

## Lipid entrapment property of polysaccharide gel (PG) extracted from fruit-hulls of durian (*Durio zibethinus* Murr. Cv. Mon-Thong)

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### Abstract

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Lipid entrapment property of polysaccharide gel (PG) extracted from fruit-hulls of durian (*Durio zibethinus* Murr. Cv. Mon-Thong)

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Lipid entrapment property of polysaccharide gel (PG) extracted from fruit-hulls of durian (*Durio zibethinus* Murr. Cv. Mon-Thong) was investigated *in vitro* by semi-permeable membrane dialysis technique using both cellulose membrane and gut sacs of dissected jejunum of rat. Lipids (cholesterol, oleic acid and stearic acid) were mixed with 0-2% W/V PG in the presence of bile salt as a surface active agent in dialysis membrane. Lipids inside and outside dialysis membrane were analyzed by HPLC method after 4-16 hours of dialysis in Ringer lactate buffer pH7. Increasing PG concentration resulted in increasing lipids trapped inside membrane and decreasing lipids released outside membrane. Two percent PG trapped about 80-90% cholesterol. The result of PG trapping cholesterol in egg yolk showed that egg yolk cholesterol released outside membrane was decreased with increasing PG concentration. A significant relationship was found between the decreasing of absorbed cholesterol into everted rat jejunum with respect to increasing concen-

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tration of PG. These results suggested that durian polysaccharide gel is able to entrap lipids and it seems to have potential use as medicinal dietary food for lipid controlling patient. Furthermore, *in vitro* study using cellulose semi-permeable membrane dialysis method may be applied for preliminary evaluation of polysaccharide effecting lipids absorption.

**Key words :** polysaccharide gel, *Durio zibethinus*, lipid entrapment property, membrane dialysis method

### บทคัดย่อ

ชุตติมา ทิพยกุล สุพันธ์ พงษ์สามารถ และ มณีวรรณ สุขสมทิพย์  
คุณสมบัติการกักเก็บสารอาหารไขมันของเจลโพลีแซคคาไรด์ (PG) สกัดจากเปลือก  
ของผลทุเรียน (*Durio zibethinus* Murr. Cv. Mon-Thong)

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การศึกษาเพื่อประเมินคุณสมบัติการกักเก็บสารอาหารไขมันของเจลโพลีแซคคาไรด์ (PG) สกัดจากเปลือกของผลทุเรียน (*Durio zibethinus* Murr. Cv. Mon-Thong) ทำการศึกษาในหลอดทดลองโดยใช้เทคนิค semi-permeable membrane dialysis ใช้เยื่อเซลลูโลส และลำไส้เล็กของหนูขาวทดสอบกับสารอาหารไขมัน : โคลเลสเตอรอล กรดโอเลอิก และกรดสเตียริก ในสารละลาย PG ความเข้มข้น 0-2% ใช้เกลือน้ำดีเป็นสารช่วยลดแรงตึงผิวให้สารไขมันผสมเข้ากันได้ดียิ่งขึ้นกับสารละลาย PG ในถุงเยื่อไดอะลิซิส หลังการแยกสารผ่านเยื่อ 4-16 ชั่วโมงนำสารละลายไขมันภายในและภายนอกถุงเยื่อไดอะลิซิส มาวิเคราะห์ปริมาณไขมันโดยเทคนิค HPLC พบว่าเมื่อเพิ่มความเข้มข้นของ PG การกักเก็บสารอาหารไขมันอยู่ในเยื่อเมมเบรนเพิ่มขึ้น และการปลดปล่อยสารอาหารไขมันออกมาภายนอกเยื่อเมมเบรนลดลงที่ความเข้มข้น 2% PG กักเก็บสารโคเลสเตอรอลได้ประมาณ 80-90% การศึกษาในลำไส้เล็กของหนูขาว ตรวจสอบการดูดซึมโคเลสเตอรอลจากสารผสมของโคเลสเตอรอลกับ PG ที่ผ่านเข้าสู่ผนังลำไส้หนูในหลอดทดลองโดยเทคนิคการแยกสารผ่านเยื่อ ได้ผลที่สอดคล้องกัน พบว่าความเข้มข้นของ PG เพิ่มขึ้นมีผลให้การดูดซึมโคเลสเตอรอลเข้าสู่ผนังลำไส้หนูลดลง จากผลการทดลองแสดงให้เห็นว่า PG มีผลลดการดูดซึมสารอาหารไขมันผ่านเข้าสู่ผนังลำไส้เล็กของหนู การศึกษานี้ทำให้คาดว่า PG สามารถนำมาใช้ประโยชน์ได้ในการเตรียมผลิตภัณฑ์อาหารควบคุมน้ำหนัก การศึกษาในหลอดทดลองเพื่อตรวจสอบผลของเส้นใยอาหารต่อการดูดซึมไขมันโดยใช้เทคนิค semi-permeable membrane dialysis อาจจะนำมาใช้เป็นวิธีการทดลองเบื้องต้นเพื่อประเมินสารโพลีแซคคาไรด์ที่มีผลกระทบต่อ การดูดซึมสารอาหารไขมัน

