbohydrate consumption is the importance of long endurance athletes. The athlete who consumed high carbohydrate nutrition, accumulate glycogen in muscle. The glycogen is the energy source of the body. The carbohydrate loading is the popular method before the competition. In the 4 days, athletes consumed a low carbohydrate and high protein with intensity exercise. The body can release accumulate glycogen to out of use. In the last 3 days, athletes consumed a high carbohydrate (amount 70% of total calories) with mild exercise. The carbohydrate loading on endurance athletes must take carbohydrate before 1 month of competition because maybe the muscle symptom during exercise. Athletes use more energy from glycogen has a inflame symptom, so, the carbohydrate intake to compensate for the body after competition or hard exercise (Jeukendrup, 2011). Consequently, crude extract and maltodextrin from sweet cassava can release energy to enhance prolong exercise.

1.4 Scope

Sweet cassava use as a source of high carbohydrate in this study will be from Nakhon Ratchasima province, Thailand. The first, study will optimize the extract condition, chemical, and physical properties. Also, the functional properties of sweet cassava extract will be investigated and then, evaluate the durability properties of sweet cassava and change gene expression with exercise in *vivo* (the rats from Experimental animal job, Suranaree University of Technology). Finally, the application of sweet cassava can prepare a supplement for long endurance athletes.

1.5 References

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CHAPTER II

LITERATURE REVIEWS

2.1 Cassava

Cassava (*Manihot esculenta* Crantz.) is the important agricultural plant in Thailand due to the fact that cassava has effects in agricultural sector and the linkages to manufactures as well (Nongnooch, 2013). The main production of the crop is mostly found in the northeast of Thailand, especially in Nakhon Ratchasima.

Cassava is the third-largest source of food carbohydrates in the tropics, rice, and maize. Emmanuel et al. (2012) reported that the proximate composition, mineral content and cyanogenic potential will be determined using standard methods. The cassava have moisture content (33.14-45.86%), protein (1.17-3.48%), ash (1.71-2.34%), crude fiber (1.38-3.20%), fat (0.74-1.49%) and carbohydrate (83.42-87.35%). Mineral contents are 0.60-1.60, 1.35-1.58 and 1.06-2.13 mg/100g for Ca, Mg and P respectively, and 0.16-0.24, 0.021-0.030, 0.04-0.13, 0.25-0.36 and 0.25-0.37 mg/100g for Fe, Mn, Zn, K and Na respectively.

The roots of the cassava plant are the main storage organ which has three distinct tissues: bark (periderm), peel (cortex), and parenchyma (starchy fresh) (figure 2.1). Cassava is classified as either sweet or bitter. Like other roots and tubers, both bitter and sweet varieties of cassava contain anti-nutritional factors and toxins, with the bitter varieties containing much larger amounts. They must be properly prepared before consumption, as improper preparation of cassava can leave enough

residual cyanide to cause acute cyanide intoxication, goiters, and even ataxia or partial paralysis.

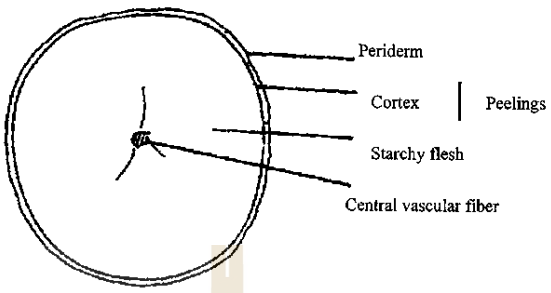

Figure 2.1 General view of cassava root (Onwueme, 1983).

2.2 Carbohydrate

There are various types of carbohydrates, including monosaccharides, disaccharides, and polysaccharides.

Monosaccharides

Monosaccharides are the simplest units of carbohydrates and the simplest form of sugar (glucose, galactose or fructose). They are the building blocks of more complex carbohydrates such as disaccharides and polysaccharides. Monosaccharides cannot be hydrolyzed to smaller carbohydrates. They are aldehydes or ketones with two or more hydroxyl groups. The general chemical formula of an unmodified monosaccharide is $(C{\bullet}H_2O)_n$, literally a "carbon hydrate". Monosaccharides are important fuel molecules as well as building blocks for nucleic acids. The smallest monosaccharides, for which n=3, are dihydroxyacetone and D- and L-glyceraldehydes.

Disaccharides

Two joined monosaccharides are called a disaccharide and these are the simplest polysaccharides. Examples include sucrose and lactose. They are composed

of two monosaccharide units bound together by a covalent bond known as a glycosidic linkage formed via a dehydration reaction, resulting in the loss of a hydrogen atom from one monosaccharide and a hydroxyl group from the other. The formula of unmodified disaccharides is $C_{12}H_{22}O_{11}$. Although there are numerous kinds of disaccharides, a handful of disaccharides are particularly notable.

Polysaccharides

Polysaccharides are polymeric carbohydrate molecules composed of long chains of monosaccharide units bound together by glycosidic linkages and on hydrolysis give the constituent monosaccharides or oligosaccharides. They range in structure from linear to highly branch. Examples include storage polysaccharides such as starch, glycogen, and structural polysaccharides such as cellulose and chitin. Polysaccharides, meanwhile, have a general formula of $C_x(H_2O)_y$ where x is usually a large number between 200 and 2500. Considering that the repeating units in the polymer backbone are often six-carbon monosaccharides, the general formula can also be represented as $(C_6H_{10}O_5)_n$ where $40 \leq n \leq 3000$.

2.3 Digestion of carbohydrates

Digestion of carbohydrate begins in the mouth, with the secretion of the enzyme salivary amylase from the serous cells of the salivary gland. This enzyme breaks starch and glycogen into disaccharides. The mucous cells of the salivary gland secrete mucus, which causes the food to stick together, and acts as a lubricant to aid in swallowing. The salivary glands are grouped into three categories: the parotid gland, submandibular glands, and sublingual, all located near the mouth. The food (bolus) is forced into the pharynx by the tongue. As the food is swallowed, it moves

into the esophagus (a straight collapsible tube), which essentially provides a passageway from the pharynx to the stomach. The mucous glands of the esophagus secrete mucus to aid in moistening and lubricating the bolus. The bolus passes through the cardiac sphincter, into the first section of the stomach. The stomach is divided into several regions: the cardiac region, body region, fundic region, and pyloric region. The stomach works to mix and churn the food, which aids in further digestion of carbohydrates. At this point, the bolus is converted into a semi-fluid paste of bolus and gastric juices called chyme.

The chyme then travels through the pyloric sphincter into the first section of the small intestines. The small intestine is divided into three sections: the duodenum, jejunum, and ileum. The majority of digestion of carbohydrates takes place in the small intestines. As the chyme moves into the duodenum, an enzyme called pancreatic amylase is released through the pancreatic duct. This enzyme splits molecules of starch and glycogen into disaccharides.

The liver plays an important role in several metabolic activities. It is responsible for changing glycogen to glucose to increase blood glucose or converting glucose to glycogen, thereby decreasing blood glucose. The liver also converts other non-carbohydrates into glucose, if needed.

The interior wall of the small intestines is covered with tiny projections called the villi. These projections increase the surface area of the intestines and play an important part in the process of absorption of the nutrients. The epithelial cells of the villi contain even smaller extensions, called microvilli. Embedded in the microvilli are digestive enzymes which are needed to further break down carbohydrates. Include sucrase, maltase, and lactase, which break down the disaccharides into monosaccharide.

The monosaccharide is then absorbed by the villi and enter the blood capillaries to be transported to other parts of the body.

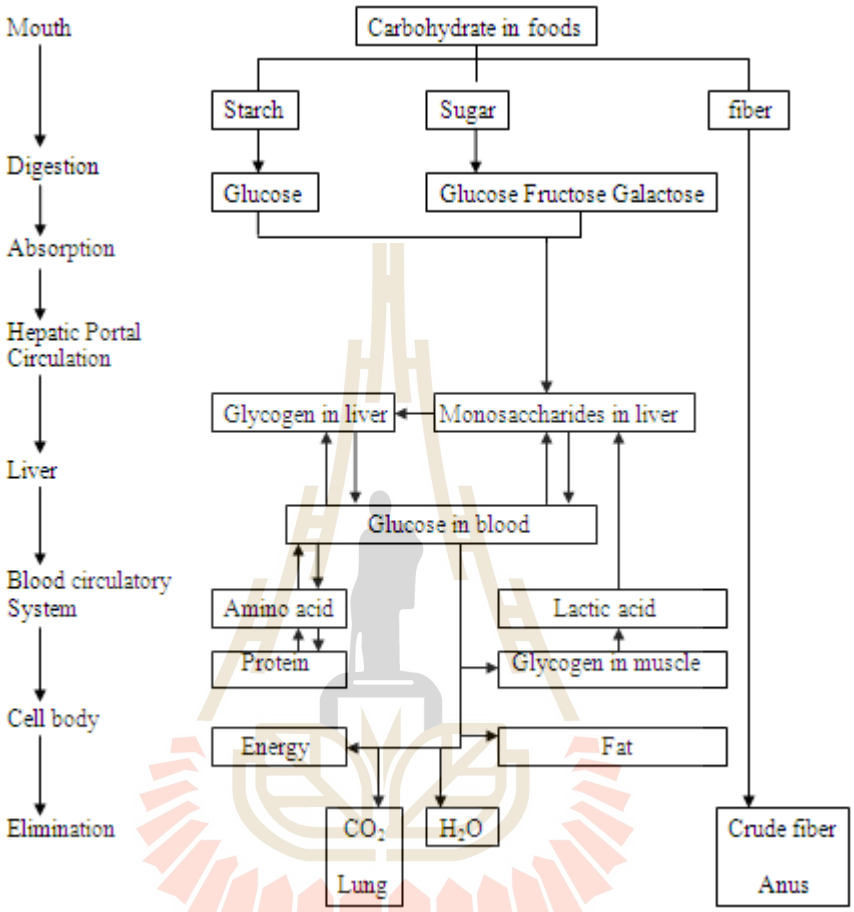

Figure 2.2 Digestion of carbohydrate.

2.4 Carbohydrate metabolism

Glucose use for ATP production occurs by two main processes. One is the glycolytic pathway and a second is the pentose phosphate pathway. Glycolysis is the major pathway for glucose oxidation (80-90%) in most tissues while the pentose phosphate pathway accounts for the remaining 10-20% (Wamelink et al., 2008). Glycolysis converts glucose into two pyruvate molecules and generates 2 ATP in the

process. Under normal aerobic conditions, glucose is oxidized to CO2 and pyruvate is converted to acetyl CoA which enters the Krebs cycle (Berg et al., 2007). Excess glucose can also store in the form of glycogen (in muscle and hepatic tissue) which can be broken down for energy production (Heppner et al., 2010).

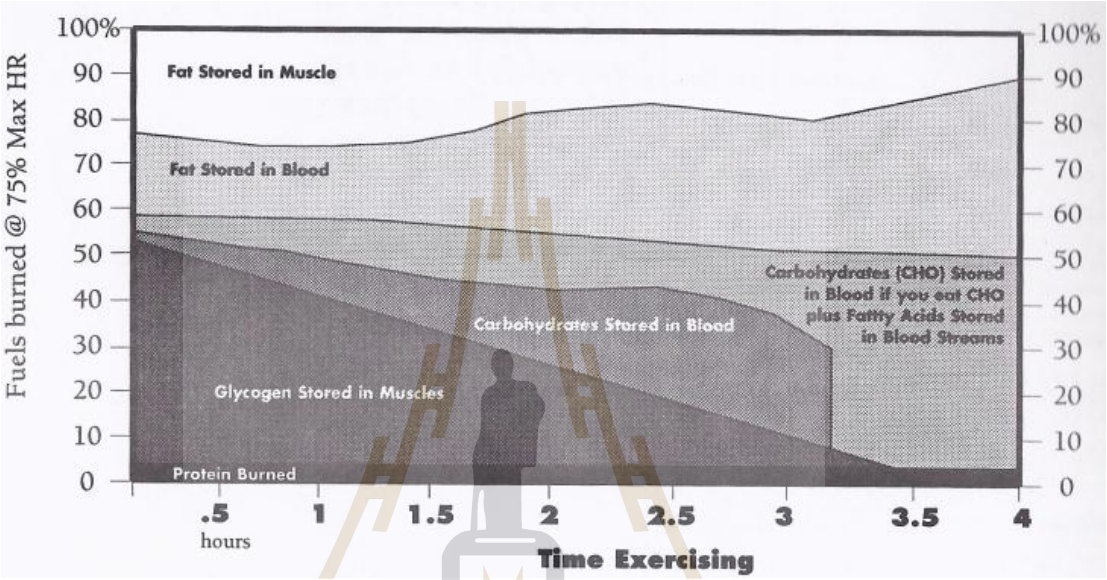

Figure 2.3 Exercise time and the fuel burned.

AMP activated Kinase

AMP-activated Kinase (AMPK) is the primary gauge of cellular energy requirements (Hardie, 2008) and is activated when AMP levels increase (i.e. energy status is low). It then acts to inhibit enzymes involved in ATP-requiring reactions to conserve cellular energy (Kemp et al., 1999). When AMPK is activated, fatty acid oxidation and glucose uptake are stimulated (Goodyear and Kahn, 1998). In the cell, AMPK can exist in a T state (less active) and an R state (more active) but also exists as dephospho-AMPK and phospho-AMPK indicating that overall AMPK can exist in four forms (Hardie and Carling, 1997). Phosphorylation by upstream kinases activates

AMPK by phosphorylating the α subunit at Thr-172 (Kemp et al., 2003). Conversely, AMPK is deactivated upon dephosphorylation (Steinberg and Kemp, 2009). The liver is largely unaffected by changes in AMPK concentrations because ATP concentrations in the liver remain relatively constant (Viollet et al., 2009). Only severe stress stimuli such as intensive exercise or fasting can cause hepatic ATP levels to decrease (Hardie, 2004). In the case that AMPK is activated in the liver, glucose output can be inhibited (Zhou et al., 2001)

The Glycaemic Index (GI)

The Glycaemic Index concept is developed to help diabetics control their blood sugar levels, it can benefit regular exercise and athletes. It is a ranking of food from 0-100 base on the immediate effect on blood sugar levels, a measure of the speed at which you digest food and convert it into glucose. If you need to get carbohydrate into your bloodstream and muscle cells rapidly, for example immediately after exercise to kick start glycogen replenishment, you would choose high glycaemic index food. Sports nutritionists find it useful to classify foods as high GI (71-100), medium GI (56-70) and low GI (0-55). The high GI, the higher the blood sugar levels after eating that food. In figure 4 shows the potato compared with a reference food, such as glucose. The GI gives a percentage of potato is 85, by the area under the curve. It's mean that potato produces a rise in blood sugar (85%) after eating an equivalent amount of glucose.

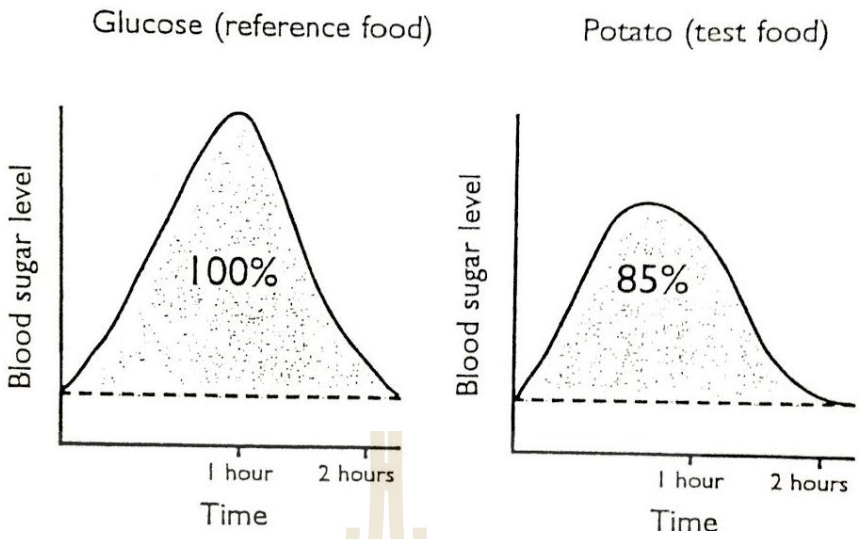

Figure 2.4 Measuring the glycaemic index of food (Jeukendrup, 2011).

2.5 Nutrition for endurance sport

Endurance sports are becoming increasingly popular and more people are running half marathon, marathon, and triathlon on competitions, lasting anywhere between 2 hours and 17 hours. Muscle glycogen and blood glucose are the importance substrates for the contracting muscle. Fatigue during prolonged exercise is often reduced blood glucose depletion and associated with muscle glycogen. Sweat losses occur because there is a need to dissipate the heat that is generated during exercise. So, the nutritional challenge is to prevent major dehydration (>2-3%) and contribute to the prevention of fatigue.

Pre-competition

1. Carbohydrate loading

The amount of dietary carbohydrate need to provide the high carbohydrate availability needs to recover glycogen store on a daily basis or promote glycogen loading depends on the duration and intensity of the athlete's exercise program such as

require 2 to 12 g/ kg/ day. The high carbohydrate intake can achieve a high glycogen store and an increase the effect on endurance performance. The glycogen storage is associated with weight gain as a result of water retention (approximately 3 g/g of glycogen).

2. Carbohydrate ingestion < 60 minutes before exercise

Glucose ingestion in 1 hour before exercise, the result in hypoglycemia and hyperinsulinemia, follow with a rapid decrease in blood glucose 15-30 minutes after exercise. Furthermore, hyperinsulinemia follows carbohydrate ingestion inhibits lipolysis and fat oxidation and rapid muscle glycogen depletion. The high insulin sensitivity rebound hypoglycemia in train athletes. When the athletes are very sensitive to low blood glucose levels from exercise induce hypoglycemia, a major contributor to fatigue. In pre-exercise, carbohydrate sources with a low glycaemic index because of blood glucose and insulin response during subsequent exercise (to delay carbohydrate feeding until 5-15 minutes before activity). The metabolic and performance effects of carbohydrate ingestion short before exercise (<15 minutes) the result as same as feed carbohydrate during activity. In conclusion, before exercise or during warm-up can take a low to moderate index carbohydrate.

3. Fluid ingestion before exercise.

The hydrating before exercise should slowly sport drink (approximately 5-7 mL/ kg body weight) at least 4 hours before exercise but produce urine or the urine is high concentration. So, the sports drink during exercise help to decrease loose body water.

During competition

1. Carbohydrate ingestion during exercise

During exercise longer than 2 hours, the effects of carbohydrates are mainly metabolic. Carbohydrate improves exercise performance when the exercise is high intensity (>75% VO2 max) and short duration (~ 1 hour), and that mechanism for the ergogenic effect during this type of activity in the central nervous system. From table 1 show a large amount of carbohydrate during exercise lasting approximately 30-60 minutes and mouth rinse with carbohydrate may be sufficient to obtain a performance benefit. The performance effects with the mouth rinse show the result same as ingesting the drink. When the exercise is more prolonged (≥2 hours), carbohydrate is very important to fuel and essential to ingest carbohydrate. So, the large amounts of carbohydrates maybe required for more prolonged exercise.

2. Carbohydrate during exercise and performance: dose response.

The exogenous glucose oxidation increased with ingestion rate and it is possible that increase in exogenous carbohydrate oxidation is directly linked with, or responsible for, exercise performance. Carbohydrate intake recommendation for more prolonged exercise can be formulated and are listed in newly proposed guidelines in Table 3.1.

3. Effect of body weight

The reason for this lack of correlation between body weight and exogenous carbohydrate oxidations is probably that the limiting factor is carbohydrate absorption (largely independent of body mass). The absorption capacity of the intestinal is modify by carbohydrate content of the diet, that intestinal transporter can be up regulate with increase carbohydrate intake.

Table 2.1 Recommendations for carbohydrate (CHO) intake during different endurance events.

Event	CHO required for optimal performance and minimizing negative energy balance	Recommended intake	CHO type	*Single carbohy-drate	**Multiple transportable carbohydrates
<30 min	None required	-	-	-	-
30-75 min	Very small amounts	Mouth rinse	Most forms of CHO		•
1-2 h	Small amounts	Up to 30 g/h	Most forms of CHO	•	•
2-3 h	Moderate amounts	Up to 60 g/h	Forms of CHO that are rapidly oxidized	•	•
			Only multiple transportable CHO	°	
>2-5 h	Large amounts	Up to 90 g/h			•

•, optimal; ° , OK, but perhaps not optimal. These guidelines are intended for athletes, exercising at a reasonable intensity (>4 kcal/min). If the (absolute) exercise intensity is below this, the figure for carbohydrate intake should be adjusted downwards. *Single carbohydrate (e.g. glucose). ** Multiple transportable carbohydrates (e.g. glucose:fructose)

4. Different advice for different endurance sports

The muscle glycogen breakdown is unaffected with carbohydrate feeding during cycling. The muscle breakdown is reduced in particular in type I muscle fibers during running. Consequently, carbohydrate feeding is improved performance in cycling and running

5. Training the gut

The absorption of carbohydrate limits exogenous carbohydrate oxidation, and exogenous carbohydrate oxidation linked with exercise performance, potential strategy to be increase the absorption capacity of the gut. High daily carbohydrate intake is an increase capacity to absorb it. Intestinal carbohydrate transporter can up regulate by expose to high carbohydrate diet. The daily carbohydrate intake affects substrate oxidation and in particular exogenous carbohydrate oxidation. The exogenous carbohydrate oxidation rates are higher after the high carbohydrate diet (a carbohydrate supplement during training) for 28 days compare with control diet. The conclusion, the gut is adaptable and can use a method to increase exogenous carbohydrate oxidation (Cox et al, 2010).

After competition

The length of time that it takes to refuel depend on 4 factors

1. Depletion

The higher the intensity exercise, the more glycogen use. The depletion of glycogen stores in the higher intensity exercise far more than low intensity exercise of equal duration. The minimum time it would take to refill muscle glycogen stores in 20 hours. The duration of workout also has a bearing on the amount of glycogen you use. So, you need to allow more time to refuel after high intensity or long workout.

