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Evaluation of *in vitro* lipid-lowering properties of 'Saba' banana [*Musa acuminata x balbisiana* (BBB group) 'Saba'] peel pectin from different extraction methods

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ABSTRACT

Introduction: This study was conducted to investigate the *in-vitro* lipid-lowering properties of 'Saba' banana peel pectin (SBP) extracted using three methods for its possible use as a dietary fibre ingredient. **Methods:** Pectin from 'Saba' banana peels were extracted using acid extraction (citric acid), enzymatic extraction (cellulase), and microwave-assisted extraction. *In-vitro* lipid-lowering assays were performed using spectrophotometry for pancreatic lipase inhibition and cholesterol binding, while liquid chromatography was used for bile acid-binding capacity. **Results:** Results revealed that all SBPs were not able to inhibit pancreatic lipase activity. However, all SBPs can notably bind to cholesterol and bile acids, taurocholate, and glycocholate. Acid-extracted pectin had the highest binding capacity to cholesterol (51.36%–55.07%) and glycocholate (27.37%), whereas all SBPs were similarly bound to taurocholate. **Conclusion:** The results of this study showed that acid-extracted SBPs can significantly bind to cholesterol and bile acids, glycocholate and taurocholate, thereby indicating a possible reduction in lipid metabolism.

Keywords: saba, pectin, pancreatic lipase inhibition, cholesterol-binding capacity, bile acid binding capacity

INTRODUCTION

Obesity and weight gain in this generation has become a global concern. According to the World Health Organization (WHO), the prevalence of overweight was 22.3%, while obesity was 4.7% in the Philippines in 2016. These may lead to the development of non-communicable diseases, such as diabetes and cardiovascular problems. Drugs have been developed to treat obesity, but some were withdrawn from the market because of various side effects (Kang & Park, 2012).

There is immense interest in the field of 'natural' products, such as dietary fibres, for the prevention of obesity and weight gain. Dietary fibre has been

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known to reduce the absorption of fat and produce a feeling of satiety, thereby reducing caloric intake (Bordicchia *et al.*, 2014).

Pectin is a soluble fibre that has a wide function as a food ingredient. Its lipid-lowering activities and possible mechanisms had also been studied. Pectin influences satiety because of its high viscosity and ability to form gels in aqueous environments. Its action on pancreatic lipase and binding of bile acid and cholesterol has been shown, thus lowering absorbed fats and increasing cholesterol and fat in faecal excretion (Adam, 2015).

Commercial pectin powders are sourced from citrus and apple pomace. In the Philippines, 'Saba' banana peel was found to be a good source. It has high pectin content and a relatively high yield (Castillo-Israel *et al.*, 2015).

'Saba' banana is second in terms of varietal production in the country, contributing to 28.2% of total banana production during the second quarter of 2020 according to the Philippine Statistics Authority. 'Saba' banana is utilised for domestic consumption and processed into chips and catsup. Banana peels constitute around 40% of the banana fruit, which is discarded as waste. This study focuses on the use of 'Saba' banana peel pectin as a natural lipid-lowering agent. As one of the many stabilisers used in the food industry, pectin can have more advantages over others because of its potential lipid-lowering property, contributing to possible actions against obesity and weight gain.

This study evaluated the *in-vitro* lipid-lowering properties of pectin extracted from 'Saba' banana peel wastes for its possible use as a dietary fibre ingredient. Specifically, it aimed to evaluate and compare pectin from 'Saba' banana peels extracted by acid (citric acid), enzyme (cellulase), and microwave in terms of *in-vitro* lipid-lowering properties, such as pancreatic lipase inhibition, cholesterol-binding capacity, and bile acid-binding capacity.

The study was only limited to the three indicators of lipid-lowering mentioned. In the bile acid binding assays, only two bile acids were used, namely sodium taurocholate and sodium glycocholate. The pectins were not subjected to *in vitro* digestion prior to analyses of the lipid-lowering properties. The pectins were extracted from unripe 'Saba' banana peels generated from a banana chips processing plant where the unripe form was used.