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Most dietary fiber is normally found in plant foods: fruits, vegetables, nuts and grains. It is classified as polysaccharide which cannot be hydrolyzed by human digestive enzymes (Brody, 1999). It has long been recognized as having benefits for health maintenance, disease prevention, and a component of medical nutritional therapy (Jenkins *et al.*, 1997; Work *et al.*, 1999; Gallahar *et al.*, 2002; Lu *et al.*, 2000). The term "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 (Prosky and De Vries, 1992). Some soluble fibers can slow down the increment of blood sugar and lipid level (Chandalia *et al.*, 2000; David *et al.*, 1984; Hong *et al.*, 1998). Effective soluble fiber must be viscous. The effect of viscosity delays the transition of chyme in the upper gastrointestinal tract, resulting in decreasing the rate of absorption, lower blood concentrations of nutrients, and alter hormonal responses to these absorbed nutrient (Terpstra *et al.*, 1998; Wood *et*

*al.*, 1994). Viscosity of dietary fiber also appears to be a requirement for fiber to lower blood cholesterol concentrations (Cynthia *et al.*, 2000). Increased intake of dietary fiber can help control obesity (David *et al.*, 1984), reduce serum cholesterol levels, reduce the risk of coronary heart disease (CHD), decrease insulin requirements in diabetic individuals (Wood *et al.*, 1994; Kiehm, 1976) and prevent colon cancer (Freudenheim, 1990). At present, dietary fiber is normally incorporated in a variety of foods and medicines for human and animals (Prosky and De Vries, 1992).

Early studies showed that polysaccharide gel (PG) extracted from fruit-hulls of durian is a soluble fiber (Pongsamart and Panmaung, 1998). It was also reported that polysaccharide gel (PG) from fruit-hulls of durian (*Durio zibethinus* Murr.) gave satisfactory results on being useful as an excipient in pharmaceutical and food preparation (Pongsamart *et al.*, 1989; Pongsamart and Panmaung, 1998). Toxicity studies showed that PG did not induce severe toxicity in mice and rats with respect to acute and subchronic toxicity test (Pongsamart *et al.*, 2001; Pongsamart *et al.*, 2002). In this study the ability to entrap lipid *in vitro* of PG extracted from fruit-hulls of durian was reported in order to gain insight into the property of polysaccharide gel for potential use as a medicinal dietary fiber.

## Materials and Methods

### Chemicals

Glacial acetic acid and absolute ethanol were obtained from BDH (England). Sodium chloride, potassium chloride, calcium chloride, sodium hydrogen carbonate, sodium phosphate monobasic were obtained from E. Merck (Darmstadt, Germany). Cholesterol, oleic acid, stearic acid, sodium taurocholate and Cellu-Sep T4 were obtained from Sigma Chemical (St. Louis, USA). Ether was from J.T. Baker (Phillipsburg, USA). Propanol-2-ol and dichromethane were from Asia Pacific Specialty Chemical Limited (Australia). Hexane, acetonitrile, methanol, chloroform, hydrochloric acid and phosphoric acid were purchased

from Mallinckrodt Chemical Co. (Paris, France).

### Plant material

Fresh whole rinds of ripen durian fruit (*Durio zibethinus* Murr. Cv. Mon-Thong) were collected during the durian season, cleaned, ground and dried. Dried ground rinds were kept in a freezer at -20°C until use.

