การศึกษาผลการกักเก็บลิปิดไว้ภายในโพลีแซคคาไรด์เจลจากเปลือกผลทุเรียนในหลอดทดลอง

นางสาวชุติมา ทิพยกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีวเวชเคมี ภาควิชาชีวเคมี
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2545

ISBN 974-17-1021-6

IN VITRO STUDY ON ENTRAPMENT OF LIPIDS WITHIN POLYSACCHARIDE GEL FROM FRUIT-HULLS OF DURIAN

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy

Department of Biochemistry
Faculty of Pharmaceutical Sciences
Chulalongkorn University

Academic Year 2002
ISBN 974-17-1021-6

Thesis Title	*In vitro* study on entrapment of lipids within polysaccharide gel from fruit-hulls of durian
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Field of Study	Biomedicinal Chemistry
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ชุติมา ทิพยกุล : การศึกษาผลการกักเก็บลิปิดไว้ภายในโพลีแซคคาไรด์เจลจากเปลือกผลทุเรียนในหลอดทดลอง (*IN VITRO* STUDY ON ENTRAPMENT OF LIPIDS WITHIN POLYSACCHARIDE GEL FROM FRUIT-HULLS OF DURIAN) อ. ที่ปรึกษา : รศ.ดร.สุนันท์ พงษ์สามารถ, อ. ที่ปรึกษาร่วม : อ.มณีวรรณ สุขสมทิพย์, 98 หน้า. ISBN 974-17-1021-6.

ศึกษาคุณสมบัติทางชีวภาพของเจลโพลีแซคคาไรด์ (PG) สกัดจากเปลือกของผลทุเรียน (*Durio zibethinus* L.) เพื่อประเมินคุณสมบัติการทนต่อการย่อยด้วยเอนไซม์อัลฟาอมัยเลสและคุณสมบัติการกักเก็บสารลิปิด โดยทำการศึกษาในหลอดทดลอง พบว่า ผงเจลโพลีแซคคาไรด์พองตัวในน้ำจับเป็นชั้นข้นหนืด 2% ของสารละลาย PG มีค่าความหนืดเท่ากับ 279.36 ± 25.87 cps. PG ทนต่อการถูกย่อยด้วยเอนไซม์อัลฟาอมัยเลสซึ่งถูกย่อยได้เพียงส่วนน้อยโดยที่โครงสร้างอัลฟาฮิลิคของ PG หายไปจากการตรวจสอบด้วยน้ำยาไอโอดีน หลังการย่อยด้วยเอนไซม์อัลฟาอมัยเลส พบปริมาณน้ำตาลรีดิวส์น้อยมาก ไม่พบน้ำตาล monosaccharides จากการตรวจสอบด้วย O-toluidine test เทียบกับ standard maltose และเทคนิค Thin Layer Chromatography ยังพบว่า PG ทนต่อการไฮโดรไลส์ในกรดเกลือเจือจางคุณสมบัติการกักเก็บลิปิดของ PG ทำการตรวจสอบในหลอดทดลอง โดยใช้เทคนิค semipermeable membrane dialysis ทดสอบกับลิปิด โคเลสเตอรอล กรดโอเลอิค และกรดสเตียริค โดยใช้ PG ในความเข้มข้น 0-2% ใช้เกลือน้ำดีเป็นสารช่วยลดแรงตึงผิวให้ลิปิดผสมเข้ากันได้ดียิ่งขึ้นกับน้ำและ PG หลังการ dialysis 4-16 ชั่วโมง นำสารละลายลิปิดภายในและภายนอกถุง dialysis membrane มาวิเคราะห์ปริมาณลิปิดโดยเทคนิค HPLC พบว่า การกักเก็บลิปิดอยู่ภายในเมมเบรนเพิ่มขึ้น และการปลดปล่อยลิปิดออกมาภายนอกเมมเบรนลดลง เมื่อเพิ่มความเข้มข้นของ PG ที่ความเข้มข้น 2% PG กักเก็บสารโคเลสเตอรอลได้ประมาณ 80-90 % ผลการทดลองเปรียบเทียบได้กับผลการทดลองเมื่อใช้กลูโคแมนแนนเป็น standard polysaccharide ความหนืดของ PG มีผลต่อการกักเก็บลิปิดไว้ใน PG การกักเก็บลิปิดใน PG เพิ่มเมื่อมีความหนืดของ PG เพิ่ม การศึกษาผลของ PG ในการกักเก็บโคเลสเตอรอลในไข่แดง พบว่า ให้ผลการทดลองที่คล้ายกัน ส่วนการศึกษาในลำไส้เล็กของหนูขาว ตรวจสอบการปลดปล่อยโคเลสเตอรอลจาก mixture ของ PG กับโคเลสเตอรอลที่ผ่านออกจากผนังลำไส้หนูในหลอดทดลองโดยเทคนิค membrane dialysis พบว่า ได้ผลที่คล้ายกัน พบว่า การเพิ่มความเข้มข้นของ PG มีผลให้ลดการปลดปล่อยโคเลสเตอรอลออกจากผนังลำไส้หนู จากผลการทดลอง แสดงให้เห็นว่า PG มีผลลดการปลดปล่อยลิปิดผ่านออกมาจากผนังลำไส้เล็กของหนู จากการศึกษาครั้งนี้ทำให้คาดว่า PG จะนำมาใช้ประโยชน์ได้ในการเตรียมผลิตภัณฑ์อาหารควบคุมน้ำหนัก การศึกษาในหลอดทดลองตรวจสอบผลของเส้นใยอาหารต่อการดูดซึมไขมันโดยใช้เทคนิค semipermeable membrane dialysis อาจจะนำมาใช้เป็นวิธีการทดลองเพื่อประเมินเบื้องต้นของสารโพลีแซคคาไรด์ที่มีผลกระทบต่อการดูดซึมอาหารพวกลิปิด

ภาควิชา……ชีวเคมี …….. ลายมือชื่อนิสิต .....……

สาขาวิชา……. ชีวเวชเคมี …….. ลายมือชื่ออาจารย์ที่ปรึกษา .....……

ปีการศึกษา…… 2545 ……..… ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....……

4276616533 : MAJOR BIOCHEMISTRY

KEY WORD: DIETARY FIBER / POLYSACCHARIDE GEL/ CHOLESTEROL/ FATTY ACID/ SEMIPERMEABLE MEMBRANE DIALYSIS TECHNIQUE

CHUTIMA TIPPAYAKUL : *IN VITRO* STUDY ON ENTRAPMENT OF LIPIDS WITHIN POLYSACCHARIDE GEL FROM FRUIT-HULLS OF DURIAN. THESIS ADVISOR : ASSO. PROF. SUNANTA PONSAMART Ph.D., THESIS CO-ADVISOR : LECTURER MANEEWAN SUKSOMTHIP, 98 pp. ISBN 974-17-1021-6.

Biological properties of polysaccharide gel (PG) extracted from fruit-hulls of durian (*Durio zibethinus* L.) were studied to evaluate its resistance to enzyme α-amylase activity and lipid entrapment property, *in vitro* study was performed. Powder of PG swelled and formed a viscous layer in water, 2%PG solution showed 279.36 ± 25.87 cps. viscosity. PG showed its resistance to α-amylase digestion and PG was only partially digested by the enzyme, α-helical structure of PG disappeared according to iodine solution test; trace amount of reducing sugar without monosaccharides end product after α-amylase digestion was received according to the O-toluidine test compared to maltose standard and TLC technique. PG was also demonstrated resistance against hydrolysis in dilute hydrochloric acid. Lipid entrapment property of PG was investigated *in vitro* by using semipermeable membrane dialysis technique. Lipids, cholesterol, oleic acid and stearic acid; were determined using PG at 0-2% concentration and bile salt being used as an addition surface active agent to help mixing lipid and water in PG homogeneously. Lipids in solution inside and outside dialysis membrane were analyzed by HPLC technique after 4-16 hours of dialysis. Increasing trapped lipids inside membrane and decreasing released lipids outside membrane were found with respect to increasing PG concentration. 2% PG trapped about 80-90% cholesterol. This result was found comparable to standard polysaccharide glucomannan. PG viscosity was also effected lipids trapping in PG, trapping of lipids in PG increased with respected to increasing viscosity of PG. PG trapping of cholesterol in egg yolk was also studied, the similar result was obtained. *In vitro* studies of cholesterol releasing from mixture of cholesterol with PG through out the membrane of dissected rat jejunum was performed by using membrane dialysis technique. The similar result was also obtained, increasing concentration of PG resulted in decreasing released cholesterol. The results indicated that PG effect to decrease lipids releasing through rat jejunum wall, according to this study, PG has expected to be use in diet food preparation. *In vitro* study the effect of dietary fiber on lipid absorption by using semipermeable membrane dialysis may be used in application as a preliminary evaluation of polysaccharide influence lipids absorption.

Department……. Biochemistry ……. Student's signature……

Field of study …... Biomedicinal Chemistry… Advisor's signature……...

Academic year ……. 2002 ……. Co-advisor's signature……..…….

ACKNOWLEDGEMENTS

I wish to express my grateful appreciation to those who assisted to me in my research and in the writing of this thesis :

To Associate Professor Dr. Sunanta Pongsamart for her helpful advices, attention, encouragement and guidance throughout the research studies, without whom none of this work would have been possible.

To Lecturer Maneewan Suksomthip, my co-advisor, for her invaluable advice and willing cooperation.

To all staff of the Biochemistry Department, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for their advice and helpful cooperation.

To the staff of the Scientific and Technological Research Equipment Center for their cooperation.

To the Graduate School of Chulalongkorn University for the provision of partial financial support.

Finally, I would like to express my infinite thanks and deepest gratitude to my family and friends for their endless love, care, understanding, and powerful encouragement.

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ABBREVIATIONS

^{o}C	=	degree celsius
cm	=	centimeter
enz.	=	enzyme
g	=	gram
Gal	=	galactose
Gal A	=	galacturonic acid
Glu	=	glucose
Glu A	=	glucuronic acid
GM	=	glucomannan
Fru	=	fructose
hr	=	hour
HPLC	=	high performance liquid chromatography
Mal	=	maltose
MD	=	maltodextrin
min	=	minute
ml	=	millilitre
mg	=	milligram
μg	=	microgram
μl	=	microlitre
PG	=	polysaccharide gel
PF	=	polysaccharide fiber
Suc	=	sucrose
TLC	=	thin layer chromatography
w/v	=	weight by volume

CHAPTER I

GENERAL BACKGROUND

1. Introduction

Dietary fiber is normally found in plant foods: fruits, vegetables, nuts and grains. It is primary the storage and cell wall polysaccharides of plants that cannot be hydrolyzed by human digestive enzymes has long been recognized as benefits for health maintenance, disease prevention, and a component of medical nutrition therapy (1,2). The term of dietary fiber is known to include a group of high-soluble fiber such as pectin, glucomannan, galactomannan, gums and insoluble fiber such as cellulose, hemicellulose, lignin (3). Some soluble fiber sources slow the appearance of glucose in blood (4). Effective soluble fiber must be viscous. The effect of viscosity slows the transition of chyme in the upper gastrointestinal tract, resulting in slower the rate of absorption, lower blood concentrations of nutrients, and alter hormonal responses to these absorbed nutrient. Viscosity of dietary fiber also appears to be a requirement for fiber to lower blood cholesterol concentrations. Most of these effects of soluble fibers are demonstrated using fiber concentrates (1). Increased intake of dietary fiber can help control obesity, reduce serum cholesterol levels, reduce the risk of coronary heart disease (CHD), decrease insulin requirements in diabetic individuals (5) and prevent colon cancer (6). At present, dietary fiber is normally incorporated in a variety of foods and medicines for human and animals (3).

Most of dietary fiber is produced from plant. Therefore, searching for such value-added product from plant waste is an interesting subject for countries exporting agricultural foods. Especially in Thailand, a lot of tons of fruit-hulls from durian is a critical agricultural residue every year in its season. Waste product of durian is a valuable new resources for deriving commercial valuable useful material in pharmaceutical and food industries.

Studies on polysaccharide gel (PG) isolated from durian waste has been reported that polysaccharide gel from fruit-hulls of durian (*Durio zibethinus* L. Bombacaceae), has given satisfactory results of being useful as an excipient in pharmaceutical and food preparation (7, 8). Toxicity studies of polysaccharide gel were recently reported that polysaccharide gel did not induce severe toxicity in mice and rats according to acute and subchronic toxicity test (9, 10)

The purpose of this study was to characterize the biological properties and lipid exclusion *in vitro* of polysaccharide gel in order to gain understanding the property of polysaccharide gel for potential use as a medical dietary fiber.

2. The purpose of this study

Polysaccharide gel from fruit-hulls of durian (*Durio zibethinus* L.) is water-soluble fiber that has potential use as dietary fiber substance as well as the dietary fiber from vegetables or other plant sources. The investigation of dietary fiber property of polysaccharide gel is studied in human enzyme digestibility and lipid exclusion properties of polysaccharide gel to gain insight into the knowledge of its biological properties. The objectives of this study are :

a) To investigate the resistance of polysaccharide gel to enzyme α-amylase digestion in comparison with maltodextrin, starch and glucomannan.

b) To evaluate the effect of polysaccharide gel on entrapment of lipids including; cholesterol, oleic acid and stearic acid in comparison to glucomannan.

c) To compare the models of *in vitro* analysis of lipid entrapment of polysaccharide gel by using membrane dialysis technique between the use of semipermeable membrane and dissecting rat jejunum.

3. Literature review

3.1 Dietary fiber (3)

Dietary fiber is a material found in or associated with food from plant, but it can not be digested and does not provide energy (calories) or building blocks for the structural growth and maintenance of the consuming organism.

Nature, however, does not provide such a simple differentiation when it comes to the human food supply. Most humans know that portions of the food they consume are nondigestible, that is, that the feces they excrete are made up not only of waste byproducts resulting from the normal conversion of digestible foodstuffs to energy, but also of materials that passed through the alimentary system untouched by digestive enzymes. What is not realized is that, in addition to the materials that are obviously fibrous in appearance, there are food components, such as pectin and β glucans, that do not appear to be fibrous at all, are even water-soluble, but that are not digested by the enzymes of the alimentary system. Therein lies the dilemma of deriving an appropriate definition for this indigestible food components.

As early as the 1800s, the term fiber was used as it related to animal forages. This fiber, term crude fiber, was the residue left over after extensive and strenuous chemical digestion of the sample. Since this material was generally considered totally nondigestible and therefore of little benefit to humans, food nutrition tables sometimes did not report the crude fiber content of the foods contained therein. When it was reported, the main purpose of the assay was to determine the portion of the food or forage totally nondigestible by nearly any means and thereby adjust the calculated energy (calories) content of the food accordingly.

Cleave, T.L. in 1956 (3), noted the types of diseases observed in the residents of underdeveloped countries these diseases states appeared related to the people's diets. Residents of underdeveloped countries had diets consisting of high

levels of whole plant materials and/or other whole foods. In contrast, people in industrialized nations consume very low levels of whole food or whole plant materials, a relationship between these consumption patterns and a number of diseases that he termed “Fibre deficiency syndromes.”

Trowell, in 1972 (3), showing the inadequacy of crude fiber figures in food nutrition tables and the importance of analyzing all the nondigestible plant cell wall materials in diet, suggested the term “dietary fibre,” a term coined earlier by Hipsley (1953). This preserved the common term of *fibre*, associated with component nondigestibility, but allowed for a definition that would include all the significant dietary components rather than the leftover residue from the harsh crude fiber measurement technique. Trowell says that the “hallmark of all the substances included in the term ‘dietary fibre’ was that they were not digested at *a*ll by the alimentary enzymes of man”.

Scientific definitions, as with all definitions, are subject to change, of course. But until the cause/effect relationships described earlier are established, the current definition will serve us well in defining and quantitating that portion of the food for which various physiological responses have been observed and reported. For the present, dietary fiber defined as “consisting of the remnants of plant cells resistant to digestion by the alimentary enzymes of the humans” is the appropriate definition.

3.1.1 Dietary fiber composition (3)

Currently, total dietary fiber is split into two main components: soluble dietary fiber and insoluble dietary fiber. Insoluble dietary fiber is plant material that is not soluble in hot water and is not digestible by enzymes that mimic the human alimentary system. Soluble dietary fiber is food material that is soluble in warm or hot water and is not digestible by appropriately chosen enzymes. Soluble

fiber is reprecipitated when that water is mixed with four parts of ethyl alcohol. The soluble fiber and the insoluble fiber each have distinct chemical characteristics and physiological effects. In terms of physiological activity, in general, soluble dietary fiber is more effective in reducing hyperlipidemias, while insoluble dietary fiber is better for alimentary system dysfunctions, such as constipation.

- **Insoluble dietary fiber components**

Cellulose -- Cellulose is probably the least soluble of all fiber components, being insoluble not only in cold or hot water, but also in hot dilute acids and alkalis as well. Cellulose, the major structural component of plants, is a glucose polymer bonded in the β-1,4 linkage configuration (as compared to starch, which has alpha 1,4 and 1,6 linkages). The 1,4 β linkage allows the cellulose polymer to crystalize in a linear configuration, with a high degree of intermolecular hydrogen bonding, which gives it substantial shear and tensile strength. Because of its chemical makeup, cellulose can be purified for use as a food ingredient.

Hemicellulose –The name hemicellulose might lead one to believe that it is a precursor or breakdown product of cellulose. Actually, the two have relatively little in common chemically. Both are insoluble in hot water, and both are polysaccharide. What distinguishes hemicellulose from cellulose is the fact that hemicellulose can be dissolved in dilute alkali. The main structure of hemicellulose is composed of a number of monosaccharides, primary xylose (xylan polymer), glucose and mannose (glucomannan polymer) , and galactose (galactan polymer). Attached to this main structure are side chains of glucose, arabinose, and glucoronic acid.

Lignin – Lignin is a highly water-insoluble polymeric material, derived in the plant by polymerization of aromatic alcohols—cinnamyl, syringyl, and guaicyl alcohol in particular. When intricately intermingled with the cellulose and

hemicellulose of the plant fiber, lignin increases resistance to degradation and subsequent solubilization.

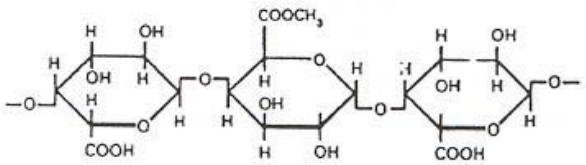
Cutin and plant waxes – These hydrophobic lipid materials are typically found in the plant structure, closely associated with the structural polysaccharide or on the outer surfaces of the plant. They are usually present in very small quantities.

- **Soluble dietary fiber components**

Gums – Basically, all soluble dietary fibers are gums from a variety of sources. They are typically (with the possible exception of β glucans and pectin) present in or used at low levels (< 0.5%) in food products. Food gums have very unique functionalities that can improve processing and eating characteristics when formulating high-fiber foods.

β *Glucans* -- β glucans are glucose polymers, wherein the individual glucose monomers are linked together with β 1,4 and β 1,3 linkages, making the polymer resistant to digestive hydrolysis. β glucans are found in significant quantities in oats, rye, and barley. Most of the β glucan present is soluble, although a small amount may be insoluble.

Pectins – Pectins are polymeric substances that are based on a polymer of α D-galacturonic acid, linked by linear sequences of 1,4 linkages. The main polymer has side chains that consist of sugars, galactose, glucose, rhamnose, and arabinose (Figure 1) (11). Pectins are primarily water-soluble, solubility being somewhat dependent on the degree of esterification of the galacturonic acid, as well as the makeup of the constituent side chains. The primary sources of pectin are citrus fruits and apples, although sugar beet pulp also has a high content of this polymer.



Pectin

Figure 1 : Structure of the dietary fiber pectin.

Glucomannan

Description : Glucomannan is a heteropolysaccharide (long chains of simple sugars, primarily mannose and glucose) that is classified as a soluble fiber (12). It is a natural dietary, calorie free, high fiber powder which is obtained from the root of the Amorphophallus Konjac, which has been safety consumed as food for over 1000 years in the Orient.

Useful of glucomannan : Glucomannan is a pectin-like, gel fiber which absorbs liquid (up to 50 time it's weight) giving a feeling of fullness. Studies of human subjects and rats have indicated that glucamannan forms a gel and greatly increases the moisture content of the food bolus during digestion (13). Like other forms of dietary fiber glucomannan is considered a "bulk-forming laxative." Glucomannan promotes a larger bulkier stool that passes through the colon more easily and requires less pressure – and subsequently less straining – to expel. In constipated individuals, glucomannan, and other bulk-forming laxatives generally help produce a bowel movement within 12 to 24 hours. The use of glucomannan for diverticular disease of the colon has also been studied in preliminary research; about one-third to one-half of the subjects were found to benefit from glucomannan (14, 15).

