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COMPARATIVE STUDY OF LIPID ENTRAPMENT PROPERTY OF POLYSACCHARIDE GEL FROM FRUIT-HULLS OF DURIAN WITH GLUCOMANNAN

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Abstract

Lipid entrapment property in vitro of polysaccharide gel (PG) from fruit-hulls of durian (*Durio zibethinus* L.) was compared with glucomannan (GM), a well studied soluble fiber which showed property of lipid lowering effect by several studies. Lipids: cholesterol, oleic acid and stearic acid entrapment *in vitro* were investigated using semi–permeable membrane dialysis method. Sample inside membrane and dialysate were analysed against respective standard for cholesterol, oleic acid and stearic acid by HPLC method. All tested lipids appeared to be trapped within PG and glucomannan. A relationship between decreasing of released lipid from dialysis membrane with respect to increasing concentration of PG or glucomannan was observed. The effect of durian polysaccharide gel on trapping lipids appears to be similar to glucomannan. The possible explanation for the ability to trap lipids of PG is also examined. The results suggest that PG from durian decreased released lipid by a viscosity-mediated interference of lipid releasing outside membrane. PG may have potential use as a supplement for treatment of hyperlipidemic patients.

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

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การศึกษาคุณสมบัติการกักเก็บสารอาหารไขมันของเจลโพลีแซคคาไรด์สกัดจากเปลือกของผลทุเรียนเปรียบเทียบกับกลูโคแมนแนน

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บทคัดย่อ

เปรียบเทียบคุณสมบัติการกักเก็บสารอาหารไขมันของเจลโพลีแซคคาไรด์ (PG) สกัดจากเปลือกของผลทุเรียน (*Durio zibethinus* L.) เปรียบเทียบกับกลูโคแมนแนน (GM) ซึ่งเป็นเส้นใยอาหารชนิดที่ละลายน้ำได้และพบว่ามีคุณสมบัติลดระดับไขมันในร่างกาย การศึกษานี้ทำในหลอดทดลองใช้เทคนิค semi-permeable membrane dialysis ทดสอบกับไขมันชนิดต่างๆ ได้แก่ โคเลสเตอรอล กรดโอเลอิค และกรดสเตียริค ในสารละลาย PG หรือสารละลายกลูโคแมนแนน วิเคราะห์ปริมาณไขมันภายในและภายนอกถุง membrane โดยเทคนิค HPLC พบว่าสารอาหารไขมันถูกกักเก็บไว้ในสารละลาย PG หรือ กลูโคแมนแนน เมื่อเพิ่มความเข้มข้นของ PG หรือกลูโคแมนแนนในถุง membrane การกักเก็บสารอาหารไขมันภายในถุง membrane จะเพิ่มขึ้น และการปลดปล่อยไขมันออกภายนอกถุงเมมเบรนลดลง ผลการกักเก็บไขมันของเจลจากเปลือกทุเรียนเทียบเคียงได้กับกลูโคแมนแนน การทดสอบคุณสมบัติซึ่งคาดว่าเป็นตัวช่วยส่งเสริมให้เกิดความสามารถในการกักเก็บไขมันของเจลจากเปลือกทุเรียนพบว่า เมื่อความหนืดของเจลจากเปลือกทุเรียนเพิ่มขึ้น การปลดปล่อยสารอาหารไขมันออกจากถุงเมมเบรนลดลง ทำให้คาดว่าความหนืดเป็นปัจจัยสำคัญประการหนึ่งที่ทำให้เจลโพลีแซคคาไรด์ (PG) สกัดจากเปลือกของผลทุเรียน สามารถกักเก็บสารอาหารไขมันไว้ได้ การศึกษาครั้งนี้ทำให้คาดว่าเจลโพลีแซคคาไรด์สกัดจากเปลือกทุเรียนน่าจะมีศักยภาพนำมาพัฒนาเป็นอาหารเสริมทางการแพทย์ เพื่อจุดประสงค์ในการลดไขมันในผู้ที่มีระดับไขมันในเลือดสูงได้เช่นเดียวกับกลูโคแมนแนน

คำสำคัญ: การกักเก็บสารอาหารไขมัน, เจลโพลีแซคคาไรด์, เปลือกผลทุเรียน, เมมเบรน ไดอะไลซิส