### Extraction of polysaccharide gel (PG) from fruit-hulls of durian.

PG was extracted from ground fresh fruit rinds of durian by hot water and followed by acid-ethanol precipitation method as described previously (Pongsamart and Panmaung, 1998).

### Animal carcasses

Male Wistar albino rat carcasses weighing 200-250 g were obtained from Department of Pharmacology, Faculty of Pharmaceutical Science, Chulalongkorn University. The dissected jejunum was used. About 10 cm of everted jejunum sac was filled with Ringer lactate buffer pH7 before dialysis in organ bath filled with mixture of lipid in PG solution.

### Lipid entrapment property of PG

#### Cholesterol entrapment property of PG

Cholesterol entrapment property of PG was evaluated by using semi-permeable membrane dialysis (M.W. cut-off 12,000). Assay of cholesterol inside and outside dialysis bag was performed by reverse phase HPLC method (Araki *et al.*, 1990; Seta *et al.*, 1990; Fenton, 1992). Briefly, stock solution of PG in Ringer lactate buffer was prepared. Aliquots of this solution were mixed with lipid micelles of 20 mg cholesterol in 1.5 ml sodium taurocholate solution (10 mg/ml in Ringer lactate buffer) and made up to final volume of 7 ml with Ringer lactate buffer pH 7 to make 5 different concentrations of PG (0, 0.5, 1.0, 1.5 and 2%). These solution mixtures were used to dialyse in 200 ml Ringer lactate buffer for 3 different periods of time (4, 10 and 16 hours) using cellulose semi-permeable membrane as a dialysis bag. To determine the amount of cholesterol entrapped by

PG in the dialysis bag, the solution in the bag was diluted to 25 ml and an aliquot of 10 ml was used to extract with ether. For determination of cholesterol released outside dialysis bag, all dialysate was used. The dialysate was evaporated until 30 ml were left and extracted with ether. After extraction, the ether layer was evaporated to dryness. The compounds recovered in the organic layer were resuspended in 1 ml mobile phase [acetonitrile:2-propanol (7:3)] filtered with 0.45  $\mu\text{m}$  membrane filter and 20  $\mu\text{l}$  was injected in the HPLC apparatus consisting of a model LC-10AD Liquid Chromatography HPLC pump and SPD 10A UV-VIS detector. The separation of compound in sample was performed at room temperature under isocratic condition (flow rate = 1.5 ml/min) using as mobile phase an acetonitrile:2-propanol (7:3) and a Symmetry C18 column (3.9x150 mm, 5  $\mu\text{m}$  particle size). The detection was performed using a model SPD - 10A UV-VIS Detector (Shimadzu Corporation, Japan) set at wavelength 210 nm.

Standard cholesterol (Sigma Chemica, St. Louis, USA) was used to prepare a calibration curve.

#### **Oleic acid and stearic acid entrapment property of PG.**

Fatty acid entrapment property of PG was evaluated by using semi-permeable membrane dialysis method as previously described. Assays of fatty acid were performed by normal phase HPLC method (Jame and Karen, 1984) at room temperature under isocratic condition (flow rate = 2 ml/min) using as mobile phase hexane: 2-propanol: acetic acid (100:0.5:0.1) and  $\mu\text{Porasil}$  column (3.9x30 cm). The UV-VIS detector was set at 206 nm.

Standard oleic acid and stearic acid (Sigma Chemica, St. Louis, USA) were used to prepare calibration curves for oleic acid and stearic acid analysis, respectively.

#### **Egg yolk cholesterol entrapment property of PG**

Two grams of egg yolk without albumin were mixed with 1.5 ml sodium taurocholate in

Ringer lactate buffer solution (10 mg/ml). These lipid micelles were mixed with aliquot of stock solution of PG in Ringer lactate buffer and made up to final volume of 7 ml with Ringer lactate buffer pH7 to make 3 different concentrations of PG (0, 1 and 2%). These mixtures were used to dialyse in 200 ml Ringer lactate buffer for 10 hr. All dialysates were collected for cholesterol analysis by HPLC method as previously described.