2. Muscle damage

The eccentric exercise (hard) can cause muscle fiber damage. The eccentric exercise is defined as the force lengthening of active muscle. Muscle damage, delays glycogen storage and complete glycogen replenishment could take long as 7-10 days.

3. Carbohydrate intake

The higher your carbohydrate intake, the faster you can refuel your glycogen stores. The glycogen storage is an increase with carbohydrate intake. Figure 3.5, the solid line show 40% carbohydrate diet and the dot line show 70% carbohydrate diet. When you consumed a low carbohydrate diet fail to replenish fully their muscle glycogen stores. Over successive days of training, their glycogen stores became progressively lower. So, who consume a high carbohydrate diet fully replaced their glycogen stores in the 22 hours between training sessions.

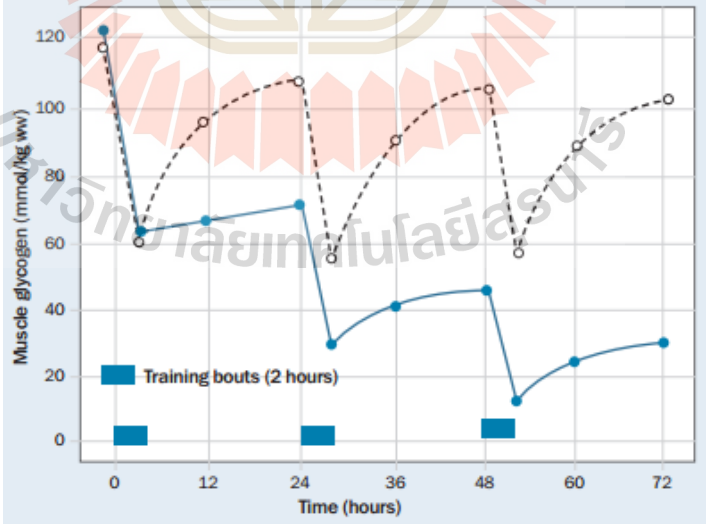

Figure 2.5 Progressive of glycogen depletion over 3 days of exercise.

4. Training experience

Adaptation to training is an increase in your glycogen storing capacity (~20%). The efficiency in refueling improves automatically with training experience and raise fitness levels. So, this is the advantage for training and competition.

The effect of carbohydrate intake on performance

Carbohydrate is an importance fuel for exercise. It stored as glycogen in your liver and muscle, and must be re-stocked each day. The purpose of liver glycogen is to maintain steady blood sugar level and muscle glycogen is to fuel physical activity. A high muscle glycogen concentration will allow you to train at your optimal intensity and achieve a greater training effect. A low muscle glycogen concentration, will lead to early fatigue, reduced training intensity and sub optimal performance. Each different carbohydrate produces a different response in the body, so base on glycaemic index (GI) and carbohydrate loading before a competition.

The relationship between glycogen and performance

The scientists discovered that capacity for endurance exercise is related to pre exercise glycogen stores and a high carbohydrate diet increases glycogen stores. Athletes eating the high carbohydrate diet stored twice as much glycogen as on the moderate carbohydrate diet and 7 times as much as eating the low carbohydrate diet. Afterwards, the athletes were instructed to cycle to exhaustion on a stationary bicycle at 75% of VO2 max. On the high carbohydrate diet managed to cycle for 170 minutes, considerably longer than the moderate carbohydrate diet (115 minutes) or the low carbohydrate diet (60 minutes) (Figure 3.6).

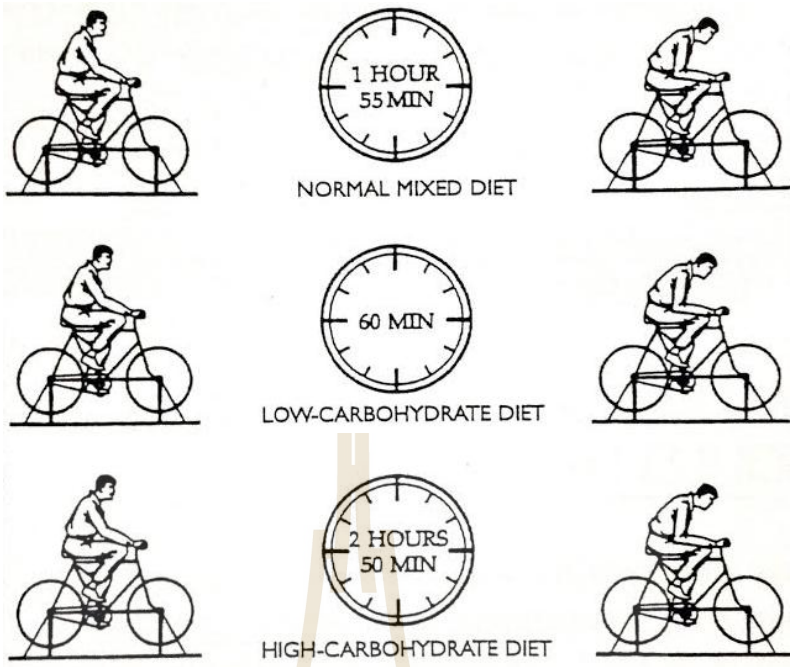

Figure 2.6 The effect of carbohydrate intake on performance (Jeukendrup, 2011).

2.6 Exercise endurance capacity

Exercise endurance is an endurance time during prolonged work. Exercise endurance is associated with enhanced lipolysis and sparing of stored glycogen, which results in delaying complete glycogen depletion. The amount of glycogen stored in working muscles is important for the endurance capacity. Glycogen deposited in liver and muscle tissue, which can readily converted to glucose as needed by the body to satisfy its energy needs for exercise endurance (Navarro et al., 2002). Increased fat utilization during endurance exercise enables athletes to improve endurance capacity. Therefore, the increase of fatty acid utilization during exercise is supposed to improve endurance capacity (Oh and Ohta, 2003).

2.7 Exercise energy supply

Muscle contraction and exercise are dependent on the breakdown of adenosine triphosphate (ATP) and the concomitant release of free energy (Glaister, 2005). The free energy release is coupled to the energy requirements of cell work. During this very short period, the major driving forces are stored high-energy phosphates and anaerobic glycolysis (Bishop, Girard, and Mendez-Villanueva, 2012). The runners can perform almost without breathing, using energy stored as ATP, creatine phosphate and glycogen (anaerobic metabolism) in the active muscles (Ghosh, 2004; Andersen, 2003). In contrast to long-distance runners, sprinters are often large, very muscular people. Sprinters have a dominance of so-called fast twitch or anaerobic muscle fibers. Those remarkably high speeds can only be maintained while stored high-energy phosphate in the form of phosphocreatine is present (Trappe et al., 2015). Almost all studies of phosphocreatine metabolism conclude that stores of phosphocreatine in skeletal muscle are emptied within the first 30 seconds of strenuous activity. After that, the very rapid rate of running must be reduced. The energy supply for those who run from about 60 seconds to three minutes is primarily glycogen stored in muscles and blood glucose (Krustrup et al., 2006). These carbohydrates can be rapidly oxidized to pyruvate, lactate and CO_2 to provide the ATP required for muscle activity (Scott, 2005). However, the rate of ATP synthesis rate is far below that seen when using phosphocreatine as the phosphate donor. These differing sources of energy are summarized in the figure. During exercise, intracellular glucose and ATP levels initially fall and AMP levels rise (Winder, 1988). AMP then markedly stimulates glucose transport by activating AMP kinase (Musi and Goodyear, 2003; Richter and Ruderman, 2009). To offset this drain on extracellular glucose and to maintain a

normal plasma glucose level, hepatic glucose production must increase up to fivefold. Initially, this occurs largely from glycogenolysis (Mutel et al., 2011). Indeed, endurance can be improved by high carbohydrate ingestion for several days before prolonged exercise (*e.g.*, a marathon run) because this increases both liver and muscle glycogen stores (Stellingwerff et al., 2007). With exercise of longer duration, however, gluconeogenesis becomes increasingly important as liver glycogen stores become depleted (Zorzano et al., 1986). Eventually, fatty acids liberated from triglycerides in adipose tissue from the predominant energy substrate, and they supply two thirds of the needs during sustained exercise. Fatty acids readily enter muscle, where they are degraded by β oxidation to acetyl CoA and then to CO2. The elevated acetyl CoA level decreases the activity of the pyruvate dehydrogenase complex to block the conversion of pyruvate into acetyl CoA. Hence, fatty acid oxidation decreases the funneling of sugar into the citric acid cycle and oxidative phosphorylation (Costill et al., 1979). Glucose is spared so that just enough remains available at the end of the marathon. The simultaneous use of both fuels gives a higher mean velocity than would be attained if glycogen were totally consumed before the start of fatty acid oxidation (Van beek et al., 2011). The mechanisms that contribute to energy sources in working muscles are summarized in Figure 3.7.

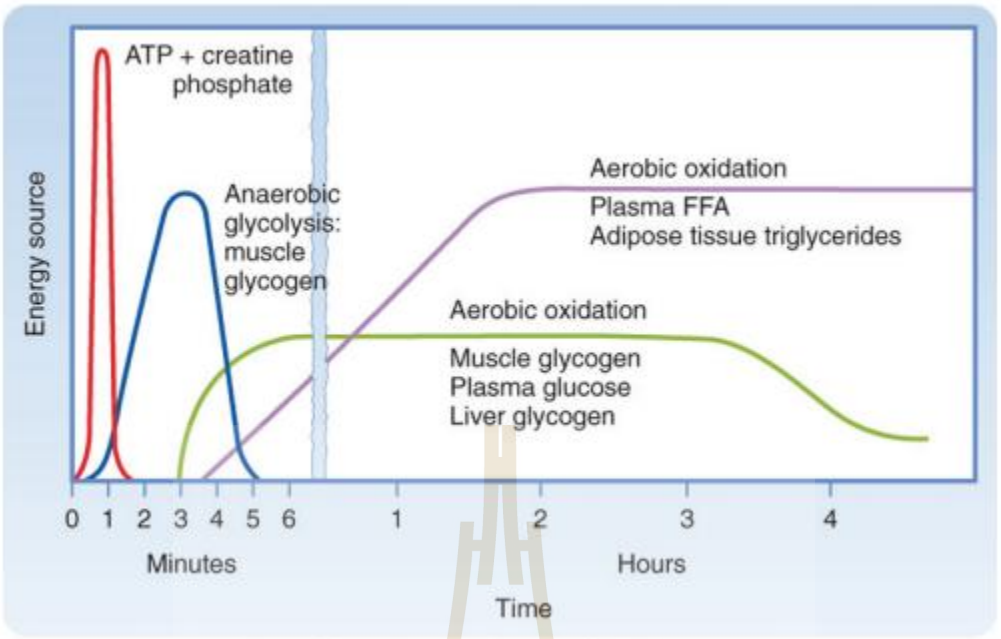

Figure 2.7 Schematic diagram illustrating the energy sources in working muscles (Koeppen and Stanton, 2008). Describe and identifies energy function, and the subsequent list indicates energy source changes occurring during exercise. (ATP,adenosine triphosphate; FFA, free fatty acid).

2.8 AMPK

The heterotrimeric protein AMPK is composed of α, β, and γ subunits. Each of these three subunits takes on a specific role for both the stability and activity of AMPK (Stapleton et al., 1996). The γ subunit includes four particular cystathione beta synthase (CBS) domains giving AMPK its ability to sensitively detect shifts in the AMP: ATP ratio. The four CBS domains create two binding sites for AMP commonly referred to as Bateman domains. Binding of one AMP to a Bateman domain cooperatively increases the binding affinity of the second AMP to the other Bateman domain (Adam et al., 2004). As AMP binds both Bateman domains the γ subunit

undergoes a conformational change which exposes the catalytic domain found on the α subunit. It is in the activation loop of the catalytic domain where AMPK becomes activated when phosphorylation takes place at threonine-172 by an upstream AMPK kinase (AMPKK) (Hawley et al., 1996). The α, β, and γ subunits can also be found in different isoforms: α1 or α2, β1 or β2, and γ1, γ2, γ3. Although the most common isoforms expressed in most cells are the α1, β1, and γ1 isoforms, it has been demonstrated that the α2, β2, γ2, and γ3 isoforms are also expressed in cardiac and skeletal muscle (Thornton et al., 1998; Chueng et al., 2000). AMPK ACTIVATION Triggering the activation of AMPK can be carried out provided that two conditions are met. First, the γ subunit of AMPK must undergo a conformational change so as to expose the active site (Thr-172) on the α subunit. The conformational change of the γ subunit of AMPK can be accomplished by an increase in (AMP). Increased concentrations of AMP will give rise to the conformational change on the γ subunit of AMPK as two AMP bind the two Bateman domains located on that subunit. It is this conformational change brought about by increased concentrations of AMP that exposes the active site (Thr-172) on the α subunit. This critical role of AMP is further substantiated in experiments that demonstrate AMPK activation via an AMP analogue 5-amino-4-imidazolecarboxamide ribotide (ZMP) which is derived from 5-amino-4-imidazolecarboxamide riboside (AICAR) (Corton et al., 1995). The second condition that must be met is the phosphorylation and consequent activation of AMPK on its activation loop at Thr-172 of the α subunit brought about by an upstream kinase (AMPKK) (Stein et al., 2000). The complex formed between LKB1 (STK 11), mouse protein 25 (MO25), and the pseudokinase STE-related adaptor protein (STRAD) has of late been identified as the major upstream kinase responsible for phosphorylation of

AMPK on its activation loop at Thr-172 (Hawley et al., 2000). AMP also makes AMPK a poorer substrate for phosphatases (Davies et al., 1995). Muscle contraction can provide the conditions mentioned above needed for AMPK activation (Winner and Hardie, 1996). As muscles contract, ATP is hydrolyzed, forming ADP. ADP then helps to replenish cellular ATP by donating a phosphate group to another ADP, forming an ATP and an AMP. As more AMP is produced during muscle contraction, the AMP: ATP ratio dramatically increases, leading to the allosteric activation of AMPK (Yamauchi et al., 2002). This fact is further authenticated with studies, such as those sited above, that used electrical stimuli as a means to contract muscle to facilitate AMPK activation (Rammusen and Winder, 1997).

Function of AMPK with exercise/ training

Many biochemical adaptations of skeletal tissue take place in response to endurance training [increased mitochondrial biogenesis and capacity (Bergeron et al., 2001), increased muscle glycogen (Holmes, Kurth-Kraczek and Winder., 1999), and an increase in factors which specialize in glucose uptake in cells such as GLUT4 and Hexokinase II (Ojuka et al., 2002) are thought to be mediated in part by AMPK when it is activated (Ojuka, 2004). Additionally, recent discoveries can conceivably suggest a direct AMPK role in increasing blood supply to exercised/trained muscle cells by stimulating and stabilizing both vasculogenesis and angiogenesis (Ouchi, 2005). Taken together, these adaptations most likely transpire as a result of both temporary and maintained increases in AMPK activity brought about by increases in the AMP: ATP ratio during single bouts of exercise and long-term training. During a single acute exercise bout, AMPK takes on immediate roles to allow the contracting muscle cells adapt to the energy challenges taking place by inducing GLUT4 translocation to the

plasma membrane (Hayashi et al., 1998) allowing for increased glucose uptake. It has also been found that AMPK can help stimulate glycolysis in heart muscle (Marsin et al., 2000). If exercise bouts continue through a long-term training regimen, AMPK and other signals will increase mitochondrial oxidative proteins, thus increasing capacity to produce ATP. AMPK accomplishes this transition to the oxidative mode of metabolism by upregulating and activating enzymes that are known to be involved (either directly or indirectly) in the oxidation of glucose and fatty-acids such as GLUT4, hexokinase II, PPARalpha, PGC-1, UCP-3, cytochrome C and TFAM, just to name a few (Merrill et al., 1997).

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CHAPTER III

ENZYMATIC DIGESTION OPTIMIZATION OF MALTODEXTRIN AND CRUDE EXTRACT FROM SWEET CASSAVA

3.1 Abstract

The purpose of this study was to obtain optimize condition for extracting sweet cassava crude extract by using enzymatic hydrolysis extraction. The optimal extraction condition to prepare sweet cassava crude extract with the highest level of maltodextrin was determined using Box-Behnken experimental design and the response surface method was applied to obtain the optimize condition. Three factors with three levels used in this experiment: α-amylase concentration, temperature, and extraction time. In addition, the proximate composition, mineral content and cyanogenic potential were determined using standard methods. The results showed that the optimum extraction conditions were the following: α-amylase concentration at 0.2% w/v, extraction temperature at 90^{o}C, and extraction time at 45 min that gave the highest percentage yield. From the experiment, the result showed that the high percentage yield (17.63%, DE=15) ($P<0.05$). Furthermore, the cassava contain moisture content 9.43±0.05% protein, 3.25±0.34% ash, 2.02±0.09% crude fiber, 2.97±0.41% fat, 1.15±0.08% carbohydrate, 85.75±0.53 and cyanogenic 0.09±0.01 mg HCN/kg. Moreover, a different infrared spectrum was observed for the sweet cassava flour, maltodextrin from sweet cassava and crude extract. This indicated that the molecular structure of

the crude extract had changed. The range peak at 1403, 1031, and 854 cm-1 corresponded to the –C=O and CH group, and maltodextrin from sweet cassava changed the peak 1000 cm-1 corresponded to the CH2 group its sugar region.

3.2 Introduction

Maltodextrin is a mixture of saccharides with a molecular weight between polysaccharides and oligosaccharides with DE lower than 20 (not sweet), which is available as white powder mostly or concentrated solution. Maltodextrin is more soluble in water than native starches and it is also cheaper in comparison with other major edible hydrocolloids (Alexander, 1992). The maximum activities of the α-amylase are usually in the pH range between 4.8 and 6.5, but the activity-pH profile and location of the pH optima differ depending on the enzyme source. For production of low DE maltodextrin, BAN 480L (a type of α-amylase) may be used. The major steps in the enzyme conversion of starch are liquefaction and saccharification. In liquefaction, the enzyme hydrolyses the α-1, 4-glycosidic bonds in starch (Bravo, 2006).

Sweet cassava (*Manihot esculenta* Crantz.) is an important agricultural plant in Thailand due to the fact that cassava has important used in effects in agricutural sector and manufactures as well. The main production of the crop is a mostly in the northeast of Thailand, especially in Nakhon Ratchasima (Thailand Tapioca starch, 2011). The mathematical modeling method seemed to be the most appropriate to apply to optimization assessment to the extraction process which is widely used in Response surface methodology (RSM). This experimental methodology combines mathematics with statistics in order to generate a mathematical model to describe the process,

analyze the effects of the independent variables and optimize the processing operations (Myers, 1971). The purpose of the study was to determine the possibility of production maltodextrin using α-amylase from sweet cassava and then evaluating the different parameters in the process (enzyme concentrations, temperatures and hydrolysis time).

3.3 Materials and methods

3.3.1 Sample preparation

Sweet cassava was obtained from Suranakhon market, Nakhon Ratchasima, Thailand. It was dried at 60°C in tray dryer and finely ground (GmbH & Co.KG D-42781, Haan, Germany) to powder, kept in vacuum package at 4°C until used.

3.3.2 Proximate analysis

Sweet cassava flour was analyzed for moisture, ash, fat, crude fiber and protein by AOAC (2000) with the following:

3.3.2.1 Moisture content (AOAC, 925.10)

The sample were put into dried moisture can and dried in a hot air oven (Memmert, Germany) at 105°C for 24 h, then transferred to a desiccator to cool and then reweighed until the weight was constant. The moisture content of the sample was calculated as:

$$\text{Moisture (\%)} = ((M0-M1)/M0) \times 100$$

M0 = weight of sample before drying (g)

M1 = weight of sample after drying (g)

3.3.2.2 Ash determination (AOAC, 900.02A)

The crucibles were burned in the muffle furnace at 550°C for 3 h and then transferred to the desiccator for cooling. The sample was put in a burned crucible and placed into a muffle furnace at 550°C for 18 h, then transferred to desiccators to cool and reweighed until the weight was constant. The ash (%) of sample was calculated as:

$$\text{Ash (\%)} = (A1/A0) \times 100$$

A1 = Ash weight (g)

A0 = Sample weight (g)

3.3.2.3 Crude protein determination (AOAC, 928.08)

Crude protein was determined by the Kjeldahl method (AOAC, 2005). The sample was weighed into a Kjedahl digestion flask and 25 ml of sulphuric, 0.5 g of copper sulphate and 5 g of potassium sulfate were added. The mixture was digest was mixed with 40% (w/v) of NaOH and distilled in a distillation apparatus for 5 min. The released ammonia was trapped with 25 ml of 4% boric acid containing a mixed indicator and titrated with HCl. The crude protein (%) calculated by using the formular below:

$$\text{Crude protein (\%)} = (N \times V \times 0.14 \times F \times 100) / \text{Sample weight (g)}$$

N = concentration of HCl (mol)

V = the volume of HCl (ml)

F = 5.55

3.3.2.4 Crude fat determination (AOAC, 963.15)

The amount of crude fat was determined using AOAC (2005). The sample was weighed on filter paper and put into a cellulose thimble. The weighed samples were extracted in the extractor (Foss tecator soxtec Avanti 2050, Hoganas, Sweden) for 4 h with 80 ml of petroleum ether. The extracted samples were placed in an oven at 105°C for 24 h, then transferred to desicator to cool and reweighed until the weight was constant. The crude fat (%) of the sample was calculated as:

$$\text{Crude fat (\%)} = ((F0-F1)/F0) \times 100$$

F0 = weight of sample (g)

F1 = weight of sample after extraction (g)

3.3.2.5 Crude fiber determination (AOAC, 978.10)

The sample was used to determine the crude fiber. 150 ml of 1.25% sulphuric acid was added to the samples and boiled for 30 min, and then the sample were filtered and washed with boiling water until the filtrate was neutral. 1.25% (w/v) of NaoH (150 ml) were added and boiled for 30 min, then the sample were filtered and washed with boiling water until the filtrate was neutral and washed twice with 95% ethanol and transferred to a crucible, then dried at 105°C in hot air oven until a constant weight was obtained. The crucible was cooled in a desiccator and weighed. The crucible was burned in a muffle furnace (Gallenkamp muffle furnace, England) at 550°C for 2 h. The crucible was transferred into a desiccator to cool and the reweighed until a constant weight was obtained. The crude fiber (%) of the sample was calculated as:

Crude fiber (%) = (CF1/ CF0) x 100

CF1 = weight of fiber (g)

CF0 = sample weight (g)

3.3.2.6 Determination of carbohydrate

The available carbohydrate was calculated by the difference between the amounts of moisture, ash, crude fat, crude protein and dietary fiber:

Available carbohydrate (%) = 100 – (Moisture (%) + Ash (%) + Crude fat (%) + Crude protein (%) +Crude fiber (%))

3.3.3 Dextrose equivalent determination

The modified Lane and Eynon titration (Corn Refiner Association-Method E-26) was used for dextrose equivalent determination. Fehling method was used to measure the content of reducing sugar. The determination of total carbohydrate was conducted using phenol-sulfuric acid method. The dextrose equivalent was then according to the following equation:

$$\text{Dextrose equivalent (DE)} = \frac{\text{Reducing sugar}}{\text{Total carbohydrate}} \text{x} 100$$

3.3.4 Production

Sweet cassava flour (250 g) was mixed with water 500 ml and stirred overnight. The mixture was centrifuged at 14,300 g, 4°C for 20 min, and the supernatant collected. The extraction was then repeated using the sediment and a second supernatant was obtained. The two supernatants were combined and freeze dry. The powder was kept in vacuum container at -20°C until used.