Glucomannan delays stomach empting, leading to a more gradual absorption of dietary sugar; this effect can reduce the elevation of blood sugar levels that is typical after a meal (14, 16). Controlled studies have found that after-meal blood sugar levels are lower in people with diabetes given glucomannan-enriched diets according to preliminary and controlled trials. One preliminary report suggested that glucomannan may also be helpful in pregnancy-related diabetes. One double-blind study reported that glucomannan (8-13 grams/day) stabilized blood sugar in people with insulin resistance syndrome (syndrome X). In a preliminary study addition of either 2.6 or 5.2 grams of glucomannan to a meal prevented hypoglycemia in adults with previous stomach surgery; a similar study of children produced inconsistent results (15).

Like other soluble fibers, glucomannan can bind to bile acids in the gut and carry them out of the body in the feces, which requires the body to convert more cholesterol into bile acids. This can result in the lowering of blood cholesterol and other blood fats (17). Controlled and double-blind studies have shown that supplementation with several grams per day of glucomannan significantly reduced total blood cholesterol LDL (“bad”) cholesterol, and triglycerides and in some cases raised HDL (“good”) cholesterol. One double-blind study reported that glucomannan (8-13 grams per day) lowered total LDL cholesterol in people with insulin resistance syndrome.

Increasing the viscosity of the intestinal contents has been shown to limit the rate of glucose absorption. This can be accomplished by using hydrocolloids, which pass undigested into the intestinal lumen. Frequently high viscosity foods are undesirble, however, introduction of konjac glucomannan is feasible by creating a mixed gel system with maltodextrin, in which the mixed gel has a relatively low viscosity but upon digestion by amylase, the mixture’s viscosity increase (18).

3.1.2 Benefits of fiber

Insoluble fiber binds; water making stool softer and bulkier (19). Therefore, fiber, especially that found in whole grain products, is helpful in the treatment and prevention of constipation, hemorrhoids and diverticulosis. Diverticula are pouches of the intestinal wall that can become inflamed and painful. In the past, a low-fiber diet was prescribed for this condition. It is now known that a high-fiber diet gives better results once the inflammation has subsided.

Low blood cholesterol levels (below 200 mg/dL) have been associated with a reduced risk of coronary heart disease. The body eliminates cholesterol through the excretion bile acid. Water-soluble fiber binds bile acids, suggesting that a high-fiber diet may result in an increased excretion of cholesterol (20). Some types of fiber, however, appear to have a greater effect than others. The fiber found in rolled oats is more effective in lowering blood cholesterol levels than the fiber found in wheat. Pectin has a similar effect that it can lower the amount of cholesterol in the blood (21).

Other claims for fiber are less well founded. Dietary fiber may help reduce the risk of some cancers, especially colon cancer. This idea is based on information that insoluble fiber increases the rate at which wastes are removed from the body. This means the body may have less exposure to toxic substances produced during digestion. A diet high in animal fat and protein also may play a role in the development of colon cancer (22).

High-fiber diets may be useful for people who wish to lose weight. Fiber itself has no calories, yet provides a “full” feeling because of its water absorbing ability. For example, an apple is more filling than a half cup of apple juice that

contains about the same calories. Food high in fiber often require more chewing, so a person is unable to eat a large number of calories in a short amount of time (23).

3.2 Carbohydrate in diet

Carbohydrates can easily be divided into three main groups called monosaccharides, disaccharides and polysaccharides (24). The general properties of these grouping are found below.

3.2.1 Monosaccharides – These are crystalline compounds, soluble in water, sweet to taste, and do not need digestion in order to be absorbed into the blood stream (25). The monosaccharides are referred to as "simple sugars". Although there are more than 200 known monosaccharides, D-glucose is the most abundant in nature. D-Glucose is an aldose or aldosugar because it has a carbonyl group at the end of the carbon chain making it an aldehyde. Other sugars may be aldosugars as well. The simplest D-aldosugar is glyceraldehydes. Its four (aldotetrose), five (aldopentose), and six (aldohexose), carbon relatives are shown in Figure 2. If, however, the carbonyl is at any position other than the terminal position, then the sugars are ketoses or ketosugars. The ketose series is shown on Figure 3.

3.2.2 Disaccharides – These are crystalline compounds, water-soluble, sweet to the taste, and must be digested to monosaccharides before they can be absorbed and used for energy (24). Disaccharides are composed of two monosaccharides connected by a glycosidic linkage. Some of the more common disaccharides are maltose, lactose, sucrose, cellobiose and gentiobiose. Disaccharides that have a free anomeric carbon are reducing sugars. Since sucrose has no free anomeric carbons, it does not have multiple conformational forms, leads to multiple conformers and thus to optical rotation in a process called inversion. Sucrose is sometimes called invert sugar as a result. Lactose is the main sugar in milk but is not found elsewhere in nature.

Upon hydrolysis of maltose, cellobiose, or gentiobiose yields two glucose units as shown in Figure 4.

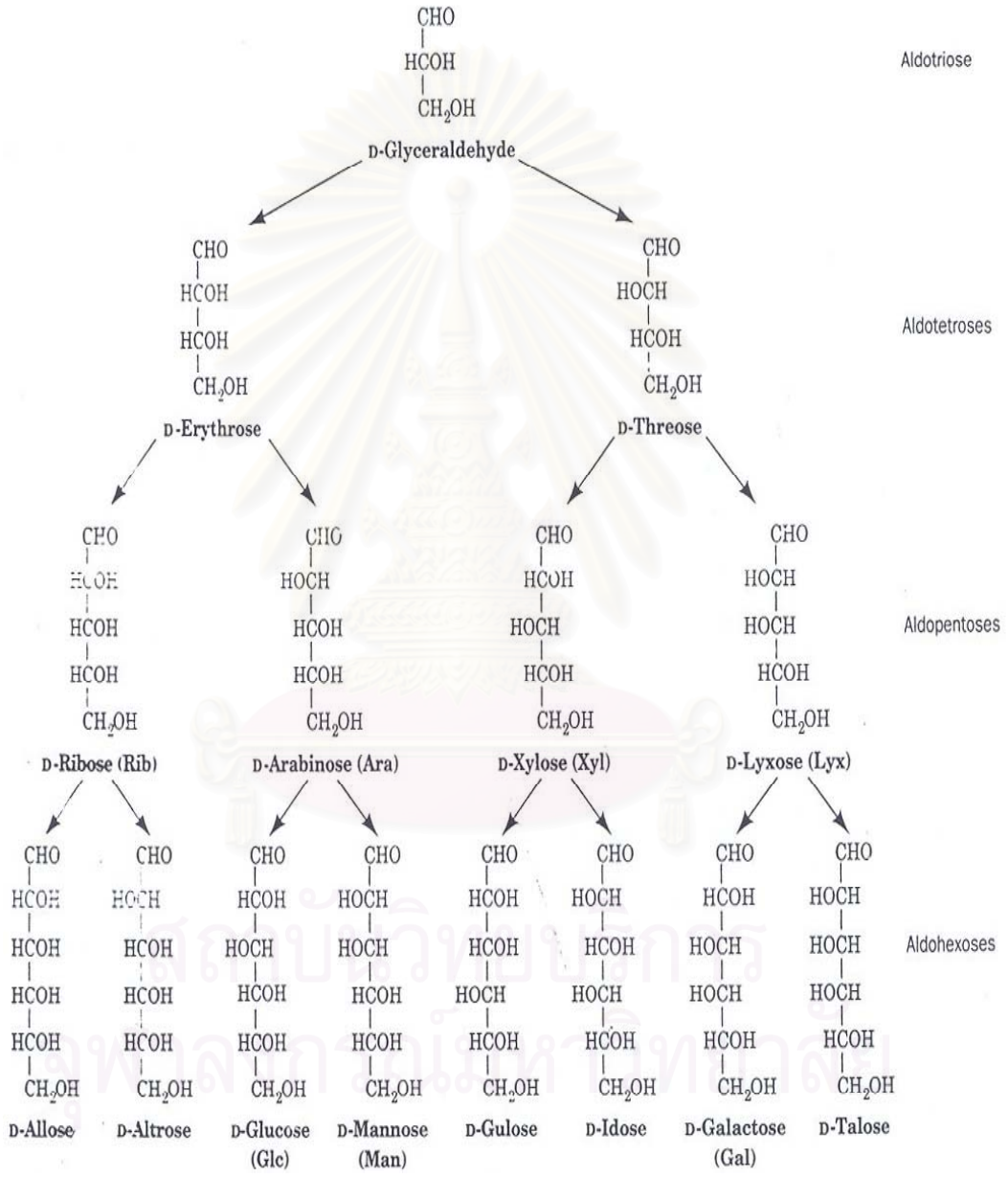


Figure 2 : D-aldoses with three to six carbon atoms.

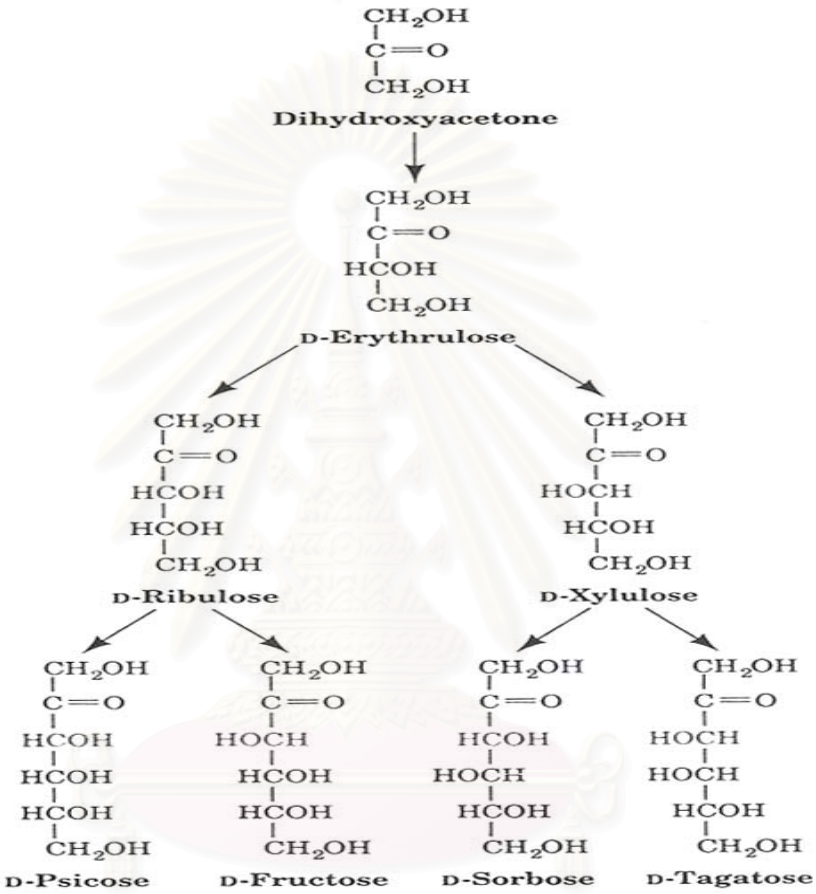


Figure 3 : D-ketoses with three to six carbon atoms.

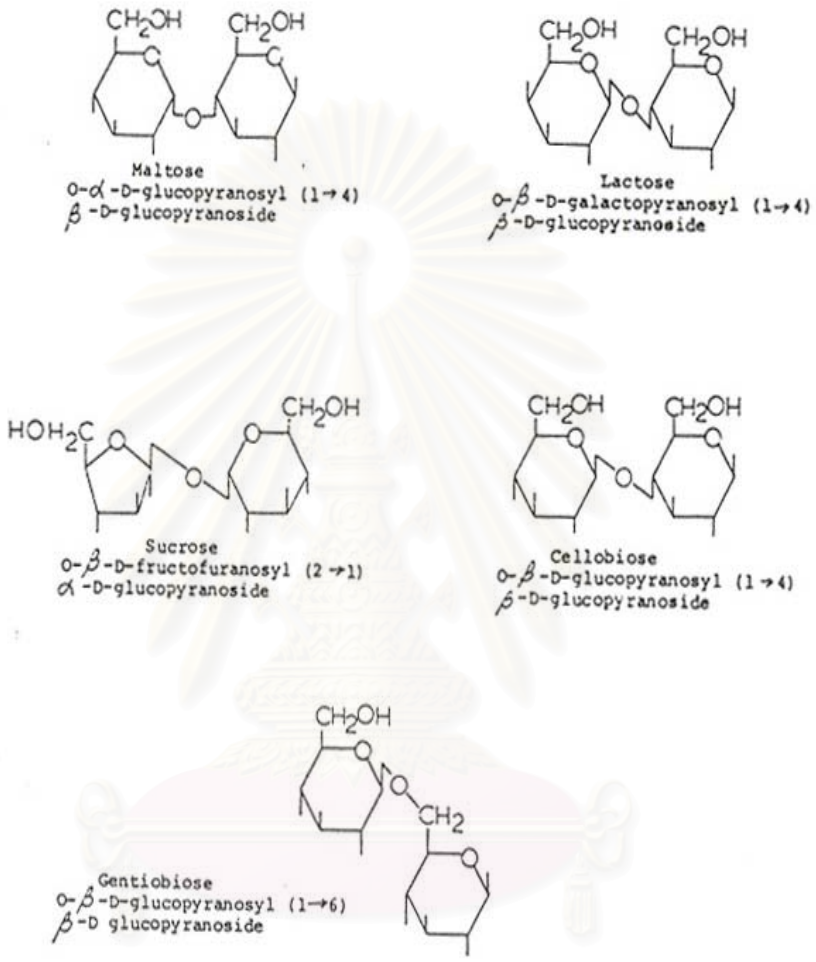


Figure 4 : Some important disaccharides.

3.3.3 Polysaccharides – These are not water soluble and are not crystalline. They form colloidal suspensions instead of solutions. They are not sweet and must be digested before being absorbed (26). Polysaccharides are homopolysaccharides if they contain only one type of monomer and heteropolysaccharide if they contain more than one type of monomer. Homopolysaccharides are called according to their repeating unit. They may be glucans, fructans, mannans, etc.

The main storage forms of polysaccharides are glycogen in animal cells and starch in plant cells. Both are deposited as granules in cells. Starch can be found in one of two forms : α-amylose or amylopectin.

Amylose consists of long unbranched chains of glucose attached to each other in α (1→4) linkages as shown in Figure 5. These chains may be from 3,000 to 500,000 molecular weight. α-Amylose readily forms hydrated micelles, and the chains form helical coils.

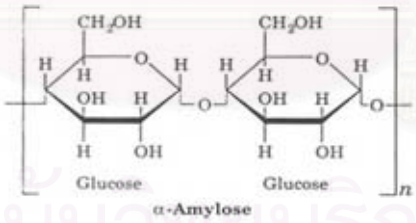


Figure 5 : α-Amylose. Its D-glucose residues are linked by α (1→4) bonds.

Amylopectin is highly branched with 24-30 residues/branch. The chains have α (1→4) linkages but the branch points consist of α (1→6) linkages as shown in Figure 6. Amylopectin forms colloidal or micellar suspensions, and its molecular weight can be as high as 100 million.

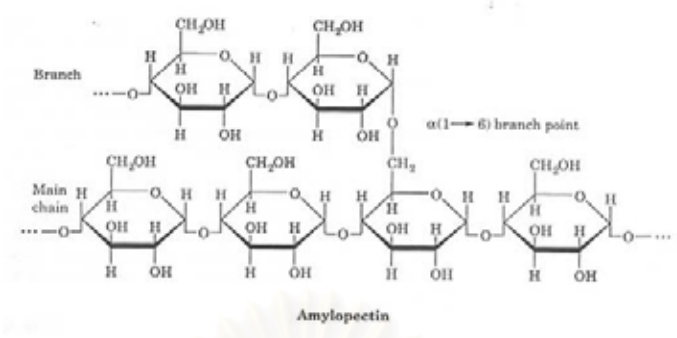


Figure 6 : Amylopectin. Its primary structure shows one of its α (1→6) branch points.

Glycogen is found to some extent in all animal cells but it is found in the largest quantities in skeletal muscle (1-2 % of the muscle wet) and in liver (up to 10 % of the liver wet weight under some conditions). On a fat-free basis, adipose tissue also has a relatively high glycogen content (about 1 %). Glycogen is structurally similar to amylopectin but is more highly branched with only 8-12 residues in a linear sequence between branches. Therefore, it is a more compact molecule than amylopectin.

There are other storage polysaccharides. These include the dextrans, which are polymers of D-glucose with glycosidic linkages other than α (1→4). The dextrans have important commercial use as chromatography supports and blood extenders. Fructans (levans) such as inulin, a β (2→1) polymer fructose, are especially useful in studies of renal function and blood volume. Mannans are found in bacteria, yeasts, molds and plants. Some of these complexes are so compact and so insoluble that they have been used to make very good buttons. Xylans and arabinans are abundant in plants.

3.3 Digestion and absorption in human gastrointestinal tract

3.3.1 Digestion and absorption of food

Secretion of digestive fluids and digestion of food were some of the earliest biochemical events to be investigated at the beginning of the era of modern science. Major milestones were the discovery of hydrochloric acid secretion by the stomach and enzymatic hydrolysis of protein and starch by gastric juice and saliva, respectively. The basic nutrients fall into the classes of proteins, carbohydrates, and fats. Many different types of food can satisfy the nutritional needs of humans, even though they differ in the ratios of proteins to carbohydrates and to fats and in the ratio of digestible to nondigestible materials. Unprocessed plant products are especially rich in fibrous material that can be neither digested by human enzymes nor easily degraded by intestinal bacteria. The fibers are mostly carbohydrates, such as cellulose (β-1,4-glucan) or pectins (mixtures of methyl esters of polygalacturonic acid, polysgalactose, and polyarabinose). High-fiber diets enjoy a certain popularity nowadays because of a postulated preventive effect on development of colon cancer.

Gastrointestinal organs have multiple functions in digestion (27, 28)

The bulk of ingested nutrients consists of large polymers that have to be broken down to monomers before they can be absorbed and made available to all cells of the body. The complete process from food intake to absorption of nutrients into the body consists of a complicated sequence of events, which at the minimum includes (Figure 7) :

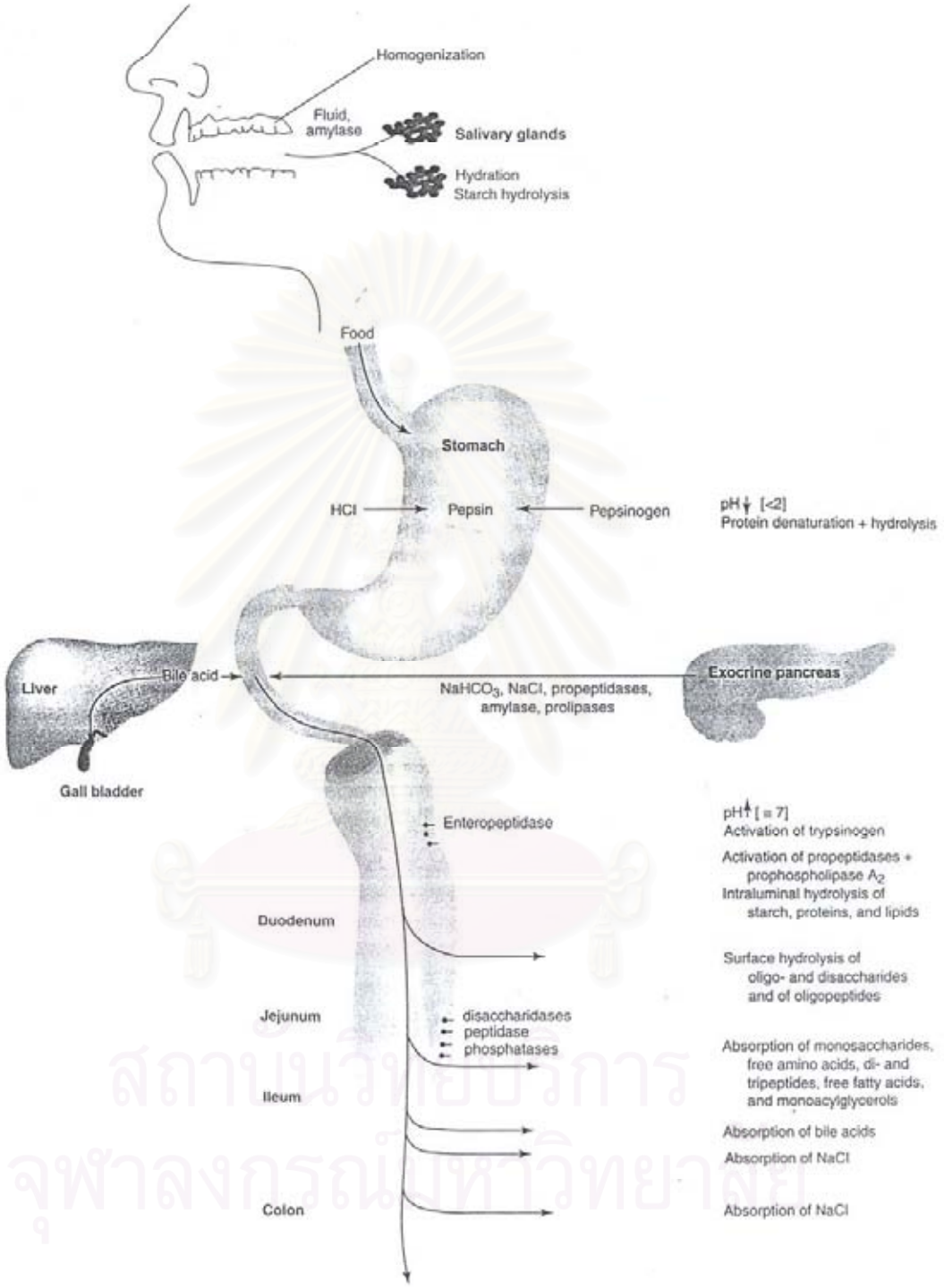


Figure 7 : Gastrointestinal organs and their functions.