#### **Analysis of cholesterol released from rat's jejunum sac.**

Preparation of rat everted jejunum sac was performed using the method described by Blackburn and Johnson (1981). The 10 cm length rat jejunum sac was filled with 1.5 ml Ringer lactate buffer and dialysed in 50 ml solution mixture of different concentration of PG solution (0, 1.0 and 2.0 % w/v in Ringer lactate buffer) mixed with lipid micelles of 0.28% W/V cholesterol in sodium taurocholate solution (10 mg/ml in Ringer lactate buffer) for 1 hr in organ bath. All solutions inside the jejunum sac were collected for analysing of cholesterol by HPLC method as previously described.

#### **Data and statistics**

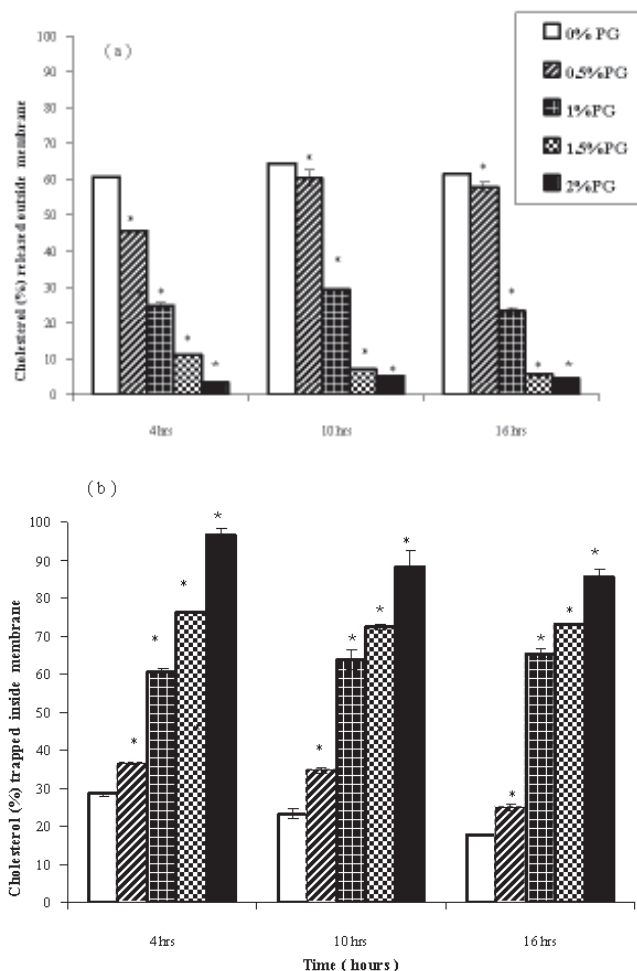
Data were expressed as mean $\pm$ SE. Difference was evaluated for statistical significance by non-parametric one-way analysis of variance (ANOVA test) followed by Post Hoc Multiple comparison. All statistical analysis was performed with SPSS 10.0 for WINDOWS (SPSS Inc, Chicago).

#### **Results**

##### **PG from fruit-hulls of durian showed the ability to entrap lipid nutrient.**

##### **The ability of PG from fruit-hulls of durian to entrap cholesterol.**

To examine the ability of PG from fruit-hulls of durian to entrap cholesterol *in vitro*, the semi-permeable membrane dialysis technique was employed, and the amount of cholesterol released outside the dialysis bag and entrapped within the