3.3.4.1 Crude extract

The crude mucilage was purified to remove starch and free soluble protein. Dispersion and hydrolysis of starch in mucilage followed the method of Southgate (1991) using a modified enzyme solution of 5000 units of α-amylase (pancreatic, EC 3.2.1.1, Merk, Darmstadt, Germany) and 5 units of pullulanase per ml (microbial, Sigma Chemical), 0.1 M sodium acetate, pH 5.2 and incubated for 16 h at 45°C. After incubation, trichloroacetic acid was added to a final concentration of 4% to precipitate free soluble protein (Lin & Huang, 1993). The mixture was stirred for 5 min, centrifuged at 10,000 g, 4°C for 10 min, and the supernatant retained. Ethanol was added into the supernatant to a final concentration of 80% and the precipitated polysaccharides were collected by centrifugation at 10,000 g for 10 min. The precipitate was repeatedly washed with 95% ethanol and acetone. Finally, the purified fraction was distilled in 4 ml water, filtered and lyophilized.

3.3.4.2 Maltodextrin

Maltodextrin was produced by combination of depolymerization i.e. hydrolysis of glycosidic linkages and trans glycoclylation. Depolymerization can be affected with either and acid (HCl or H_2SO_4) or enzymes or by combination treatment. A suspension containing 30% dry matter was liquefied to make the starch susceptible to further enzymatic breakdown by α-amylase (pancreatic, EC 3.2.1.1, Merk, Darmstadt, Germany). Modified as recommended by Kachenpakdee et al. (2016) for starch hydrolysis by α-amylase including adjusted to pH 6 was carried out. The reaction was stopped by HCl 0.1 N at pH 4.2. The hydrolysate was separated by centrifugation (Hettich, Universal 32R, DJB labcare Ltd) at 8,000 rpm for 20 min to

separate the soluble fraction from the insoluble fraction. The soluble fraction was dried by spray dryer. The powder was kept in vacuum container at -20°C until used.

3.3.5 Experimental Design

Response surface method is an empirical optimization technique for evaluating the relationship between experimental outputs (or responses) and factors. This method is usually used in combination with factorial design methods such as Box–behnken designs. The Box-behnken designs can sharply reduce the number of experimental sets without decreasing the accuracy of the optimization compared with traditional factorial design methods (Kocabas, 2001). This study design consisted of 15 experimental runs. Replication the center points was used to estimate the experiment error. In general, the first order polynomial is as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \varepsilon$$

In the equation;

Y = response variable (dependent variable)

X_i = predictor variable (independent variable)

β_0 = constant

β_i= partial regression coefficients

K = represents the number of independent variables

ε = error

The response surface method was applied to identify the optimum levels of the three variables of the extraction enzyme α-amylase concentration (w/v), extraction temperature (°C) and extraction time (min) regarding the responses extract yields. The

design independent and dependent variables include enzyme α-amylase concentration (X_1; 0.1, 0.2 and 0.3 %w/w), extraction temperature (X_2; 90, 95 and 100°C) and extraction time (X_3; 15, 30 and 45 min). The experiments used the Box-behnken design. The order of the experiments was fully randomized. Data were analyzed by One-Way ANOVA. The data was fitted to first-order model to obtain regression coefficient. The model use in the response surface analysis as follow:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + e$$

The quality of the model was evaluated with R^2 and analyzed by ANOVA. The validity of the developed mathematic model was confirmed by three additional experiments that were performed under the optimum conditions.

3.3.6 Determination of the functional properties

3.3.6.1 Water holding capacity (WHC)

The water holding capacity of the dietary fiber was determined using the method of Jasberg et al. (1989) with some modifications. A dried sample (3 g) was mixed with an excess of deionized and distilled water and allowed to hydrate for 2 h. The excess water was then removed by allowing the wet sample to drain through a fine-meshed wire screen. A portion of the wet sample on the screen was carefully removed, weighed and dried to constant weight (±0.05 mg) in a hot-air oven (110°C). WHC was defined as follows: (Jasberg et al., 1989)

WHC = (dry weight –wet weight)/ dry weight

3.3.6.2 Oil binding capacity (OBC)

The oil binding capacity (OBC) was determined by the method of Caprez et al. (1986). A dried sample (5 g) was mixed with soybean oil in a centrifugal

tube and left for 1 h at room temperature (25°C). The mixture was then centrifuged at 1500 g for 10 min, the supernatant was decanted and the pellet recovered by filtration through a nylon mesh. OBC was expressed as follows:

OBC = (dry weight - pellet weight)/ dry weight

3.3.6.3 Water solubility index (WSI)

The water solubility index (WSI) was determined by AACC, method No. 44-19 (AACC, 2000). The powder (S1, g) was dispersed in a centrifuge tube by adding water with a powder/water ratio of 0.02/1 (w/w) at ambient temperature. Then the dispersion was incubated in a water bath (WB22, Memmert,Germany) at 80°C for 30 min, followed by centrifugation at 6000 rpm for 10 min. The supernatant was carefully collected in a pre-weighed evaporating dish (S2, g) and was dried at 103±2°C, and the evaporating dish with the residue was weighed again (S3, g). WSI was calculated using the following formula:

WSI (%) = ((S3-S2)/ S1) x 100

3.3.7 Fourier transform infrared spectrophotometer (FTIR)

FTIR spectroscopy analysis is the method used for identifying functional groups and to acquire more information about materials and products. Two mg of samples were prepared in tube. IR spectra (4000-400 cm^{-1}) were recorded using a Bruker T27/HYP 2000 (Germany) spectrometer with a resolution of 4 cm^{-1} and 64 scans per sample (Oh et al., 2005).

3.3.8 Statistical analysis

All experiments were performed in triplicate and reported as the mean ± SD and the *p*-value < 0.05 level of significance. The experimental data were analyzed using Design Expert® Software (Version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN, USA) and an analysis of variance (ANOVA). SPSS® software (SPSS Inc., Chicago, IL, USA) was used to perform all the statistical calculations. Statistical analysis for each parameter assessed was performed using analysis of variance (ANOVA) followed by Turkey's post hoc test.

3.4 Results and Discussion

3.4.1 Chemical composition of sweet cassava flour

Sweet cassava flour was determined for crude protein, ash, moisture, fat, carbohydrate, and cyanogenic potential. The results are shown in Table 3.1.

Table 3.1 Chemical composition of sweet cassava.

Components	**Content (%) dried basis**
Moisture	9.425 ±0.05
Ash	2.02± 0.09
Protein	3.25 ± 0.34
Fat	1.15 ± 0.03
Crude fiber	2.97 ± 0.41
Carbohydrate	81.185±0.53
Cyanogenic	0.09±0.011 (mg HCN/kg)

Table 3.1 shows the chemical composition of sweet cassava contain moisture content: 9.43±0.05% protein, 3.25±0.34%ash, 2.02±0.09% crude fiber, 2.97±0.41%fat, 1.15±0.08%carbohydrates, 81.815±0.53 and cyanogenic potential at 0.09±0.01 mg HCN/kg. In a previous study, Emmanuel et al. (2012) reported that the proximate composition was determined using standard methods. The cassava have moisture content (33.14-45.86%), protein (1.17-3.48%), ash (1.71-2.34%), crude fiber (1.38-3.20%), fat (0.74-1.49%) and carbohydrates (83.42-87.35%). The acute lethal dose of hydrogen cyanide for human beings is reported to be 0.5-3.5 mg/kg body weight. Approximately 50-60 mg of free cyanide from cassava and its processed products constitutes a lethal dose for an adult man (WHO, 1965).

3.4.2 Optimization of maltodextrin production

3.4.2.1 Dextrose equivalent

From the dextrose equivalent obtained from sweet cassava hydrolysis with vary α- amylase (0.1, 0.2 and 0.3% (w/v)) at 100°C. is presented in Table 3.2.

Table 3.2 Dextrose equivalent (DE) obtained from sweet cassava hydrolysis with vary α- amylase (0.1, 0.2 and 0.3% (w/v)) at 100°C.

Hydrolysis time	**%DE**		
(min)	**0.1%**	**0.2%**	**0.3%**
15	14.43±0.15	14.89±0.58	16.45±0.75
30	17.02±0.39	17.32±0.80	18.14±0.35
45	19.56±0.69	20.04±0.32	22.80±0.12
60	29.28±0.70	29.42±0.18	35.59±0.60
75	42.64±0.81	52.13±0.31	54.07±0.19
90	44.16±0.56	53.74±0.62	62.53±0.32

The dextrose equivalent (DE) obtained from sweet cassava hydrolysis with varying α- amylase concentrations (0.1, 0.2 and 0.3%) at 100°C as showed in the Table 1. Once the relationship between the DE and the hydrolysis time for each source under the hydrolysis conditions was established, one fraction was removed every 15 min from the water bath until 90 min of hydrolysis was completed. The results show that the hydrolysis time ranged from 15-45 min, DE<20 produced maltodextrin, but over 45 min, DE>20 produced syrup. So, this study selected the hydrolysis time limit to 45 min. Moore et al. (2005) reported that soluble solids suspension of cassava and corn starch were evaluated during the enzyme action (α- amylase, THERMAMYL- 120L- NOVO Nordisk). A desirable DE was achieved after 15 min of hydrolysis at 100°C. During, 30 min cassava starch and corn starch were used to produce maltodextrin.

3.4.2.2 Optimization of maltodextrin production by response surface methodology

Table 3.3 Experiment design and response of independent variables to the extract parameters.

Exp .No.[a]	Independent variables			%Yield (Y, %(w/w))	
	α-amylase%(w/v) X_1	Temperature (°C) X_2	Time (min) X_3	Observed	Predicted
1	0.3	90	30	14.6±0.29[b]	14.48
2	0.1	100	30	3.98±0.22[g]	4.68
3	0.1	95	45	12.60±0.37[c]	12.74
4	0.2	95	30	11.01±0.05[d]	9.58
5	0.2	100	15	1.70±0.05[h]	1.53
6	0.1	90	30	12.51±1.15[c]	12.28
7	0.3	100	30	5.87±0.35[f]	6.89
8	0.2	100	45	8.59±0.02[e]	10.04
9	0.2	95	30	10.53±0.11[d]	9.58
10	0.1	95	15	4.05±0.20[fg]	4.22
11	0.2	90	15	9.37±1.26[de]	9.12
12	0.2	90	45	14.94±0.21[b]	17.63
13	0.2	95	30	11.40±0.06[d]	9.58
14	0.3	95	45	17.34±0.03[a]	14.93
15	0.3	95	15	5.02±0.11[f]	6.42
	X_1	X_2	X_3		
p-value	0.0608	<0.0001	<0.0001		

[a] Experiments were conducted in a random order.

Table 3.4 ANOVA result for the response surface linear model on the yield of maltodextrin.

variables	**coefficient**
Intercept	+9.58
X_1	+1.10
X_2	-3.80**
X_3	+4.26**
R^2	0.92
Adjusted R^2	0.89
F	40.61**
Adeq .Prec.	20.95
Lack of fit	24.01

*p< 0.05 indicates statistical significance.

**p< 0.01 indicates statistical significance

The production of maltodextrin (Y) from sweet cassava was obtained from all the experiments listed in Table 3.3. According to Myer (1971), R^2 should be at least 0.80 for a good fit to the model. The high value of the coefficient of multiple determination (R^2 = 0.9172) exhibited that the model adequately represents the experimental results.

RSM was used to determine the regression coefficients and statistical significance of the model terms. The model F-value (14.67) and Adequate Precision (20.95) showed that the model can be used to predict maltodextrin yield. All the variables of extraction to determine correlation between independent and dependent variables at the interactive level had a significant effect ($p<0.05$), including the optimal extraction condition for obtaining the highest percentage yield as shown in Table 3.3.

Table 3.3 and 3.4 showed The ANOVA results for the suggested linear models for the maltodextrin responses. It can be seen that there was high statistical significance between the multiple regression relationships, the independent variables and the maltodextrin responses. The probability (*p*) values of the regression models were less than 0.01, which was statistically significant. The R^2 values, adjusted R^2 values and predicted R^2 for the response were 91.72, 89.46 and 84.39 respectively. This demonstrates good correlation between the independent variables and the response. The model was stronger and the predicted responses better as the R^2 value became closer to 1.0000. A regression model, with R^2 value greater than 0.8000, was considered to have a high correlation (Jaya et al., 2010). The ANOVA for the lack of fit test for response was insignificant ($p > 0.05$) which demonstrates that the model was adequately fitted to the experimental data for the responses. The highest percentage of maltodextrin production was obtained when the enzyme concentration at 0.2 %w/v, extraction temperature at 90°C and extraction time at 45 min that produced highest maltodextrin level at 14.93 %w/w. While, replacing the value of optimum condition into regression equation obtained.

$$Y = 9.58 + 1.10X_1 - 3.80X_2 + 4.26X_3; \text{ linear}$$

The equation showed predicted yield value is 17.63 %w/w. The results from the experiment show that the extraction temperature and extraction time affected to the maltodextrin percentage yield ($p < 0.01$) more than enzyme concentration. In addition, data was analyzed for correlation between independent variables and maltodextrin percentage. A statistical analysis showed *p* -value less than 0.01 which is significant.

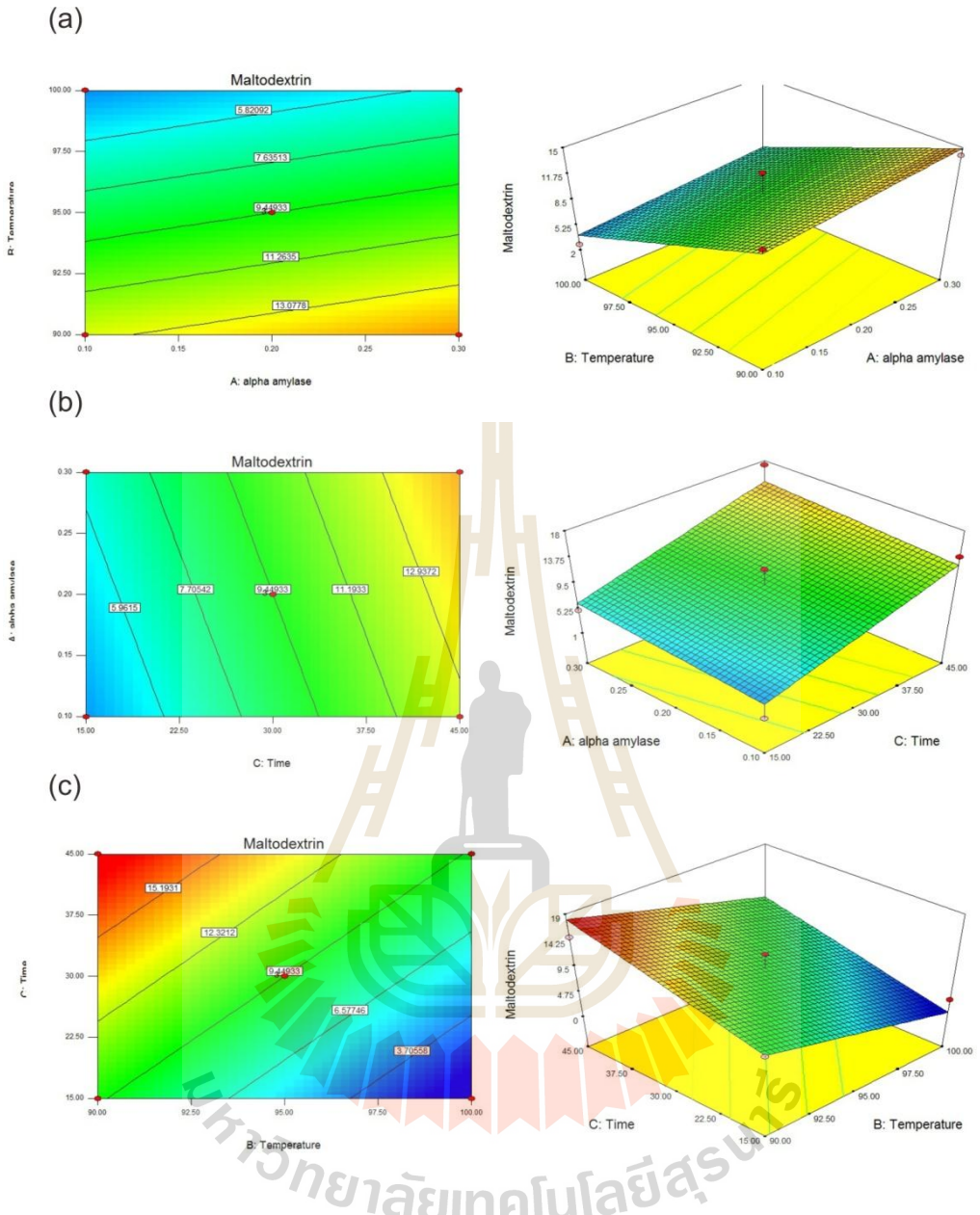

Figure 3.1 Response surface plots (a) the interaction effect of α-amylase concentrationsand extraction temperature on maltodextrin production at extraction time 45 minutes, (b) effect of α-amylase concentrations and extraction time on maltodextrin production at extraction temperature 90°C, (c) effect of extraction temperature and extraction time on maltodextrin production at α-amylase concentrations 0.2%w/v.

The response surfaces are illustrated with three-dimensional plots which represent the responses according to two factors and fixed for constant. The highest maltodextrin level showed in relation to α-amylase concentration, extraction temperature, and extraction time are shown in Figures 3.1 This shows that maltodextrin decreased as the temperature increased from 90°C to 100°C because α-amylase Termamyl 120L. Some studies on starch hydrolysis have used using Bacillus a-amylases which showed reasonable activity at temperatures between 70°C and 100°C (Manoj et al., 2005; Barkar et al., 2013). Maltodextrin started to increase when the enzyme concentration and extraction time increased.

3.4.2.3 Model verification

Table 3.5 Experimental and predicted values of maltodextrin response from optimized paramaters.

Response	**Experimental**	**Predicted**	***p*-value**
Maltodextrin	10.99±0.07	9.58	0.52

$p < 0.05$ indicates statistical significance.

Optimization was carried out to determine optimal parameters in the process of maltodextrin extraction using the response surface method optimization procedure as shown in Table 3.5 The selected optimum parameters in this study were an enzyme concentration at 0.2 %w/v, the extraction temperature at 95°C and extraction time of 30 min. Based on the triplicate runs by using recommended optimum parameters, the mean values was 10.99±0.07 for maltodextrin production. The experimental values and predicted values and their *p* values were analyzed by using a t-test. The results

showed that there were no statistical significant ($p > 0.05$) in the experimental and predicted values of the responses, which indicates that the models were sufficient to predict the maltodextrin response. These results indicate that there is an excellent correlation between experimental and predicted results which in turn proves the validity of the model.

3.4.3 Maltodextrin and crude extract properties

Table 3.6 Properties of maltodextrin and crude extract from sweet cassava.

Components	Crude extract	Maltodextrin
Moisture	6.15± 0.09	5.25± 0.04
Ash	0.25±0.03	0.18±0.02
Total carbohydrate (%)	87.60±0.29	94.57±0.54
% yield	6.84±0.47	14.25±0.77
Oil binding capacity (OBC) (g oil/g sample)	0.11±0.05	0.03±0.01
Water holding capacity (WHC) (%)	15.47±1.05	18.14±0.98
Water solubility index (WSI) (%)	97.50±0.83	98.40±0.94

Table 3.6 showed the chemical position of maltodextrin and crude extract from sweet cassava in percentages. Moisture, total carbohydrate, and % yield were 5.25, 94.54, and 14.25 respectively and crude extract were 6.15, 87.60, and 6.84 respectively.

The functional properties of the maltodextrin and crude extract showed In Table 3.6 maltodextrin is that oil binding capacity is 0.03 g oil/g sample, water holding capacity 18.14 and water solubility index 98.40 respectively and crude extract oil binding capacity is 0.11 g oil/g sample, water holding capacity 15.47 and water solubility index 97.50 respectively.

Total carbohydrate, mainly consisted of sucrose. The rest were glucose, glucose 6 phosphate and fructose. The sweet cassava contained a possible source of Fructo-oligosaccharide (FOS). FOS typically consisted of a sucrose molecule (glucose-fructose disaccharide) with additional fructose unit added by 2-1 glycosidic linkage to fructose unit of sucrose. Fructose is a dietary sugar found in natural fruits. FOS is indigestible sugar, found in wheat, barley and banana.