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Introduction

Dietary fibers is a polysaccharide that is indigestible by mammalian digestive enzymes. It may be broad divided into two classes: soluble-fiber such as pectin, glucomannan, galactomannan, gums and insoluble - fiber such as cellulose, hemi cellulose, lignin[1]. It has been suggested that high-soluble fiber diets may be beneficial for health in many ways since they delay the rate of gastric emptying[2]. Increased intake of dietary fiber help control obesity, reduce serum cholesterol levels, reduce the risk of coronary heart disease (CHD), decrease insulin requirement in diabetic individuals and prevent colon cancer[3]. Most dietary fiber is derived from plants. Therefore, searching for source of soluble fiber, a value added product from plant waste is an interesting subject for agricultural countries such as Thailand. Durian (*Durio zibethinus* L., Bombacaceae family) is one of the most favorite fruits in Thailand. Durian fruit-hulls are large pericarp, heavy and become a huge waste product. Early studies have concluded that polysaccharide gel (PG) extracted from fruit-hulls of durian is composed of sugar; glucose, rhamnose, arabinose, fructose and galacturonic acid; in large complex of polysaccharide (apparent molecular weight 500 to 1,400 k Dalton)[4,5]. PG also showed swelling property and forming a viscous layer in water as a soluble fiber and resist to enzyme α- amylase hydrolysis and dilute hydrochloric acid hydrolysis at 37^0C for 30 minutes and 4 hours, respectively[5]. Toxicity studies showed that PG did not induced sub-chronic toxicity in male and female mice[6]. In addition, PG did not induce severe toxicity in male mice and rats after high oral dose feeding[5].

Glucomannan, a viscous polysaccharide, is a highly branched polysaccharide derived from the tuber *Amorphophallus konjac*. It has demonstrated hypocholesterolemic effects by several studies[7,8]. However, how this fiber reduces cholesterol, remains uncertain. One possible properties of glucomannan which could mediate its hypo-cholesterolemic effect is its viscosity. In this study, in vitro lipid entrapment ability of PG from fruit-hulls of Durian was compared with glucomannan, a well studied soluble fiber which showed the property of hypocholesterolemic effect to assess its potential use as medicinal supplement food for lipid lowering purpose. Furthermore, a possible explanation for the observation of lipid entrapment property of durian polysaccharide gel was also examined.

Materials and Methods

Chemicals

Glacial acetic acid and absolute ethanol were obtained from BDH (England). Sodium chloride, potassium chloride, calcium chloride, sodium hydrogen carbonate and 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 obtained from J.T. Baker (Phillipsburg, USA). Propanol-2-ol and dichromethane were obtained from Asia Pacific Specialty Chemical Limited (Australia). Hexane, acetonitrile , methanol, chloroform, hydrochloric acid and phosphoric acid were purchased from Mallinckrodt Chemical Co., Ltd. (Paris, France).

Plant materials

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

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

PG was extracted by method previously described[4] by using hot water and followed by acid – ethanol precipitation.

Lipid entrapment property of PG comparable with glucomannan

Cholesterol entrapment property

The cholesterol entrapment property was evaluated by using semi-permeable membrane dialysis. Assay of cholesterol inside and outside dialysis membrane was performed by reverse phase HPLC method.[9-11] Briefly, stock solution of PG and glucomannan in Ringer lactate buffer were prepared. Aliquot of these solutions 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 and glucomannan (0,0.5,1.0,1.5 and 2.0%). These solution mixtures were used to dialyse in 200 ml Ringer lactate buffer for 10 hours using cellulose semi-permeable membrane as a dialysis bag. To determine the amount of cholesterol trapped by PG and glucomannan in the dialysis membrane, the

solutions inside membrane were diluted to 25 ml. An aliquot of 10 ml were used for analysis of cholesterol by HPLC. For determination of cholesterol released outside membrane, all dialysate were used. The samples were evaporated until small amounts (30 ml) were left and extracted with ether. 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 μm membrane filter and 20 μl 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 (3.9 x 150 mm, 5 μm particle size). The detection was performed using a model SPD – 10A UV-VIS Detector (Shimadzu Corporation, Japan) set wavelength at 210 nm.