1. Mechanical homogenization of food and mixing of ingested solids with fluids secreted by the glands of the gastrointestinal tract.

2. Secretion of digestive enzymes that hydrolyze macromolecules to oligomers, dimers, or monomers.

3. Secretion of electrolytes, acid, or base to provide an appropriate environment for optimal enzymatic digestion.

4. Secretion of bile acid as detergents to solubilize lipids and facilitate their absorption.

5. Hydrolysis of nutrient oligomers and dimers by enzymes on the intestinal surface.

6. Transport of nutrient molecules and of electrolytes from the intestinal lumen across the epithelial cells into blood or lymph.

3.3.2 Digestion and absorption carbohydrates in human

Dietary carbohydrates provide a major portion of the daily caloric requirement. They consist of mono-, di-, oligo-, and polysaccharides. Monosaccharides (simple carbohydrates) need not be hydrolyzed for absorption. Disaccharides require the small intestinal surface enzymes for hydrolysis into monosaccharide, while oligosaccharides and polysaccharides must be hydrolyzed to their component monosaccharides before being absorbed. The digestion of starch begins with salivary amylase, but this activity is much less important than that of pancreatic amylase in the small intestine. Amylase hydrolyzes starch, with the primary end products being maltose, maltotriose, and α-dextrins, although some glucose is also produced. The products of α-amylase digestion are hydrolyzed into their component monosaccharides by enzymes expressed on the brush border of the small intestinal cells, the most important of which are maltase, sucrase, isomaltase and lactase (27, 29).

Monosaccharides : Only D-glucose and D-galactose are actively absorbed in the human small intestine. D-fructose is not actively absorbed, but has a rate of diffusion greater than would be expected by passive diffusion.

Di-, oligo-, and polysaccharides : These are not hydrolyzed by amylase and/or intestinal surface enzymes cannot be absorbed; therefore they reach the lower tract of the intestine, which from the lower ileum on contains bacteria. Bacteria can utilize many of the remaining carbohydrates because they possess many more types of saccharidases than humans. Monosaccharide that are released as a result of bacterial enzymes are predominantly metabolized anaerobically by the bacteria themselves, resulting in degradation products such as short-chain fatty acids, lactate, hydrogen gas (H_2), methane (CH_4), and carbon dioxide (CO_2). These compounds can cause fluid secretion, increased intestinal motility, and cramps, either because of increased intraluminal osmotic pressure, and distension of the gut, or a direct irritant effect of the bacterial degradation products on the intestinal mucosa.

3.3.3 Digestion and absorption of lipids

Lipid digestion requires overcoming the limited water solubility of lipids. An adult man ingests about 60 – 150 g of lipid per day. Triacylglycerols constitute more than 90% of the dietary fat. The rest is made up of phospholipids, cholesterol, cholesterol esters, and free fatty acids. In addition, 1 – 2 g of cholesterol and 7 – 22 g of phosphatidylcholine (lecithin) are secreted into the small intestine lumen as constituents of bile.

Lipids are lack of solubility in aqueous solutions. The poor water solubility presents problems for digestion because the substrates are not easily accessible to the digestive enzymes in the aqueous phase. In addition, even if ingested lipids are hydrolyzed into simple constituents, the products tend to aggregate to larger

complexes that make poor contact with the cell surface and therefore are not easily absorbed. These problems are overcome by increases in the interfacial area between the aqueous and lipid phase and “solubilization” of lipids with detergents. Thus changes in the physical state of lipids are intimately connected to chemical changes during digestion and absorption (28).

At least five different phases can be distinguished (Figure 8) : 1) hydrolysis of triacylglycerols to free fatty acids and monoacylglycerols; 2) solubilization by detergents (bile acids) and transport from the intestinal lumen toward the cell surface; 3) uptake of free fatty acids and monoacylglycerols into the cell and resynthesis to triacylglycerols; 4) packaging of newly synthesized triacylglycerols into special lipid-rich globules, called chylomicrons; and 5) exocytosis of chylomicrons from cells and release into lymph.

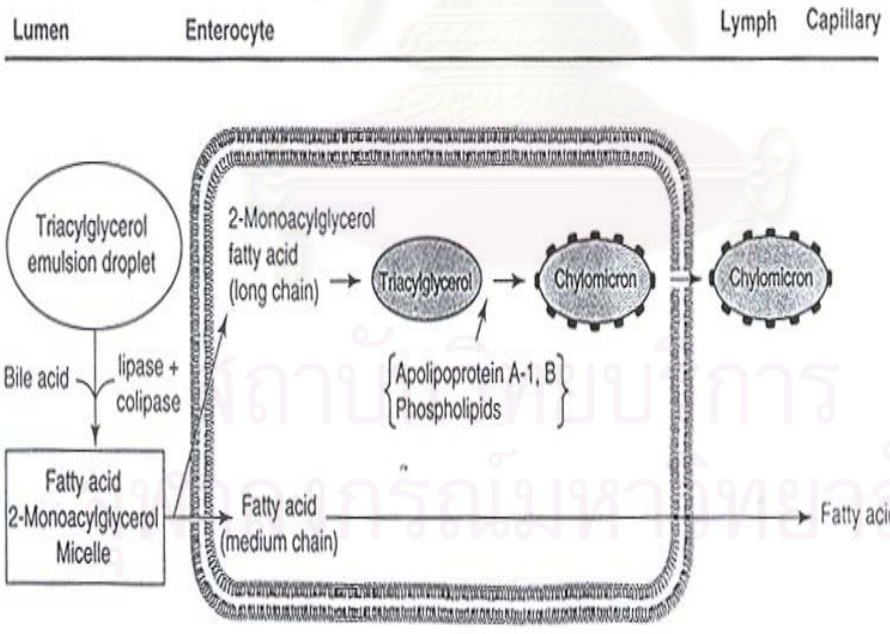


Figure 8 : Digestion and absorption of lipids.

Lipids are digested by gastric and pancreatic lipases

Digestion of lipids is initiated in the stomach by an acid-stable lipase, most of which is thought to originate from glands at the back of the tongue. However, the rate of hydrolysis is slow because the ingested triacylglycerols form a separate lipid phase with a limited water-lipid interface. The lipase adsorbs to that interface and converts triacylglycerols into fatty acids and diacylglycerols (Figure 9). The importance of the initial hydrolysis is that some of the water-immiscible triacylglycerols are converted to products that posses both polar and nonpolar groups. Such surfactive products spontaneously adsorb to water-lipid interfaces and confer a hydrophilic surface to lipid droplets thereby providing a stable interface with the aqueous environment. At constant volume of the lipid phase, any increase in interfacial area produces dispersion of the lipid phase into smaller droplets (emulsification) and provides more sites for adsorption of more lipase molecules.

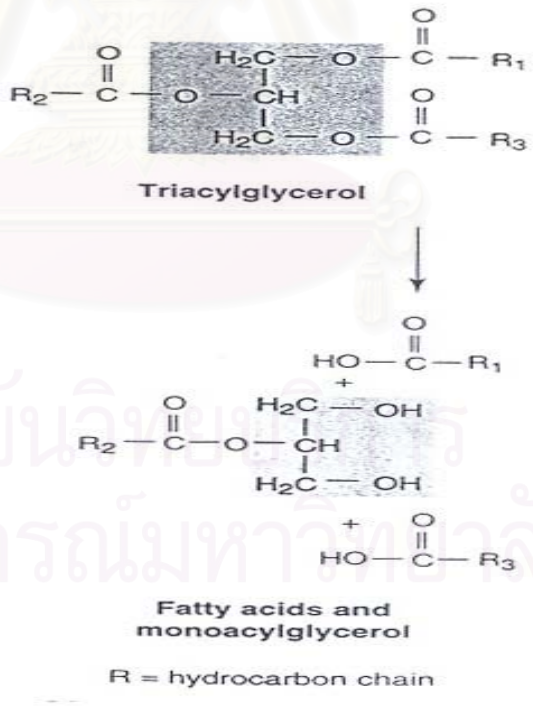


Figure 9 : Mechanism of action of lipase.

The major enzyme for triacylglycerol hydrolysis is the pancreatic lipase (Figure 10). This enzyme is specific for esters in the α-position of glycerol and prefers long-chain fatty acids with more than ten carbon atoms. Hydrolysis by the pancreatic enzyme also occurs at the water-lipid interface of emulsion droplets. The products are free fatty acids and β-monoacylglycerols. The purified form of the enzyme is strongly inhibited by the bile acids that normally are present in the small intestine during lipid digestion.

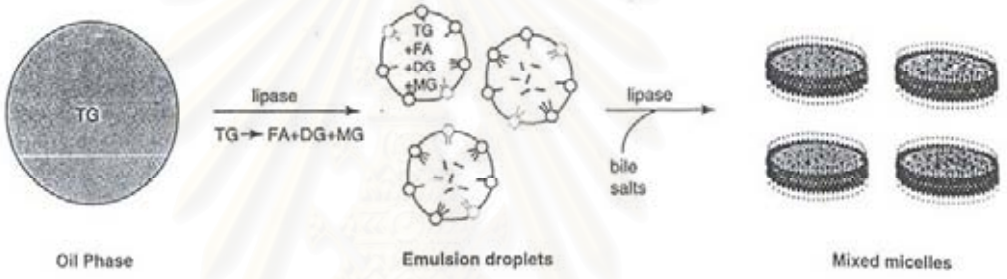


Figure 10 : Changes in physical state during triacylglycerol digestion. Abbreviations : TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; FA, fatty acid.

Bile emulsifies, neutralizes and excretes cholesterol and bile pigments (30, 31)

In addition to many functions in intermediary metabolism, the liver, by producing bile, plays an important role in digestion. The gallbladder stores bile produced by the liver between meals. During digestion, the gallbladder contracts and supplies bile rapidly to the duodenum by way of the common bile duct. The pancreatic secretions mix with the bile, since they empty into the common duct shortly before its entry into the duodenum.

Properties of bile :

(1) Emulsification : The bile salts have considerable ability to lower surface tension. This enables them to emulsify fats in the intestine and to dissolve fatty acids and water-insoluble soaps. The presence of bile in the intestine is an important adjunct to accomplish the digestion and absorption of fats as well as the absorption of the fat-soluble vitamins A, D, E, and K (Figure 11). When fat digestion is impaired, other foodstuffs are also poorly digested, since the fat covers the food particles and prevents enzymes from attacking them. Under these conditions, the activity of the intestinal bacteria causes considerable putrefaction and production of gas (32).

Figure 11 : Role of mixed micelles in fat absorption.

(2) Neutralization of acid : In addition to its function in emulsification, the bile, having a pH slightly above 7, neutralizes the acid chyme from the stomach and prepares it for digestion in the intestine.

(3) Excretion : Bile is an important vehicle for bile acid and cholesterol excretion, but it also removes many drugs, toxins, bile pigments and various inorganic substances such as copper, zinc, and mercury.

(4) Bile pigment metabolism : The origin of the bile pigments from hemoglobin. Bilirubin is a metabolite of the heme portion of heme proteins, mainly hemoglobin. Bilirubin is excreted into the intestine and bile from the liver. The site of the catabolism of hemoglobin is the reticuloendothelial system (RES). Bilirubin is then released into the blood steam where it binds tightly to albumin and is transported to the liver. Upon uptake by the liver, bilirubin is conjugated with glucuronic acid to form bilirubin mono and diglucuronide, which are water-soluble metabolites. The metabolites are then excreted in the bile.

Ultimately, cholesterol must enter the liver and be excreted in the bile as cholesterol and as bile acids (salts) (33)

About 1 g of cholesterol is eliminated from the body per day. Approximately half is excreted in the feces after conversion to bile acids. The remainder is excreted as neutral steroids. Much of the cholesterol secreted in the bile is reabsorbed, and it is believed that at least some of the cholesterol that serves as precursor for the fecal sterols is derived from the intestinal mucosa. A large proportion of the biliary excretion of bile salts is reabsorbed into the portal circulation, taken up by the liver, and reexcreted in the bile. This is known as the enterohepatic circulation. The bile salts not reabsorbed, or their derivatives, are excreted in the feces.

Bile acids are formed from cholesterol (27, 34)

Bile acids are biological detergents that are synthesized in the liver from cholesterol (Figure 12) and secreted as conjugates of glycine or taurine with bile into duodenum. Since bile contains significant quantities of sodium and potassium and the pH is alkaline, it is assumed that the bile acids and their conjugates are actually in a salt form – hence the term “bile salts.”

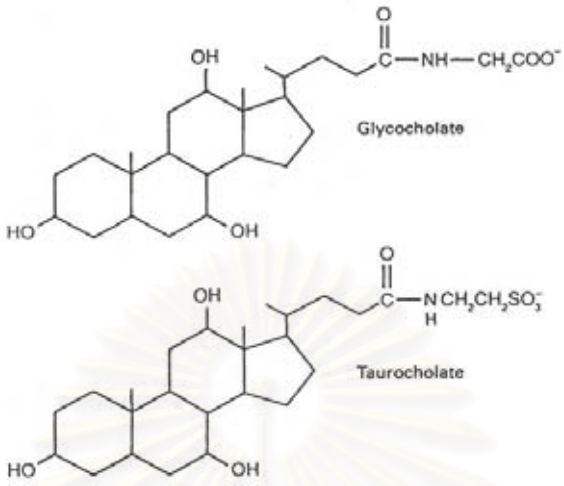


Figure 12 : Glycocholate and taurocholate. Glycocholate contains a molecule of glycine, while taurocholate contains a molecule of taurine.

Most bile acids return to liver in the enterohepatic circulation (35, 36)

A small fraction of the bile salts – perhaps only as 500 mg/d – escapes absorption and is therefore eliminated in the feces. Even though this is a very small amount, it nonetheless represents a major pathway for the elimination of cholesterol. The enterohepatic circulation of the bile salts is so efficient that each day the relatively small pool of bile acids (about 3-5 g) can be cycled through the intestine 6 – 10 times with only a small amount lost in the feces. However, each day, an amount of bile acid equivalent to that lost in the feces is synthesized from cholesterol by the liver, so that a pool of bile acids of constant size is maintained. This is accomplished by a system of feedback control.

3.4 Principle for chemical and biological analysis of PG

3.4.1 Reduction test for sugar

Carbohydrates that have a potentially free aldehyde or ketone group exist in solution at equilibrium with the enediol form. At a slightly alkaline pH this conversion is favoured and the resulting enediol is an active reducing agent. Reduction methods can be used for disaccharides provided that the aldehyde or ketone group of at least one of the monosaccharides has not been eliminated in the glycosidic bond. Sucrose is an example of a disaccharide in which the anomeric carbon atoms of both monosaccharides are involved in the glycosidic bond and the reducing power is lost. However, this distinction between reducing and non-reducing disaccharides can sometimes be used to advantage in qualitative tests. Hydrolysis of glycosidic linkage between sugars to give C1 hydroxyl of anomeric carbon which is a reactive reducing end its preferable to reduction test.

The methods involves the reduction of cupric ions (Cu^{+2}) to cuprous ions (Cu^{+}), which in alkaline solution form yellow cuprous hydroxide, which is in turn converted by the heat of the reaction to insoluble red cuprous oxide (Cu_2O). In the qualitative tests based on this reaction, the production of a yellow or orange-red precipitate indicates the presence of a reducing carbohydrate. It is necessary to keep the cupric salts in solution and to this end Benedict's reagent incorporates sodium citrate while Fehling's reagent uses sodium potassium tartrate. Under carefully controlled reaction conditions, the amount of cuprous oxide formed may be used as a quantitative indication of the amount of reducing carbohydrate present, although different carbohydrates will result in the formation of different amounts of cuprous oxide (37).

Quantitative analysis for reducing sugar by O-toluidine test.

O-toluidine reagent reacts with the aldosugars (glucose, maltose) in the presence of acid to form a blue-green colored complex (38, 39) light absorption at 630 nm. is measured to quantitative analysis of sugar.

3.4.2 Iodine solution test for polysaccharide

Glycosidic bond of polysaccharide at alpha - 1,4 linkages between glucose chain in starch cause the helical structure of the polysaccharide chain. The inner diameter of the helix is big enough for elementary iodine to become deposited inside helical structure, thus forming a blue complex (evidence for starch). When starch is mixed with iodine in water, an intensely colored starch / iodine complex is formed clear. It seems that the iodine (in the form of 15-ions) gets stack in the coils of beta amylose coil. There is some transfer of charge between the starch and the iodine. That changes the way electrons are confined, and so, energy levels. The iodine / starch complex has energy level spacings that are just so for absorbing visible light-giving the complex its intense blue color (40, 41). Different colors form (blue → purple → pink → pink-purple → pink → red brown) when the iodine reagent is added to various decreasing chain length of sugar in carbohydrates : starch = blue, dextrin = blue-purple, purple or pink-purple, glycogen = orange or amber (42).

3.5 Dialysis technique for the analysis of PG on lipid exclusion

Dialysis is dependent upon the use of a dialyzing membrane : either a synthetic membrane with fixed pore size, as in hemodialysis, or a naturally according peritoneal membrane, as in peritoneal dialysis. The movement of solutes is largely determined by the size of these molecules in relation to the pore size of the membrane. As a general rule, smaller molecular weight substance will pass through the membrane more easily than larger molecular weight substances. A common assumption is that pore size of the peritoneal membrane is somewhat larger than that of the hemodialysis membrane;

this would explain the observation that larger molecular weight substances appear to cross the peritoneal membrane to a greater extent than they cross the hemodialysis membrane (43, 44, 45).

3.6 Lipids analysis

3.6.1 Cholesterol analysis by colorimetric method

Colorimetric determinations have been commonly used in the past to determine cholesterol. One of the more popular cholesterol determinations first introduced by Zlatkis et al. (1953) involves the reaction of cholesterol with a $FeCl_3$ reagent (46). The colour is developed independently of whether the cholesterol is free or esterified. In the most commonly used colorimetric reaction (Liebermann-Burchard), the production of a green colour with acetic anhydride and concentrated sulphuric acid, the esters react more rapidly than the free sterol. Since the colour intensity passes through a very transient maximum, a standard procedure applied to free cholesterol or its esters can give falsely high values for the esters. Most workers with the Liebermann-Burchard method include a saponification procedure, although it is claimed that use of a selective colour filter makes this unnecessary. Both the Zlatkis and Liebermann-Burchard procedures are further disadvantaged by the use of caustic reagents. Cholesterol may be precipitated with digitonin followed by determination with a colorimetric assay although precipitated with substances other than cholesterol may occur (47).