That starch granules mainly contain varying amounts of two types of glucose polymers: amylose and amylopectin, which differ in molecular structure. In amylose, glucose units are linked in a linear structure by α1,4 glycosidic links while some glucose units in amylopectin are linked by α1,6 bonds, resulting in branched structures (Buléon et al., 1998; Tester et al., 2004). Most starches contain approximately 70–80% amylopectin and roughly 20-30% amylose. The latter is known to be less rapidly digested by pancreatic α-amylase (Topping et al., 1997; Englyst and Englyst, 2005). Thus, depending on the amylose content of the native starch (some specific selected crops have a high amylose starch content of up to 70%) differences in blood glucose response will occur. Accordingly, high amylose rice has a lower glycemic index (GI = 38) than low amylose rice (GI = 57) (Atkinson et al., 2008).

(a) (b) (c)

Figure 3.2 Photographs of (a) sweet cassava powder, (b) maltodextrin and (c) crude extract.

3.4.4 Component content determination (FTIR)

The main goal of the study was to monitor sweet cassava changes occurring during extraction. A study of the sweet cassava flour, maltodextrin from sweet cassava, maltodextrin commercial, and crude extract from sweet cassava using FTIR microspectroscopy technique showed differences in individual structure of sweet cassava (Yan et al., 2009). The results of the FTIR analysis are shown in Table 3.7 and Figure 3.4.

The broad band in this region is due to the OH-stretching vibrations arising from hydrogen bonding in cellulose (Oh et al., 2005). The vibrations at 3317 cm^{-1} (Figure 3.4) were assigned to the hydroxyl groups (Atef et al., 2014). The natural fiber or polymer composites due to the hydroxyl groups (-OH) appearedin the cellulose and lignin structure of the fiber molecule (Soom et al., 2009). The O–H stretching mode of starch was observed in the region of 3000–3600 cm^{-1} (Mathew et al., 2008). The band in this region is attributed to the stretching vibration of O-H in the constituent

sugar residues (Kanmani et al., 2011). Crude extract is water soluble. So, showed high available to bind with water.

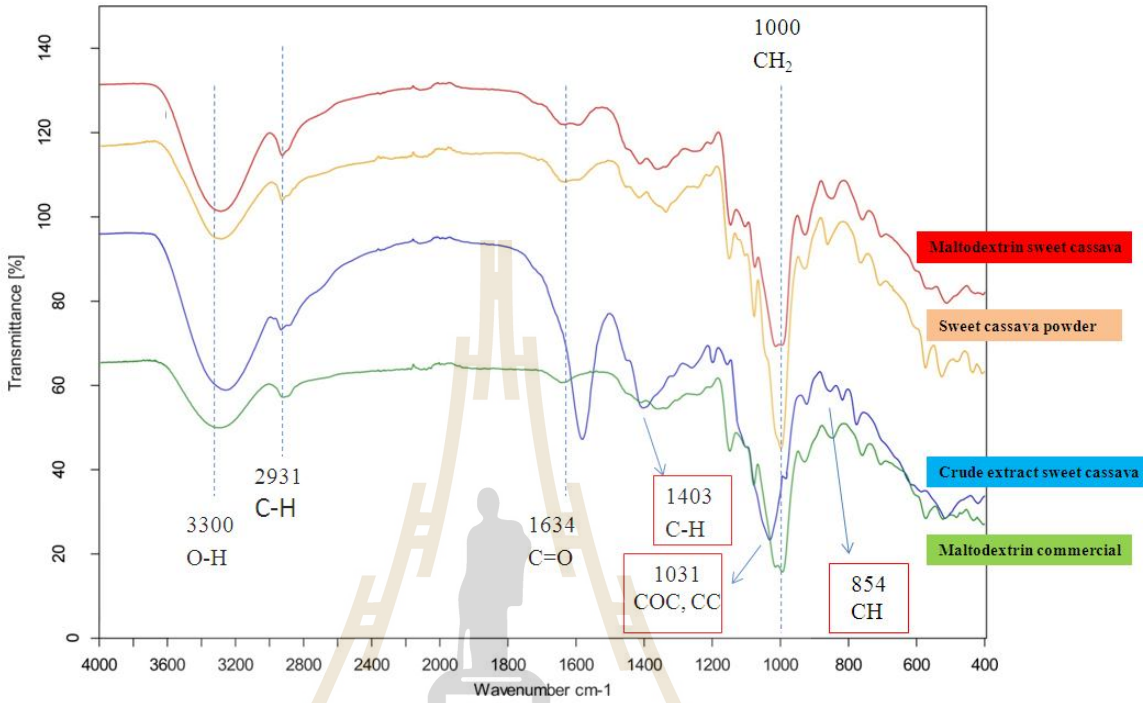

Figure 3.3 FTIR spectra of sweet cassava flour, maltodextrin commercial, maltodextrin from sweet cassava, and crude extract from sweet cassava. The IR region is between 400-4000 cm^{-1}.

Table 3.7 IR assignments of the main vibrations in the FTIR spectra.

IR region (cm^{-1})	Vibrations (cm^{-1})	Assignments
3700-3000	3300	Hydrogen bonded OH bond stretching vibrations OH Hydroxyl group
3000-2800	2931	C-H stretching of lignocellulosic components
1800-1500	1634	C=O stretching vibrations in conjugated
1500-1200	1365	carbonyl o
	1403	C-H bending
		C=O stretching in carboxamide functional groups
1200-650	1000	CH_2 stretching vibrations
	1031	COC, CC stretching
	854	CH stretching vibrations

- IR region 3700-3000 cm^{-1}

The broad band in this region is due to the OH-stretching vibrations arising from hydrogen bonding in cellulose (Oh et al., 2005). The vibrations at 3317 cm^{-1} (Figure 3.4) were assigned to the hydroxyl groups (Atef et al., 2014). The natural fiber or polymer composites due to the hydroxyl groups (-OH) appearedin the cellulose and lignin structure of the fiber molecule (Soom et al., 2009). The O–H stretching mode of starch was observed in the region of 3000–3600 cm^{-1} (Mathew et al., 2008). The band in this region is attributed to the stretching vibration of O-H in the constituent sugar residues (Kanmani et al., 2011). Crude extract is water soluble. So, showed high available to bind with water.

- IR region 3000-2000 cm^{-1}

The characteristic absorption peak at 2923 cm^{-1} suggests C-H stretching vibrations in lignocellulosic components such as cellulose, hemicellulose and lignin (Ibrahim et al., 2011). The FTIR spectra in the region 2800- 3000 cm^{-1} was due to the C–H stretching mode. The sharp bands at 2920-2928 cm^{-1} were related to the CH_2 vibration for the native hydrolyzed and hydrolyzed HHP-treated potato starch with 0.06% (w/v) α-amylase. This could be attributed to changes in conformation and crystallinity (Tai-Hua et al., 2015).

- IR region 1800-1500 cm^{-1}

The infrared absorption region 1630-1640 are related with the tightly water. The peak at ~1640 cm^{-1} was a sign of firmly bound water present in the native, enzyme-treated and hydrolyzed HHP-treated starch under different conditions (Tai-Hua et al., 2015). The spectrum of the acetyl group is observed by the C=O band at 1732 cm^{-1} of fiber (Himmelsbach et al., 2002; Proniewicz et al., 2001). The band at 1640 cm^{-1} C=O and –CHO showed the stretching vibrations in conjugated carbonyl of oat ß-glucan (Xu et al., 2013). The adsorption capacity of metal ions is dependent on pH and ionic strength. When the pH value increases, the carboxyl group (COOH) which are dissociated from the carboxyl anions (RCOO-) show stronger interaction with the toxic cations resulting in higher binding capacity of the dietary fiber (Ilharco and Brito de Barros, 2000). The strong absorbance peak of the two ester carbonyl stretches at 1550 and 1650 cm^{-1} indicated high protein content in crude extract (Lin et al., 2003).

-IR region 1500-1200 cm^{-1}

The band at 1365 cm^{-1} can be assigned to C-H, which presents characteristic peaks of cellulose nanofibrils (Robert et al., 2004). CH_2 bending vibrations are related to

the structure of cellulose and aromatic skeletal vibrations (Rosu et al., 2010; Penttilä et al., 2013; Marques et al., 2006; Kizil et al., 2002). The absorption bands at 1020-1250 cm^{-1} are related with CH_2OH mode as well as the C–O–H deformation mode. The C-O stretch (1150-1000 cm^{-1}) mostly from the carbohydrate structure, the crude extract is mainly a carbohydrate compound.

- IR region 1200-650 cm^{-1}

Infrared absorption at 928-930 cm^{-1} was attributed to the glycosidic linkages in starches. The band at ~850 cm^{-1} was sensitive to changes of crystallinity compared to NPS, the native hydrolyzed and hydrolyzed HHP-treated potato starches with 0.02% and 0.04% (w/v) α-amylase showed no significant change of peak position or intensity but exhibited significant changes in the fingerprint area. Peaks became sharper with increased enzyme concentration (Tai-Hua et al., 2015).

3.5 Conclusion

The optimum conditions for the maximum production of maltodextrin from sweet cassava was determined using randomized Box-Behken design and the data was statistically analyze using ANOVA. The condition that show that enzyme concentration at 0.2%w/v, extraction temperature at 90°C, and extraction time at 45 min produce the highest %yield (17.63, DE = 15). Moreover, a different infrared spectrum was observed for the sweet cassava flour, maltodextrin from sweet cassava and crude extract. This indicated that the molecular structure of the crude extract had changed. The range peak at 1403, 1031, and 854 cm^{-1} corresponded to the –C=O and CH group. Maltodextrin from sweet cassava changed the peak 1000 cm^{-1} corresponded

to the CH_2 group its sugar region. It is recommended that future research should study the supplement for endurance exercise.

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CHAPTER IV

EFFECT OF CRUDE EXTRACT AND MALTODEXTRIN FROM SWEET CASSAVA ON EXERCISE ENDURANCE CAPACITY IN MALE WISTAR RAT

4.1 Abstract

The exercise endurance capacity of maltodextrin and crude extract from sweet cassava was investigated in male Wistar rat. Fifty rats were devided into exercise training (swimming without load) and non-exercise training groups that received control (1 ml/kg double deionized distilled (DDD)), crude extract and maltodextrin (250 and 500 mg/kg) orally once daily for 16 days (n=5, each). The time to exhaustion after the weight loaded forced swimming observed on day 16 on all rats. Body weight gain, relative organ weight of liver and hind limb skeletal muscles (soleus and gastrocnemius), biochemical parameters (glucose, triglyceride, apartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, blood urea nitrogen (BUN), and lactate dehydrogenase (LDH)), liver glycogen content and gastrocnemius muscle glycogen content were determined. In non-exercise and exercise training feeding maltodextrin at dose 500 mg/kg showed time to exhaustion higher than all groups. Maltodextrin and crude extract at dose 250 and 500 mg/kg significantly increased liver and gastrocnemius muscle glycogen content as compared to control group ($P<0.05$). In exercise training and non-exercise group showed no significantly changed in glucose, BUN, triglyceride and insulin levels in all groups. Moreover, at

crude extract dose 250 mg/kg significantly increased in AST and ALT, LDH levels in exercise training group ($P<0.05$). In addition, the creatinine level significantly increased in exercise training group compared with non-exercise training group ($P<0.05$). The present finding suggested that maltodextrin and crude extract appeared to have potential to support exercise endurance capacity. Crude extract and maltodextrin may increasing assist in the glycogen contents in liver and gastrocnemius muscle in both non-exercise and exercise training group by improving glycogen reserve and glycogen sparing effect. In conclusion, the maltodextrin at dose 500 mg/kg could be used as supplement for endurance sport assistant. However, further study still needed to elucidate the mechanism underlying this action of maltodextrin and crude extract from sweet cassava.

4.2 Introduction

Sweet cassava (ManihotesculentaCrantz.) is the important agricultural plants in Thailand because cassava shows effects in agricultural sector and the linkages to manufactures as well. The main production of the crop is a mostly found in the northeast of Thailand, especially in NakhonRatchasima (Thailand Tapioca starch, 2011). Sweet cassava is a major food or food ingredient in many countries. The composition of this tuber is 38% carbohydrate and 60% water. The carbohydrates in cassava tubers contain monosaccharides (fructose, arabinose, and galactose) and polysaccharides. It has been reported that the intake of high-carbohydrate foods increased muscle glycogen content, which can prolong exercise time and delay fatigue (Coyle and Coggan, 1984). Many sports, such as soccer, tennis, and track and field events, require athletes to compete repeatedly within the space of a few days. In addition, athletes train almost

every day. If an athlete can maintain muscle glycogen via dietary supplementation, he/she can recover efficiently and engage in subsequent training or competition. Consequently, studies showed the effects of regimens and substance supplementation on muscle glycogen and sports performance, for example, carbohydrate loading (Hawley et al., 1997). Recently, several studies have indicated that extracted polysaccharides provide the following benefits: enhancing muscle glycogen and sports performance, extending endurance times, resistance to fatigue, decreasing oxidative stress after strenuous exercise (Yoa and Li., 2010; Zheng et al., 2010) and detoxifying the body (Charles and Huang, 2009). Yen et al. (2013) reported that of sweet cassava had effected on endurance exercise in rats. The result showed the running time to exhaustion of supplement group (took cassava supplement) was significantly longer than normal group at 49% (enhanced 64 min from 43 min).

Maltodextrin is a mixture of saccharides with a molecular weight between polysaccharides and oligosaccharides with DE lower than 20, which is available as most white powder or concentrated solution. Maltodextrin is more soluble in water than native starches, also cheaper when comparison with other major edible hydrocolloid (Alexander, 1992). The maximum activity of the α-amylase is usually in the pH range between 4.8 and 6.5, but the activity-pH profile and location of the pH optima differs depending on the enzyme source. For production of low DE maltodextrin, BAN 480L (a type of α-amylase) may be used. The major steps in the enzyme conversion of starch are liquefaction and saccharification. In liquefaction, the enzyme hydrolyses the α-1, 4-glycosidic bonds in starch (Bravo, 2006).

Although sweet cassava is a staple food in many countries, and the literature indicates that it contains abundant carbohydrates and seems beneficial for sports

performance, the aim of this study was to examine the effects of maltodextrin and crude extract sweet cassava on sport performance using a rat model .In addition the experimental designed for investigate time to exhaustion with and without, glycogen storage in liver and muscle, and blood chemistry.

4.3 Materials and methods

4.3.1 Preparation of maltodextrin and crude extract solution

Maltodextrin and crude extract from sweet cassava from 3.3.4 were used in all experiments performed in this reserch. On the day of each experiment, maltodextrin and crude extract were freshly prepared by dissolving in DDD water at the desired concentrations.

4.3.2 Animals

The eight weeks old male Wistar rats (weighting 250-300 g) were housed under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 25±1°C)with free access to food and water .The experiments performed following the animal care and use committee guidelines of Suranaree University of Technology (SUT) .All studies conducted under permission of the SUT Animal Care and Use Committee.

4.3.3 Chemicals

95%Ethanol solution

95%ethanol solution (v/v)) C_2H_5OH, Analytical Grade ≤99.8%, CarloErba Reagents, Frances)was prepared by adding 95 ml of ethanol to 5 ml of DI water.

5%Phenol solution (w/v)

5%Phenol solution was prepared by adding 50 g of phenol (C_6H_5OH; Sigma Chemical Co., St .Louis, MO, USA) to 900 ml of DI water .This solution was slightly adjusted volume to 1000 ml with DI water in a volumetric flask.

30% KOH/Na_2SO_4 solution

30%KOH/Na_2SO_4 solution was prepared by adding 180 g of potassium hydroxide (KOH; Sigma Chemical Co., St .Louis, MO, USA (to 500 ml of DI water.This solution was slightly adjusted volume to 600 ml with DI water and saturated with sodium sulfate (Na_2SO_4; Sigma Chemical Co., St .Louis, MO, USA).

4.3.5 Preparation of glycogen standard solutions

Stock glycogen standard solution 5 mg/ml (w/v)(10 ml) was prepared by adding 50 mg glycogen powder from bovine (Sigma, St .Louis, MO, USA)to 9 ml of DDD water .This solution was slightly adjusted volume to 10 ml with DDD water.

200 µg/ml standard glycogen solution (20 ml):

Preparation :mixed 0.8 ml of stock glycogen standard solution 5 mg/ml with 10 ml of DDD water and then adjusted the final volume to 20 ml.

175 µg/ml standard glycogen solution (20 ml):

Preparation :mixed 17.5 ml of 200 µg/ml standard glycogen solution with 1 ml of DDD water and then adjusted the final volume to 20 ml.

150 µg/ml standard glycogen solution (20 ml):

Preparation :mixed 17.143 ml of 175 µg/ml standard glycogen solution with 1.5 ml of double deionized distilled (DDD)water and then adjusted the final volume to 20 ml.

125 µg/ml standard glycogen solution (20 ml):

Preparation :mixed 16.667 ml of 150 µg/ml standard glycogen solution with 2 ml of DDD water and then adjusted the final volume to 20 ml.

100 µg/ml standard glycogen solution (20 ml):

Preparation :mixed 16 ml of 125 µg/ml standard glycogen solution with 3 ml of DDD water and then adjusted the final volume to 20 ml.

75 µg/ml standard glycogen solution (20 ml):

Preparation :mixed 15 ml of 100 µg/ml standard glycogen solution with 4 ml of DDD water and then adjusted the final volume to 20 ml.

50 µg/ml standard glycogen solution (20 ml):

Preparation :mixed 13.333 ml of 75 µg/ml standard glycogen solution with 5 ml of DDD water and then adjusted the final volume to 20 ml.

25 µg/ml standard glycogen solution (20 ml):

Preparation :mixed 10 ml of 50 µg/ml standard glycogen with 8 ml of DDD water and then adjusted the final volume to 20 ml.

4.3.4 Preparation of sweet cassava extract solution

500 mg/ml crude extract (20 ml):

Preparation :dissolved 10 g of crude extract in DDD water and then adjusted the final volume to 20 ml.

250 mg/ml crude extract (20 ml):

Preparation :dissolved 5 g of crude extract in DDD water and then adjusted the final volume to 20 ml.

500 mg/ml maltodextrin(20 ml):

Preparation :dissolved 10 g of maltodextrin in DDD water and then adjusted the final volume to 20 ml.

250 mg/ml maltodextrin (20 ml):

Preparation :dissolved 5 g of maltodextrin in DDD water and then adjusted the final volume to 20 ml.

Sweet cassava extract was prepared every 3 days and stored at 4°C.

4.3.5 Experimental designs

After adaptation period for one week, rats (n=50)were randomly divided into five groups :control group (1 ml/kg DDD water)and four treatment groups.

The rats in the treatment groups were gavaged with two different doses of maltodextrin and crude extract at 250 mg/ml/kg and 500 mg/ml/kg for low and high dose group, respectively. These doses selected according to the study of Yen et al. (2013) which calculated by converting human dose used in normal folklore to rodent dose based on following criteria:

Rat dose (mg/kg) = (human dose mg/kg x 70 kg x 0.018)/ 200

4.3.6 Exercise endurance capacity

The exercise training groups (received vehicle, maltodextrin and crude extract at doses of 250 and 500 mg/ml) were subjected to swimming training without any load for 15 days (6 days a week). Exercise endurance capacity was observed by weight loaded forced swimming test as previously described by Kumar et al .(2011). The rats were trained for 30 min/day with subsequent increase of 10 min/day till it reaches 1 h .Swimming was performed in groups of three in an acrylic plastic pool 90

cm x45 cm x 45 cm (filled with water up to 60 cm and maintained at a temperature between 34-36°C .During the exercise protocol, the rats in non-exercise groups received vehicle, crude extract and maltodextrin at doses of 250 and 500 mg/ml were kept in plastic cage containing about 3 cm of water maintained the same temperature to exclude potential stress and other potential confounding effects .On day, 16 after administered 30 min all rats allowed to swim until exhaustion with load of 3 %body weight attached to the tails .The uncoordinated movement and stay under the water for 10 s without swimming at the surface accepted as the exhaustion criteria of the rats .At this point, the rats rescued and swimming time was recorded for each rat.

4.3.7 Determination of blood biochemical variables

Immediately after exhaustion, the rats were anesthetized with carbon dioxide. Blood samples (10 ml) were collected by cardiac puncture. Hind-limb skeletal muscles (soleus, extensor digitorumlongus (EDL) and gastrocnemius) from both sides and liver were quickly excised, dried with filter paper and weighed. Tissue samples were then frozen on dry ice and stored at -20°C until further used. Whole blood obtained by cardiac puncture was collected into the tube with and without anticoagulant. Blood samples in the tube without anticoagulant was cooled for about 3.5 h at 4°C, the serum was prepared by centrifugation (Labconco Corporation Ltd., Missouri, USA) at a speed of 1,000 g at 4°C for 20 minutes and the levels of serum lactate dehydrogenase (LDH) were determined by an automatic analyzer (Wang et al., 2012). Blood samples in the tube with anticoagulant were centrifuged at 2,000 g at 4°C for 5 min (Hemmings and Song, 2004), the plasma levels of blood urea nitrogen (BUN), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, and

creatinine and serum lactate dehydrogenase (LDH) were determined by an automatic analyzer (Wang et al., 2012).