MATERIALS AND METHODS

'Saba' banana peel pectin extraction

Preparation of 'Saba' banana peel powder followed the methods of Castillo-Israel *et al.* (2015). Peels of green mature unripe 'Saba' banana were sliced into approximately 2 x 2 cm pieces and soaked in 0.05% sodium metabisulfite for 1 hour. The peels were oven-dried at 55°C for 24 hours, cooled at ambient temperature, and ground into flour until it can pass through a no. 80 mesh, then stored in polyethylene bags.

Acid extraction

Ten grams of the dried 'Saba' banana peels were mixed with 250 mL distilled water, and 0.50 N hydrochloric acid (HCl) was added until pH of 1.5 was reached. The mixture was heated with continuous stirring at 90±5°C in a stirring hot plate for 4 hours. The solution was filtered using a 1-mm mesh screen with two layers of cheesecloth. The filtrate was added with absolute ethanol at 1:2 (v/v). The precipitate was filtered through a miracloth. The collected residue (pectin) was washed with aqueous ethanol (75%), followed by absolute ethanol, then oven-dried for 5 hours at 50°C.

Enzymatic extraction
The powder was swelled using distilled water (8.0 mL/g 'Saba' banana peel powder) at room temperature. For the enzyme solution, cellulase, with an initial concentration of 200 to 400 U/mL was used to extract pectin. One hundred mL of the enzyme was diluted with 50 mL distilled water. Then, 100 mL of the diluted sample was added with distilled water to a final volume of 600 mL to make the enzyme solution.

Extraction of pectin
Enzyme solution was added to the swollen sample at 8.0 mL/g peel powder and extracted at 41–50°C for 3 hours. The solution was filtered using cheesecloth, then the filtrate was collected and weighed. The filtrate was heated to 50°C for 1 hour to deactivate the enzyme. Pectin was precipitated by slowly adding absolute ethanol to the filtrate at a ratio of 1:2 (v/v) with stirring for 1 hour. After standing, the precipitate was washed with 95% ethanol. Pectin was then collected and oven-dried at 50°C for 5 hours.

Microwave-assisted extraction
'Saba' banana peel powder was weighed and placed in a 250-mL beaker. Diluted HCl solution with pH 3.0 was added to the powder in a solid-liquid ratio of 8.0%. The mixture was then placed in a rotating disc of the microwave digester (Ethos UP, High-Performance Microwave Digestion System, Torre Boldone, Italy) and heated to 195°C for 60 seconds. Microwave power was set at 1,000 W. The mixture was cooled to room temperature and filtered using a Grade 1 qualitative filter paper (11 µm). The filtered extract was slowly added with an equal volume of 95% (v/v) ethanol with continuous mixing. The mixture was allowed to stand for 2 hours at 4°C. The coagulated pectin mass was filtered and washed twice with 95% (v/v) ethanol. The residue was oven-dried at 40°C until constant weight.

Pectin yield
Pectin yield was calculated for each extraction method using the formula:
Pectin yield (%), dry basis = P/Bi x 100
Where, P = extracted pectin, g
Bi = weight of alcohol-insoluble-residue, g

***In-vitro* assays for the lipid-lowering activity of pectin**
All sample preparations were done at the Institute of Food Science and Technology, University of the Philippines Los Baños (UPLB), while analyses were done at the Institute of Chemistry, UPLB. All chemicals and reagents were analytical reagent grade and purchased from Thermo Fisher Scientific, USA.

Pancreatic lipase inhibition assay
Methods of Chedda *et al.* (2016) with modifications were used.

Sample preparation
Pectin powders (0.05 g) from different extraction methods were dissolved in 30 mL 100 mM phosphate buffer with pH of 7.2 in a 50-mL beaker. The pectin solution was heated to 50°C with continuous stirring for 30 minutes. After heating, the pectin solution was transferred to a 50-mL volumetric flask and added with 100 mM phosphate buffer to a volume of 50 mL. This served as the stock solution (1000 µg/mL). Concentrations of 20, 40, and 60 µg/mL were prepared from the stock solution.

The positive controls were commercial citrus pectin and Orlistat. Exactly 0.05 g of citrus pectin was dissolved in 50 mL 100 mM phosphate buffer as the stock solution. For the Orlistat, 0.05 g was dissolved in 50 mL dimethyl sulfoxide (DMSO).

Buffer solution
100 mM phosphate buffer was prepared with 0.5% (v/v) of Triton-X-100. The solution pH was adjusted to 7.2.