3.6.2 Cholesterol analysis by HPLC method

Although HPLC has become a very useful analytical tool in cholesterol analysis in general and can offer a non-destructive alternative to GC techniques, its specific application to cholesterol determination has been limited because cholesterol does not have a strong absorption peak in the UV region. Cholesterol and related sterols do, however, have an unsaturation centre and a

functional group that absorbs in the range 203-214 nm with a maximum at 210 nm for cholesterol (48). HPLC offers the advantage that many separations can be achieved at ambient temperature and the separated compounds can be recovered from the mobile phase for further analysis by complementary techniques such as GC and MS (49). Many HPLC methods have been published for the determination of cholesterol esters and cholesterol in plasma (50, 51) and other biological sample (48). Beyer and Jensen used C_{18} reversed-phase HPLC to determine cholesterol in egg yolk (46).

3.6.3 Fatty acids, oleic acid and stearic acid, analysis by HPLC method

Normal phase columns have been used to separate triglycerides, diglycerides, sterols, free fatty acids and monoglycerides after removal of phospholipids by column chromatography but reversed-phase separations have generally been preferred in the determination of cholesterol (52). Isocratic mobile phase has been used that is suitable for UV detection at 206 nm. The mixture of hexane : 2-propanol : acetic acid (100 : 0.5 : 0.1, v/v/v) for the determination of total fatty acids (53). The higher retention unsaturated lipids compared to saturated lipids (49).

CHAPTER II

EXPERIMENTAL

1. Materials

The following substances were commercially available, glacial acetic acid and absolute ethanol of analytical grade obtained from BDH, England. Potassium iodate, potassium fluoride, thiourea, copper sulfate, potassium hydroxide, sodium chloride, potassium chloride, calcium chloride, sodium hydrogen carbonate, ferric chloride, 1-naphthol and O-toluidine all of GR grade, were obtained from E. MERCK, Darmstadt, Germany; potassium iodide from Vidhayasom, Bangkok, Thailand. Maltose, cholesterol, triton x-100 and sodium taurocholate are analytical grade, were obtained from Sigma Chemical Co, USA; soluble starch, glucose and sulfuric acid all of analytical grade were obtained from E. MERCK, Darmstadt, Germany; ether analytical grade J.T. Baker, Phillipsburg, USA. Anhydrous disodium phosphate and disodium hydrogen phosphate all of analytical grade, were obtained from Fluka-Garantie, Switzerland; propanol-2-ol and dichromethane HPLC grade from Asia Pacific Specialty Chemical Limited, Australia; hexane, acetonitrile and methanol HPLC grade from Mallinckrodt Chemical Co, Paris, France. Oleic acid and stearic acid are cosmetic grade from Shrichand United Dispensary Co, Bangkok, Thailand; sodium potassium tartrate tetrahydrate and trichloroacetic acid analytical grade from Farmitalia Carlco Erba; chloroform, hydrochloric acid and phosphoric acid analytical grade from Mallinckrodt Chemical Co, Paris, France.

2. Equipment

- Magnetic stirrer type KMO 2 electronic (Janke & Kunkel, KIKA-WERK)
- HPLC pump (LC-10AD Liquid Chromatograp, Shimadzu Corporation, Japan)
- HPLC detector (SPD-10A UV-VIS Detector, Shimadzu Corporation, Japan)
- Integrator (C-R6A Chromatopac, Shimadzu Corporation, Japan)
- Column type Symmetry C_{18} (3.5 x 150 mm., 5 μ) (Waters, USA.)
- Column type μ Porasil (3.9 mm. X 30 cm.) (Waters, USA.)
- Spectrophotometer type Spectronic 21 (Bausch & Lamb, Germany)
- Spectrophotometer type Spectronic® GENESYS™ 5 (SPECTRONIC INSTRUMENT, INC., New York, USA.)
- Membrane filter sterile , pore size 0.45 μm (Whatman®, England)
- Water bath (EDELSTAHL, Rostfrei)
- Separatory funnel (Kimax, USA.)
- Syringe Filter, Nylon filter media, 13 mm., 0.45 μm (ORANGE SCI)
- Vortex type Retsch Mixer
- Pipetman (GILSON, France)
- Circuit Breaker (NISSHIN DENKO Co. Ltd., Japan)
- pH meter type MP 230 (Mettler Toledo, Switzerland)
- Transsonic Digital (Elma®, Germany)

3. Methods

Biological properties of polysaccharide gel (PG) from fruit-hulls of durian were determined to gain insight into the effect of PG on lipid exclusion in order to understand its potential in application as a medical diet food. The methods below were employed to examine *in vitro* study on entrapment of lipids within PG and α-amylase activity on PG extracted from fruit-hulls of durian.

3.1 Plant material

Fresh durian fruit-hulls waste was collected during the durian season, cleaned, ground and dried. Dried hulls were kept in dried and cold place until used.

3.2 Extraction of polysaccharide gel (PG)

PG was extracted by using hot water and followed by acid-ethanol precipitation. The procedure was modified from the method previously report by Pongsamart and Panmaung (1998) (54).

3.3 Evaluation of biological properties of PG

3.3.1 Determination of enzyme α-amylase digestion of PG

Incubation 1 ml of 4% PG with 50 U enzyme α-amylase (1 ml) at 37^0C for 0 and 30 min. End products after α-amylase digestion were analyzed for polysaccharide with I_2 test, and reducing sugars with Fehling's test and O-toluidine method. Using starch as positive control and water as blank.

3.3.1.1 I_2 solution test for polysaccharide

- Stock iodine solution (0.1 M)

Dissolve 0.3567 g potassium iodate and 4.5 g potassium iodide in 80 ml distilled water. Add 9.0 ml of concentrated HCl and diluting with distilled water to a final volume of 100 ml. This solution may be stored in a dark bottle in refrigerator and being used within two months.

- Working iodine solution

Dissolve 59 g potassium fluoride ($KF.2H_2O$) in 350 ml distilled water. Add 50 ml stock iodine solution and diluting with distilled water to a final volume of 500 ml. This solution may be stored in a refrigerator within a dark bottle for at least two months.

Reaction mixture

Add 0.5 ml of working iodine solution to 1 ml test digestive end product and observe the blue-purple colour appearance of polysaccharide.

3.3.1.2 Fehling's test for reducing sugars (37)

A. Fehling's reagent

- Dissolve 6.92 g $CuSO_4.5H_2O$ in 60 ml distilled water and add one drop of concentrated H_2SO_4. Make up the solution to 100 ml with distilled water. (a)

- Dissolve 12.0 g KOH and 34.6 g sodium potassium tartrate tetrahydrate (Rochelle salt) in 60 ml distilled water. Make up the solution to 100 ml with distilled water. (b)

- Mix (a) and (b) immediately before use.

B. Solution of starch substrate in buffer solution. (40 mg/ml)

- Dissolve 2.66 g anhydrous disodium phosphate and 0.86 g benzoic acid in 50 ml distilled water. Heat the mixture until boiling, and cool at room temperature. (c)

- Dissolve 4.0 g soluble starch in 40 ml cooled water. (d)

- Add (c) to (d) and heat the mixture until boiling for 2 min.

- After cooling to room temperature, adjust the pH to 7.0 and make up the solution 100 ml with distilled water.

C. Solution of PG substrate in buffer. (40 mg/ml).

Prepare the test solution of PG in the same procedure as reagent B.

Reaction mixture

- Add 1.0 ml Fehling's reagent to 1 ml test digestive end product, mix and heat the solutions for 5 min in boiling water.

- Observe an appearance of red precipitate of cuprous oxide within 1 min of reducing sugars.

3.3.1.3 O-toluidine reagent test for quantitative analysis of reducing sugars (37, 38)

A. O-toluidine reagent

Dissolve 0.15 g thiourea in 94 ml glacial acetic acid. Add 6.0 ml O-toluidine and keep in the dark bottle.

B. Standard maltose (1000 mg%)

Dissolve 0.5 g maltose in 100 ml distilled water.

C. 3% TCA

Dissolve 3.0 g TCA in 100 ml distilled water.

D. 6% TCA

Dissolve 6.0 g TCA in 100 ml distilled water.

E. Freshly prepared solution of test substrate (starch, PG, glucomannan and maltodextrin) in buffer. (40 mg/ml)

Procedure

(1) A standard curve of maltose

- A series of standard maltose containing 0 – 4 mg of maltose.
- Add 0.4 ml of 3% TCA and adjust to 0.5 ml with distilled water.
- Add 4 ml of reagent A, and mix carefully.
- Heat the solution in boiling water for 8 min and observe an absorbance at 630 nm.
- Plot graph of absorbances at 630 nm vs maltose concentrations.

(2) Test digestive end products of sample for reducing sugar

- Incubate 0.75 ml of 4% solution of test PG substrate with 50 U enzyme α-amylase from saliva 0.75 ml at 37^0C for 0 and 30 min.
- Add 1 ml 6% TCA to precipitate protein and macromolecule in 1 ml mixture of digestive end products after α-amylase digestion, centrifugation at 2,000 x g for 15 min.
- Mix 0.5 ml of the filtrate with 0.5 ml distilled water and 4 ml of O-toluidine reagent. Reducing sugars were determined by O-toluidine method comparing to standard maltose, reducing end products were calculated using the ploted standard curve of maltose concentrations.

3.3.1.4 Determination of component sugars, end product

PG solutions (20 mg/ml) were digested by α-amylase (salivary, 25 U) at 37^0 C for 30 min. After complete digestion, the solution was precipitated with 60% ethanol. Clear filtrate was seperated by centrifugation at 2,000 x g, 15 min and evaporated. Aqueous solution was loaded on a TLC plate of silica gel 60 G to determine sugar components, using solvent mixture of acetone : water : chloroform : methanol (75 : 5: 10 : 10) as the mobile phase. Spray reagent was 5% 1-naphthol in ethanol and H_2SO_4 (55). The results were compared to standard sugars.

3.3.2 Determination of mild acid digestion of PG

Reagents

A. Dissolve 1.0 g of PG in 50 ml distilled water to make 20 mg/ml concentration.

B. 1 M HCl (made up by adding 8.3 ml of concentrated HCl to 100 ml of distilled water).

C. I_2 solution (Working iodine solution)

Reaction mixture

(1) Mix the substrate solutions (1000 μl) with diluted HCl (10, 50 and 100 μl of reagent B) to make 0.01, 0.05 and 0.1 M HCl. Control was PG solution mix with distilled water.

(2) Incubate the test and control solutions at 37^0 C for 0, 0.5, 1 and 4 hours, cool immediately after incubation.

(3) Add 0.5 ml of reagent C and observe purple colour appearance of polysaccharide.

3.3.3 Study the in vitro effect on lipids entrapment of PG

3.3.3.1 Preliminary study on cholesterol entrapment property of PG using spectrophotometric method for cholesterol analysis (47)

Reagents

A. Ringer lactate buffer (Clark-forg Ring), pH 7.0

Prepare the reagent by dissolving

NaCl	6.5	g
KCl	0.14	g
$CaCl_2.2H_2O$	0.16	g
$NaH_2PO_4.2H_2O$	0.012	g
$NaHCO_3$	0.2	g
Glucose	2.0	g

Adjust the pH to 7.0 and make up the solution to 1 litre distilled water.

B. Triton X-100 in Ringer lactate buffer solution. (1% w/v)

C. Ferric chloride reagent consists of 80 ml of solution of 2.5% $FeCl_3.6H_2O$ in conc. H_3PO_4 and 920 ml of conc. H_2SO_4 per litre. Transfer this potent reagent by an automatic dispenser.

Procedure

(1) A standard curve for cholesterol

a) A series of standard solutions containing 0-200 μg of cholesterol.

b) Add sufficient absolute alcohol to a) to bring the volume to 2.0 ml.

c) Add 2.0 ml of ferric chloride reagent, and mix carefully at the lowest setting of the vortex mixer.

d) Incubate the reaction mixture for 30 min at room temperature, and record an absorbance of wavelength 550 nm.

e) Plot graph of OD. vs cholesterol conc.

(2) Cholesterol entrapment analysis

a) Preparation of test solutions

- Prepare a series of test polysaccharide gel solutions containing 0(control), 0.5, 1.0, 1.5, and 2.0% with 5.0 ml triton X-100 (1% w/v) in Ringer lactate buffer. Test solution of PG was mixed with 20 mg cholesterol and made up a final volume to 7.0 ml with triton X-100 (1% w/v) in Ringer lactate buffer.

- Dialysis tubing (M.W.CO. 12,000) was treated in Ringer lactate buffer overnight before use.

- Fill the test solutions in the dialysis tubings, tightly tie and dialyse in 200 ml triton X-100 (1% w/v) in Ringer lactate buffer in 250 ml beakers for 10 hours.

b) Cholesterol analysis by color reaction test

After dialysis, sample in dialysis bag and a solution of dialysate in beaker was collected and cholesterol was extracted with ether. Measure cholesterol content entraped within PG inside dialysis bag and cholesterol infusion outside dialysis bag by color reaction of cholesterol with a $FeCl_3$ reagent (46) and read an absorbance at 550 nm. Cholesterol in the test solution was measured using the ploted standard curve.

3.3.3.2 Study on cholesterol entrapment properties of PG using HPLC technique for cholesterol analysis

Reagents

A. Sodium taurocholate in Ringer lactate buffer solution. (10 mg/ml)

B. Mobile phase was acetonitrile : 2-propanol (7 : 3)

Procedure

(1) Preparation of the test sample

- Prepared a series of PG solutions containing 0 (control), 0.5, 1.0, 1.5 and 2.0% in 4.0 ml Ringer lactate buffer.

- Twenty milligram of cholesterol was made in micellular-solubilizing with 1.5 ml bile salt (sodium taurocholate solution) (56, 57, 58), mixed well in different PG solution and made up to final volume at 7.0 ml with Ringer lactate buffer.

- Filled the test solutions in dialysis bags, tightly tie and dialysed in 200 ml Ringer lactate buffer in 250 ml beakers for 4, 10 and 16 hours, respectively.

- Test solution inside dialysis bag was collected and made up to final volume at 25 ml with Ringer lactate buffer. An aliquot of 10 ml was used to extract for cholesterol with ether and then evaporated to dryness. The cholesterol residue was dissolved in 1.0 ml of mobile phase.

- The solution of dialysate of each test was collected, evaporated until the final volume about 30 ml and extracted for cholesterol with ether. The ether layer was evaporated to dryness. The residue of cholesterol was dissolved in 1.0 ml of mobile phase.

(2) Cholesterol analysis using HPLC technique(48, 51)

Chromatographic condition :

Reverse phase HPLC column was used : Symmetry C_{18} (3.9 X 150 mm., 5 μm). Mobile phase for HPLC was acetonitrile : 2-propanol (7 : 3), filtered through a 0.45 μm membrane filter. The column was eluted at a flow rate of 1.5 ml/min at ambient temperature and monitored at 210 nm.

Procedure analysis :

Cholesterol (2.5-12.5 μg) was used as standard. Twenty μl of standard cholesterol and sample were injected into injector, respectively. Each value was determined by at least two injections of the same sample.

Calculation :

Identification and quantification of the eluted cholesterol standards and samples were examined by comparing its retention time and integration the standard curve of the average peak area of cholesterol.

Statistical analysis :

Results are presented as mean $\pm$ SDs. Data were analyzed using One-way ANOVA followed by Post Hoc Multiple Comparisons. In all analyses a value of $P < 0.05$ was considered significant. All statistical analyses were performed by using SPSS 10.0 for WINDOWS (SPSS Inc, Chicago).

3.3.3.3 Analysis on fatty acids, oleic acid and stearic acid entrapment properties of PG using HPLC technique for fatty acid analysis

Reagents

A. Sodium taurocholate in Ringer lactate buffer solution. (10 mg/ml)

B. Mobile phase was hexane : 2-propanol : acetic acid (100 : 0.5 : 0.1).

Procedure

(1) Preparation of test solution

Test solution should be prepared in exactly the same manner as analysis of cholesterol using HPLC (3.4.2)

(2) Oleic acid analysis (53)

Chromatographic condition :

Normal phase HPLC column was used : μ Porasil (3.9 x 30 cm.). Mobile phase for HPLC was hexane : 2-propanol : acetic acid (100 : 0.5 : 0.1), filtered through a 0.45 μm membrane filter. The column was eluted at a flow rate of 2.0 ml/min at ambient temperature and monitored at 206 nm.

Procedure analysis, calculation, and statistical analysis :

Oleic acid standard curve (2.5-12.5 μg) was prepared. Oleic acid was measured and calculated in the same way as cholesterol measurement, calculation and statistical analysis were followed.

(3) Stearic acid analysis (53)

Chromatographic condition :

The chromatographic condition for stearic acid analysis was the same condition as oleic acid analysis.

Procedure analysis, calculation, and statistical analysis :

Stearic acid (5 –25 μg) standard was prepared. Stearic acid content in the test solution was determined and presented as statistical analysis using the same procedure for oleic acid analysis.

3.4 Analysis on cholesterol, oleic acid and stearic acid entrapment of glucomannan (GM) using HPLC

Glucomannan (konjak mannan) was a commercial dietary fiber using in comparison to PG on the effect of lipids entrapment.

Preparation of test solution was prepared in the same manner as PG preparation, the dialysing time was 10 hours.

Chromatographic condition, procedure for lipids analysis, calculation, and statistical analysis of lipids entrapment of glucomannan were the same as previously described in PG.

3.5 Analysis on lipid entrapment of the mixture of 1.5 % polysaccharide gel with 0.25 % polysaccharide fiber (polysaccharide fiber isolated from durian fruit-hulls) in comparison to 1.5% polysaccharide gel at 4, 10 and 16 hours by using dialysis technique and HPLC technique for lipid analysis

Preparation of test solution was prepared, reaction mixture contaning 1.5 % polysaccharide gel + 0.25 % polysaccharide fiber. Mixing with lipids such as cholesterol, oleic acid and stearic acid. The test mixtures were dialysed for 4, 10 and 16 hours, respectively.

Chromatographic condition, procedure analysis, calculation, and statistical analysis of lipids entrapment of 1.5% polysaccharide gel + 0.25 % polysaccharide fiber were maintained by the same method of lipids entrapment of 1.5 % polysaccharide gel.

3.6 Evaluation the relationship of viscosity of PG to lipid entrapment property

Viscosity of PG solutions (0, 0.5, 1.0, 1.5 and 2.0 % w/v) were determined by Viscometer (Rheology international®), shear rate at 100 rpm at room temperature. All apperent viscosity values were presented in unit of miliPascal seconds (mPa.s). One mPa.s is equal to one centipoise (59, 60).

3.7 Study on entrapment of cholesterol in egg yolk of PG by semi-permeable membrane dialysis method and using HPLC technique for cholesterol analysis

A. Preparation of test solution

- An egg was weighed. Egg yolks were separated from albumen and blended to mix thoroughly (46).

- Two gram of yolk was prepared in micellular-solubilizing with 1.5 ml sodium taurocholate in Ringer lactate buffer solution (10 mg/ml), mixed well in test

concentrations of PG (0, 1 and 2 %) and made up to a final volume at 7.0 ml with Ringer lactate buffer.

- Filled the test mixtures in dialysis bags and dialysed in 200 ml buffer for 10 hours.

- After dialysis the dialysate mixture was collected, evaporated until the volume was about 30 ml and extracted for cholesterol with ether. The ether layer was evaporated to dryness. The cholesterol residue was dissolved in 1.0 ml of mobile phase.

B. Chromatographic condition, method of analysis, calculation, and statistical analysis of cholesterol content of egg yolk releasing from PG into outside membrane were determined by the same procedure as cholesterol (commercial grade) entrapment analysis of PG (3.3.3.2).

3.8 Study of PG property effecting on releasing of cholesterol from everted jejunal sacs of rats

A. Preparation of test solution

- 0.14 gram of cholesterol (0.28% w/v) was prepared in micellular-solubilizing with 10.71 ml sodium taurocholate in Ringer lactate buffer (10 mg/ml), mixed well in test concentrations of PG (0, 1 and 2 %, w/v), and made up to a final volume at 50 ml with Ringer lactate buffer in 50 ml cylinders.

- Male Wistar strain rats (≈ 250 g) were allowed food and water until being terminated by ether and all blood was withdrawed. Everted jejunal sacs (10 cm. of length) were prepared from each rat (61, 62) in Ringer lactate buffer, filled with 1.5 ml Ringer lactate buffer, and dialysed for 1 hour in prepared solution mixture of cholesterol in PG.

- After dialysis the sacs were rinsed in Ringer lactate buffer. The solutions within the everted sacs were collected, adjusted to final volume at 2 ml with Ringer

lactate buffer and extracted for cholesterol with ether. The ether layer was evaporated to dryness. The cholesterol residue was dissolved in 1.0 ml of mobile phase.