4.3.8 Determination of glycogen levels in tissue samples

The procedure for determination of glycogen levels in liver and gastrocnemius muscle samples was used as previously described (Lo et al., 1970) with some modifications. Briefly, the tissue samples were cut and weighted 25 mg in an eppendorf tube and kept on ice. The solution of 750 µl of 30% KOH saturated with Na2SO4 was added to samples, making sure that the tissue was absolutely immersed in the solution. The tubes were put in a boiling water bath (model WB-22, WiseBath, Korea) for 30 min until homogeneous solutions was obtained. After that, tubes were removed from the boiling water bath, swirled and cooled in ice. The 95% ethanol (900 µl) was added to precipitate the glycogen from the alkaline digestate, swirled and left samples in ice for 30 min. The sample tubes were centrifuged (model Z 233M-2, HermleLabortechnik GmbH., Germany) at 840×g or 3873 rpm for 30 min. The supernatant was carefully aspirated and dried tubes upside down (5 min). The obtained of glycogen precipitate was dissolved with 1.5 ml of DDD water (vortex until went into solution) and appropriate aliquot of the glycogen solution was pipette into three 16 × 100 mm test tubes (0.5 ml each). The 0.5 ml of 5% phenol solution was added to the above. Immediately after added phenol solution, rapidly added 2.5 ml of 96-98% H_2SO_4 (within 10-20 sec), the stream of acid being directed against the liquid surface rather than against the side of the test tube (15 ml size), to ensure good mixing. The tubes were allowed to stand for 10 minutes. Then, they were shaken and placed in a water bath at 25-30°C for 10-20 min, before readings were taken. The glycogen standard solutions were prepared at various concentration (0, 25, 50, 75, 100, 125, 150, 175, and 200 µg/ml)

and blank was prepared with 500 µl of DDD water. The absorbance of mixtures was measured by a spectrophotometer (CECIL 1011, England) at 490 nm. All tests were performed in a triplicate to minimize errors resulting from accidental contamination and reading carried out within 30 min.

The equation use to calculate the tissue content of glycogen was assessed by following formula:

$$\text{grams of glycogen}/100\text{ g tissue} = \frac{A490}{k} x \frac{V}{v} x \frac{10^{-4}}{W}$$

Where : V =total volume of glycogen solution

v =volume of aliquot used in the color reaction

A490 =absorbance at 490 nm

W =weight of tissue sample in gram

k =slope of standard cure

Units =1 per microgram glycogen.

4.3.9 Relative organ weight

Each organ to body weight ratio (relative organ weight: ROW) was calculated as following: (Aniagu et al., 2005; Abdullab et al., 2009).

ROW = (Weight of organ (g) /body weight of rat (g) on the day of sacrifice) × 100%

4.3.10 Statistical analysis

Data expressed as mean ± S.E.M. Statistical analysis assessed by analysis of variance (ANOVA) using SPSS 18.0 (SPSS, Inc., Chicago, IL). Post hoc testing performed for inter-group comparisons (Duncan's). P-Value less than 0.05

($P<0.05$) was considered statistic significant. All graphs created by Sigma Plot software (version 10, Systat Software Inc., USA).

4.4 Results and discussion

4.4.1 Exercise endurance capacity

Effect of 16 days administration of maltodextrin and crude extract from sweet cassava with and without exercise training on exercise endurance capacity in male wistar rat are shown in Fig 4.1.

In non-exercise training group, the swimming time to exhaustion in rats received maltodextrin at dose 500 mg/kg (42 min) was significantly higher than other group ($P<0.05$). In exercise training group, the swimming time to exhaustion in rats received maltodextrin at dose 500 mg/kg (82 min) was significantly higher than crude extract 500 (67 min), maltodextrin 250 mg/kg (63 min), and 250 mg/kg (54 min), respectively ($P<0.05$). In comparison to non-exercise training, the rat received maltodextrin and crude extract at 250 and 500 mg/kg exercise training groups showed swimming time to exhaustion significant higher than non-exercise training group, except, control group. All groups, swimming time to exhaustion of all exercise training group were significant increased when compared to their respective non-exercise treatment groups. In consistent with the increasing in swimming time to exhaustion, increasing in plasma glucose levels were also found in rat treated with maltodextrin and crude extract. These changes may be a result of the increased demanding for glucose by contracted muscle ledding to an increasing of glucose uptake in working skeletal muscle (blood glucose) during prolong exercise (Wu et al., 2013). The result of blood sugar profile might relate with the result of liver glycogen content (Sanchez et al., 1971). The increasing in plasma glucose correlates with the reduction in liver glycogen content,

promote increased breakdown of liver glycogen to glucose. The literature showed that muscle glycogen content is associated with running time to exhaustion in both human and animal studies (Akermark et al., 1996; Dohm et al., 1983). As a result, increased muscle glycogen delays fatigue and/or extends time to exhaustion.

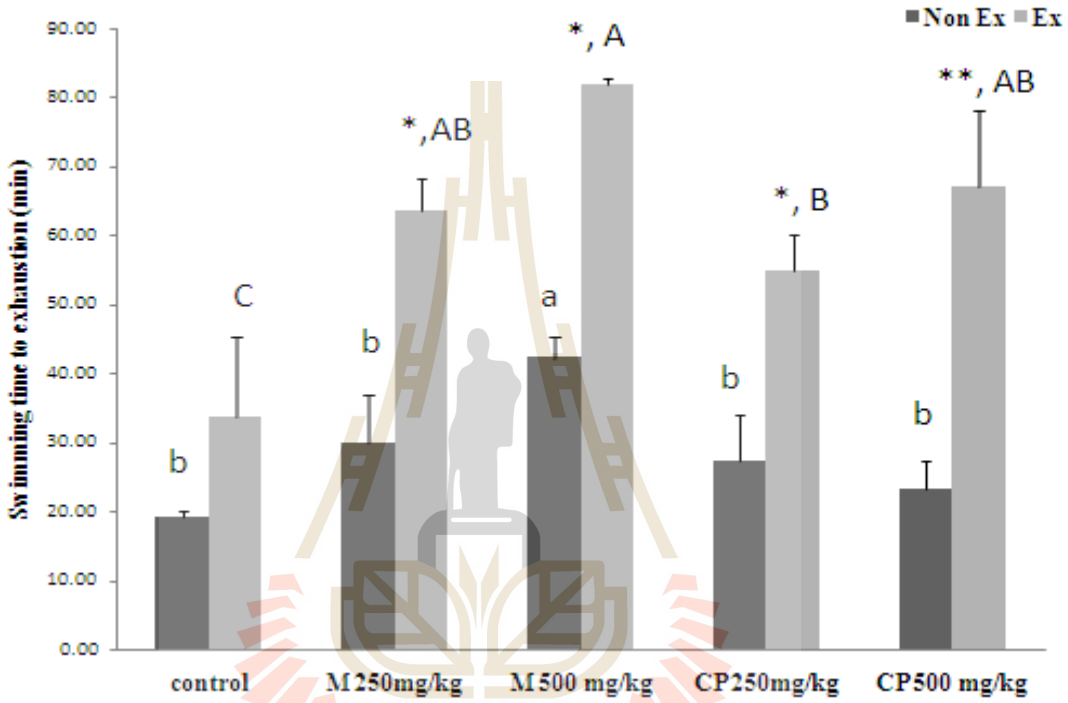

Figure 4.1 Effects of 16 days administration of maltodextrin (M) and crude extract (CP) from sweet cassava on exercise endurance capacity. Value are expressed as mean±S.E.M.; n=5 per group. *Indicates significant difference compare between Non Ex and Ex with same treatment.Small letters indicate significant difference within Non Ex.Capital letters indicate significant difference within Ex. Mean sharing the same superscript are not significantly different from each other (*P<0.05*, two-way ANOVA; Ducan's Method).

4.4.2 Body weight gain

The body weight gain of male wistar rats on day 16 from day 1 after administration of crude extract and maltodextrin with and without exercise training are shown in Fig 4.2.

The weight gain, in non-exercise training group, maltodextrin and crude extract group were decreased the weight gain when compared with control group. In exercise training group, the weight gain in rats received crude extract at dose 500 mg/kg was significantly higher than other group ($P<0.05$). In comparison to non-exercise training, control group was significant difference in body weight gain between exercise training and non-exercise training rat. Although, rat treated with maltodextrin and crude extract showed the weight gain decreased in exercise training group when compared with non-exercise training group. Reduction of body weight gain by crude extract may be a result of direct and indirect activity suppression of adipocyte lipoprotein lipase, as well as other enzymes involved in triglycerol synthesis and storage including enhancement of growth hormone dependent lipolysis (Engelson, Pi-Sunyer, and Kotler, 1999). The effect of carbohydrate on body weight observed due to enhanced energy intake and weight gain in rodents. In contrast, consumption of 32% sucrose solution was found to accelerate weight gain with no corresponding increase in calories intake (Sclafani and Xenakis, 1984), while lower concentrations such as 10% sucrose may or may not alter weight gain. In mice, caloric intake and weight gain vary significantly according to the type of sugar and concentration of sugar solution used, although some strains remain altogether resistant to sugar-induced obesity (Glendining et al., 2010). The result similarly found that wistar strain do not show accelerated weight gain following sucrose consumption (Kending et al., 2011; Sheludiakova et al., 2011). Energy balance is a process through which the body attempts to establish homeostasis.

Most individuals spend much of their lives in the same weight range without daily focus on caloric intake and output. The two parts of the equation for weight maintenance are energy intake (eating and drinking) versus energy output (nonexercise thermogenesis + exercise). To achieve the weight loss, the American Diabetes Association (ADA) (Colberg et al., 2016), American Academy of Clinical Endocrinologists (AACE) (Handelman et al., 2015), and National Academy of Nutrition and Dietetics (Seagle et al., 2009) all recommend exercise as an integral part of any weight loss program. Physical activity and exercise are often used interchangeably. However, correctly defined, physical activity is all movement that creates energy expenditure, whereas exercise is planned, structured physical activity (Colberg et al., 2016). Encouraging individuals to exercise for longer periods of time each day may help to enhance weight loss. However, it is challenging for some patients to consistently achieve even small of exercise daily. In counseling patients, it is important not to focus on the potential for weight loss as the sole outcome from exercise, but rather to suggest that exercise may contribute to weight loss efforts and does result in a myriad of other health-related benefits. That was reduced the likelihood of patients using the lack of weight loss as a reason to discontinue their exercise program (Carla, 2017).

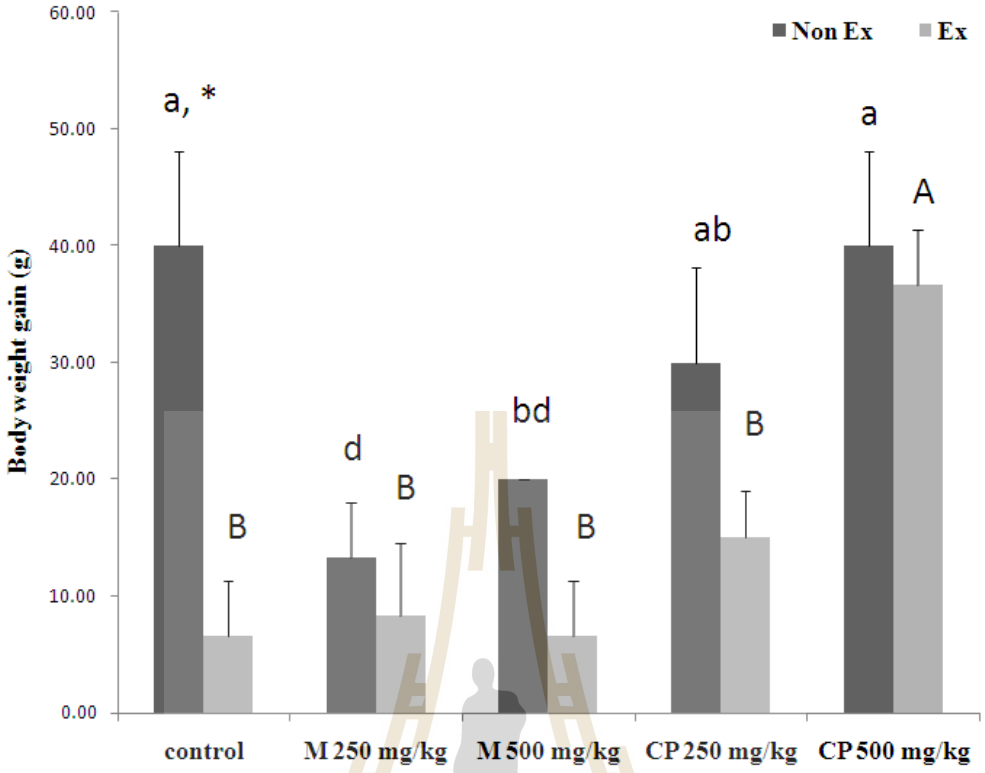

Figure 4.2 Effects of 16 days administration of maltodextrin (M) and crude extract (CP) from sweet cassava on body weight gain. Value are expressed as mean±S.E.M..; n=5 per group. * Indicates significant difference compare between Non Ex and Ex with same treatment. Small letters indicate significant difference within Non Ex.Capital letters indicate significant difference within Ex. Mean sharing the same superscript are not significantly different from each other (*P<0.05*, two-way ANOVA; Ducan's Method).

4.4.3 Relative organ weight

Table 4.1 Effects of 16 days administration of maltodextrin (M) and crude extract (CP) from sweet cassava on relative organ weight of liver, soleus, extensor digitorumlongus (EDL) and gastrocnemius.

Group	Relative organ weight (g/100 g BW)			
	Liver	**Soleous**	**EDL**	**Grastrocnomius**
Control				
Non Ex	2.71±0.03	0.06±0.01	0.07±0.01	1.10±0.03
Ex	2.97±0.11	0.07±0.01	0.08±0.03	1.13±0.04
250 mg/kg M				
Non Ex	3.04±0.10	0.05±0.00	0.06±0.01	1.12±0.03
Ex	3.01±0.16	0.05±0.00	0.06±0.01	1.16±0.04
500 mg/kg M				
Non Ex	3.06±0.19	0.05±0.01	0.05±0.01	1.10±0.03
Ex	2.89±0.18	0.04±0.00	0.05±0.00	1.13±0.02
250 mg/kg CP				
Non Ex	3.25±0.24	0.06±0.01	0.06±0.01	1.13±0.02
Ex	3.04±0.39	0.05±0.00	0.06±0.00	1.13±0.02
500 mg/kg CP				
Non Ex	3.05±0.27	0.05±0.01	0.06±0.01	1.14±0.03
Ex	2.84±0.29	0.05±0.01	0.06±0.01	1.15±0.03

Values are expressed as mean ± S.E.M.M =maltodextrin, CP =crude extract, Ex = exercise training, Non Ex =non exercise training .

Table 4.1 Effects of 16 days administration of maltodextrin from sweet cassava on relative organ weight of liver, soleus and gastrocnemius.There was no significant difference in relative organ weight of the liver, soleous and gastrocnemius muscle of all groups in both exercise and non-exercise training group. Individual muscles tend to be a mixture of various fiber types, but their proportions vary depending on the action of

that muscle and the species .For instance, in human, the soleus muscle contain type I fibers, the EDL muscles contain type II fibers, while the gastrocnemius muscles contain mixed type fibers .In this study, relative organ weights of the liver, soleus muscle, gastrocnemius muscle and EDL muscle of maltodextrin, crude extract and control were not change .The result similarly the percentage difference of muscle weight between the suspended and control group was similar in each group of muscle (soleus, gastrocnemius, and EDL) (Ohira et al., 2002). Previous studies, that atrophy in skeletal muscles following spaceflight (Martin, Etgerton and Grindeland, 1988) and/or hind limb suspension is greater in slow-twitch red than in fast twitch white muscles (Ohira, 1989).

4.4.4 Blood chemical parameters

The blood biochemical parameter levels (glucose, triglyceride, LDH, AST, ALT, creatinine, BUN and insulin) of male wistar rats after administration of DDD, maltodextrin and crude extract with and without exercise training show in table 4.2 Glucose, BUN, triglyceride and insulin in rats administered with in exercise training and non-exercise training were not statistically different.

Glucose

There was no significant difference in glucose level in non-exercise and exercise training group. In comparison to non-exercise training group, exercise-training groups that received maltodextrin and crude extract showed reduction of glucose level. The exercise training could enhance reduction of plasma glucose level compared with non-exercise training group. These result indicated the increased demanding for glucose by contracting muscle causes and increased glucose uptake to working skeletal muscle during prolong exercise (Wu et al., 2003). Blood glucose and insulin

concentrations are important markers of carbohydrate metabolism during exercise. Regarding insulin, despite a tendency to be lower in the Ex group compared to the control group. The maintenance of normal blood glucose levels during exercise by ingesting carbohydrate-containing foods before or during exercise can prolong the exercise time and delay fatigue. In this study, blood glucose concentrations after exercise in the crude extract ex group were higher than the control-Ex group. This result suggests that crude extract benefited the maintenance of blood glucose levels (Yen et al., 2013).

BUN

There was no significant difference in BUN level in non-exercise and exercise training. The exercise training could enhance reduction of plasma glucose level compared with non-exercise training group. Blood urea nitrogen (BUN) is a product of energy metabolism, is another sensitive index of fatigue status. The less an animal is adapted to exercise, the more the BUN level increases (Zhang, Yao and Bao, 2006). The lower BUN level observed in this study indicated the positive effect of ß- glucan on enhancing endurance (Jia and Wu, 2008; Wang et al., 2008). It indicated that possessed the ability to lower or retard the formation of BUN after exercise. Sokal et al. (2013) suggested that in term during exercise affects protein metabolism, resulting in a positive nitrogen balance. This phenomenon has fundamental importance in understanding human metabolic processes and may have indications in training programs for athletes. The possible causes of high BUN was increased production (muscle and other protein breakdown, increased consumption of protein in diet). Furthermore, the possible causes of high BUN was influenced muscle damage and other protein breakdown after

exhaustive exercise in associated with the early stage of muscle hypertrophy process in the group receive IGF-1 (Adams et al., 1998).

Triglyceride

There was no significant difference in triglyceride level in non-exercise and exercise training. In exercise training, triglyceride levels in rat received maltodextrin and crude extract were lower than non-exercise group. Triglyceride levels reduced at the beginning of the exercise and thereafter they returned to the control level despite the exercise continued. Triglyceride reduced again during the exhaustive exercise (Stankiewicz-chorozucha and Gorski, 1980). Moreover, Dall et al. (1983) suggested the ability of exercise training to attenuate diabetic hyper-triglyceridemia, in chow-fed and sucrose-fed rats that allow running spontaneously in exercise wheel cages, related to lower plasma free fatty acid concentrations, and decreased in plasma triglyceride secretion.

AST and ALT

In non-exercise training group, AST level, there was no significant difference in all groups. In exercise training group, AST level at 250 mg/kg of crude extract fedding was significantly higher than other groups. In comparison to non-exercise training group, all exercise training groups showed significant enhancement in AST and ALT. ALT levels in 250 mg/kg of crude extract (exercise training group) was significantly higher than non-exercise training group ($P<0.05$).There was no significant difference in exercise and non-exercise groups in ALT. ALT and AST measurement are important for the assessment of liver damage. These finds highlight the importance of imposing relevant restrictions on strenuous exercise prior to and during clinical studies and exhibit the need to consider of intense muscular activity as possible cause of

asymtomatic elevations of liver function test in daily clinical practice (Pettersson et al., 2008).

LDH

In non-exercise training, there was no significant difference in LDH level. In exercise training, LDH level in 250 mg/kg of crude extract higher than other group. In comparison, between non exercise training and exercise training group LDH levels in 250 mg/kg of crude extract (exercise training group) and control (exercise training group) was significantly higher than non-exercise training group ($P<0.05$).

LDH is an accurate indicator of muscle damage as it catalyzes the inter conversion of pyruvate and lactate (Kim et al., 2003). Our result showed that rat fed maltodextrin and crude extract have a higher level in LDH activity after exercise than non-exercise group. Chao et al. (2013) suggested that ß-glucan prevent fatigue by accelerating metabolic rate of lactic acid in muscle and it also consistent with our observation that rat fed oat ß-glucan had low lactate level after exercise. Therefore, exercise induced damage caused elevated LDH level could be suppressed by treatment with maltodextrin and crude extract. The result suggested that the enhancement effect of crude extract on exercise endurance was likely through the protection of the corpuscular membrane by modifying several enzyme activities.

Creatinine

In non-exercise training, the control group was significant lower in creatinine levels than all groups. In exercise training group, creatinine level there was no significant difference in all groups. Creatinine is an accurate indicator of muscle damage. The normal function of creatinine in cells is to add a phosphate group to creatine, turning it into the high-energy molecule phosphocreatine. In tissue and cell

that consume ATP rapidly, especially skeletal muscle, phosphocreatine serves as an energy reservoir for the rapid buffering and regeneration of ATP. Thus, creatine, a breakdown product of phosphocreatine, is an important indicator for rapid consumption of ATP (Willimann et al., 1992). Majority of the creatinine kinase normally exists in the muscle. However, during the process of muscle degeneration, the muscle cells lyse and their content find their way into the blood stream (Coombers and McNaughton, 2000). Thus, an increase in blood creatinine kinase indicates that muscle damage has occurred or is occurring. This effect may be due to the increased of creatinine kinase level, that responsible for the breaking down of creatinine phosphate into creatinine in muscle.In addition, this effect in the exercise-training group showed higher than non-exercise group. Furthermore, intense exercise can increase creatinine by increasing muscle break down (Hamilton et al., 1972).

Insulin

There was no significant difference in triglyceride level in non-exercise and exercise training. Insulin concentrations are important markers of carbohydrate metabolism during exercise. Regarding insulin, despite a tendency to be lower in the exercise training. The maintenance of normal blood glucose levels during exercise by ingesting carbohydrate-containing foods before or during exercise can prolong the exercise time and delay fatigue (Jeukendup, 2004; Suh, Paik and Jacobs, 2007). In this study, although the blood glucose concentrations lower in exercise group after exhaustive exercise than non-exercise group no significant difference. Several studies indicate that deteriorations in sport performance related to hyperglycemia in several prolonged types of exercises (Bosch, Dennis and Noakes, 1994; Shephard and Leatt, 1987). As a result, maintaining euglycemia in crucial during the later stages of exercise. In this study, blood glucose and insulin after exercise in exercise group were

Table4.2.Effects of 16 days administration of crude extract from sweet cassava on blood biochemical parameters of male Wistar rats .