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

Oleic acid and stearic acid entrapment property

Fatty acid entrapment property was evaluated by using semi-permeable membrane dialysis method as previously described. Assay of fatty acid was performed by normal phase HPLC method[12] 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 μPorasil column (3.9 x 30 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.

Relationship between viscosity and lipid entrapment property of PG

Viscosity of 5 different concentrations of PG solution samples (0, 0.5, 1.0, 1.5 and 2.0% W/V) were determined by using Viscometer (Rheology international). The shear rate was set at 100 rpm at room temperature. Viscosity values were presented in mili Pascal second (mPa.s). One mPa.s is equal to one centipoises.

Data and statistics

Data were expressed as mean ± SE. Difference were evaluated for statistical significance by non-parametric one-way analysis of variance (ANOVA test) followed by

Post Hoc Multiple comparison. All statistical analyses were performed with SPSS 10.0® for WINDOWS (SPSS Inc, Chicago).

Results

Cholesterol entrapment property of PG comparable with glucomannan

Glucomannan, a soluble fiber which has been well studied and showed lipid lowering effect was used for comparative study with PG on lipid entrapment ability by using semi-permeable membrane dialysis method. Quantitative analysis of lipid was accomplished by HPLC method. The result showed that in the presence of PG or glucomannan (GM), the amount of cholesterol trapping inside membrane was higher than in the absent of PG or GM. Furthermore, the amount of cholesterol released from membrane in the presence of PG or GM was lower than the amount released in the absence of PG or GM (Fig. 1). The result also showed the relationship between the concentration and cholesterol entrapment property of PG and GM. Increasing PG or GM concentration resulted in increasing trapped cholesterol inside membrane and decreasing released cholesterol outside membrane (Fig. 1).

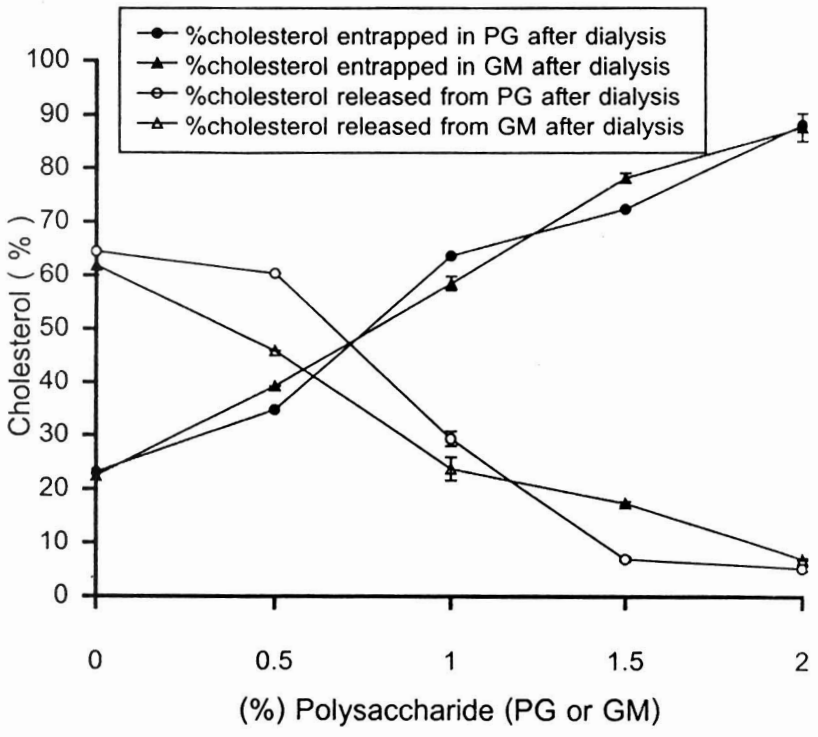


Figure 1. Effect of PG on trapping cholesterol inside dialysis membrane and releasing cholesterol outside membrane in comparison with GM at 0 - 2% conc. Dialysis time 10 hr. PG = polysaccharide gel and GM= glucomannan. Values are means with SE represented by vertical bars.