B. Chromatographic condition, method of analysis, calculation and statistical analysis of cholesterol absorbed through outside layer of jejunum sac were examined by the same procedure as cholesterol (commercial grade) entrapment analysis of PG (3.3.3.2).

CHAPTER III

RESULTS

Polysaccharide gel (PG) was prepared from dried fruit-hulls of durian, pale yellowish powder was obtained. The powder swelled and formed a viscous layer when suspended in water. In addition, the result of biological and lipid entrapment properties studied *in vitro* are illustrated.

1. Biological Properties of Polysaccharide Gel

1.1 α-amylase digestion of PG

PG solution was incubated for digestion test by enzyme ∝- amylase for 30 min, 37^{o}C. Then the property of digested PG was identified for polysaccharide and reducing sugars by using various method as follows:

I_2 test for ∝- helix structure : The digested PG was no longer given purple coloration with iodine solution compared with control starch and maltodextrin, the blue of starch and purple-red brown color of maltodextrin were disappeared. Glucomannan gave negative test, before and after digestion test (Table 1).

Fehling's test for reducing end of sugars : The digested PG did not show orange-red precipitation with Fehling's test in comparison to control starch (Table 2), by this investigation.

O-toluidine test for quantitative test of reducing end of sugars : The digested PG showed green color with O-toluidine reagent in comparison to starch, and maltodextrin. Maltose was represented a reducing sugar standard. The amount of reducing sugar in digested substrates (starch, PG, glucomannan, and maltodextrin)

after reacted with O-toluidine reagent were calculated using maltose standard curve. It was found that the amount of reducing sugar were found at high to low concentration as followed, maltodextrin > starch > PG > glucomannan (Table 1). Positive test of glucomannan was possibly obtained according to acid and heat hydrolysis of glucomannan as well as in PG. However, this appearance possibly due to trace of starch impurity.

TLC method (sugar components) : Sugar components of the digested PG were determined by TLC method. The result showed two bands that identical to maltose and sucrose standard (Figure 13).

1.2 Mild Acid Hydrolysis of PG

PG was incubated for hydrolysis in various concentrations of hydrochloric acid (0.01 M HCl, 0.05 M HCl, and 0.1 M HCl) for 0, 0.5, 1, and 4 hours, respectively. The PG solutions at hydrolysis were determined by iodine test. Purple colour was still appeared in comparison with control polysaccharide gel solution before acid hydrolysis (Table 3).

Table 1: ∝-Amylase digestion of polysaccharide gel in comparison with starch, maltodextrin and glucomannan. Enzyme, 25 U ; substrate, 20 mg/ml ; incubation, 37 °C

Reagent (Reaction)	**Time digestion** (min)	**Blank (Distilled water,DW)**	**Control Starch (S)**		Polysaccharide gel (PG)		**Glucomannan** (GM)		Maltodextrin (MD)	
		DW+Enz.	**S+DW**	S+Enz.	**PG+DW**	PG+Enz.	**GM+DW**	GM+Enz.	**MD+DW**	MD+Enz.
I_2 Solution (∝ -helix test)	0	⊖ **ve**	⊕ **ve** (blue)	⊕ **ve** (blue)	⊕ **ve** (purple)	⊕ **ve** (purple)	⊖ **ve**	⊖ **ve**	⊕ **ve** (purple+red brown)	⊕ **ve** (purple+red brown)
	30	⊖ **ve**	⊕ **ve** (blue)	⊖ **ve**	⊕ **ve** (purple)	⊖ **ve**	⊖ **ve**	⊖ **ve**	⊕ **ve** (purple+red brown)	⊖ **ve**
O-Toluidine (reducing end test)	0	⊖ **ve**	⊖ **ve**	⊕ **ve(green)** eq. to 0.6 mg maltose	⊖ **ve**	⊖ **ve**	⊖ **ve**	⊖ **ve**	⊖ **ve**	⊕ **ve(green)** eq. to 0.63 mg maltose
	30	⊖ **ve**	⊖ **ve**	⊕ **ve(green)** eq. to 17.52 mg maltose	⊖ **ve**	⊕ **ve(green)** eq. to 6.3 mg maltose	⊖ **ve**	⊕ **ve(green)** eq. to 4.8 mg maltose	⊖ **ve**	⊕ **ve(green)** eq. to 19.20 mg maltose

Table 2 : Fehling's test for reducing sugar after α- amylase digestion of PG. Enzyme α- amylase = 25 U; control (soluble starch) = 20 mg/ml; substrate (PG) = 20 mg/ml

Test	Time (min) hydrolysis at 37^0 C	Fehling's test
Blank (distilled water)	After digestion by α - amylase 0 min	⊖ ve (5 min heated)
	After digestion by α- amylase 30 min	⊖ ve (5 min heated)
Control (Soluble starch)	After digestion by α- amylase 0 min	⊖ ve (5 min heated)
	After digestion by α- amylase 30 min	⊕ ve (1 min heated) orange-red precipitation
PG	After digestion by α- amylase 0 min	⊖ ve (5 min heated)
	After digestion by α- amylase 30 min	⊖ ve (5 min heated)

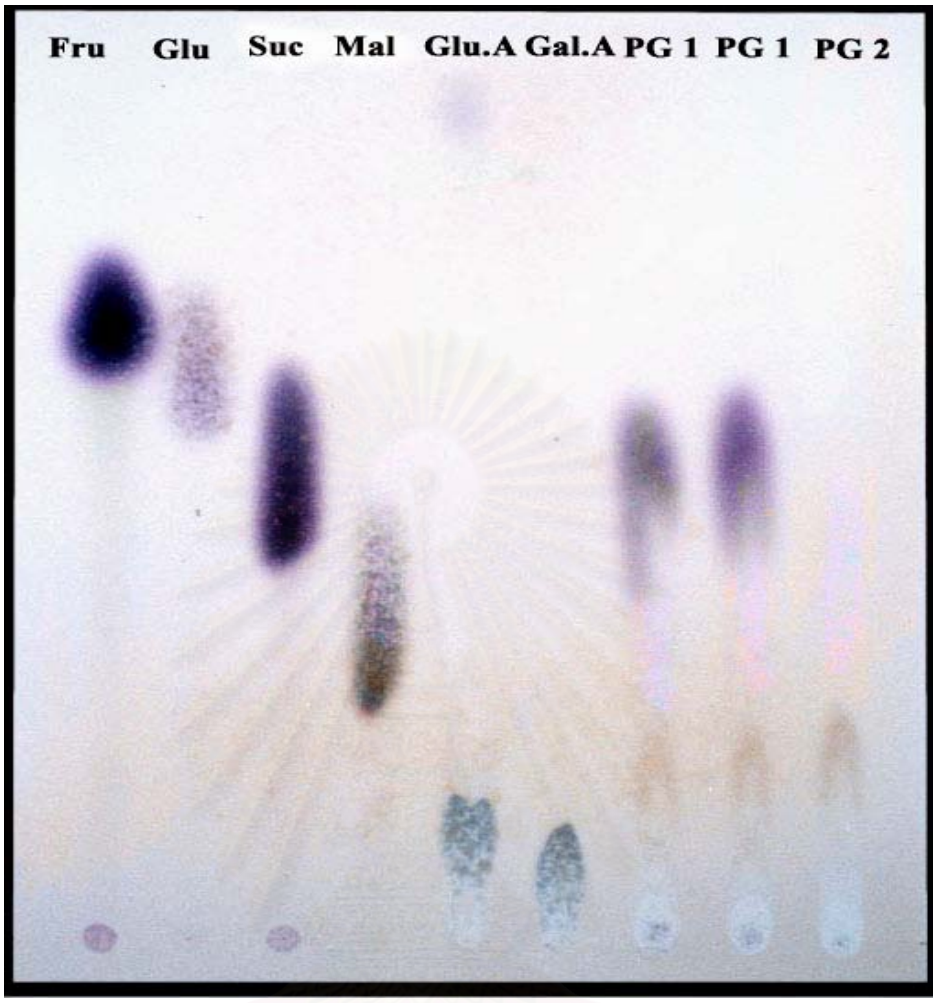


Figure 13 : Thin-layer chromatography of α-amylase hydrolyzate of polysaccharide gel (PG) . Standard sugars Fru = fructose; Glu = glucose; Suc = sucrose; Mal = maltose; Glu A = glucuronic acid; Gal A = galacturonic acid; PG1 = α-amylase hydrolyzate of PG for 30 min digestion (50 μl). PG2 = α-amylase hydrolyzate of PG for 0 min (50 μl). TLC Plate, silica gel 60 F_{254} aluminium sheet; solvent system, acetone – water – chloroform – methanol (75 : 5 : 10 : 10, v/v/v/v); spray reagent, mixture of 5% 1-napthol in ethanol and sulfuric acid.

Table 3 : Acid hydrolysis of polysaccharide gel (PG) in various concentrations of dilute hydrochloric acid (0.01 M HCl, 0.05 M HCl, and 0.1 M HCl) at digestion time 0, 0.5, 1, and 4 hours. Substrate (PG), 20 mg/ml in dilute HCl; Control = PG in water; ⊕ ve = purple color.

Time (hr.) hydrolysis at 37^0 C	control (PG in water)	I_2 test for polysaccharide after hydrolysis in dilute HCl		
		PG in 0.01 M HCl	PG in 0.05 M HCl	PG in 0.1 M HCl
0	⊕ ve	⊕ ve	⊕ ve	⊕ ve
0.5	⊕ ve	⊕ ve	⊕ ve	⊕ ve
1	⊕ ve	⊕ ve	⊕ ve	⊕ ve
4	⊕ ve	⊕ ve	⊕ ve	⊕ ve

2. Lipid Entrapment Property of Polysaccharide Gel

2.1 Preliminary Study on Cholesterol Entrapment Property of PG

Each PG concentration (0-2% PG) was evaluated for the property of cholesterol trapping inside membrane and releasing outside membrane after dialysis for 10 hours using spectrophotometric method to determine the amount of cholesterol. The results showed that cholesterol trapping inside membrane increased significantly ($p < 0.05$) with respect to increasing PG concentration. Furthermore, cholesterol releasing outside membrane decreased significantly ($p < 0.05$) with respect to increasing PG concentration (Figure 14). At 2% PG trapped 70% of cholesterol.

2.2 Study on Lipid Entrapment Properties of PG Using Dialysis Technique and Using HPLC Technique for Lipids Analysis

Cholesterol : Cholesterol releasing outside membrane decreased as well as cholesterol trapping inside membrane increased with respect to increasing PG concentration (Figure 15 a, b) after dialysis for 4-16 hours. At 2%, 1.5% and 1 % PG trapped 85.68 $\pm$ 1.81 %, 73.37 $\pm$ 0 % and 65.35 $\pm$ 1.3 % of cholesterol after 16 hours dialysis, respectively.

Oleic acid : Oleic acid releasing outside membrane decreased as well as oleic acid trapping inside membrane increased with respect to increasing PG concentration (Figure 16 a, b) after dialysis for 4-16 hours. At 2%, 1.5% and 1% PG trapped 68.75 $\pm$ 1.41 %, 53.41 $\pm$ 0.46 % and 42.3 $\pm$ 2.76 % of oleic acid after 16 hours dialysis, respectively.

Stearic acid : Stearic acid releasing outside membrane decreases as well as stearic acid trapping inside membrane increased with respect to increasing PG concentration (Figure 17 a, b) after dialysis for 4-16 hours. At 2 %, 1.5% and 1% PG trapped 66.76 ± 1.34 %, 54.32 ± 3.66 % and 43.75 ± 0.45 % of stearic acid after 16 hours dialysis.

2.3 Study on Lipid Entrapment of PG Compared with Glucomannan Using Dialysis Technique and Using HPLC Technique for Lipids Analysis

Lipids (cholesterol, oleic acid and stearic acid) releasing outside membrane decreased with respect to increasing glucomannan concentration as well as lipids trapping inside membrane increased with respect to increasing glucomannan concentration after dialysis for 10 hours, the results lipids trapping profile of glucomannan were comparable to the results of PG on trapping of lipids (Figure 18, 19 and 20).

Cholesterol released outside membrane and trapped inside membrane profiles of increasing concentrations of PG were shown in Figure 18, the similar results of PG and standard polysaccharide glucomannan were obtained.

Oleic acid released outside membrane and trapped inside membrane profiles of increasing concentrations of PG were shown in Figure 19, the similar results of PG and standard polysaccharide glucomannan were obtained.

Stearic acid released outside membrane and trapped inside membrane profiles of increasing concentrations of PG were shown in Figure 20, the similar results of PG and standard polysaccharide glucomannan were obtained.

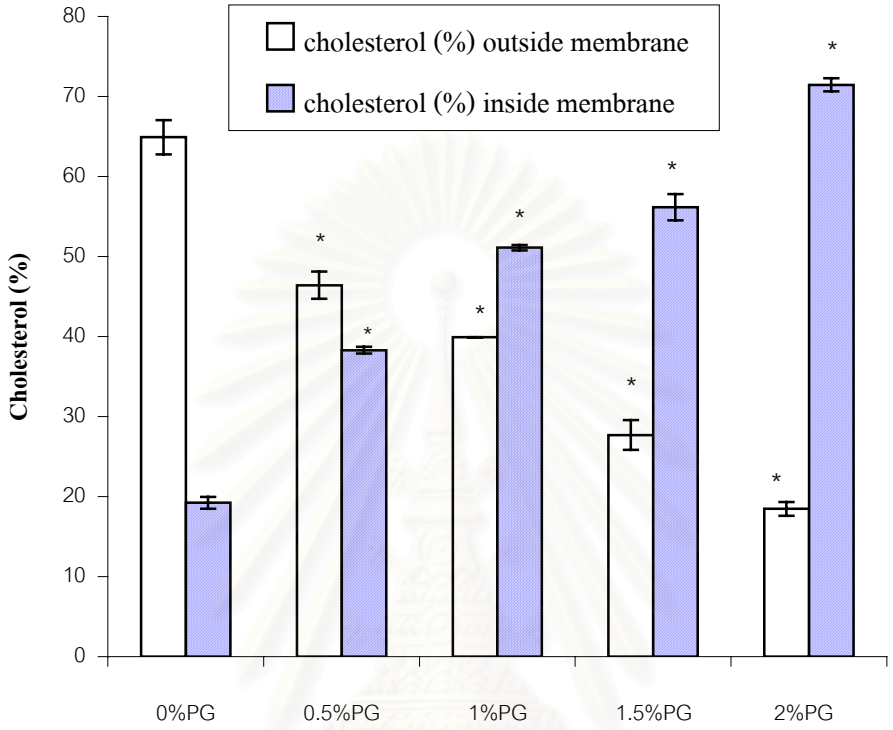


Figure 14 : Preliminary *in vitro* study of the effect of PG on releasing and trapping of cholesterol outside and inside membrane after dialysis for 10 hours. The analysis of cholesterol is accomplished by color reaction test. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars. * = mean values were significantly different from control values (0% PG), $P < 0.05$.

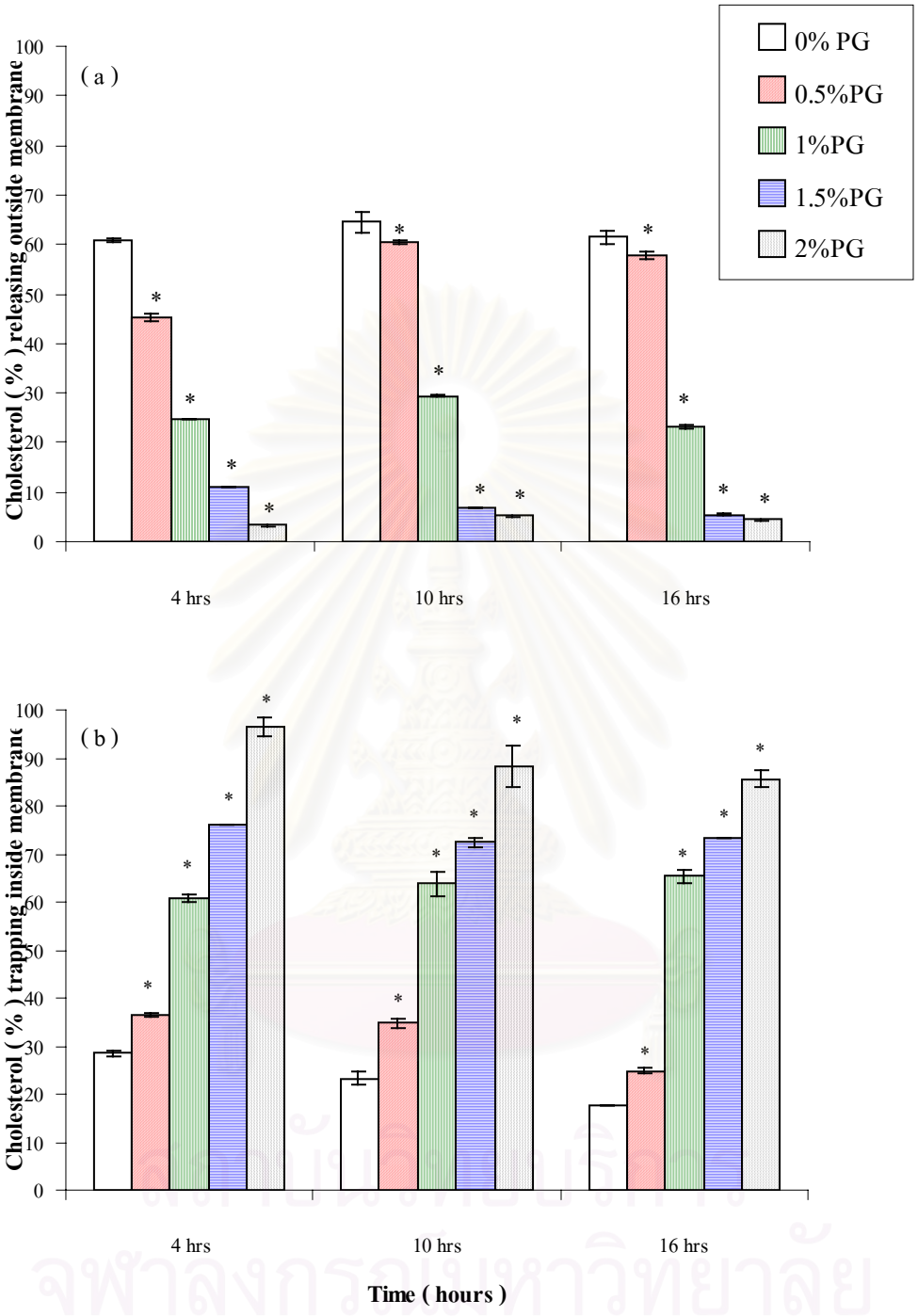


Figure 15 : Effect of PG on releasing (a), and trapping (b) of cholesterol after dialysis for 4, 10 and 16 hours. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars. * = mean values were significantly different from control values (0% PG), $p < 0.05$.