Enzyme solution
The porcine pancreatic lipase enzyme solution was prepared by dissolving 6 mg porcine pancreatic lipase in 10 mL 100 mM phosphate buffer by gentle vortexing.

Substrate solution
The substrate used was *p*-nitrophenylbutyrate (PNPB). PNPB working solution was prepared using 8.493 µL of PNPB. The solution was made up to 10 mL with acetonitrile.

In-vitro pancreatic lipase inhibition assay
The total assay volume was 200 µL. Approximately 25 µL of test solution or positive control was added with 50 µL of the enzyme solution, 100 µL of the buffer solution, and 25 µL of PNPB solution in a 96-well microplate and mixed thoroughly. Blank was made by substituting the test solution with 100 mM phosphate buffer. The reaction was allowed to stand for 30 minutes at 37°C. Lipase activity was determined by measuring the hydrolysis of PNPB to *p*-nitrophenol at 400 nm using a microplate plate reader (Multiskan™ GO, Waltham, MA, USA). Percent inhibition was calculated using the formula:

$$\%\ \text{inhibition} = \frac{|\text{Absorbance of blank} - \text{absorbance of test}|}{\text{Absorbance of blank}} \times 100$$

Cholesterol-binding capacity
Methods of Boungoura, Wenshui & Jiali (2009) were employed with modifications.

Sample preparation
Pectin powders (0.05 g) from different extraction methods were dissolved in 30 mL 15 mM phosphate buffer with pH of 7.4 in a 50-mL beaker. The pectin solution was heated to 50°C with continuous stirring for 30 minutes. After heating, the pectin solution was transferred to a 50-mL volumetric flask and added with 15 mM phosphate buffer to a volume of 50 mL. This pectin solution (1000 µg/mL) was used as the stock solution from which 20, 40, 60, 80, and 100 µg/mL were prepared.

The positive controls were commercial citrus pectin and cholestyramine. Citrus pectin (0.05 g) and cholestyramine (0.05 g) were dissolved in 50 mL 15 mM phosphate buffer as the stock solution.

Cholesterol micellar solution
Reagents used were analytical grade bought from Sigma-Aldrich (St. Louis, MO, USA). Cholesterol micellar solution containing 10 mM sodium taurocholate, 0.4 mM cholesterol, 1 mM oleic acid, 132 mM NaCl, and 15 mM phosphate buffer (pH 7.4) was sonicated for 20 minutes.

In-vitro cholesterol micellar solubility inhibition assay
Approximately 450 µL of the cholesterol micellar solution was added with 450 µL of sample. The solutions were incubated at 37°C for 24 hours. Then, the solutions were centrifuged for 10 minutes. The supernatant was collected and used for cholesterol concentration determination.

From the supernatant, 80 µL of each sample was pipetted into a 96-well microplate. Then, 100 µL of glacial acetic acid was added, followed by 120 µL Zak's reagent and mixed thoroughly. For the control, 80 µL of cholesterol micellar solution was added instead of the sample. The solution was further incubated for 15 minutes at room temperature, and absorbance was measured at 560 nm using a microplate reader.

Standard calibration curve
Cholesterol standards in glacial acetic acid with the following concentrations: 20, 25, 30, 35, 40, and 45 µg/mL were

prepared and absorbance was read at 560 nm.

Bile acid binding assay

Bile acid binding was performed according to the methods of Kongo-Dia-Moukala *et al.* (2011) with modifications.

Sample preparation

Pectin powders (0.002 g) from different extraction methods were dissolved in 60 mL 50 mM phosphate buffer with pH of 6.5 in a 50-mL beaker. The pectin solutions were heated to 50°C with continuous stirring for 30 minutes. After heating, the pectin solutions were transferred to a 100-mL volumetric flask and added with 50 mM phosphate buffer to a volume of 100 mL. The positive controls used were commercial citrus pectin and cholestyramine. Positive controls were prepared by dissolving 0.002 g of the powder in 100 mL 50 mM phosphate buffer.

Bile acid solution

The bile acids used for this assay were sodium taurocholate and sodium glycocholate. Two millimolar of each bile acid was prepared in 50 mM phosphate buffer of pH 6.5. The control that was used to calculate bound bile acid was 2 mM bile acid without sample.