Fatty acid entrapment property of PG comparable with glucomannan

To investigate further for the ability of PG from fruit-hulls of durian to entrap other lipid nutrient in comparison with glucomannan, both saturated and unsaturated fatty acid were included. In the presence of PG or glucomannan, the amount of trapped oleic acid was higher than in the absent of PG or GM. The amount of released oleic acid was also reduced in the present of PG or GM inside membrane (Fig. 2).

The effect of GM or PG on saturated fatty acid entrapment was also shown in fig. 3. In the presence of PG or GM, the amount of stearic acid released outside membrane reduced compared with control value (in the absence of PG or GM). Similar result was observed when analyzing stearic acid trapped inside membrane (Fig. 3).

All results suggested that PG from fruit-hulls of durian has lipid entrapment property and it is likely that this property of PG from fruit-hulls of durian is similar to glucomannan.

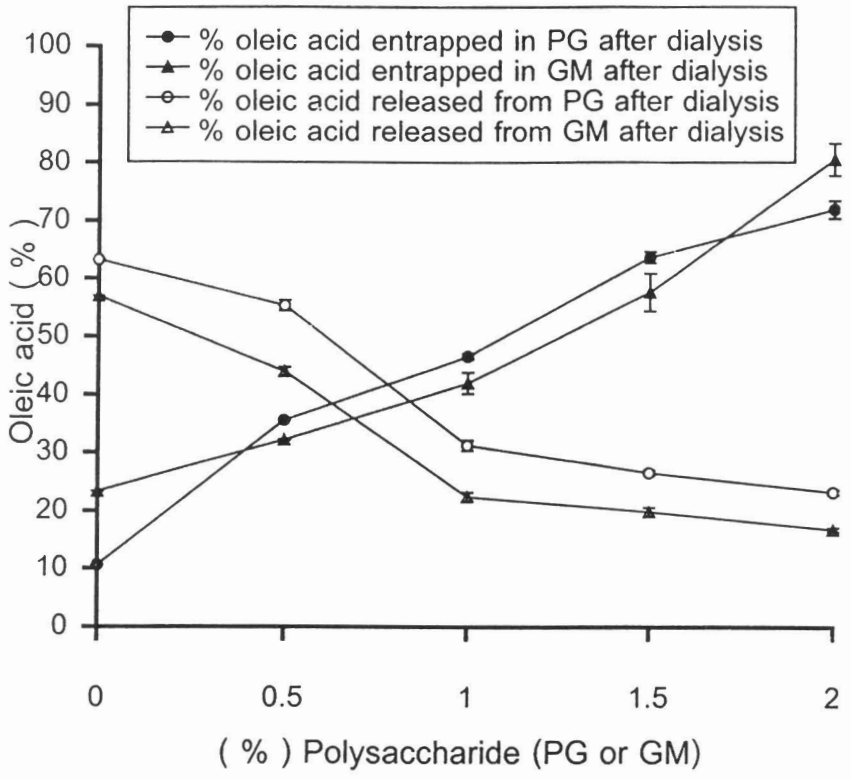


Figure 2. Effect of PG on trapping oleic acid inside dialysis membrane and releasing cholesterol outside membrane in comparison with GM at 0 - 2% conc. Dialysis time 10 hr. PG = polysaccharide gel and GM= glucomannan. Values are means with SE represented by vertical bars.