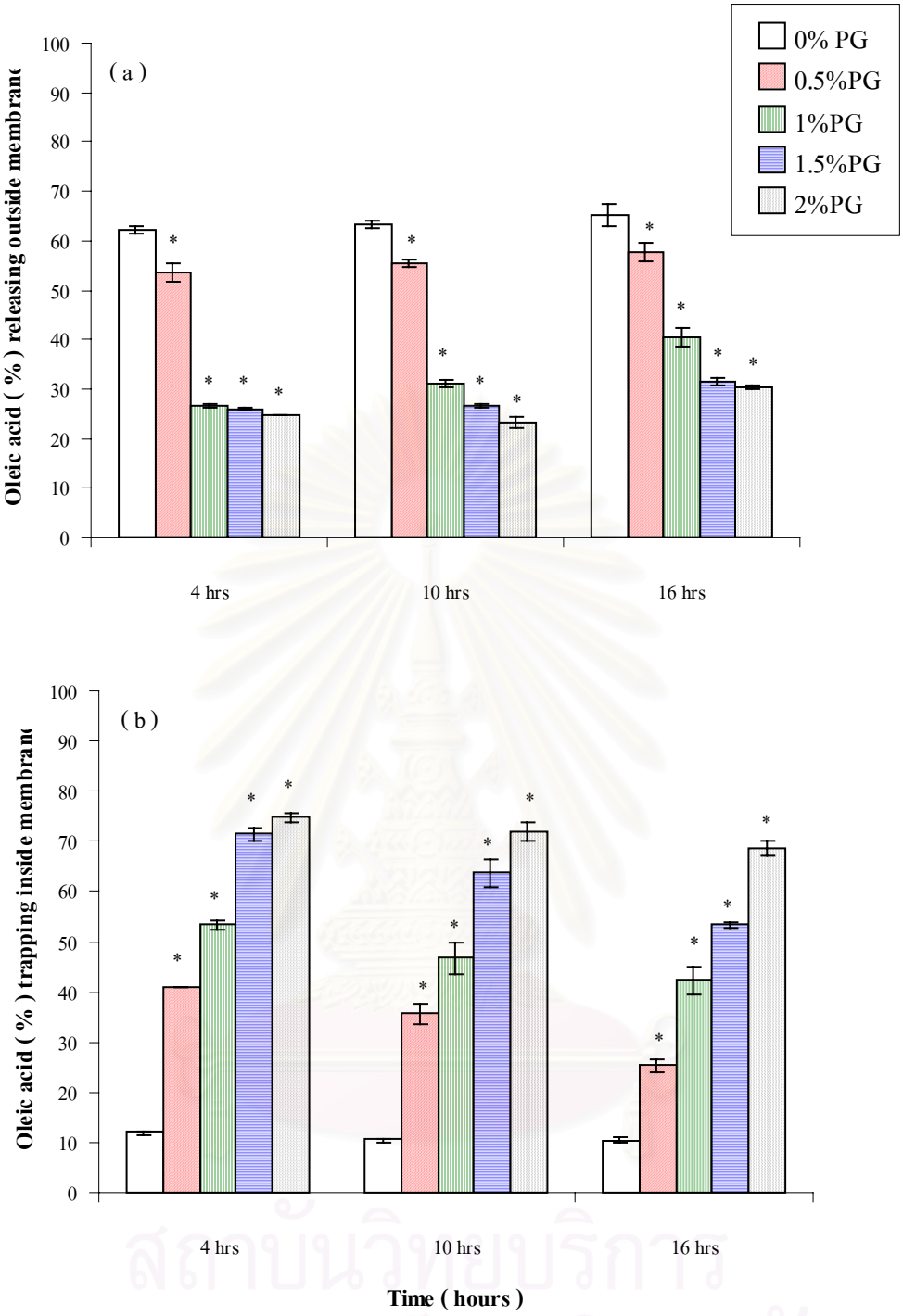


Figure 16 : Effect of PG on releasing (a), and trapping (b) of oleic acid after dialysis for 4, 10 and 16 hours. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars. * = mean values were significantly different from control values (0% PG), $p < 0.05$.

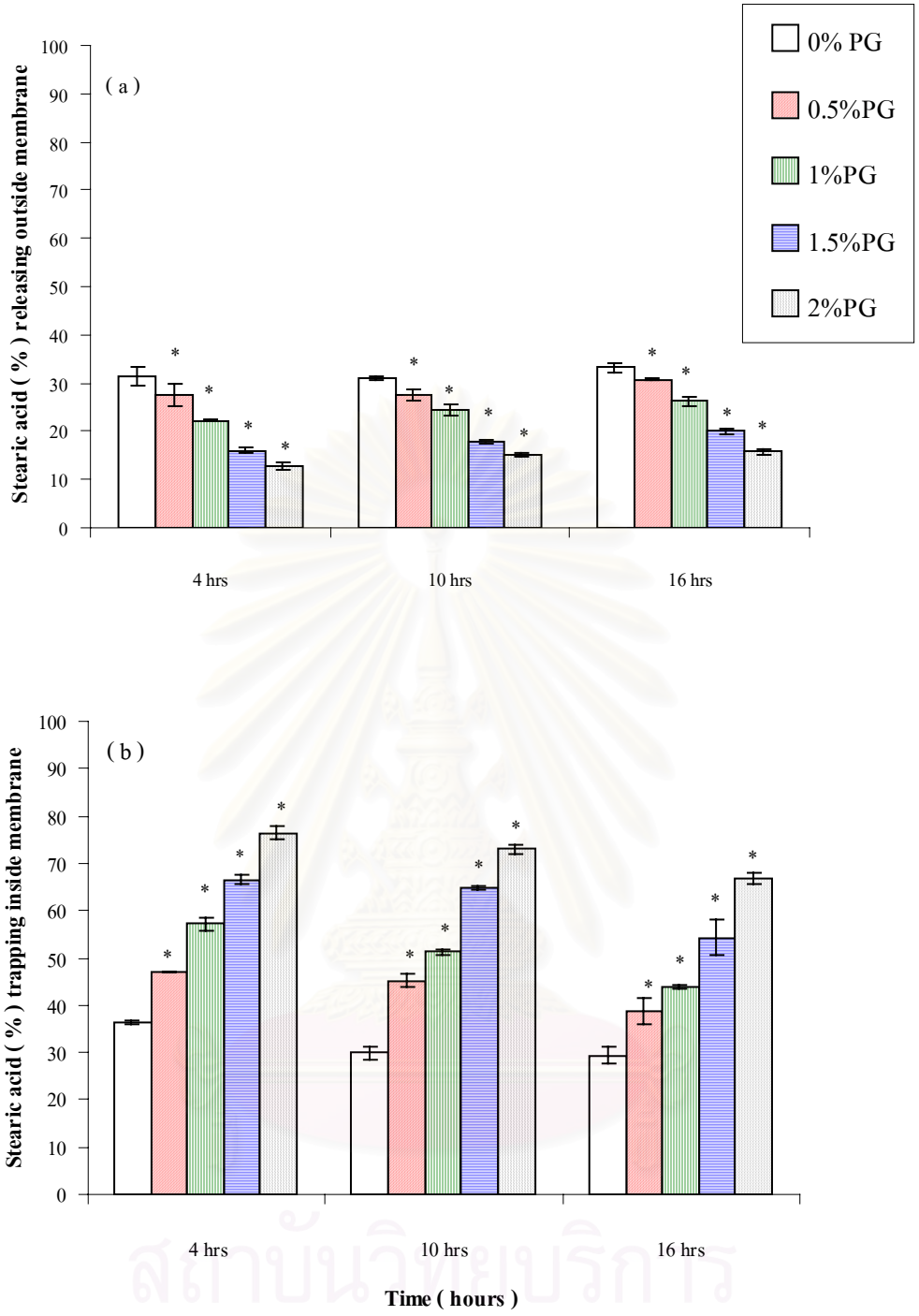


Figure 17 : Effect of PG on releasing (a), and trapping (b) of stearic acid after dialysis at 4, 10 and 16 hours. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars. * = mean values were significantly different from control values (0% PG), $p < 0.05$.

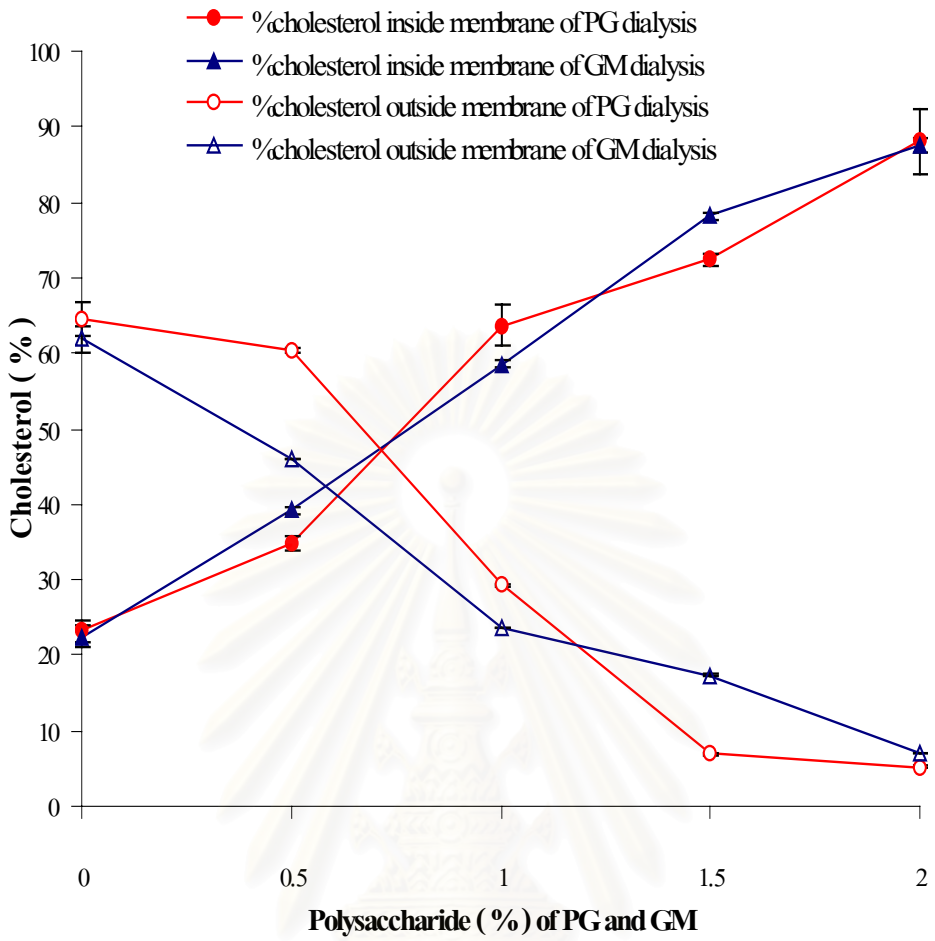


Figure 18 : Effect of PG on trapping cholesterol inside dialysis membrane and releasing cholesterol outside membrane in comparison with GM at 0-2% concentrations. Dialysis time 10 hours. PG = polysaccharide gel and GM = glucomannan. Values are means with standard errors represented by vertical bars.

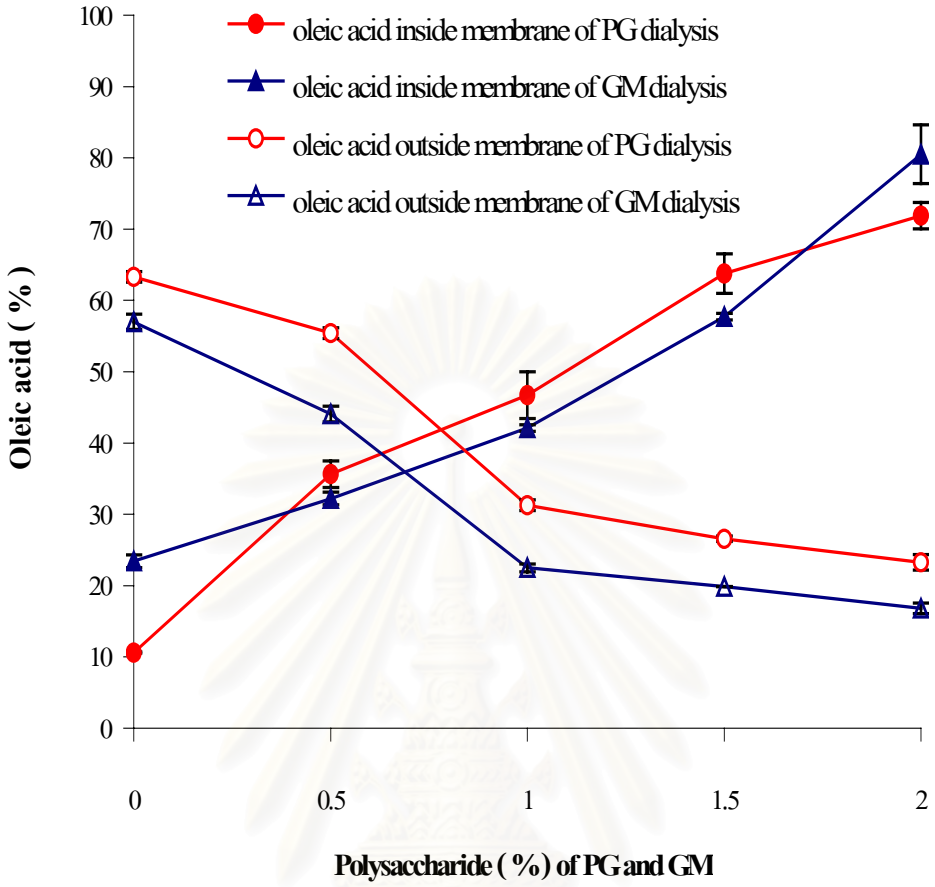


Figure 19 : Effect of PG on trapping oleic acid inside dialysis membrane and releasing oleic acid outside membrane in comparison with GM at 0-2% concentrations. Dialysis time 10 hours. PG = polysaccharide gel and GM = glucomannan. Values are means with standard errors represented by vertical bars.

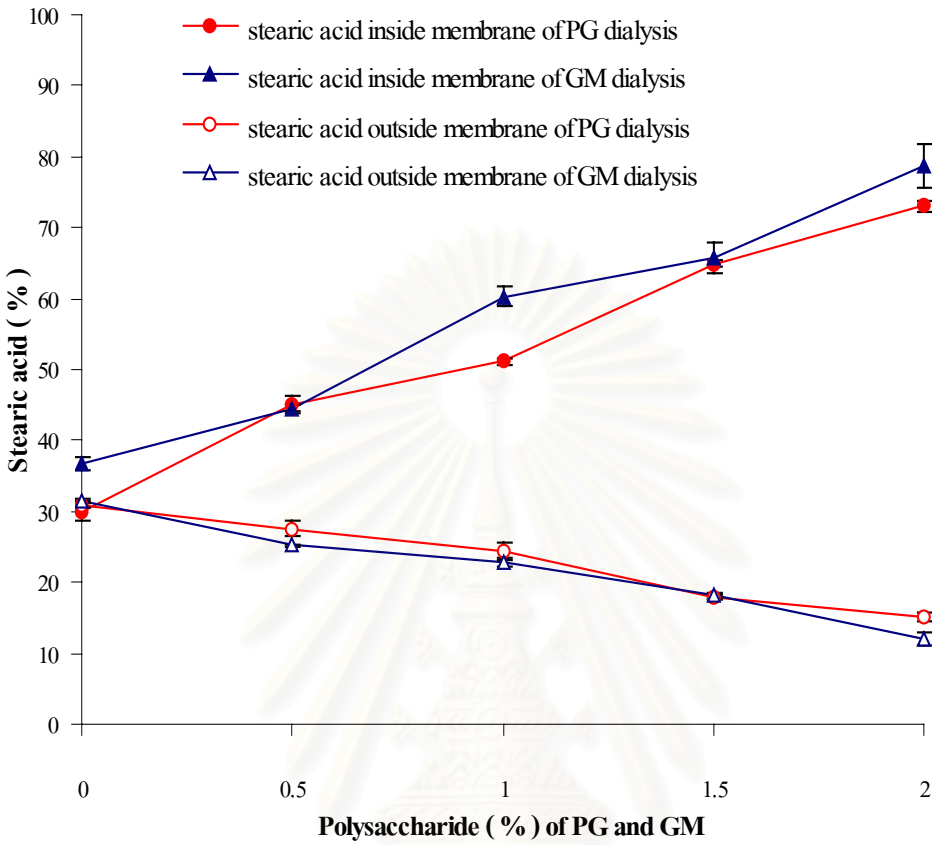


Figure 20 : Effect of PG on trapping stearic acid inside dialysis membrane and releasing stearic acid outside membrane in comparison with GM at 0-2% concentrations. Dialysis time 10 hours. PG = polysaccharide gel and GM = glucomannan. Values are means with standard errors represented by vertical bars.

2.4 Analysis on Lipid Entrapment Property of the mixture of 1.5% w/v of PG and 0.25% w/v of PF and 1.5% w/v of PG by Dialysis Technique and Lipid Analysis Using HPLC technique

To evaluate whether PF could enhance the lipid entrapment property of PG, the mixture of PG and PF and PG alone had been used in this study. The result showed that the amount of lipids (cholesterol, oleic acid, and stearic acid) released from dialysis membrane or retained inside the membrane in the presence of 1.5% w/v of PG with 0.25% w/v of PF or 1.5% w/v PG alone were comparable after dialysis 4-16 hours (Figure 21, 22, and 23). The results after dialysis 4, 10 and 16 hours were similar.

Cholesterol released outside membrane and trapped inside membrane profiles were shown in Figure 21, the same profiles were obtained with 1.5% w/v of PG and the mixture of 1.5% w/v of PG with 0.25% w/v of PF.

Oleic acid released outside membrane and trapped inside membrane profiles were shown in Figure 22, the same profiles were obtained with 1.5% w/v of PG and the mixture of 1.5% w/v of PG with 0.25% w/v of PF.

Stearic acid released outside membrane and trapped inside membrane profiles were shown in Figure 23, the same profiles were obtained with 1.5% w/v of PG and the mixture of 1.5% w/v of PG with 0.25% w/v of PF.

These results showed that lipid released outside membrane and trapped inside membrane of the mixture of 1.5% w/v of PG with 0.25% w/v of PF were similar to 1.5% w/v of PG (Figure 21, 22 and 23).

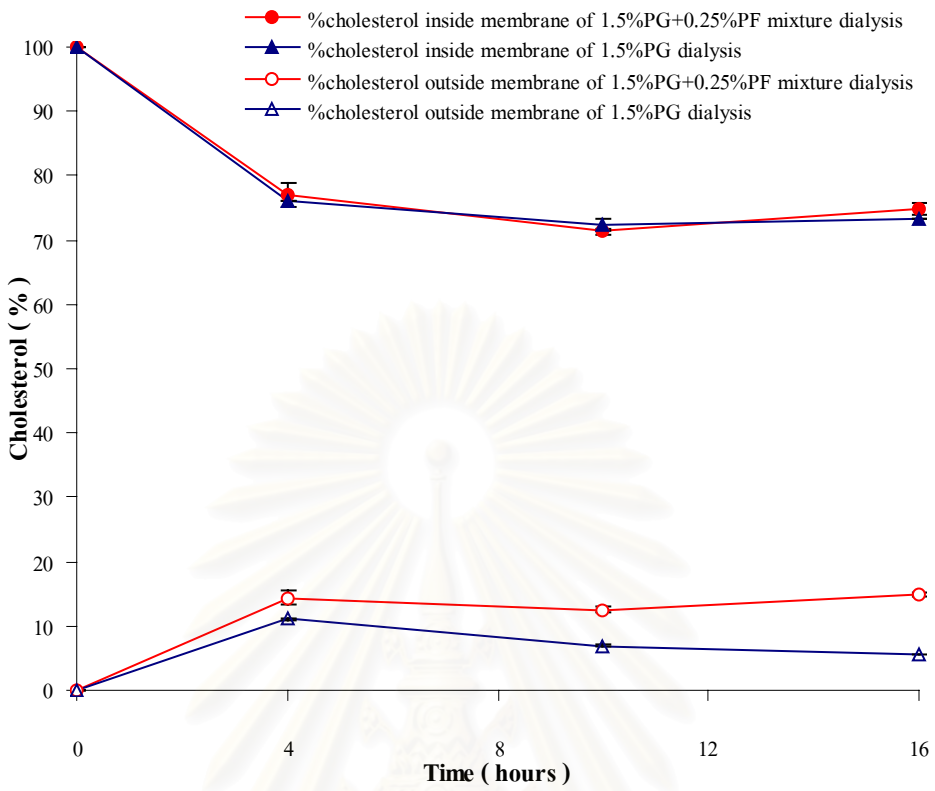


Figure 21 : Effect of mixture 1.5% w/v of PG and 0.25% w/v of PF on trapping cholesterol inside or releasing cholesterol outside dialysis membrane in comparison to 1.5% w/v of PG. Dialysis time 4-16 hours. PG = polysaccharide gel and PF = polysaccharide fiber. Values are means with standard errors represented by vertical bars.