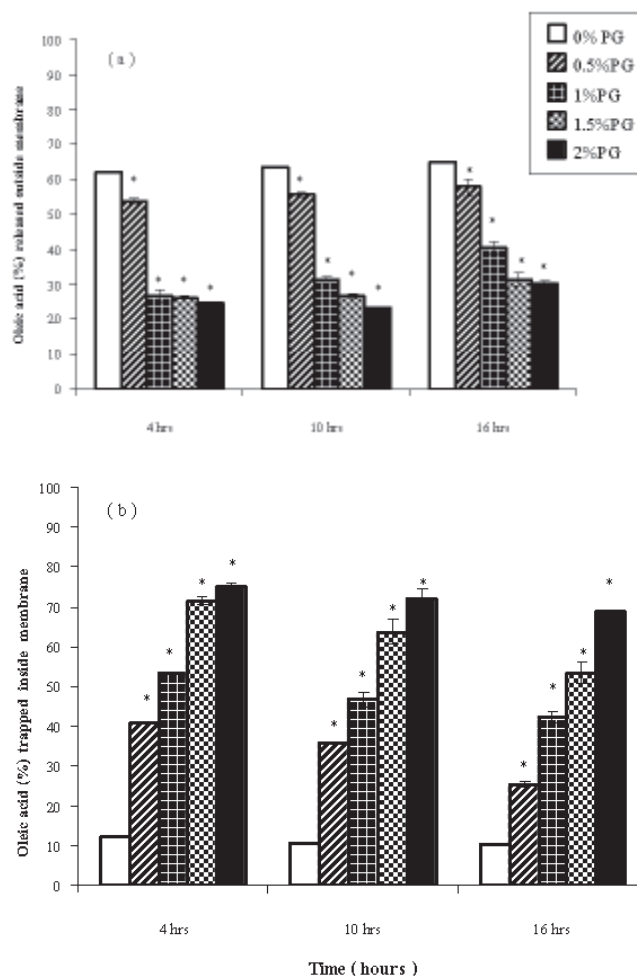
**Figure 1.** Effect of PG on releasing (a), and trapping (b) of cholesterol after dialysis for 4, 10 and 16 hours. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars. \* = mean values were significantly different from control values (0% PG),  $p < 0.05$ .

bag were analysed by HPLC method. The result showed that PG inside the dialysis bag has an effect on the release of cholesterol from dialysis bag. The amount of cholesterol released from dialysis bag in the presence of PG inside the bag is lower than the amount released in the absence of PG (Figure 1a). Furthermore, the amount of cholesterol entrapped inside dialysis membrane in the presence of PG is higher than that in the absence of PG (Figure 1b). These results showed the same direction and suggested that PG has ability to entrap cholesterol inside dialysis

membrane. Moreover, it is likely that the ability to entrap cholesterol of PG increased with increasing concentration of PG (Figure 1b).

**The ability of PG from fruit-hulls of durian to entrap fatty acid.**

To investigate further for the ability of PG from fruit-hulls of durian to entrap other lipid nutrient, both saturated and unsaturated fatty acid were included. The results showed that in the presence of PG inside dialysis bag, oleic acid released from dialysis membrane was reduced



**Figure 2. 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 2a). This result was confirmed by analysis of the amount of oleic acid trapped by PG inside dialysis bag. The results were in agreement with those obtained with analysis of oleic acid outside dialysis bag (Figure 2b).

The effect of PG on saturated fatty acid entrapment was also examined. In the presence of PG inside dialysis bag, the amount of stearic acid released outside dialysis membrane was reduced significantly compared with control value (in the absence of PG) and a similar result was obtained when analysing the amount of stearic acid en-

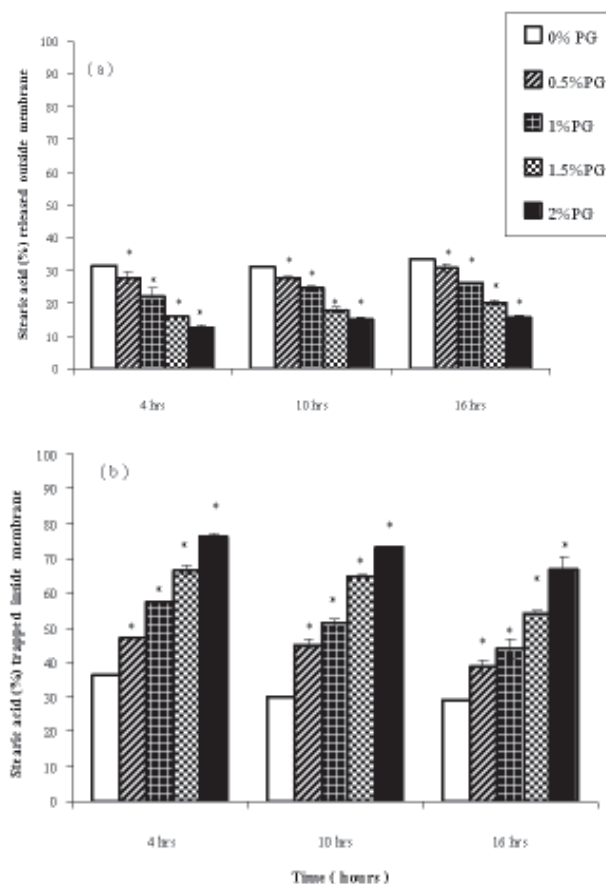
trapped inside dialysis membrane (Figures 3 a,b respectively).