Group	Parameters							
	Glucose (mg/dl)	**BUN (mg/dl)**	**Creatinine (mg/dl)**	**TG (mg/dl)**	**AST (U/L)**	**ALT (U/L)**	**LDH (U/L)**	**Insulin (uiU/L)**
Control								
Non Ex	94.33±27.42	19.46±1.20	0.36±0.01(b)	81.66±9.86	183±58.20	31±1.00	2410±796.99	2±0.00
Ex	100±8.88	22.96±1.25	0.51±0.09	142±63.37	163.66±58.15(B)	40.66±6.02	6424±1733.56(B,*)	2±0.00
250 mg/kg M								
Non Ex	113±23.38	16.7±4.07	1.05±0.10(a)	111±47.69	98.33±18.77	30±3.46	2753.33±782.09	2±0.00
Ex	88.66±9.29	19.56±7.42	0.70±0.31	77±20.95	109.66±28.36(B)	33.33±3.05	5867.33±3599.64(B)	2±0.00
500mg/kg M								
Non Ex	97±5.00	15.4±6.24	0.95±0.16(a)	154±21.63	142.66±52.91	37.66±13.42	4344±953.17	2±0.00
Ex	71.33±28.02	25.2±3.05	0.54±0.24	112.33±17.89	240±110.36(B)	38.5±0.07	5656.66±2964.91(B)	2±0.00
250 mg/kg CP								
Non Ex	89±20.88	13.46±2.13	1.05±0.05(a)	146±54.83	719±947.43	35.5±10.60	3722±1858.27	2±0.00
Ex	59±8.88	27±3.56	0.4±0.24	136±29.13	453.66±203.06(A)	37±7.78(*)	9237.66±660.73(B,*)	2±0.00
500mg/kg CP								
Non Ex	118±58.00	17.26±4.02	0.83±0.28(a)	165±53.32	147.66±93.07	34.66±7.63	5152.66±3340.09	2±0.00
Ex	86.33±11.67	20.66±0.83	0.49±0.11	102.66±18.58	133±86.13(B)	36±9.16	5947.33±3946.2(B)	2±0.00

Values are expressed as mean ± S.E.M * .indicates significant difference compare between Non Ex and Ex with same treatment.Small letters indicate significant difference within Non Ex .Capital letters indicate significant difference within Ex .M =maltodextrin, CP =crude extract, Ex =exercise training, Non Ex =non exercise training .

similar to those in non-exercise group .This result suggests that maltodextrin and crude extract benefited the maintenance of blood glucose and insulin level

4.4.5 Glycogen tissue content

The effect of 16 days administration of maltodextrin and crude extract on liver glycogen levels in male wistar rats with and without exercise training are shown in Fig 4.3.

In exercise training and in non-exercise training, liver glycogen level in 250 and 500 mg/kg of crude extract and 500 mg/kg of maltodextrin ware higher than maltodextrin at dose 250 mg/kg and control group. In comparison to non-exercise training, exercise-training groups that received water (control group) showed significant reduction in liver glycogen levels than treated with maltodextrin and crude extract group.

Energy storage and supply is an important factor related to exercise performance. In terms of energy, expenditure with exercise, rapid ATP consumption and energy deficiency is a critical cause of physical fatigue. Glycogen is the predominant source of glycolysis for ATP production. Therefore, glycogen storage directly affects exercise ability. Previous study, that the more glucose was metabolized, the more liver glycogen stores reduced. The increases in muscle glycogen content following the administration of maltodextrin and crude extract at dose of 250 and 500 mg/kg found in the present study. This finding suggested the capability of the maltodextrin and crude extract in maintenance muscle during exhaustive exercise causing muscle fatigue. Raising muscle glycogen increases the capacity for prolonged exhausting exercise, while a low initial glycogen concentration is associated with more rapid development of fatigue (Hermansen, Hultman, and Saltin, 1967).

Endurance was improved by high carbohydrate ingestion for several days before prolonged exercise because this increases both liver and muscle glycogen stores (Stellingwerff et al., 2007). With exercise of longer duration, however, gluconeogenesis becomes increasingly important as liver glycogen stores become depleted. Glucose is spared so that just enough remains available at the end of the marathon. The simultaneous use of both fuels gives a higher mean velocity than would be attained if glycogen were totally consumed before the start of fatty acid oxidation. In addition, protein and carbohydrate supplement has been reported to potentiate the plasma insulin response of the supplement following a fast or prolonged aerobic exercise (Spiller et al., 1987; Zawadzki, Yaspelkis, and Ivy, 1992; Van Loon et al., 2000). Thus, it is possible that a carbohydrate and protein supplement may be more effective than a carbohydrate supplement for the sparing of muscle and possibly liver glycogen during variable intensity exercise (Bowtell et al., 1999).

The effects of 16 days administration of maltodextrin and crude extract on gastrocnemius muscle glycogen level in male wistar rats with and without exercise training show in Fig 4.4.

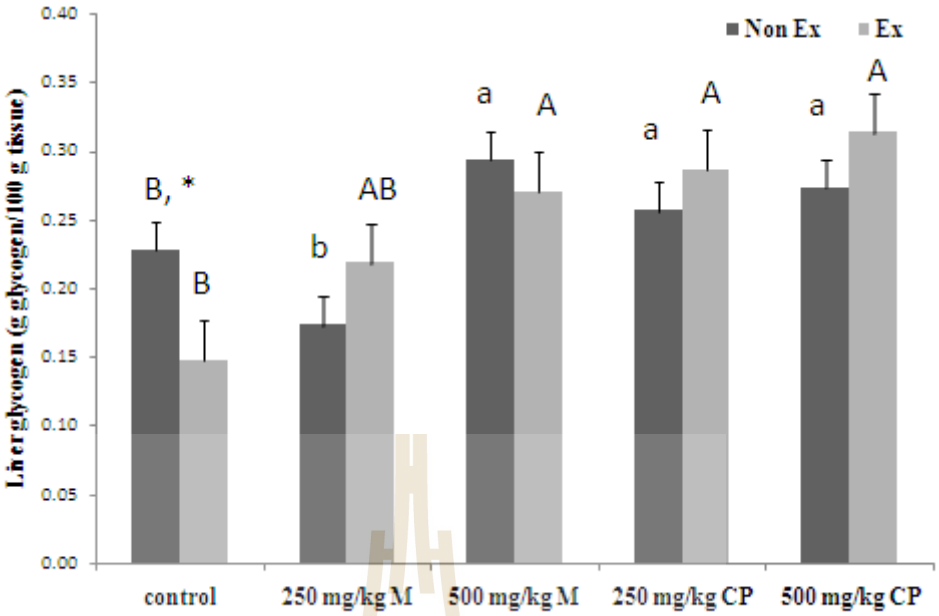

Figure 4.3 Effects of 16 days administration of maltodextrin (M) and crude extract (CP) from sweet cassava on liver glycogen content. Value are expressed as mean±S.E.M.; n=5 per group. * Indicates significant difference compare between Non Ex and Ex with same treatment. Small letters indicate significant difference within Non Ex.Capital letters indicate significant difference within Ex. Mean sharing the same superscript are not significantly different from each other ($P<0.05$, two-way ANOVA; Ducan's Method).

In non-exercise training group and exercise training group, the gastrocnemius muscle glycogen level in 250 and 500 mg/kg group of maltodextrin and crude extract were significantly higher than control groups .Increasing muscle glycogen through diet, and before exercise, is one method to enhance endurance capacity. Some studies reported that sweet cassava has abundant carbohydrates such as monosaccharides and polysaccharides (Charles et al., 2008). In addition, several studies

reported that supplementation with extracted polysaccharide is beneficial for increasing glycogen levels. The effect of maltodextrin and crude extract from sweet cassava on boosting sport performance was similar to that seen in the above-mentioned studies. However, the physiological characteristics of the two muscles (i.e. slow-twitch fibers in the soleus muscle and fast-twitch (FT) fibers in gastrocnemius muscle. Hamilton et al. (2010) reported that post-exercise muscle glycogen storage can be augmented by creatine and carbohydrate supplementation following exercise compared with carbohydrate ingestion alone. Thereafter, result showed that creatine supplemented subjects, during a phase of rehabilitation from immobilization-induced muscle atrophy, had larger muscle glycogen content when compared with non-supplemented subjects (650 versus 520 mmol/kg dry weight) (Op'tEijnde et al., 2001). Accordingly, an 18% increase in muscle glycogen content was reported as a result of 5 days of concomitant creatine and carbohydrate supplementation compared with placebo ingestion (van Loon et al., 2004). It was showed that performing a glycogen loading protocol (exhaustive exercise followed by a high carbohydrate diet for 3 days) after creatine loading resulted in a 10% greater glycogen content when compared to a glycogen loading before creatine loading protocol (Nelson et al., 2001). In light of these findings, it was speculated that creatine supplementation could beneficially affect performance by modulating pre-exercise muscle glycogen content. Furthermore, it has been speculated that creatine loading could also affect performance during exercise by increasing PCR content and consequently decreasing the reliance on glycolysis and muscle glycogen (Brannon et al., 1997; Yquel et al., 2002).

Crude extract and maltodextrin may increase the glycogen content in both liver and gastrocnemius muscle in both exercise and non-exercise training by improving glycogen reserve and glycogen-sparing effects. Glycogen-sparing effect can improve time to exhaustion because glycogen depletion is associated with physical exhaustion. Delayed utilization of glycogen resulted in endurance exercise capacity improvement (Holloszy et al., 1998; Oh and Ohta, 2003). The major factor in determining the steady-state glycogen concentration in resting muscle appears to be the rate of muscle glucose uptake (Ren et al., 1993). The study of Ren et al. (1994) demonstrated that the adaptive increase in glucose transporter 4 (GLUT4), by potentiating insulin-stimulated glucose transport, might function to increase the rate of muscle glycogen repletion. The rapid adaptive increase in GLUT4 expression could provide a survival advantage by making possible more rapid replenishment of muscle glycogen stores when consume carbohydrate between exercise bouts or even when exercise continued at a lower intensity. The rapid adaptive increase in GLUT4 may also be help to explain the data that a few days of exercise training can result in a significant enhancement of insulin action on glucose disposal (Rogers et al., 1988). It is well documented that the adaptations induced by endurance exercise training, primarily, the increase in muscle mitochondria, have a potent glycogen-sparing effect during exercise that plays a major role in improving endurance (Holloszy and Coyle, 1984).

It was known that endurance capacity of body was markedly decreased if the energy was exhausted. As glycogen was the important resource of energy during exercise, the increasing of glycogen stored in liver is an advantage to enhance the endurance of the exercise (Yu et al., 2008). Wilber (1959) reported that severe depletion of liver glycogen was noted in all guinea pigs that swam to exhaustion. Liver glycogen

depletion might be an important factor in the development of fatigue because as liver glycogen is depleted during exercise there is an inability to maintain blood glucose level, and the ensuing hypoglycemia could result in impaired nervous function (Dohm et al., 1983). Dohm et al. (1983) also demonstrated the importance of muscle glycogen levels in endurance exercise and suggested that depletion of muscle glycogen was a factor in fatigue and exhaustion (Jung et al., 2004).

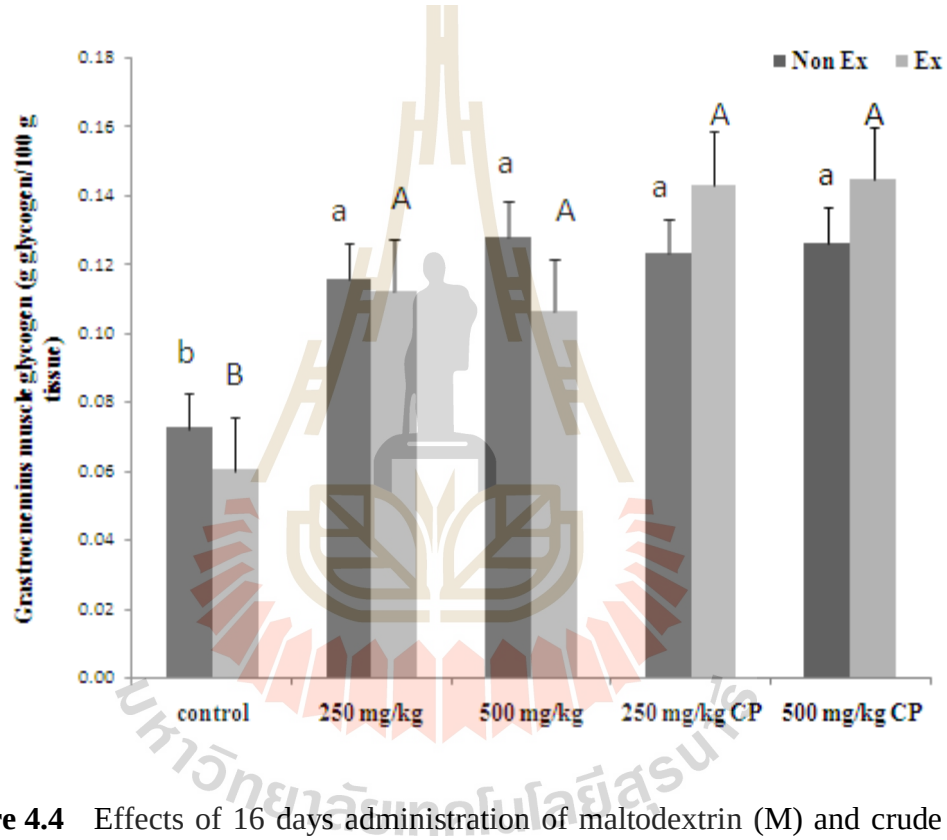

Figure 4.4 Effects of 16 days administration of maltodextrin (M) and crude extract (CP) from sweet cassava on muscle glycogen content. Value are expressed as mean±S.E.M.; n=5 per group. * Indicates significant difference compare between Non Ex and Ex with same treatment. Small letters indicate significant difference within Non Ex.Capital letters indicate significant difference within Ex. Mean sharing the same superscript are not significantly different from each other ($P<0.05$, two-way ANOVA; Ducan's Method).

In the present study, the data of glycogen are shown in figure 4.3 and 4.4. After swimming, the concentration of liver glycogen of all the treatment groups were higher than that of control group *(P<0.05)*. The concentration of muscle glycogen of all the treatment groups were higher than that of the control group *(P<0.05)*. These data indicated that crude extract and maltodextrin can significantly increase the concentration of liver and muscle glycogen of male wistar rat after swimming.

4.5 Conclusion

The present finding suggested that crude extract and maltodextrin appeared to have potential to support exercise endurance capacity. Crude extract and maltodextrin from sweet cassava may increase the glycogen contents in liver and gastrocnemius muscle in both non-exercise and exercise training by improving glycogen reserve and glycogen sparing effect. But, crude extract at low dose showed high level in AST and ALT. The result, suggest high dose maltodextrin from sweet cassava is a good supplement for endurance sport. However further study are need to elucidate the mechanism underlying this action of maltodextrin from sweet cassava.

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CHAPTER V

EFFECTS OF MALTODEXTRIN AND CRUDE EXTRACT FROM SWEET CASSAVA ON AMPK EXPRESSION IN TISSUE WISTAR RAT

5.1 Abstract

The exercise endurance capacity of maltodextrin and crude extract from sweet cassava was investigated in male Wistar rats. Fifty rats were devided into exercise training (swimming without load) and Non-exercise training groups that received control (1 ml/kg double deionized distilled), crude extract and maltodextrin (250 and 500 mg/kg) orally once daily for 16 days (n=5, each). On day 16, all rats were observed antioxidant enzyme in organ of liver and hind limb skeletal muscles (soleus and gastrocnemius), and 5′-AMP-activated protein kinase (AMPK α1, 2) and receptor γ coactivator-1 α (PGC-1 α) gene expression were determined. This study aims to investigate exercise performance of maltodextrin and crude extract in rat model. Two-week, maltodextrin and crude extract significantly enhances the exercise performance in swimming test. In exercise group showed increased levels of antioxidative enzymes superoxide dismutase (SOD), glycogen, but the reduced levels of malondialdehyde (MDA) and reactive oxygen species (ROS) in liver. Further data show that maltodextrin and crude extract have strongly enhanced AMPK, and the expressions of peroxisome proliferator have activated PGC-1α in liver and muscle.

Our data suggest that maltodextrin and crude extract possess antifatigue effects related to AMPK-linked antioxidative pathway.

5.2 Introduction

The heterotrimeric, 5'-AMP-activated protein kinase (AMPK) is composed of α, β, and γ subunits. Each of these three subunits takes on a specific role for both the stability and the activity of AMPK. The γ subunit plays a particularly important role during exercise because it includes four cystathione beta synthase (CBS) domains. The four CBS domains create two binding sites for AMP, commonly referred to as Bateman domains. Binding of one AMP to a Bateman domain cooperatively increases the binding affinity of the second AMP to the other Bateman domain (Thomson et al., 2006). The α, β, and γ subunits can be found in different isoforms: α1 or α2: β1 or β2: and γ1, γ2, γ3. Although the α1, β1, and γ1 isoforms are the most common expressed in most cells, it has been demonstrated that the α2, β2, γ2, and γ3 isoforms are also expressed in cardiac and skeletal muscle (Hutber et al., 1997). The α2-containing AMPK complex (AMPKα2) is considered the foremost AMPK isoform responsible for the metabolic adaptations to training and exercise in contracting skeletal muscle (Chueng et al., 2000). The energy-sensing capability of AMPK can be attributed to its ability to detect and react to fluctuations in the AMP: ATP ratio that take place during rest and exercise (muscle contraction) (Holmes et al., 1999). AMPK is sensitive to the fluctuating (increasing) AMP: ATP ratio during exercise because AMP acts as an allosteric activator of AMPK, while ATP inhibits AMPK activation by binding to the Bateman domains on the γ subunit. As AMP binds both Bateman domains, the γ subunit undergoes a conformational change that exposes the catalytic domain found on

the α subunit. Subsequently, this action allows AMPK to become activated in the activation loop of the catalytic domain when phosphorylation takes place at threonine-172 by an upstream AMPK kinase (AMPKK) (Kurth-Kraczek et al., 1999). Because increases in AMP concentrations in contracting skeletal muscle usually correlate with small decreases in ATP, AMPK will become activated either when energy demands needed for exercise are not met or when they are in short supply. Upon activation by AMPKK, AMPK serves as a metabolic master switch regulating several intracellular adaptations to muscle contraction. Major responses to exercise and adaptations to endurance training effectuated by AMPK include the cellular uptake of glucose (Hurst et al., 2005), the β-oxidation of fatty acids, and the biogenesis of GLUT4 and mitochondria. Adaptations of skeletal tissue that take place in response to endurance training increased muscle glycogen (Hawley et al., 2003), increased PGC-1 , hexokinase II, UCP-3, cytochrome C (Ojuka et al., 2004), as well as increased activities of mitochondrial enzymes such as citrate synthase, succinate dehydrogenase, and malate dehydrogenase are also thought to be mediated in part by activated AMPK. Furthermore, recent discoveries suggest a direct AMPK role in increasing blood supply to exercised/trained muscle cells by stimulating and stabilizing both vasculogenesis and angiogenesis (Zong et al., 2000). Aside from up regulating these pathways that result in the generation of ATP during exercise, AMPK also acts to down regulate synthetic pathways that consume ATP (Wojtaszewski et al., 2000). Together, these adaptations serve to decrease the high energy demands in muscle tissue felt during exercise and endurance training. Both the rapid/acute and chronic/long-term effects of AMPK activation most likely transpire in part as a result of both temporary and maintained increases in AMPK activity brought about by

increases in the AMP: ATP ratio during single bouts of exercise and long-term training. During a bout of exercise, muscle AMPK activity increases in response to metabolic stress brought about by an extreme cellular demand for ATP. This AMPK response to exercise has been found to notably decrease in red quadriceps (RQ) following training, while putative AMPK roles such as increased GLUT4 and hexokinase II expression and mitochondrial biogenesis are maintained (Thornton et al., 1998). One possible explanation for the attenuating effects of training on the AMPK response to exercise is that perhaps the metabolic stress of trained muscle fibers during stimulation has been reduced due to the cellular adaptations to exercise that took place during training (increased GLUT4, blood supply, and mitochondria). Adaptations such as these increase cellular capacity to produce ATP. Thus, the increased AMP: ATP ratio required to maximize AMPK activation would be expected to be lower in trained rats than in untrained rats that lack these adaptations. It is also possible that the AMPK activity threshold needed to elicit such changes is lower than expected or that it is decreased with training. Despite what is actually taking place to account for the endurance-trained attenuation of the AMPK response to exercise, it is also interesting to note that although AMPKK protein abundance has been shown to increase in skeletal tissue with endurance training, its level of activity has been shown to decrease with endurance training in both trained and untrained tissue (Woods et al., 2003; Winder et al., 2000). Currently, the activity of AMPKK immediately following a 2-hr bout of exercise of an endurance-trained rat is unclear. It is possible that there exists a direct link between the observed decrease in AMPKK activity in endurance-trained skeletal muscle and the attenuated AMPK response to exercise with endurance training. These hypothesize that these necessary high-intensity conditions for AMPK activation

will need to increase higher still with training. This study will investigate the effects of maltodextrin and crude extract from sweet cassava on the AMPK response to exercise in wistar rat and its effects on other antioxidant enzymes that take on key metabolic roles as well. This will include a comparative study that will experiment the AMPK response to exercise.

5.3 Materials and Methods

5.3.1 Animals

The eight weeks old male Wistar rats (weighting 250-300 g) were housed under standard laboratory conditions (12:12 h dark-light cycle, ambient temperature 25±1°C) with free access to food and water. The experiments were performed following the animal care and use committee guidelines of Suranaree University of Technology (SUT). All studies were conducted under permit of the SUT Animal Care and Use Committee.

5.3.2 Experimental designs

After adaptation period for one week, rats (n=50) were randomly divided into five groups: control group (1 ml/kg DDD water) and four treatment groups.