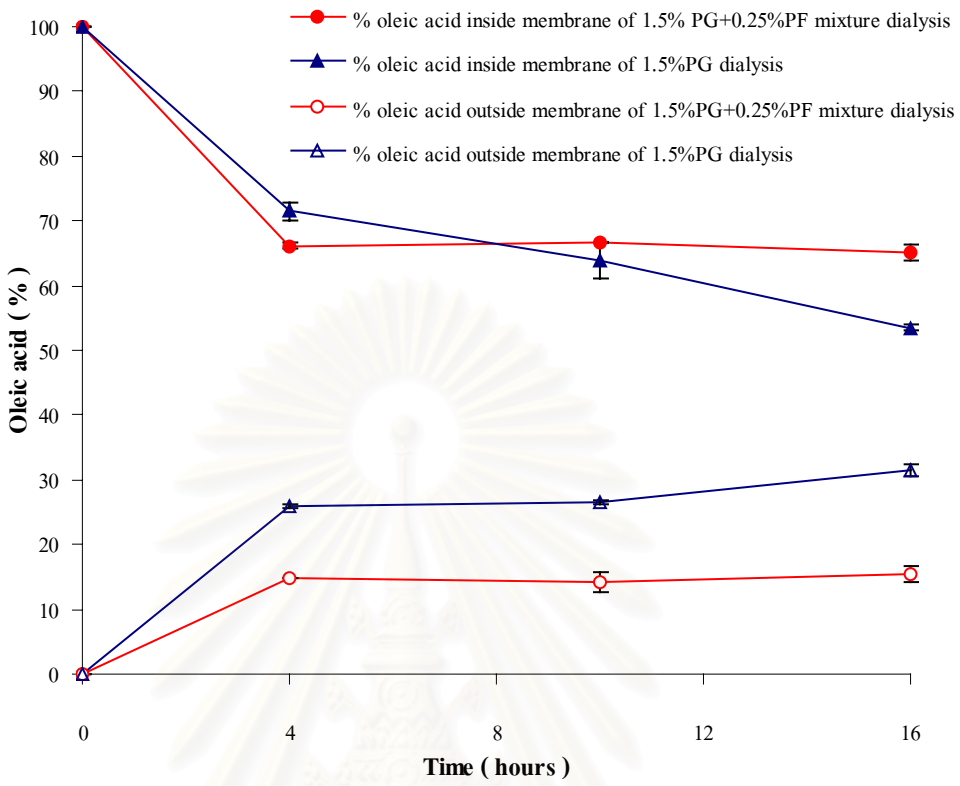


Figure 22 : Effect of mixture 1.5% w/v of PG and 0.25% w/v of PF trapping oleic acid inside or releasing oleic acid outside dialysis membrane in comparison to 1.5% w/v of PG. Dialysis time at 4-16 hours. PG = polysaccharide gel and PF = polysaccharide fiber. Values are means with standard errors represented by vertical bars.

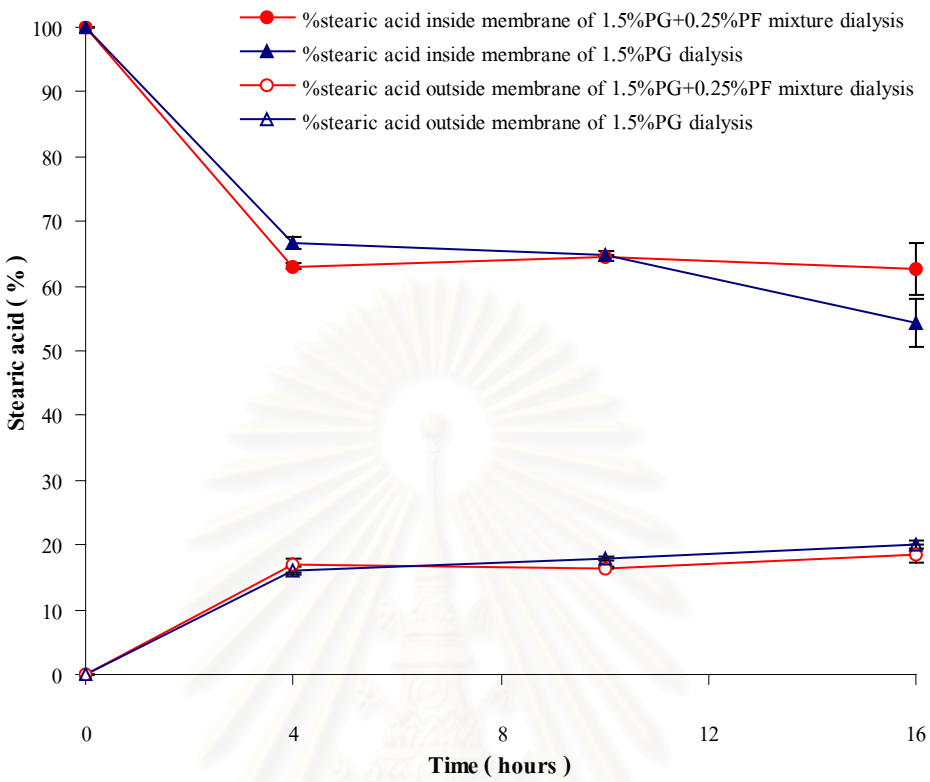


Figure 23 : Effect of mixture 1.5% w/v of PG and 0.25% w/v of PF trapping stearic acid inside or releasing stearic acid outside dialysis membrane in comparison to 1.5% w/v of PG. Dialysis time 4-16 hours. PG = polysaccharide gel and PF = polysaccharide fiber. Values are means with standard errors represented by vertical bars.

3. The Relationship Between the Viscosity of PG and Lipids Entrapment Property

The effect of viscosity of PG on lipids entrapment property of the gel had been evaluated. Figure 24, 25, and 26 showed the relationship between the viscosity of the gel and the lipids entrapment property. The results showed that the increasing of viscosity of PG effected, the increasing of lipids (cholesterol, oleic acid, and stearic acid) entrapped inside the membrane. Increasing of dialysis time was found less effect to decrease trapping of lipids by PG.

4. Entrapment of Cholesterol in Egg Yolk of PG by Semipermeable Membrane Dialysis Method and Using HPLC Technique for Cholesterol Analysis

Cholesterol in egg yolk released significantly ($p < 0.05$) outside semeipermeable membrane decreased with respect to increasing PG concentration. The result showed in Figure 27, 15.89 $\pm$ 0 % and 24.71 $\pm$ 0.25 % cholesterol released from 2% and 1% PG, respectively; after 10 hours of dialysis.

5. Effect of PG on Releasing of Cholesterol into Outside Intestinal Wall of Dissected Invert Jejunal Sacs of Rats

The presence of PG was associated with a significant ($p < 0.05$) reduction of cholesterol released into outside intestinal wall of dissected invert jejunal sacs of rats (Figure 28).

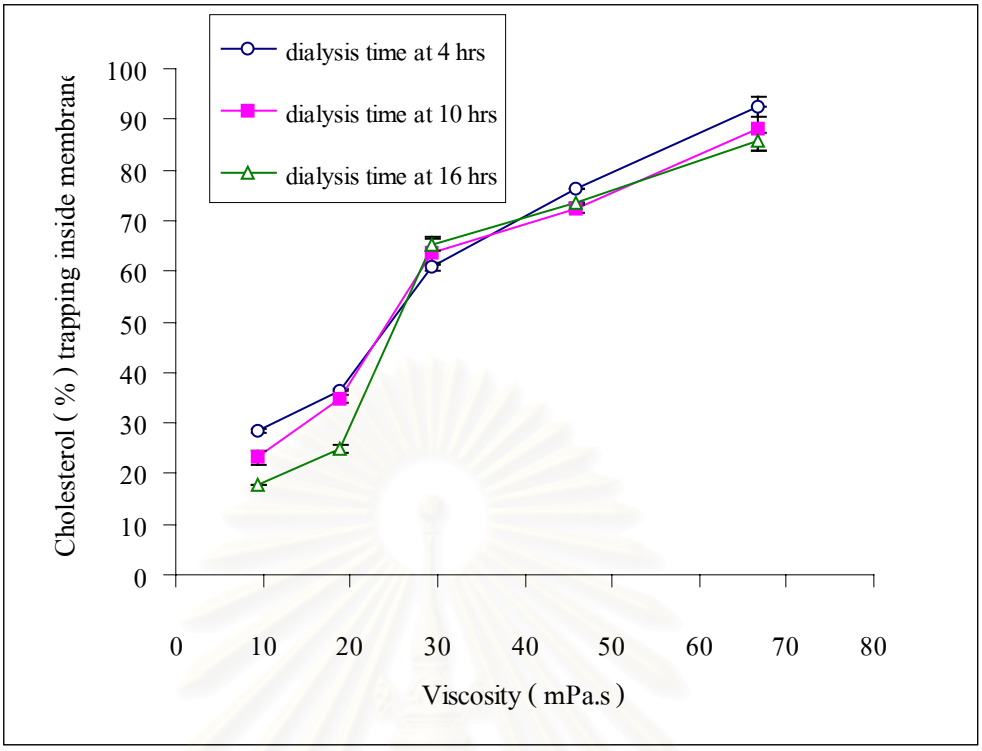


Figure 24 : Effect of viscosity of PG on trapping cholesterol inside dialysis membrane after dialysis for 4-16 hours. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars.

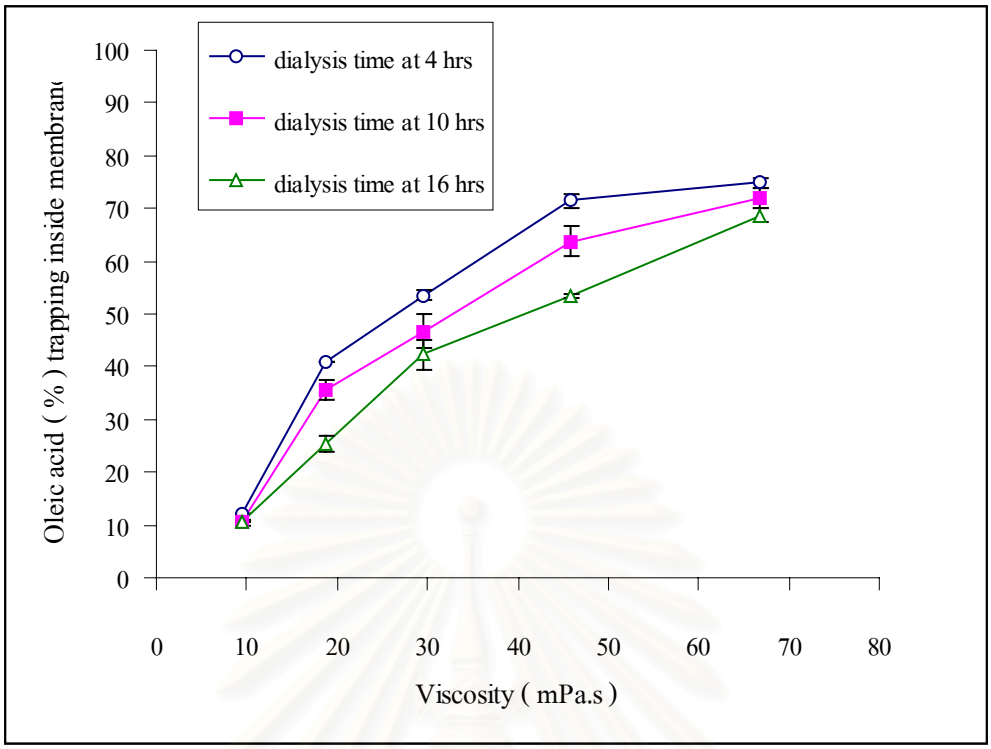


Figure 25 : Effect of viscosity of PG on trapping oleic acid inside dialysis membrane after dialysis for 4-16 hours. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars.

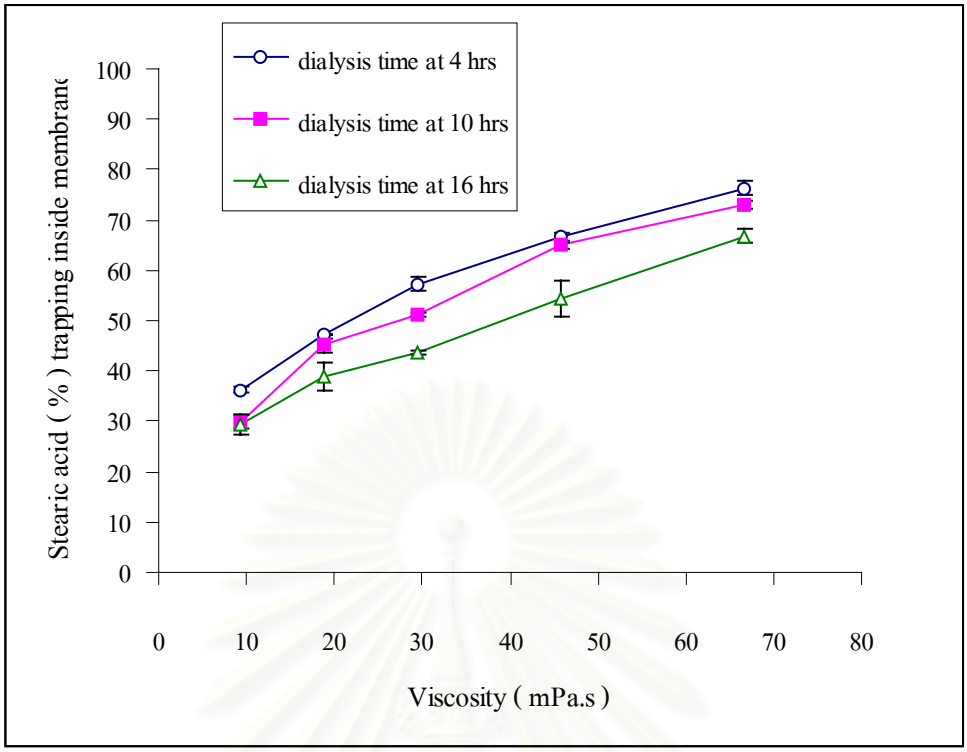


Figure 26 : Effect of viscosity of PG on trapping stearic acid inside dialysis membrane after dialysis for 4-16 hours. PG = polysaccharide gel. Values are means with standard errors represented by horizontal bars.

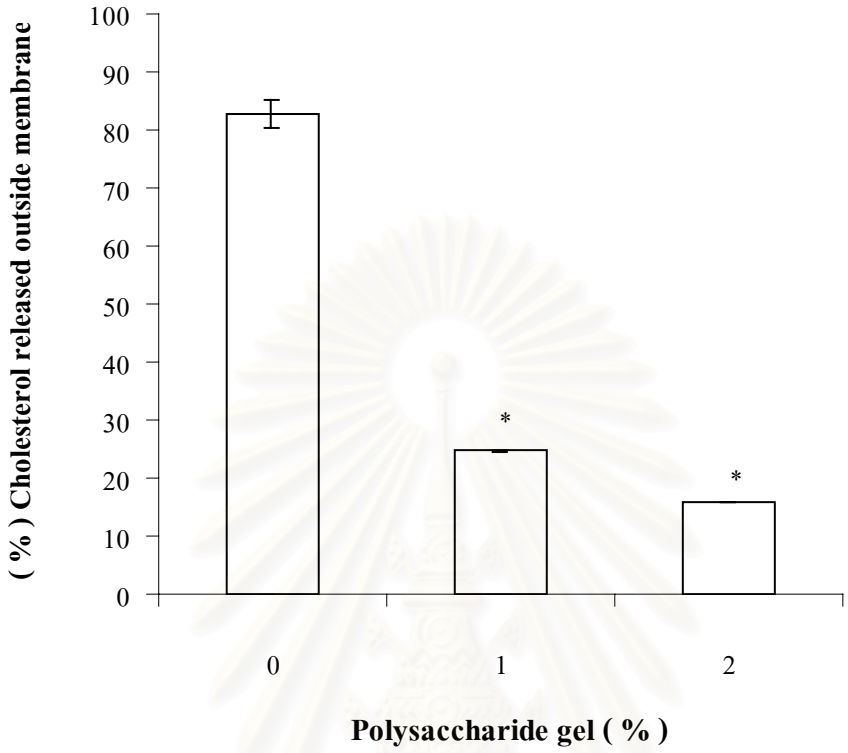


Figure 27 : Effect of PG on the releasing of cholesterol from egg yolk outside the membrane after dialysis for 10 hours. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars. * = mean values were significantly different from control values (0% PG), $p < 0.05$.

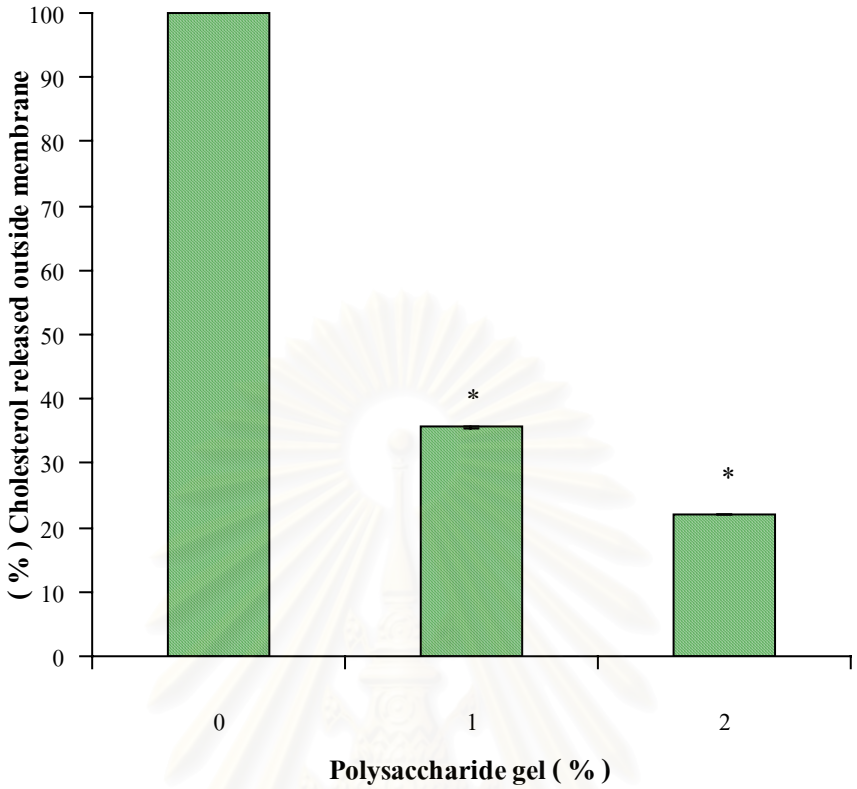


Figure 28 : Effect of PG on releasing of cholesterol from inside through outside wall of dissected invert jejunum of rat after dialysis for 1 hour. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars. * = mean values were significantly different from control value (0% PG), $p < 0.05$.

This result showed that increasing PG concentration effected the reduction of released cholesterol into outside wall of rat jejunum after dialysis 1 hour, the amount of 21.39 $\pm$ 0.03 % and 35.6 $\pm$ 0.1 % of cholesterol were released from inside into outside rat jejunal wall in the presence of 2% and 1% PG, respectively.

CHAPTER IV

DISCUSSION AND CONCLUSION

Biological properties of polysaccharide gel (PG)

Digestion of starch, the main carbohydrate source in human diet, begins in the mouth. Saliva contains ∝- amylase, an enzyme randomly hydrolyzes the ∝ (1→4) glycosidic linkage of starch, was used for PG digestion test. Then ∝- helical structure of starch was the positive control, because of having ∝ (1→4) glycosidic linkages as a substrate, for enzyme ∝- amylase digestion test.

Polysaccharide gel, formed ∝-helical structure according to iodine test, was partially digested by α- amylase in saliva which hydrolyze the α (1→4) glycosidic linkage between monosaccharide units. Purple coloration, a specific for the ∝ - helix linkages the positive test of hydrolyzed of PG with iodine reagent, was disappeared which indicate ∝ helical structure was destroyed.

The enzyme ∝ - amylase digested α (1→4) glycosidic linkage in polysaccharide gel to give reducing end products. The qualitative test for reducing sugar based on Fehling's reagent and observe the digestive products of polysaccharide gel. Result showed negative test (no orange-red precipitation) in comparison to the digestive starch (positive control). Although the qualitative method of measuring reducing end of polysaccharide gel was shown negative. Quantitative method, O-toluidine reagent, was used to investigate the amount of reducing end of digestive polysaccharide gel compared to digested starch, glucomannan, and maltodextrin. Maltose was represented a reducing sugar standard for this experiment. In addition, the reducing end product was found in maltodextrin > starch > polysaccharide gel >

glucomannan, respectively. This result indicated that polysaccharide gel may be partially digested by ∝ - amylase.

The composition of sugars after digestion of PG was identified by TLC technique. The hydrolysis product showed 2 components of sugars identical to standard sucrose and maltose. This result shows that disaccharide sugar is the major component after digestion of PG with ∝ - amylase. So polysaccharide gel was not completely hydrolyzed to give any monosaccharide by ∝ - amylase.

The acid hydrolysis product of polysaccharide gel (pH < 1) gives purple coloration with iodine solution. Clearly, this study also demonstrates that a helical chain structure of polysaccharide gel is not degraded after incubation for acid hydrolysis for 4 hours. The result suggests that polysaccharide gel is fairly stable to be digested by hydrochloric acid in the stomach.