In-vitro bile acid binding assay

Solutions of 1 mL of 2 mM bile acid solution and 1 mL of each sample were prepared. The solution was incubated for 1 hour at 37°C in an oven incubator. The solution was centrifuged for 20 minutes. The supernatant was collected, filtered with a 0.45-micron filter, and transferred into UPLC vials. After the reaction, unbound taurocholate and glycocholate were measured using Ultra Performance Liquid Chromatographic (UPLC) with a BEH C18 column (2.1 x 50mm, 1.7 µm) (ACQUITY H Class UPLC System, Prague, Czech Republic).

Standard calibration curve

Bile acid solutions with the following concentrations: 0, 0.4, 0.8, 1.2, 1.6, and 2.0 mM were prepared. Peak area at 210 nm was recorded using UPLC. Assays using taurocholate and glycocholate were performed in separate experiments.

Ultra-Performance Liquid Chromatography (UPLC)

Three injections of 3 µL of the sample supernatant or bile acid standard (sodium taurocholate or sodium glycocholate) were injected into the UPLC. The sample was eluted with methanol: 0.4% KH_2PO_4 (65:35) at a flow rate of 0.15 mL/min for three minutes. Peak area at 210 nm was recorded and used to calculate the concentration of unbound bile acids. The concentration of unbound bile acids was calculated from the standard calibration curve. Percent bound bile acids were calculated:

$$\% \text{ bound bile acid} = \frac{Cc\text{-}Cs}{Cc} \times 100,$$

where C_c = concentration of bile acid in control

C_s = concentration of bile acid in the samples

Statistical analysis

All data obtained were expressed in mean±standard deviation (*SD*) and were subjected to one-way analysis of variance (ANOVA) at $p \leq 0.05$, followed by Tukey's Honest Significant Difference (HSD) test at $p \leq 0.05$. Statistical analyses were performed using SPSS Version 25.0 (SPSS, Chicago, IL, USA).

RESULTS

Acid extraction of pectin produced the highest yield at 20.02%, followed by microwave-assisted extraction (14.22%), while enzymatic extraction had the lowest yield (6.18%). All pectins were fine powders and of the same brown

colour (Figure 1) with a distinct smell after drying and grinding, which was similar to pectins extracted using acids by Castillo-Israel *et al.* (2015) from 'Saba' banana peel.

The action of 'Saba' banana peel pectin (SBPs) extracted using different methods, citrus pectin (CP), and Orlistat (OS) was investigated using the UV-Visible spectrophotometry method (Chedda *et al.*, 2016). The substrate, *p*-nitrophenylbutyrate, was reacted with porcine pancreatic lipase and pectin at 37°C. The activity of lipase was measured by the absorbance of the hydrolysis product, *p*-nitrophenol, at 400 nm. Pancreatic lipase inhibition was measured by percent difference in absorbance of reaction without pectin.

All SBPs showed lower inhibition compared to CP and OS at all concentration levels (Figure 2). At 20 μg/mL, SBP inhibited lipase activity in the range of 11.43%–14.19%. CP and OS had a peak percent inhibition of 57.52% and 46.10%, respectively, which were not significantly different ($p \leq 0.05$). On the basis of concentration, there was no trend in the inhibition of lipase activity among SBPs. In contrast, the inhibition action of CP and OS were decreasing as concentration increased.

For cholesterol-binding capacity, SBPs were incubated with the cholesterol micellar solution composed of cholesterol, bile acid, fatty acid, and buffer at pH 7.4. Cholesterol-binding capacity was measured by the percent difference of cholesterol concentration from cholesterol micellar solution alone and that incubated with pectin. Generally, the effect of concentration varied among different SBPs, CP, and cholestyramine (CH). For the standard CH, comparable binding was observed at concentrations of 20, 60, 80, and 100 μg/mL, whereas binding was slightly lower at 40 μg/mL (Table 1). For CP, at 60 μg/mL, binding was slightly lower compared to the rest of the concentration levels. For AP, similar binding was observed at 40–80 μg/mL, and slightly lower binding at 20 and 100 μg/mL. For EP, all concentration levels showed no significant difference in binding. Lastly, for MP, slightly lower binding was observed at 40 and 60 μg/mL ($p \leq 0.05$).