All results obtained so far suggested that PG from fruit-hulls of durian has ability to entrap lipid nutrient (cholesterol, oleic acid and stearic acid). Furthermore, this ability increased in correlation with increasing concentration of PG.

**The ability of PG from fruit-hulls of durian to entrap egg yolk cholesterol.**

To determine the ability of PG from fruit-hulls of durian to entrap cholesterol from daily





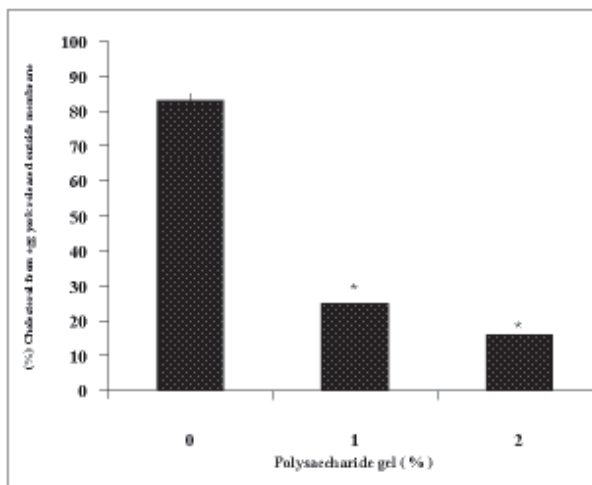
**Figure 3. Effect of PG on releasing (a), and trapping (b) of stearic acid after dialysis for 4, 10 and 16 hours. PG = polysaccharide gel. Values are means with standard errors represented by vertical bars. \* = mean values were significantly different from control values (0% PG),  $p < 0.05$ .**

intake food, egg yolk was used in stead of purified cholesterol. Semi-permeable membrane dialysis was employed, and quantitative analysis of cholesterol was accomplished by HPLC method. A significant relationship was observed between the decrease of released egg yolk cholesterol from dialysis bag and increasing concentration of PG inside dialysis membrane (Figure 4). This result confirmed the ability of PG from fruit-hulls of durian to entrap cholesterol from daily intake food.

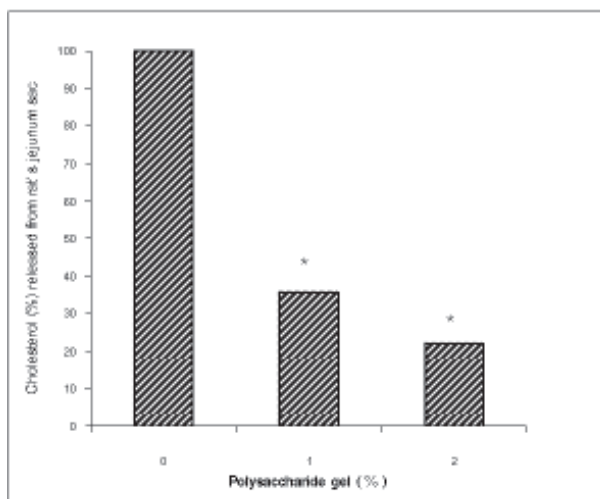
**Effect of PG on absorbing cholesterol into rat's jejunum sac.**

To confirm the ability of PG on lipid entrapment, *in vitro* dialysis method with biological

membrane (everted rat jejunum sac) was used instead of cellulose semi-permeable membrane and HPLC method was used to analyse the amount of cholesterol. The result was similar to those obtained from using cellulose semi-permeable membrane. In the presence of PG, the amount of cholesterol (%) absorbed from rat jejunum sac decreased. Moreover, a significant relationship was observed between decreasing absorbed cholesterol and increasing concentration of PG (Figure 5). This result suggested that cellulose semi-permeable membrane can be used as well as biological membrane to evaluate lipid entrapment property of potential dietary fiber.