***In vitro* study the effect on lipids entrapment of polysaccharide gel**

At present, there is no clear evidence for blood lipid controlling ability of soluble polysaccharide. Although the mechanism of action is not well established, it is generally accepted that the effects are according to a reduction rate of lipids absorption. The presence of a various polysaccharide solution in gut lumen may slow the releasing of lipids to absorption surface.

Polysaccharide makes the mechanical disruption of food in the stomach slower and then impede the delivery of nutrients to the small intestine. This effect may control the obesity in humans.

This experiment was designed in order to study the *in vitro* effect on lipids entrapment of polysaccharide gel. Cellulose membrane was used in comparison with membrane from some other organs such as stomach, small intestine and large intestine. Dialysis technique to study the effect on lipids entrapment of polysaccharide gel at various concentration and times in Ringer Lactate buffer, pH 7.0 was employed.

Preliminary study on cholesterol entrapment property of polysaccharide gel using dialysis technique and spectrophotometric method for cholesterol analysis. Triton X-100 as emulsifier to mix cholesterol and PG homogeneously, the result shows that the higher the concentration of PG (from 0 – 2 % w/v of PG), the higher cholesterol trapping in dialysis membrane after dialysis for 10 hours.

By the same experiment, the HPLC technique of analysis which is more accuracy was used to analyze lipids (cholesterol, oleic acid, and stearic acid) entrapment property of PG. Sodium taurocholate (bile salt) was applied as an emulsifier.

The result shows that the higher the concentration of PG, the more amount of cholesterol entrapment inside the dialysis membrane or the smaller amount of cholesterol releasing from the dialysis membrane. The results of fatty acids (oleic acid and stearic acid) entrapment property of PG are in similar direction with the property of cholesterol entrapment.

These results suggest that lipid absorption reduce upon higher intake of polysaccharide gel. So the consumption of polysaccharide gel may influence reduction of the absorption of lipids.

There are also essential fatty acid such as oleic acid which is very important for body function and we can get these kind of lipid only from food. Therefore, polysaccharide gel consumption should not be overdose in order to get adequately essential fatty acid for health.

The effect on lipid entrapment of polysaccharide gel compare with glucomannan

Glucomannan (commercial product) swelled in water, like polysaccharide gel. Lipids entrapment properties were compared between glucomannan and polysaccharide gel. The results show that lipid entrapment property of PG and glucomannan are comparable.

The effect on lipid entrapment of polysaccharide gel compare with the mixture of polysaccharide gel and polysaccharide fiber (PF)

Dietary fiber has been defined in analytical terms as either soluble or insoluble. Polysaccharide fiber was one part of durian-fruit hulls extraction, which is an insoluble cellulose. A product, polysaccharide fiber (approximately 0.25 %w/v of PF) was previously reported accelerate to suspend polysaccharide gel (1.5 %w/v of PG) in food preparation. In the present study, the mixture of polysaccharide fiber (0.25 %w/v of PF) and polysaccharide gel (1.5 %w/v of PG) was determined for the effect on lipids entrapment property compared with polysaccharide gel (1.5 % w/v of PG). However, there were no differences on lipid trapping profiles between PG and the mixture of PG and PF after 4, 10, and 16 hours of dialysis, respectively.

The effect of viscosity of polysaccharide gel on lipids entrapment property

There is an evidence for the effectiveness of soluble polysaccharide as means of controlling lipids levels in humans according to viscosity of polysaccharides. Polysaccharide gel, a soluble fiber can form viscous solutions. Thus the experiment was designed to follow the magnitude of the effects of viscosity of polysaccharide gel on lipids levels. The present results show that high viscosity of polysaccharide gel increase lipid trapping inside membrane after 4, 10, and 16 hours of dialysis, respectively.

So the consumption of polysaccharide gel may decrease the dispersion of food, and influence on gastric emptying. These factors effect to reduce the rate at which lipids nutrients enter the small intestine. Therefore, the absorption of lipid from alimentary tract decrease and lipid entrapped in fiber excrete out of the body together with fecal excretion.

Effect of polysaccharide gel on entrapment of cholesterol in egg yolk

Excessive energy consumption is the risk factor of diseases such as coronary heart disease and obesity, which is the more serious nutritional problems. Lipids accumulation was an important factor in these diseases. Cholesterol in egg yolk was found in high level (150-200 mg / egg yolk). The effect of polysaccharide gel on entrapment of cholesterol in egg yolk was investigated. It was found that the higher concentration of PG decreased the amount of cholesterol released outside the dialysis membrane significantly. This result suggests that PG intake may control cholesterol level after egg yolk ingestion, since egg is one of the composition of food frequently intake.

Effect of polysaccharide gel on the releasing of cholesterol into outside jejunum of rat

Dissected rat's jejunum was used as a model instead of semi-permeable dialysis membrane. The results show that the higher PG concentration effect to reduce the releasing of cholesterol from the jejunum. This result is in agreement with the result obtained from using semi-permeable membrane dialysis. So semi-permeable membrane dialysis may be applied as a model for testing animal intestinal absorption.

Conclusion

Plants produce a wide variety of polysaccharide which is an important part of the human diet. Polysaccharide gel (PG) is considered as a polysaccharide that is extracted from fruit-hulls of durian (*Durio zibethinus* L.). It seems interesting to investigate the property of PG for further use as a dietary fiber.

The results of this study confirm that polysaccharide gel has potent lipid-lowering effect. In addition, saliva α- amylase serve to hydrolyze only the α-1,4 glycosidic linkage of polysaccharide gel helical structure which identified by iodine test. Consequently the result of the saliva amylase digestion is reducing end products of the mixture of long-chain, disaccharide or partial digested polysaccharide gel that was investigated using O-toluidine test, and TLC test. Polysaccharide gel was also appeared to resist to dilute hydrochloric acid hydrolysis. This result suggests that PG will resist to acid hydrolysis within the stomach.

Semi-permeable membrane and dissected rat's jejunum were used as a model for *in vitro* study on lipid entrapment property of polysaccharide gel. The result was investigated using membrane dialysis and HPLC technique. The result shows that PG has an effect on lipid entrapment which might be associated with a reduction in lipid absorption and further lipid lowering level.

However, proposed a quite different mechanism. It was assumed that polysaccharide gel may be formed a polysaccharide gel-lipid complex. This complex was hypothesized to gel in the small intestine, entrapping the lipid and thereby preventing lipolysis, with subsequent excretion of the undigested lipid. Inhibition of lipase activity within the small intestine would lead to accumulation of a lipid emulsion. In the presence of substantial amounts of unabsorbed lipid within the small

intestine, lipid will partition into the lipid phase, leading to greater excretion of fecal lipid.

Furthermore, higher concentration of polysaccharide gel intake was also showed reduction of cholesterol from egg yolk outside dialysis membrane. This result presented that polysaccharide gel may be able to prevent obesity or coronary heart disease.

These results summarized that semi-permeable membrane might be used a model in stead of animal organ. Polysaccharide gel was also suggested in order to use as a dietary fiber and medical food.

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APPENDICES

Appendix A

HPLC technique for lipid analysis

1. HPLC technique for cholesterol analysis

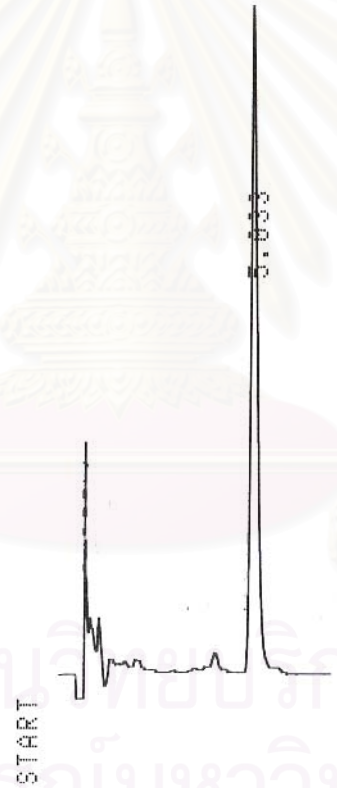

Figure 29 : Liquid chromatogram of cholesterol used 2-propanol : acetronitrile (7 : 3) as mobile phase. Retention time at 5.033 min.

2. HPLC technique for oleic acid analysis

Figure 30 : Liquid chromatography of oleic acid used hexane : 2-propanol : acetic acid (100 : 0.5 : 0.1) as mobile phase, retention time at 4.402 min.

3. HPLC technique for stearic acid analysis

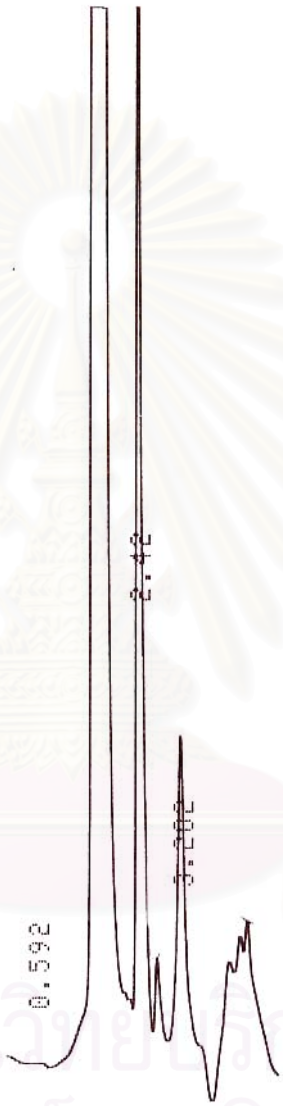

Figure 31 : Liquid chromatogram of stearic acid used hexane : 2-propanol : acetic acid (100 : 0.5 : 0.1) as mobile phase, retention time at 3.202 min.

Appendix B

Lipid Standard Curve Using HPLC Technique

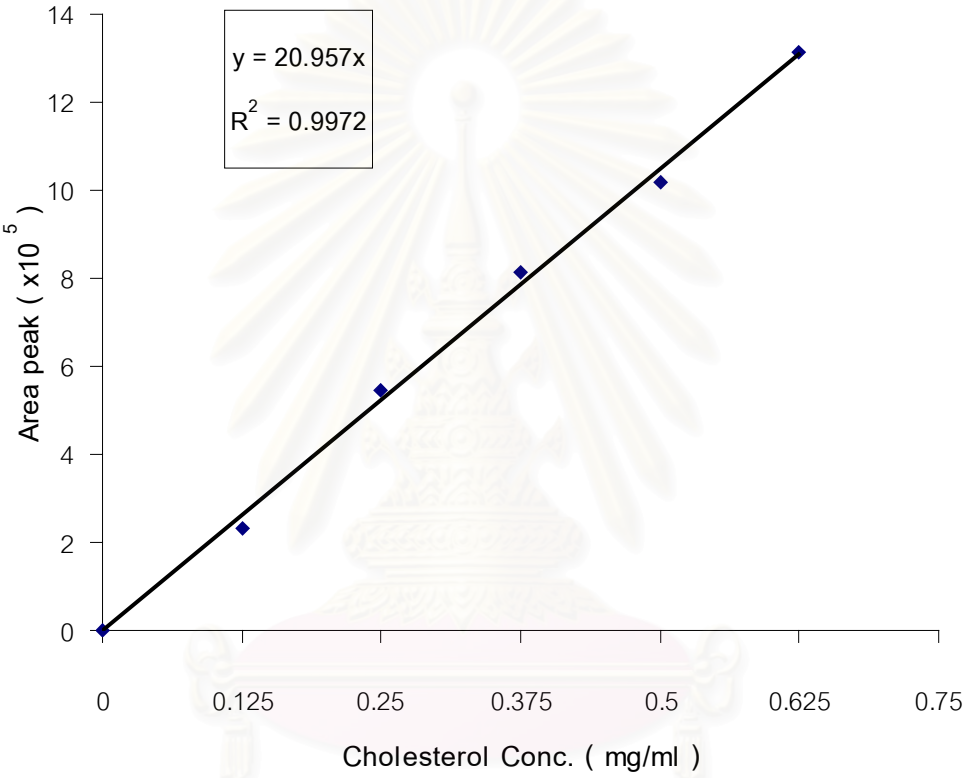

Figure 32 : Cholesterol Standard Curve

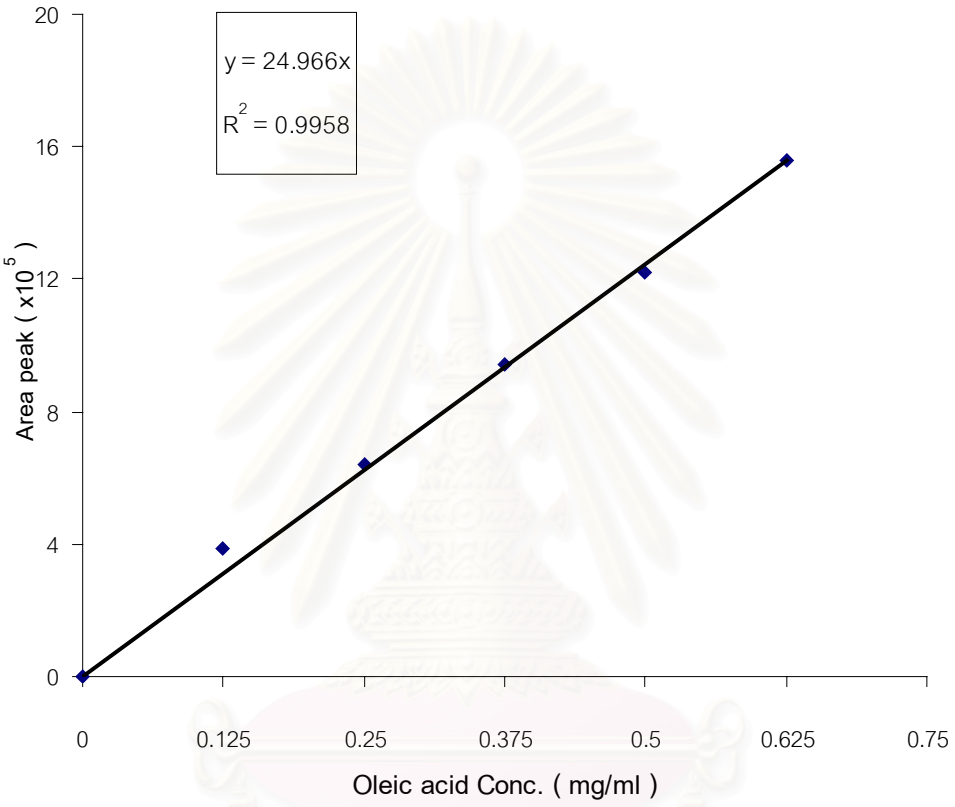

Figure 33 : Oleic acid Standard Curve

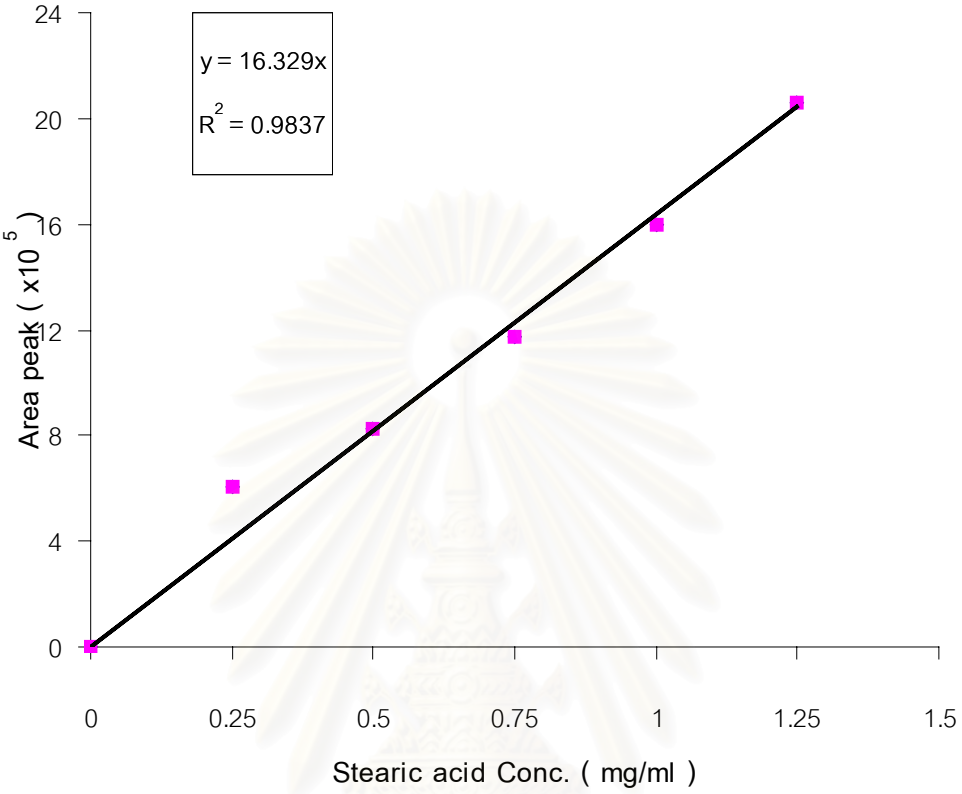

Figure 34 : Stearic acid Standard Curve

Appendix C

Cholesterol **($C_{27}H_{46}O$)**

M.W. = 386.66

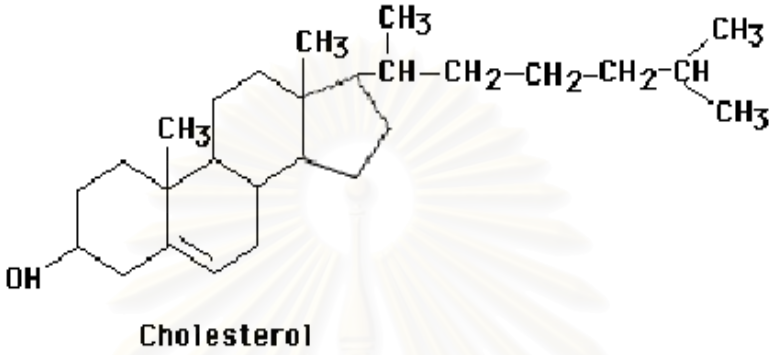

Figure 35 : Cholesterol Structure

Oleic acid ($CH_3(CH_2)_7CH=CH(CH_2)_7COOH$)

M.W. = 282.2368

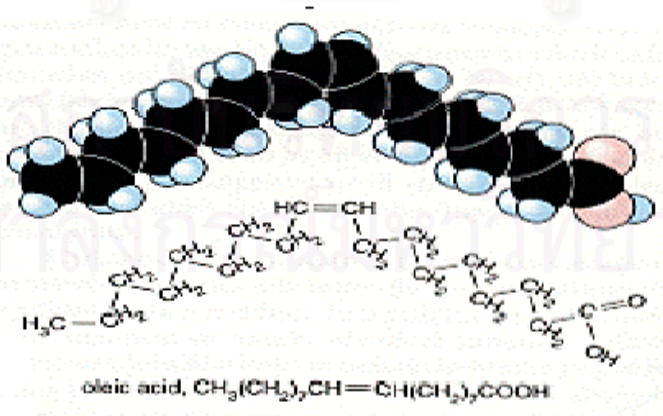

Figure 36 : Oleic acid Structure

Stearic acid **($CH_3(CH_2)_{16}COOH$)**

M.W. = 284.48

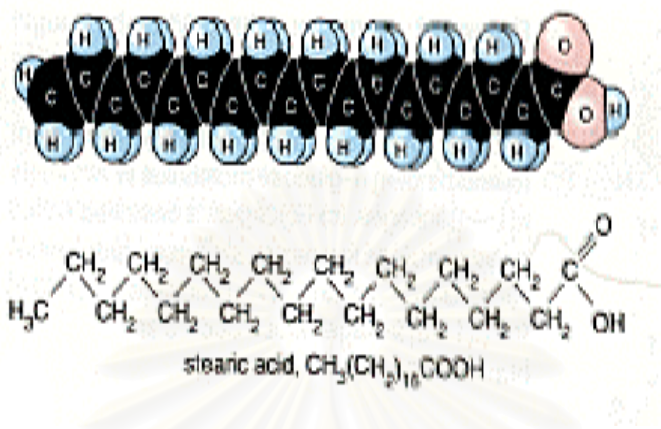

Figure 37 : Stearic acid Structure

VITAE

Miss Chutima Tippayakul was born on August 30, 1975 in Trang, Thailand. She graduated with Bachelor Degree of Biochemistry in 1999 from Faculty of Science, Chulalongkorn University.