The rats in the treatment groups were gavaged with 2 diferrent doses of crude extract and maltodextrin: 250 mg/ml/kg for low dose group and 500 mg/ml/kg for the high dose group, respectively. These doses are selected according to the study of Yen et al. (2013) which were calculated by converting human dose used in normal folklore to rodent dose based on following criteria:

$$\text{Rat dose (mg/kg)} = (\text{human dose mg/kg} \times 70\ \text{kg} \times 0.018)/200$$

5.3.3 Exercise endurance capacity

The exercise training groups (received vehicle, crude extract and maltodextrin at doses of 250 and 500 mg/ml) were subjected to swimming training without any load for 15 days (6 days a week). Exercise endurance capacity was observed by weight loaded forced swimming test as previously described by Kumar et al. (2011). The rats were trained for 30 min/day with subsequent increase of 10 min/day till it reaches 1 h. Swimming was performed in groups of three in an acrylic plastic pool (90 cm x45 cm x 45 cm) filled with water up to 60 cm and maintained at a temperature between 34-36°C. During the exercise protocol, the rats in Non- exercise groups (received vehicle, crude extract and maltodextrin at doses of 250 and 500 mg/ml) were kept in plastic cage containing about 3 cm of water maintained the same temperature to exclude potential stress and other potential confounding effects. On day 16 after administered 30 min all rats were allowed to swim till exhaustion with load of 3% body weight attached to the tails. The uncoordinated movements and stay under the water for 10 s without swimming at the surface were accepted as the exhaustion criteria of the rats. At this point, the rats were rescued and swimming time was recorded for each rat.

5.3.4 Oxidative stress- related parameter analysis

Immediately after exhaustion, the rats were anesthetized with carbon dioxide. Hind-limb skeletal muscles (soleus, extensor digitorum longus (EDL) and gastrocnemius) from both sides and liver were quickly excised, dried with filter paper and weighed. Tissue samples were then frozen on dry ice and stored at -20°C until further used. The liver and muscle of scarified rat were dissected, and they were

homogenized in double distilled water after wash three times in ice-cold physiological saline solution. The levels of ROS MDA, and SOD in liver were detected.

5.3.4.1 Reactive oxygen species (ROS)

Hydrogen peroxide (H_2O_2) production was quantified by the method of Holland and Storey (1981). In brief, 0.1 ml liver extract was added to an assay mixture containing KCl (1.13 mol), 0.1 ml potassium phosphate (150 mmol), 0.05 ml $MgCl_2$ (60 mmol), 0.05 ml EDTA (8 mmol), 0.1 ml Tris-HCl (200 mmol, pH 7.4), and 0.1 ml acetylated ferrocytochrome c (1 mmol). The oxidation of ferrocytochrome c, which provides a measure of H_2O_2 production, was then evaluated at 550 nm, in a spectrophotometer.

5.3.4.2 Malondialdehyde (MDA)

Lipid peroxidation (LPO) was determined by the procedure of Weerawan et al. (2006). Malondialdehyde (MDA), formed as an end product of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a color product that can be measured optically at 532 nm. The level of MDA was expressed as mmol MDA per mg protein.

5.3.4.3 Superoxide dismutase (SOD)

The chromic acetate thus produced is measured colorimetrically at 450 nm. Superoxide dismutase (SOD) was assayed according to the method of Marklund (1984).

5.3.5 RNA isolution and reverse transcription (RT)-PCR

Total RNA was extracted from 400 mg of liver and grastrocnemius muscle using NucleoSpin RNA kit (Macherey-Nagel, Dueren Germany), according to

the producer's method. The isolated RNA synthesized complementary DNA (cDNA) using qPCR RT Master mix (Vivantis Technologies Sdn. Bhd, Malaysia), according to producer's method. The gene expression used qPCRBIO SyGreen Mix Lo-ROX (PCR Biosystems, Canada), according to the producer's method and was performed using the Biorad/C1000Touch Thermocycle (Biorad, CA, USA) with specific primer. The relative expression levels of mRNAs were determined with the GAPDH gene. Primer sequences are shown in Table 5.1. The quantitative RT-PCR was performed using DNA Engine Optical 2, in which the mixture was heated to 50°C for 30 min for reverse transcription, heated to 95°C for 10 min, and then cycled 40 times at 95°C for 30 s, 56°C for 1 min, and 72°C for 50 s for AMPK α1 and AMPK α2. The mixture was heated to 50°C for 30 min for reverse transcription, heated to 95°C for 10 min, and then cycled 40 times at 95°C for 30 s, 60°C for 1 min, and 72°C for 50 s for PGC-1α and GAPDH.

Table 5.1 Primer sequences.

Gene name	Primer	Primer Sequence (5' to 3')
AMPK α1	Reverse	GGG AGG TCA CGG ATC AGG
	Forward	GGG ATC CAT CAG CAA CTA TCG
AMPK α2	Reverse	TGT CGT ATG GTT TGC TCT GG
	Forward	TCG CAG TGG CTT ATC ATC TC
PGC-1α	Reverse	GGCCTGCAGTTCCAGAGAGT
	Forward	GACCCCAGAGTCACCAAATGA
GAPDH	Reverse	GGC ATG GAC TGT GGT CAT GAG
	Forward	TGC ACC ACC AAC TGC TTA GC

5.3.6 Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was assessed by analysis of variance (ANOVA) using SPSS 18.0 (SPSS, Inc., Chicago, IL). Post hoc testing was performed for inter-group comparisons (Duncan's). P-Value less than 0.05 ($P<0.05$) was considered statistically significant.

5.4 Results and discussion

5.4.1 Effects of maltodextrin and crude extract from sweet cassava on antioxidant enzyme

Reactive oxygen species (ROS) plays an essential role during human normal physiological and pathological process such as exercise and metabolism (Alfadda et al., 2012). Over production of ROS unbalanced cellular redox status and led to tissue damage (Yin et al., 2016). Important, excessive oxidative stress can be elicited by strenuous exercise and further lead to irreversible oxidize damage (Armstrong., 1990). Antioxidant enzymes, such as superoxide dismutase (SOD) is responsible for cell damage and muscle fatigue. SOD can prevent biomacromolecules such as DNA, protein and lipids from attacks of ROS (Zhao et al., 2017). Malondialdehyde (MDA) is one of the end-product resulting from degradation of cell membrane by radical (Nallamuthu., 2014) and biomarker of oxidative stress (Giera et al., 2012).

The antioxidant parameter levels (SOD, MDA and ROS) of liver male wistar rats after adminitration of DDD, maltodextrin and crude extract with and without exercise training were shown in table 5.2.

In Non- exercise group and exercise group, maltodextrin from sweet cassava enhanced the activities of SOD and reduced the levels of MDA and ROS in liver after exhaustive. In comparison to Non-exercise training, there was no significant difference in SOD, MDA and ROS in all groups ($p<0.05$). Similary, crude extract displayed positive regulatory effects on levels of SOD and MDA of exercise and Non-exercise group ($P<0.05$). The reduced ROS levels of liver was observed in maltodextrin and crude extract exercise group ($p<0.05$).

The regulatory effects on ROS, MDA and SOD in organs of maltodextrin and crude extract in exercise and Non-exercise rat indicated the important role of oxidative system in preventing exercise induced fatigue. The accumulation of oxygen free radicals is one of the risk factor which are responsible for oxidative stress and muscle fatigue (Whaley-Connnell et al., 2011), and they can be cleared by antioxidant enzymes. Antioxidants can successfully prevent or reduce oxidative stress and further improve exercise performance (Peternelj and Coombes, 2011). Proteins, separated from *Panax quinquefolium*, improve behavioural alterations linked to chronic fatigue via inhibition oxidative damage (Qi et al., 2014). Furthermore, *Cordyceps militaris* displays antifatigue property via scavenging ROS and enhancing activities of SOD and GSH-Px (Dong et al., 2015). MDA is recognized as the end product of lipid peroxidation, which can be used to assess the degree of the lipid peroxidation for free radical (El-Maghrabey et al., 2014). SOD prevents lipid peroxidation via catalyzing the conversion of superoxide into hydrogen peroxide and oxygen. GSH forms a formidable defense with glutathione peroxidase to prevent lipid peroxides (Sun et al., 2010).

ROS are produced by the plant's normal metabolic processes such as photosynthesis and respiration. However, during stress situations, the metabolic imbalance leads to the formation of ROS in excess, causing oxidative stress. Among the various types of ROS, singlet oxygen ($^1O_2$), superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and radical hydroxyl ($OH^{\cdot}$) may be formed (Gupta and Huang, 2014). When the production of ROS is much higher than the volume that can be metabolized, they may react with several cellular components. When in excess, it may cause damage to lipids, proteins and DNA, leading to a structural function change and/or inhibition thereof (Ferreira et al., 2007). On the other hand, plants have a complex antioxidant system whose function is to protect cells from damage caused by ROS. The main enzymatic components of the defense system are SOD, appears to be the first to act in the line of defense regarding the elimination of ROS, performing the dismutation of superoxide radicals into hydrogen peroxide. In mammals, the highest SOD activity is found in liver 14,400 unit/g wet weigh but other enzyme such as catalase and GPx found 670 and 85 unit/g wet weigh. So, antioxidant enzyme study only SOD in liver. The general belief is that these enzymatic and non-enzymatic antioxidants present in the organelle (circulatory and tissue) systems have the capacity to break down free radical formations by directly scavenging the free radical forming agents such as H_2O_2 or chelate the metal ions serving as an oxidative base such as Fe^{2+}, which in turn minimise the development of reactive oxygen species. The latter process indeed avoids lipid, protein and DNA damage in the cellular systems, improves immune responses and protect animals (organisms) from oxidative stress and disease development (Bekhit et al., 2013; Chauhan et al., 2014).

Table 5.2 The antioxidant enzyme effects of maltodextrin and crude extract in swimming rat.

Groups		Parameter		
		MDA (Mmol/mg)	**SOD (U/mg)**	**ROS (FI/g)**
Control	**Non Ex**	12.2± 0.9 (b)	270.5±17.8 (b)	1516.5±59.9 (b)
	Ex	11.8±0.5 (B)	240.9±19.8 (B)	1606.7±80.0 (B)
250 mg/kg	**Non Ex**	9.8±0.6 (ab)	369.6±18.9 (a)	1495.6±32.8 (b)
Maltodextrin	**Ex**	9.6±0.9 (AB)	316.4±15.8 (AB)	1446.3±51.2 (AB)
500 mg/kg	**Non Ex**	9.3±0.7 (ab)	365.1±23.8 (a)	1360.3±53.8 (ab)
Maltodextrin	**Ex**	8.7 ±0.9 (A)	388.6±32.2 (A)	1210.2±35.6 (A)
250 mg/kg	**Non Ex**	9.7±0.4 (ab)	318.4±18.2 (ab)	1361.2±32.3 (ab)
Crude Extract	**Ex**	8.8±0.9 (A)	303.8±20.2 (AB)	1356.5±43.2 (AB)
500 mg/kg	**Non Ex**	8.8±0.4 (a)	296.5±12.3 (b)	1273.3±42.5 (a)
Crude Extract	**Ex**	8.5±0.7 (A)	332.4±15.3 (AB)	1289.8±52.7 (A)

Value are expressed as mean ± SEM; n=5 per group.*indicated significant difference compared between Non Ex and Ex with the same treatment. Small letters indicate significant differences within Non Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other ($p<0.05$, two way ANOVA; Duncan's method). Ex = exercise training, Non Ex = Non exercise training.

Maltodextrin and crude extract were to prevent lipid peroxidation and further protect cells from oxidative injury via suppressing hyper level of MDA and ROS and enhancing SOD activities, which are involved in maltodextrin and crude extract effect on enhancing exercise endurance.

5.4.2 RNA isolation and reverse transcription (RT) - PCR

The expressions of AMPK α1, AMPK α2, and PGC-1α were detected to further analyze the molecular mechanisms under maltodextrin and crude extract effects. After exhaustive swimming, over twofold enhancement of AMPK expression was observed in liver and muscle tissue of maltodextrin and crude extract treated (Figure 5.1, 5.2 and 5.3).

AMPK α1

From the figure 5.1 the expression of AMPK α1 showed in liver, in Non-exercise training group, 500 mg/kg of maltodextrin showed relative fold AMPK α1 expression higher than other group. There was no significant relative fold AMPK α1 expression in exercise training group. In comparison with Non-exercise and exercise group, 500 mg/kg of crude extract was significant lower relative fold AMPK α1 expression in exercise group ($p<0.05$).

From the figure 5.1 the expression of AMPK α1 showed in muscle, in Non-exercise training group, 500 mg/kg of crude extract showed relative fold AMPK α1 expression higher than other group. There was no significant relative fold AMPK α1 expression in exercise training group. Following exhaustive exercise, McArdle mice display increased AMPK phosphorylation in the tibialis anterior and EDL muscles, while WT mice display no significant increase in AMPK activity (Neilsen et al., 2018). While increased AMPK activity in McArdle patients and rodent models

seems contrary to previous findings, the authors hypothesized that since McArdle disease results in an inability to break down glycogen, there is a subsequent increase in the AMPK activity in order to maintain an energy balance via increased glucose uptake (Krag et al., 2016). Other rodent models have directly targeted muscle glycogen syntase, which is affected in patients with Glycogen Storage Disease 0 (Kollberge et a., 2007). Muscle-specific glycogen synthase knock-out models display increased AMPK phosphorylation (Pederson et al., 2005) and markedly reduced glycogen content in skeletal muscle in the basal state, likely due to the retained capacity to break down but an inability to resynthesize glycogen.

AMPK was proposed to also play an important role in regulating exercise-induced fatty acid oxidation in skeletal muscle (Ruderman et al., 1999). During exercise in the rat, AMPK phosphorylates acetyl-CoA carboxylase (ACC), leading to a decrease in its activity, a fall in malonyl-CoA, deinhibition of carnitine palmitoyltransferase I, and a subsequent increase in fatty acid oxidation (Rasmussen and Winder, 1997). Activating AMPK with AICAR has similar inhibitory effects on ACC, decreasing malonyl-CoA levels and increasing fatty acid oxidation (Merill et al., 1997). Recently, it has been shown that similar to the effects in rodents, acute exercise in humans causes a significant decrease in ACC activity in association with augmented fatty acid oxidation rates (Chen et al., 2000). Therefore, activating AMPK with exercise or pharmacological agents could potentially result in not only increased glucose disposal in muscle but also decreased malonyl-CoA content; these two mechanisms might ameliorate insulin resistance and improve glycemia. The isoform-specific changes in AMPK α1 and α2 activity during exercise suggest that these isoforms may play different physiological roles in human skeletal muscle. As

mentioned previously, AMPK α1 is widely distributed, and AMPK α2 is primarily expressed in skeletal muscle, heart, and liver (Stapleton et al., 1996). In INS-1 cells, AMPK α1 is localized predominantly in the cytosol, whereas AMPK α2 is localized in the cytosol and nucleus (Salt et al., 1998). The nuclear localization of AMPK α2 raises the possibility that this isoform might regulate gene expression in response to cellular stress. Besides differences in tissue distribution and subcellular localization, substrate specificity for downstream targets between isoforms seems to differ as well (Woods et al., 1996). An interesting possibility is that the differential activation of the AMPK isoforms during exercise could be related to the magnitude of changes in cellular energy levels. Compared with the α1 isoform, liver AMPK α2 activity is more dependent on AMP concentrations. In young nondiabetic humans, significant depletion of phosphocreatine and a small decrease in ATP during exercise at 70% VO_{2max} lead to an increase in muscle AMPK α2 activity but no change in α1 (Fujii et al., 2000). A similar finding on differential AMPK activation in humans during moderate-intensity exercise has been confirmed by Wojtaszewski et al.(2000). These findings suggest that with this intensity of exercise, α2-containing rather that α1 isoform–containing AMPK complexes are primarily responsible for activating AMPK-stimulated metabolic changes in skeletal muscle of both healthy and subjects with type 2 diabetes. However, it is also possible that under conditions of extreme exercise intensity, α1-containing complexes are critical metabolic regulators, because a recent report has shown that 30 s of supramaximal sprint exercise significantly increased the activity of both isoforms (Chen et al., 2000).

AMPK *α*2

From the figure 5.2 the expression of AMPK *α*2 showed in liver, in Non-exercise training group, 500 mg/kg of maltodextrin showed relative fold AMPK *α*2 expression higher than other group. In exercise training, 500 mg/kg of maltodextrin and 250 mg/kg crude extract showed relative fold AMPK *α*2 expression higher than other group. In comparison between exercise and Non-exercise training, 250 mg/kg of maltodextrin and crude extract were significant higher relative fold AMPK α2 expression in exercise training group ($p<0.05$), except 500 mg/kg of maltodextrin showed significant lower relative fold AMPK α2 expression in exercise training group ($p<0.05$).

From the figure 5.2 the expression of AMPK *α*2 showed in muscle, in Non-exercise training group, there was no significant relative fold AMPK α2 expression. In exercise training group, 500 mg/kg of maltodextrin and 250, 500 mg/kg crude extract showed significant lower relative fold AMPK α2 expression ($p<0.05$).

While AMPK α2 activity is readily increased by exercise and muscle contraction in rodents (Musi et al., 2004), increases in AMPK α1 activity after exercise/contraction are less consistent. For example, AMPK α1 activity in mouse quadriceps muscle was approximately four times higher immediately after 90 min of treadmill running at 13–17 m/min (Jogensen et al., 2005), but was not activated at all after running at 10–15 m/min for 60 min (Dzamko et al., 2008). Similarly, 30 min of treadmill running at 30% of maximum running capacity activated AMPK α2 in mouse skeletal muscle, but not AMPK α1, while running at 70% of maximum activated both isoforms (Maarbjerge et al., 2009). In vitro contraction of the extensor digitorum longus (EDL) muscle for 25 min activated AMPK α1, while 20 min of in situ

contraction of the tibialis anterior (TA) failed to do so (Dzamko et al., 2008). The data from rodents is confirmed in human studies where cycling for 1 h at 50% and 70% VO_2max failed to activate AMPK α1 (Fuji et al., 2000), while a single 30 s sprint (Chen et al., 2000) or high intensity interval cycling (4 × 30 s bouts of cycle sprints) (Gibala et al., 2009) activated both AMPK α1 and α2 isoforms. Thus, activation of AMPK α1 isoforms by exercise requires greater intensity work and/or duration than for the activation of AMPK α2. As discussed below, this has important implications in relation to AMPK's impact on muscle growth and repair, as AMPK α1 appears to be critical in the regulation of anabolism.

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase that responds to cellular energy status by inhibiting ATP-consuming pathway and activating ATP-producing pathway (Hardie and Sakamoto. 2006; Aschenbach et al. 2004), and is active as a heterotrimer consisting of one catalytic subunit (*α)* and two non-catalytic subunit *(*β, and γ*) (*Hardie. 2004). The *α*2 isoform is by far the most abundant in skeletal muscle, representing at least 66% of total AMPK activity (Vioolet et al. 2003). In skeletal muscle, the activity of AMPK is thought to be mediated mainly through the AMPK *α*2 isoform (Wojtaszewski et al., 2002; Musi et al.2001). The majority of human studies showed that AMPK is activated in an exercise-intensity-dependent manner and there is more pronounced response of AMPK *α*2 activation compared with AMPK *α*1 (Chen et al., 2003; Wojtaszewski et al., 2000). Fuji et al. (2000) reported that AMPK *α*2 activity was significantly increased in vastus lateralis biopsy samples following 60 min of cycle ergometry at 70% VO_2peak but not at 50% VO_2peak. No changes in AMPK *α*1 activity were observed at either exercise intensy. In mice, it was reported that after 30 min of moderate-intensity-swimming exercise,

AMPK α2 activity increased by 80%, but AMPK α1 activity did not change significantly in muscle (Nakano et al., 2006). The studies attempted to address the relation between exercise duration and activity of AMPK α2, and found that when exercise time is prolonged, AMPK α2 activity is increase correspondingly (Lee-Young et al., 2006; Stephens et al., 2002).

PGC-1*α*

PGC-1α is a transcriptional co-activator known to target transcription factors regulating mitochondrial metabolism, fatty acid oxidation enzymes, and angiogenesis in skeletal muscle (Yan et al., 2011). PGC-1α function is highly sensitive to exercise, and has been suggested to co-ordinate the mitochondrial post-exercise transcriptional response, although this process can occur independently of PGC-1α in both rodent (Rowe et al., 2012) and human (Keller et al., 2011; Timmons et al., 2010) exercise models. Consistent with this concept, PGC-1α muscle-specific transgenic mice display a muscle energetic profile similar to highly trained athletes and an increased expression of genes known to be involved in lipid utilization and oxidation (Calvo et al., 2008).

From the figure 5.3 the expression of PGC-1*α* showed in liver In Non-exercise and exercise training group, there was no significant relative fold PGC-1α expression. In comparison between exercise and Non-*exercise* training group, 250 mg/kg maltodextrin showed significant lower relative fold PGC-1α expression in exercise training group ($p<0.05$)

From the figure 5.3 the expression of PGC-1*α* showed in muscle, in Non-exercise and exercise training group, there was no significant relative fold PGC-1α expression. In comparison between exercise and Non-*exercise* training group, 250 and

500 mg/kg crude extract showed significant lower relative fold PGC- 1α expression in exercise training group ($p<0.05$).

The mRNA of PGC-1α increases during prolonged exercise and is influenced by carbohydrate availability. It is unknown if the increases in mRNA reflect the PGC-1 α protein or if glycogen stores are an important regulator. Seven male subjects (23 +/- 1.3 yr old, maximum oxygen uptake (Vo_2 max)) 48.4 +/- 0.8 ml.kg(-1).min(-1)) exercised to exhaustion (approximately 2 h) at 65% Vo_2 max followed by ingestion of either a high-carbohydrate (HC) or low-carbohydrate (LC) diet (7 or 2.9 g.kg(-1).day(-1), respectively for 52 h of recovery. Glycogen remained depressed in LC while returning to resting levels by 24 h in HC. PGC-1α mRNA increased both at exhaustion (3-fold) and 2 h later (6.2-fold) but returned to rest levels by 24 h. PGC-1α protein increased 23% at exhaustion and remained elevated for at least 24 h. While there was no direct treatment effect (HC vs. LC) for PGC-1α mRNA or protein, there was a linear relationship between the changes in glycogen and those in PGC-1α protein during exercise and recovery (r = -0.68). In contrast, PGC-1beta did not increase with exercise but rather decreased below rest level at 24 and 52 h, and the decrease was greater in LC. PGC-1α protein content increased in prolonged exercise and remained up-regulated for 24 h. (Anila et al., 2008).