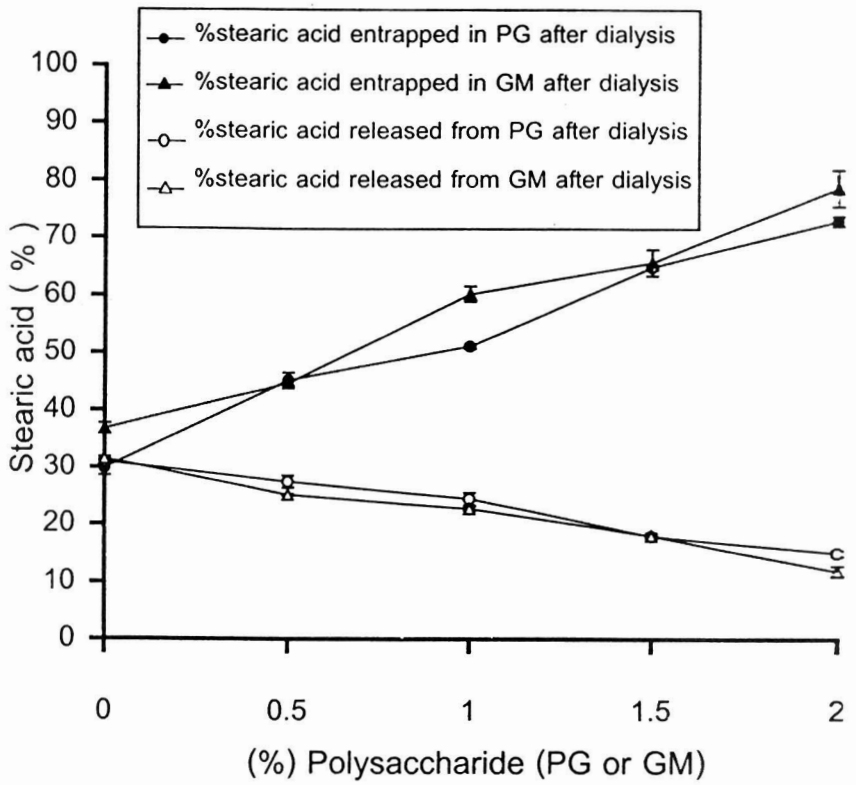


Figure 3. Effect of PG on trapping stearic acid inside dialysis membrane and releasing cholesterol outside membrane in comparison with GM at 0 - 2% conc. Dialysis time 10 hr. PG = polysaccharide gel and GM= glucomannan. Values are means with SE represented by vertical bars.

The relationship between viscosity and lipid entrapment property of PG.

There are many possible chemical and physical properties which contribute to lipid entrapment property of dietary fiber. There is a report showed that the cholesterol-lowering effect of glucomannan is due to an increase in the viscosity of intestinal contents[13]. To evaluate the effect of viscosity on lipid entrapment property of PG, the viscosity of PG at different concentrations was measured, and the relationship between viscosity and lipid entrapment property of PG was evaluated. The results showed that higher viscosity of PG could increase trapped lipid (cholesterol, oleic acid and stearic acid) inside membrane after 4,10 and 16 hours of dialysis. The higher the viscosity of PG, the larger the amount of lipid (cholesterol, oleic acid and stearic acid) trapped inside membrane (Fig. 4-6).

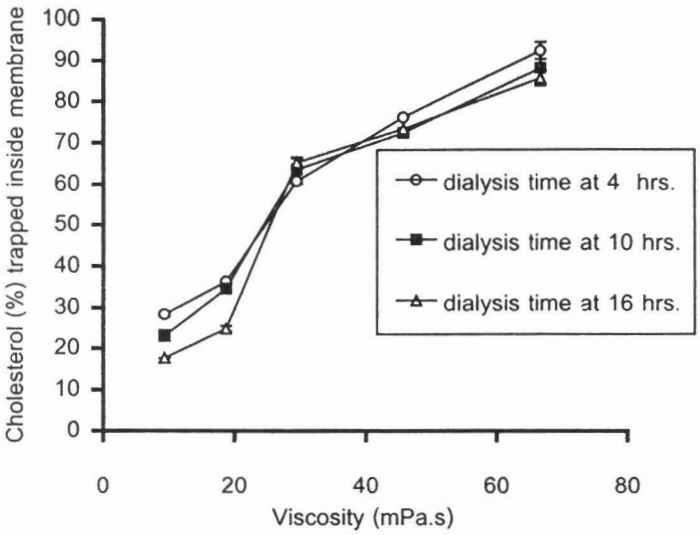


Figure 4. Effect of viscosity of PG on trapping cholesterol inside dialysis membrane after dialysis for 4 – 16 hrs. PG=polysaccharide gel. Values are means with SE represented by vertical bars.