**Figure 4.** Effect of PG on releasing of cholesterol from egg yolk outside 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 5.** Effect of PG on releasing of cholesterol through the 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),  $p < 0.05$ .

### Discussion

At present, the usefulness of dietary fiber as lipid lowering agent is generally recognized, although the mechanism of action for this property

remains uncertain. It is generally accepted that the effects are from a reduction in lipids absorption. The presence of various polysaccharide solution in gut lumen may delay the release of lipids to the absorption surface.



This experiment was designed to examine *in vitro* effect of polysaccharide gel (PG) from fruit-hulls of durian on lipids entrapment. Various lipids:cholesterol, oleic acid and stearic acid were determined using PG at 0-2% concentration and bile salt as surface active agent to help solubilize lipid in PG mixture. *In vitro* lipid entrapment property was investigated by using semi-permeable membrane. Both cellulose membrane as well as gut sacs of dissected jejunum of rat were used. HPLC method was used for quantitative analysis of lipid after 4-16 hr of dialysis. The results of analyzing the amount of lipids inside dialysis bag were in agreement with those obtained from outside dialysis bag which showed that in the presence of PG, the amounts of lipids trapped inside membrane were higher than in the absence of PG. Moreover, increasing concentration of PG resulted in increasing lipid entrapment ability. Surprisingly, the sum of the amounts of lipids inside and outside dialysis bag was not equal to 100 % as expected. In this regard, it can be explained that because of the difficulty of removing solution mixture inside dialysis bag for lipid analysis, some of the samples remained inside the bag. After taking these amounts of lipids into consideration, the result showed that total amounts of lipids were approximately 100% (data not shown). The ability of PG to entrap cholesterol from egg yolk, the general daily intake food, was also observed. All these results suggested that PG from fruit-hulls of durian may be potential dietary fiber to be developed as medicinal supplement food for blood lipid lowering purpose, including weight control agent. Furthermore, *in vitro* studies of effect of PG on cholesterol absorption through the membrane of dissected rat jejunum was also performed by dialysis technique. The results were in agreement with those obtained from cellulose dialysis membrane. The result indicated that PG has negative effect on cholesterol absorption throughout rat jejunum wall. In the presence of PG, cholesterol absorbed through rat jejunum wall decreased. So the simple and *in vitro* method of semi-permeable membrane dialysis may be used for studying the effect of dietary fiber on lipid

absorption to preliminarily estimate the influence of polysaccharide on lipid absorption. Reduction in food dispersion will influence gastric emptying which will reduce the rate at which lipid nutrients enter the small intestine. Therefore, the absorption of lipid from food will decrease and most lipid which is associated with fiber will be excreted out of body in the form of fecal excretion (Cynthia *et al.*, 2000). The result also showed the ability of PG to entrap essential fatty acid (such as oleic acid). Therefore, polysaccharide gel consumption should not be overdone in order to get adequate levels of essential fatty acid for good health.

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### References

- Araki, N., Horiuchi, S., Rahim, AT., Takata, K., and Morino, Y. 1990. Microquantification of cholesterol and cholesteryl esters in rat peritoneal macrophages by reverse-phase high-performance liquid chromatography. *Anal. Biochem.* 185(2): 339-345.
- Blackburn, N.A., and Johnson, I.T. 1981. The effect of guar gum on the viscosity of the gastrointestinal contents and on glucose uptake from the perfused jejunum in rat. *J. Nutr.* 46: 239-246.
- Brody, T. 1999. Nutrients that resist or escape digestion. pp. 53-61. **In:** Tom Brody (ed.), *Nutritional Biochemistry*. 2<sup>nd</sup> edition, Academic Press, San Diego.
- Chandalia, M., Garg, A., Lutjohann, D., von Bergmann, K., Grundy, S.M., and Brinkley, L.J. 2000. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N. Engl. J. Med.* 342: 1392-1398.