ATP, known as a rapid energy source, can be influenced by levels of muscle (H^+) and myofibrillar ATPase during exercise (Zhang and Wan 2006). However, the half-life of ATP is <1 second, and it makes glycogen become an indirect energy source for ATP synthesis. Long-term endurance exercise, which is related to muscle mitochondria dysfunction, results in a reduction of muscle glycogen depletion (Kim et al., 2015). The regulatory effect of PGC-1α on mitochondrial biogenesis has been well

reported, and exercises have influenced the expression of PGC-1α (Baar et al., 2002). PGC-1α is involved in exercise-induced down regulation of the expression of glycogenolytic and glycolytic enzymes. Another study reports that fatigue and exhaustion are viewed as a multicomponent biochemical process related to PFK-1 linked glycolytic pathway (Dobson, Parkhouse and Hochachka 1987). Although the enhanced expression of both PGC-1α and PFK-1 were observed in the liver of maltodextrin and crude extract treated wistar rat after exhuastive exercise, it is hard to conclude that they are involved in the antifatigue effects. Based on our data, the enhanced ATP and glycogen levels in serum and organs by maltodextrin and crude extract contribute to improve exercise endurance. AMPK serves as the key factor on regulation of glucose and lipid metabolism (Sun et al., 2010) and helps to maintain the levels of ATP in various conditions (Ceddia, 2013). The activated AMPK via falling cellular energy status enhanced ATP generation, whilst inhibiting ATP consumption (Rios et al., 2014). AICAR, an AMPK agonist, significantly enhanced running endurance in animals (Narkar et al., 2008). On the other hand, except for the fatigue situation, during oxidative stress, the activated AMPK successfully promoted cell survival by inhibiting free radical accumulation (Bonini and Gantner, 2013). Liraglutide reduced oxidative stress by the alteration of AMPK/SREBP1 pathway in Raw264.7 cells (Wang and Yang 2015). AMPK contributed to the antioxidant activity via regulating the levels of SOD and GSH (Lee et al., 2014; Zhao et al., 2015).

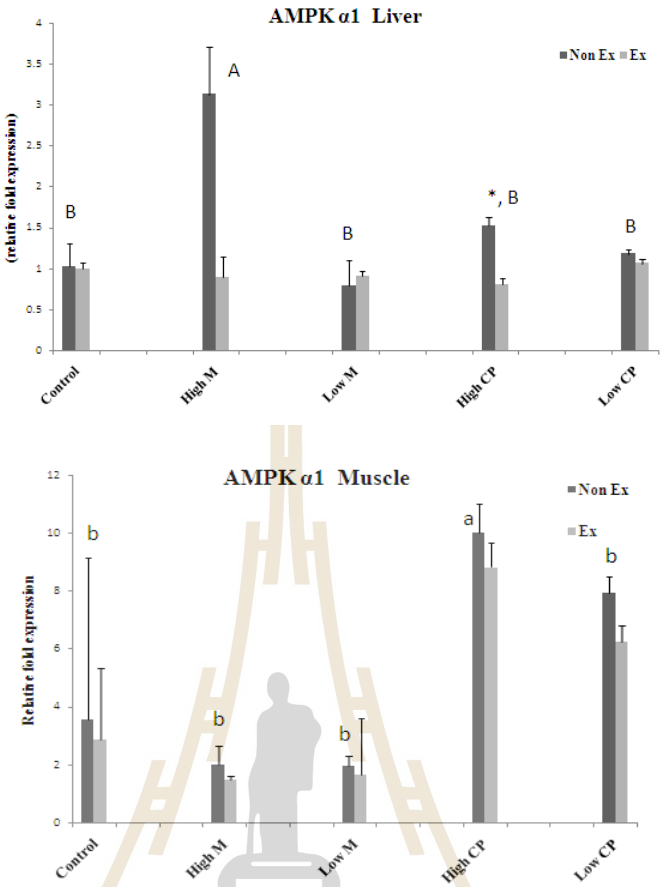

Figure 5.1 At the end of 2 week maltodextrin (M) and crude extract (CP) administration, exhaustive swimming was performed. After exercise, the mRNA expression levels of gene, AMPK α1 in liver (top) and muscle tissue (bottom) were evaluated by semi-quantitative RT-PCR to present the relative expression of AMPK α1 gene. Values are expressed as mean± S.E.M. (n=3). .*indicated significant difference compared between Non Ex and Ex with the same treatment. Small letters indicate significant differences within Non Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other ($p<0.05$, two way ANOVA; Duncan's method). Ex = exercise training, Non Ex = Non exercise training.

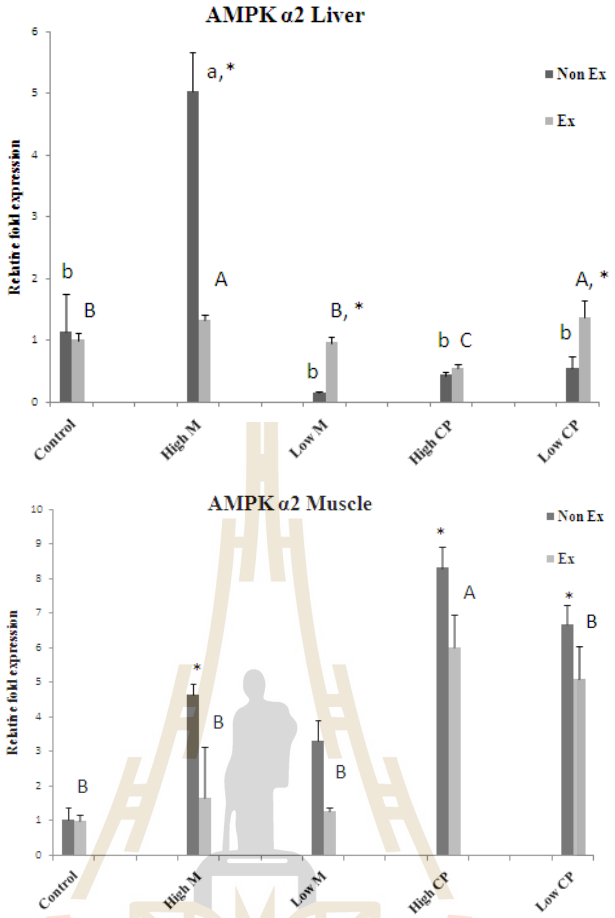

Figure 5.2 At the end of 2 week maltodextrin (M) and crude extract (CP) administration, exhaustive swimming was performed. After exercise, the mRNA expression levels of gene, AMPK α2 in liver (top) and muscle tissue (bottom) were evaluated by semi-quantitative RT-PCR to present the relative expression of AMPK α2 gene. Values are expressed as mean± S.E.M. (n=3). .*indicated significant difference compared between Non Ex and Ex with the same treatment. Small letters indicate significant differences within Non Ex. Capital letters indicate significant differences within Ex. Means sharing the same superscript are not significantly different from each other ($p<0.05$, two way ANOVA; Duncan's method). Ex = exercise training, Non Ex = Non exercise training.

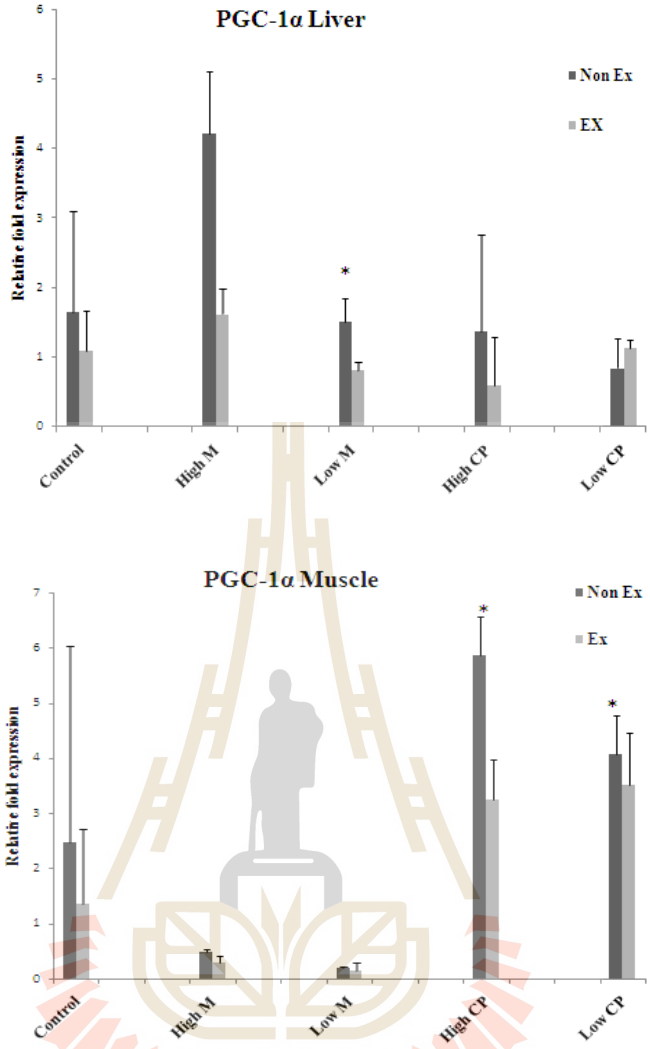

Figure 5.3 At the end of 2 week maltodextrin (M) and crude extract (CP) administration, exhaustive swimming was performed. After exercise, the mRNA expression levels of gene, PGC 1α in liver (top) and muscle tissue (bottom) were evaluated by semi-quantitative RT-PCR to present the relative expression of PGC 1α gene. Values are expressed as mean± S.E.M. (n=3). .*indicated significant difference compared between Non Ex and Ex with the same treatment. Means sharing the same superscript are not significantly different from each other ($p<0.05$, two way ANOVA; Duncan's method). Ex = exercise training, Non Ex = Non exercise training.

5.5 Conclusion

In conclusion, our data indicate that maltodextrin and crude extract from sweet cassava elevate the endurance capacity at least in part via activating AMPK- linked antioxidant pathway. That maltodextrin and crude extract have strongly enhanced the activation of 5′-AMP-activated protein kinase (AMPK), and the expressions of peroxisome proliferator have activated receptor γ coactivator-1 α (PGC-1α) in liver and muscle. Our data suggest that maltodextrin and crude extract possess effects related to AMPK-linked antioxidative pathway.

5.6 References

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CHAPTER VI

CONCLUSION

6.1 Conclusion

The present study, demonstrate for exercise endurance capacity of maltodextrin and crude extract from sweet cassava in *vivo*. Male Wistar rat were used to evaluate these properties swimming test, the summary of data obtained from the study of exercise endurance capacity of maltodextrin and crude extract shown in Table 6.1, 6.2, 6.3 and 6.4.

The extraction process requires the maltodextrin by enzyme application. The enzyme reaction conditions for the solubilization were optimized via a response surface methodology (RSM). The selected dependent variable was a percentage yield. The highest yield (17.63%, Dextrose equivalent=15) of maltodextrin obtained from enzymatic digestion conditions was 0.2% of α-amylase (w/v), temperature 90 °C and time 45 min. Crude extract and matodextrin from sweet cassava (*Manihot esculenta* Crantz.) contains many components such as mainly carbohydrate consisted of glucose, maltose, and sucrose. The Fourier transform infrared (FT-IR) spectra showed 3 types (sweet cassava flour, maltodextrin and crude extract). The absorptions show the highest peaks were due to sugar region. The range peak at 1403, 1031, and 854 cm^{-1} corresponded to the –C=O and CH group, and maltodextrin changed the peak 1000 cm^{-1} corresponded to the CH_2 group its sugar region.

The exercise endurance capacity effect of maltodextrin and crude extract from sweet cassava was investigated in male Wistar rats. Fifty rats were divided into exercise training (swimming without load) and non exercise training groups that received control (1 ml/kg double deionized distilled), crude extract and maltodextrin (250 and 500 mg/kg) orally once daily for 16 days (n=5, each). The present finding suggested that crude extract and maltodextrin appeared to have potential to support exercise endurance capacity. Crude extract and maltodextrin may increase the glycogen contents in liver and gastrocnemius muscle in both non exercise and exercise training by improving glycogen reserve and glycogen sparing effect. But, crude extract at low dose showed high level in AST and ALT. The result, suggest high dose maltodextrin from sweet cassava is a good supplement for endurance sport.

The effects on enhancing exercise performance of maltodextrin and crude extract in rat model. All rats were observed antioxidant enzyme in organ of liver and hind limb skeletal muscles (soleus and gastrocnemius), and 5′-AMP-activated protein kinase (AMPK α1,2) and activated receptor γ coactivator-1 α (PGC-1 α) gene expression. In exercise group showed increased levels of antioxidative enzymes (SOD), and glycogen and the reduced levels of malondialdehyde (MDA) and reactive oxygen species (ROS) in liver. Further data show that maltodextrin and crude extract have strongly enhanced the activation of 5′-AMP-activated protein kinase (AMPK), and the expressions of peroxisome proliferator have PGC-1α in liver and muscle. Our data suggest that maltodextrin and crude extract possess anti fatigue effects related to AMPK-linked antioxidative pathway.

In conclusion, this study demonstrated enhancing effect on exercise endurance capacity of maltodextrin and crude extract in *vivo*. Its compound can inhibition role in

tumor cell proliferation and hepaprotective function in liver and reduction of reactive oxygen species. Its compound (mainly carbohydrate) may be responsible for the potent improve liver and enhancing effect on exercise endurance capacity of crude extract. Further studies are needed to clarify the understanding mechanism.

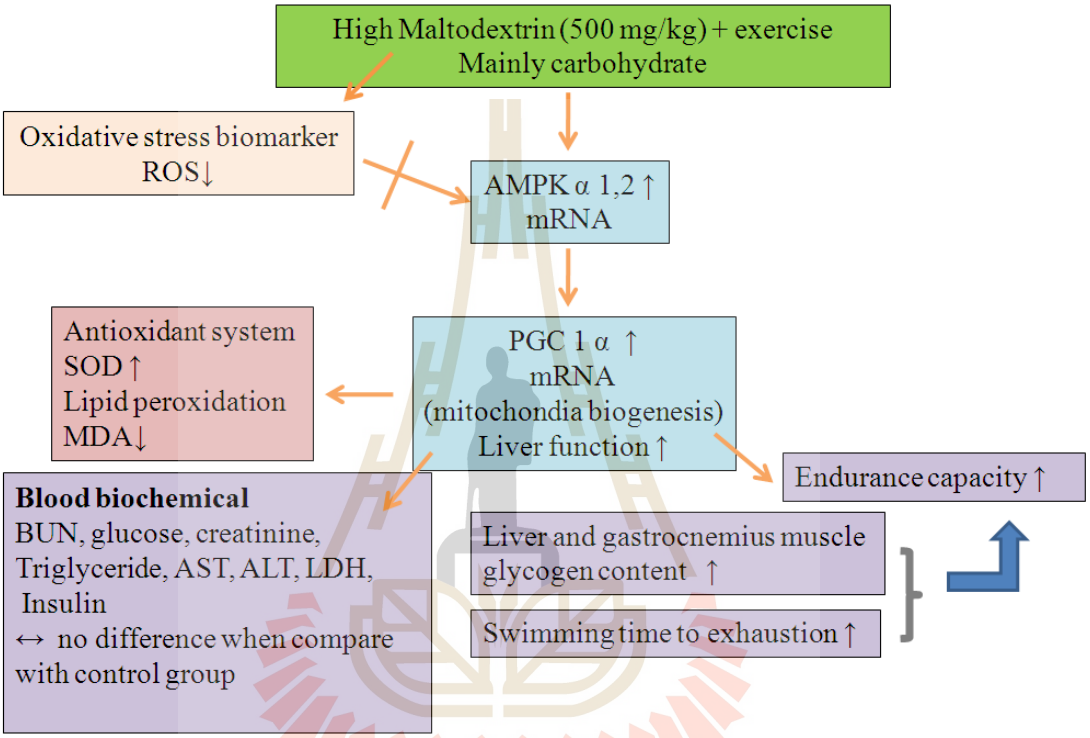

Figure 6.1 Mechanism of endurance capacity actions of maltodextrin.

Table 6.1 Effect of 16 days administration of maltodextrin (M) and crude extract (CP) on observation parameter in exercise and non exercise training.

Parameters	Exercise training				Non Exercise training			
	250 M	**500 M**	**250 CP**	**500 CP**	**250 M**	**500 M**	**250 CP**	**500 CP**
Swimming time to exhaustion	↑	↑	↑	↑	↔	↑	↔	↔
Body weight gain	↔	↔	↔	↑	↓	↓	↓	↑
Liver ROW	↔	↔	↔	↔	↔	↔	↔	↔
EDL ROW	↔	↔	↔	↔	↔	↔	↔	↔
Soleus ROW	↔	↔	↔	↔	↔	↔	↔	↔
Grastrocnemius Row	↔	↔	↔	↔	↔	↔	↔	↔
Liver glycogen	↔	↑	↑	↑	↔	↑	↑	↑
Gastrocnemius glycogen	↑	↑	↑	↑	↑	↑	↑	↑
Glucose	↓	↓	↓	↓	↑	↑	↓	↑
Triglyceride	↓	↓	↔	↓	↑	↑	↑	↑
LDH	↔	↔	↑	↔	↔	↔	↔	↔
AST	↔	↔	↑	↔	↔	↔	↔	↔
ALT	↔	↔	↔	↔	↔	↔	↔	↔
Creatinine	↔	↔	↔	↔	↑	↑	↑	↑
BUN	↔	↔	↔	↔	↔	↔	↔	↔
Insulin	↔	↔	↔	↔	↔	↔	↔	↔

250 M + Ex, 500 M + Ex, 250 CP + Ex, 500 CP + Ex compared with Ex control group.

250 M + Non Ex, 500 M + Non Ex, 250 CP + Non Ex, 500 CP + Non Ex compared with Non Ex control group. (↔ no change, ↑ increase, and ↓ decrease).

Table 6.2 Effect of 16 days administration of maltodextrin (M) and crude extract (CP) on observation parameter in exercise and non exercise training.

Parameters	Exercise training				Non Exercise training			
	250 M	**500 M**	**250 CP**	**500 CP**	**250 M**	**500 M**	**250 CP**	**500 CP**
SOD	↔	↑	↔	↔	↑	↑	↔	↔
MDA	↔	↔	↔	↓	↔	↓	↓	↓
ROS	↔	↓	↔	↓	↔	↔	↔	↓
AMPK α 1 Liver	↔	↔	↔	↔	↔	↑	↔	↔
AMPK α 1 Muscle	↔	↔	↑	↑	↔	↔	↑	↑
AMPK α 2 Liver	↔	↑	↑	↔	↔	↑	↔	↔
AMPK α 2 Muscle	↔	↔	↑	↑	↔	↔	↑	↑
PGC 1 α Liver	↔	↔	↔	↔	↔	↑	↔	↔
PGC 1 α Muscle	↔	↔	↑	↑	↔	↔	↑	↑

250 M + Ex, 500 M + Ex, 250 CP + Ex, 500 CP + Ex compared with Ex control group.

250 M + Non Ex, 500 M + Non Ex, 250 CP + Non Ex, 500 CP + Non Ex compared with Non Ex control group.

(↔ no change, ↑ increase, and ↓ decrease).

Table 6.3 Comparative effect of 16 days administration of maltodextrin and crude extract between exercise and non exercise training.on observation parameters.

Parameters	**Exercise training**			
	250 M	**500 M**	**250 CP**	**500 CP**
Swimming time to exhaustion	>	>	>	>
Body weight gain	↔	↔	↔	↔
Liver ROW	↔	↔	↔	↔
EDL ROW	↔	↔	↔	↔
Soleus ROW	↔	↔	↔	↔
Grastrocnemius Row	↔	↔	↔	↔
Liver glycogen	↔	↔	↔	↔
Gastrocnemius glycogen	↔	↔	↔	↔
Glucose	↔	↔	↔	↔
Triglyceride	↔	↔	↔	↔
LDH	↔	↔	>	↔
AST	↔	↔	↔	↔
ALT	↔	↔	>	↔
Creatinine	↔	↔	↔	↔
BUN	↔	↔	↔	↔
Insulin	↔	↔	↔	↔

(↔ no difference, > more than, and < less than).

Table 6.4 Comparative effect of 16 days administration of maltodextrin and crude extract between exercise and non exercise training.on observation parameters.

Parameters	**Exercise training**			
	250 M	**500 M**	**250 CP**	**500 CP**
SOD	↔	↔	↔	↔
MDA	↔	↔	↔	↔
ROS	↔	↔	↔	↔
AMPK α 1 Liver	↔	<	↔	<
AMPK α 1 Muscle	↔	↔	↔	↔
AMPK α 2 Liver	>	<	>	↔
AMPK α 2 Muscle	↔	<	<	<
PGC 1 α Liver	↔	<	↔	↔
PGC 1 α Muscle	↔	↔	<	<

(↔ no difference, > more than, and < less than).

BIOGRAPHY

Ms. Kakanang Posridee was born in January 29, 1986 in Buriram Province, Thailand. She received Master's degree in M.Sc. (Food Technology) from school of Food Technology, Institute of Agricultural Technology, Suranaree University of Technology in 2012. In 2013, she attended degree of Doctor of Philosophy in Food Technology at Suranaree University of Technology. During her graduate study, she obtained opportunities to oral presentation her research work at the International Food Reserch Conference (UPM, Malaysia, 25-27th July, 2017). She also published her research work under the title of "Optimization of sweet cassava (*Manihot esculents* crantz.) crude extract with high maltodextrin level using Response Surface Methodology" in International Food Research Journal (25(Suppl. 1): s51-s56) in December 2018. Furthermore, she obtained opportunities to poster presentation her research work at the Food Innovation Asia Conference 2019 (Bangkok, Thailand, 13-15th June, 2019).