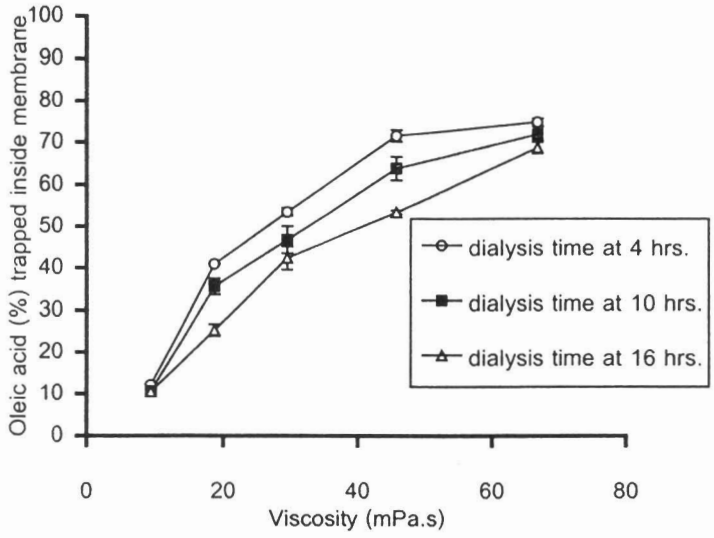


Figure 5. Effect of viscosity of PG on trapping oleic acid inside dialysis membrane after dialysis for 4 – 16 hrs. PG=polysaccharide gel. Values are means with SE represented by vertical bars.

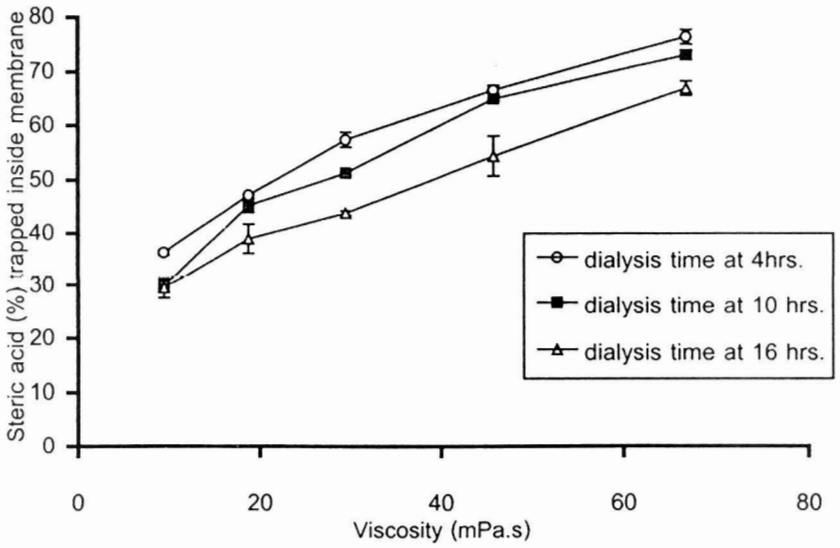


Figure 6. Effect of viscosity of PG on trapping stearic acid inside dialysis membrane after dialysis for 4 – 16 hrs. PG=polysaccharide gel. Values are means with SE represented by vertical bars.

Discussion and conclusion

In this study, lipid entrapment property of durian polysaccharide gel was compared with glucomannan. All tested lipid including cholesterol, oleic acid and stearic acid appeared to be trapped within PG and glucomannan, low amount of tested lipids was released outside dialysis membrane. Increasing concentration of both fibers resulted in increasing trapped lipids and decreasing released lipids outside membrane. The effect of PG from durian on trapping lipids is similar to glucomannan, a well studied soluble fiber which showed property of lipid lowering effects. It is possible that the cholesterol – lowering effect of glucomannan is due to an increase in the viscosity of intestinal contents. Increased intestinal contents supernatant viscosity is highly correlated with reduced plasma and liver cholesterol[14,15] and reductions in cholesterol absorption[16] in hamsters. Polysaccharide gel extracted from fruit-hulls of durian showed swelling property and forming a viscous layer in water as a soluble fiber, thus the viscosity of PG may be a possible explanation for lipid entrapment ability of PG. So the relationship between viscosity and lipid entrapment property of PG was also evaluated. A significant relationship was observed between increasing of trapped lipid inside membrane with

respect to increasing viscosity of PG. These results suggest that PG lowered released lipids by a viscosity-mediated interference of lipids releasing outside membrane. The results also suggest potential use of durian polysaccharide gel as a supplement for the treatment of hyperlipidemic patients.

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