- Cynthia, M.G., Jessa M., Robert, H., and John, W. 2000. Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. *J. Nutr.* 130: 2753-2759.
- David, E.W., Vaghoubian, V., and Behforooz, A. 1984. Effect of glucomannan of obese patients: A clinical study. *J. Int. Obesity* 8: 289-293.
- Fenton, M. 1992. Chromatographic separation of cholesterol in foods. *J. Chromatogr.* 624: 369-388.
- Freudenheim, J.L., Graham, S., Horvath, P.J., Marshall, J.R., Haughey, B.P., and Wilkinson, G. 1990. Risks associated with source of fiber and fiber components in cancer of the colon and rectum. *Cancer Res.* 50: 3295-3300.
- Gallaher, D.D., Gallaher, C.M., Mahrt, G.J., Carr, T.P., Hollingshead C.H., Hesslink, Jr. R., and Wise, J. 2002. A glucomannan and chitosan fiber supplement decreases plasma cholesterol and increases cholesterol excretion in overweight normocholesterolemic humans. *J. Am. Coll. Nutr.* 21(5): 428-433.
- Hong, S., Lin, H., Ralph, L.P., and Fernandez, M.L. 1998. Dietary soluble fiber lowers plasma LDL cholesterol altering lipoprotein metabolism in female guinea pigs. *J. Nutr.* 128: 1434-1441
- Jame, G.H., and Karen, C. 1984. Separation of neutral lipids and free fatty acids by high-performance liquid chromatography using low wavelength ultraviolet detection. *J. Lipid Res.* 25: 1142-1148.
- Jenkins, D.J., Wolever, T.M., Vidgen, E., *et al.* 1997. Effect of psyllium in hypercholesterolemia at two monounsaturated fatty acid intakes. *Am. J. Clin. Nutr.* 65(5): 1524-1553.
- Kiehm, T.G., Anderson, J.W., and Ward, W. 1976. Beneficial effects of a high carbohydrate, high fiber diet on hyperglycemic diabetic men. *Am. J. Clin. Nutr.* 29: 895-899.
- Lu, X.Z., Walker, Z. K., Muir, G. J., Mascara, T., and O'Dea, K. 2000. Arabinoxylan fiber, a by-product of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. *Am. J. Clin. Nutr.* 71: 1123-1128.
- Pongsamart, S., Tawatsin, A., and Sukrong, S. 2002. Long-term consumption of polysaccharide gel from durian fruit-hulls in mice. *Songklanakarin J. Sci. Technol.* 24(4): 555-567.
- Pongsamart, S., Sukrong, S., and Tawatsin, A. 2001. The determination of toxic effects at a high oral dose of polysaccharide gel extracts from fruit-hulls of durian (*Durio zibethinus* L.) in mice and rats. *Songklanakarin J. Sci. Technol.* 23(1): 55-62.
- Pongsamart, S., and Panmaung, T. 1998. Isolation of polysaccharides from fruit-hulls of durian (*Durio zibethinus* L.). *Songklanakarin J. Sci. Technol.* 20(3): 323-332.
- Pongsamart, S., Dhumma-upakorn, R., and Panmaung, T. 1989. Extraction of pectin-like substance from durian-rind to use for pharmaceutical preparation and food products. Research Report. Faculty of Pharmaceutical Sciences, Chulalongkorn University,
- Prosky, L. and De Vries, J. W. 1992. Properties of food fibers/fibers in food product. In *Controlling Delivery Fiber in Food Product*, Van Nosrand Reinhold, New York. pp. 6-10.
- Seta, K., Nakamura, H., and Okuyama, T. 1990. Determination of  $\alpha$ -tocopherol, free cholesterol, esterified cholesterols and triacylglycerols in human lipoproteins by high-performance liquid chromatography. *J. Chromatogr.* 515: 585-595.
- Terpstra, A.H.M., Lapre, J.A., DeVries, H.T., and Beynen, A.C. 1998. Dietary pectin with high viscosity lowers plasma and liver cholesterol concentration and plasma cholesteryl ester transfer protein activity of hamsters. *J. Nutr.* 128: 1944-1949.
- Wolk, A., Manson, J.E., Stampfer, M.J., Colditz, G.A., Hu, F.B., Speizer, F.E., Hennekens, C.H., and Willett, W.C. 1999. Long term intake of dietary fiber and decreased risk of coronary heart disease among women. *JAMA.* 281(21): 1998-2004.
- Wood, P.J., Braaten, J.T., Scott, F.W., Riedel, K.D., Wolynetz, M.S., and Collins, M.W. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. *British J. Nutr.* 72(5): 731-743.