The comparison of binding activity among different SBPs, CP, and CH is also shown in Table 1. AP had the highest cholesterol-binding activity (51.36%–55.07%), which was statistically comparable to CP (49.06%–53.02%) and CH (45.65%–52.43%). EP (41.69%–45.82%) and MP (39.93%–45.01%) showed similar binding, but were significantly lower than AP ($p \leq 0.05$). Nevertheless, the binding action of SBPs

Figure 1. Pectin samples extracted from 'Saba' banana peels using various extraction methods. (L-R: acid-extracted, enzyme-extracted, microwave-assisted extracted pectin)

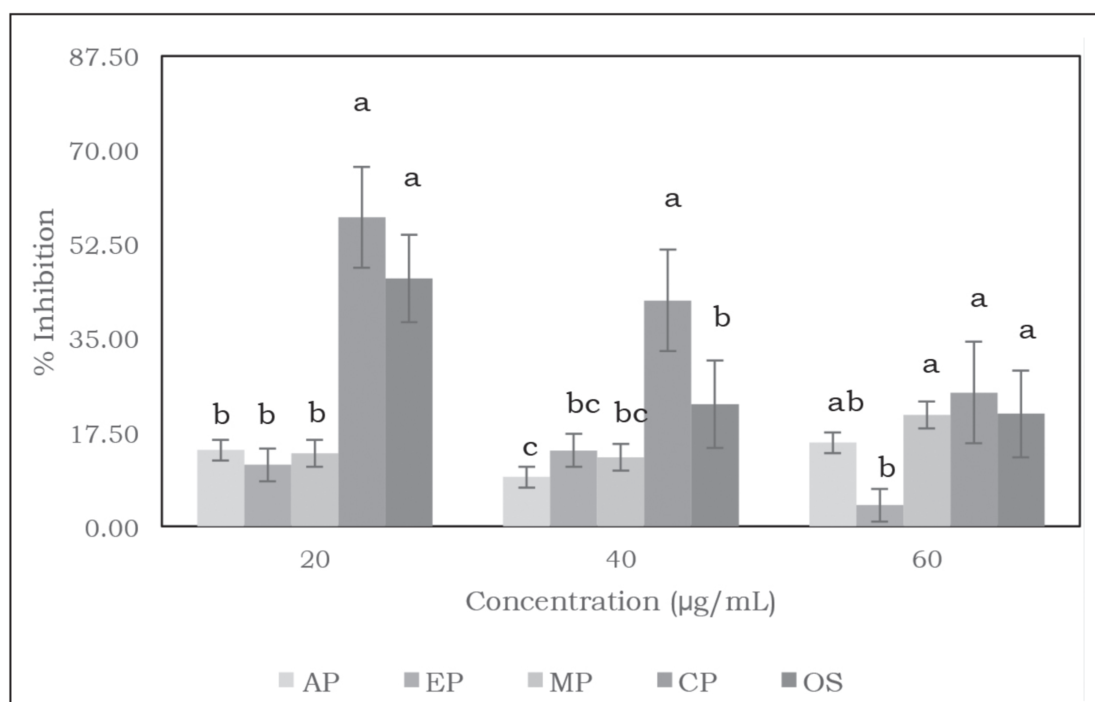

Figure 2. Pancreatic lipase inhibition of 'Saba' banana peel pectin extracted using three different methods. AP=acid extraction; EP=enzymatic extraction; MP=microwave-assisted extraction; CP=commercial citrus pectin; OS=Orlistat

on cholesterol was comparable to CP or CH.

The capacity of SBPs to bind to two bile acids, namely taurocholic and glycocholic acids, in comparison to commercial pectin and cholestyramine was examined. Figure 3 shows that all SBPs, CP, and CH were able to bind bile acids at concentration of 20 μg/mL. Further, taurocholate bound to pectin better than glycocholate. For taurocholate binding, CH had the highest

Table 1. Cholesterol binding capacity of 'Saba' banana peel pectin extracted using different methods at varying concentrations

Samples†	*Cholesterol binding capacity (%)*‡				
	20 μg/mL	*40 μg/mL*	*60 μg/mL*	*80 μg/mL*	*100 μg/mL*
AP	$52.48^{B,a}$±1.10	$53.26^{AB,a}$±1.18	$54.77^{A,a}$±0.93	$55.07^{A,a}$±1.31	$51.36^{B,a}$±1.43
EP	$44.13^{A,b}$±4.98	$41.69^{A,b}$±2.81	$43.28^{A,bc}$±0.65	$45.82^{A,cd}$±1.70	$45.55^{A,b}$±1.28
MP	$45.01^{A,b}$±2.02	$39.93^{B,b}$±1.34	$40.42^{B,c}$±2.15	$41.69^{AB,d}$±2.09	$41.79^{AB,b}$±2.09
CP	$51.16^{AB,ab}$±0.80	$53.02^{A,a}$±1.00	$49.06^{B,abc}$±1.17	$50.04^{AB,b}$±3.61	$49.99^{AB,a}$±1.80
CH	$48.33^{AB,ab}$±6.43	$45.65^{B,ab}$±3.59	$51.65^{AB,ab}$±2.33	$48.82^{AB,bc}$±2.38	$52.43^{A,a}$±2.78

†AP=acid-extracted pectin; EP=enzyme-extracted pectin; MP=microwave-assisted-extracted pectin

‡Means with the same uppercase letter within rows are not significantly different based on Tukey's HSD at 5% level of significance; means with the same lowercase letter within columns are not significantly different based on Tukey's HSD at 5% level of significance

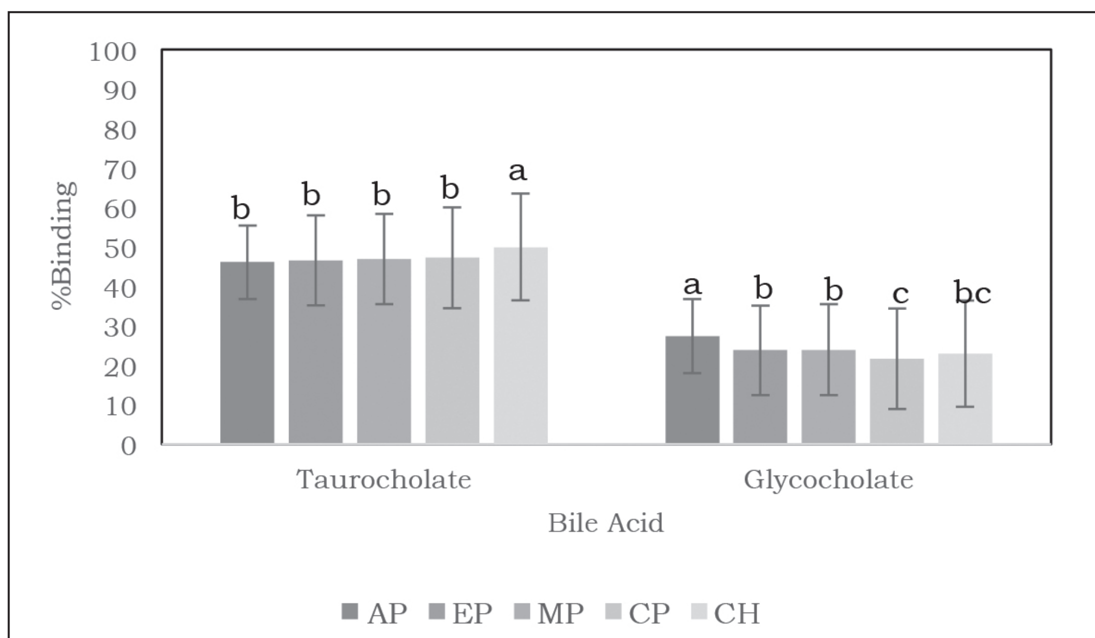

Figure 3. Taurocholate (L) and glycocholate (R) binding of Saba banana peel pectin extracted using three different methods. AP=acid extraction; EP=enzymatic extraction; MP=microwave-assisted extraction; CP=commercial citrus pectin; CH=cholestyramine

binding at 49.95%. All SBPs and CP showed similar binding to taurocholate ($p \leq 0.05$). SBP taurocholate binding was in the range of 46.10%–46.90%, which was not much difference with CH binding. On the other hand, AP had the highest binding capacity to glycocholate at 27.37%. EP, MP, and CH then followed with comparable binding at 23.81%, 23.93%, and 22.95%, respectively. These results suggested that SBP, regardless of extraction methods, could bind effectively to taurocholate and glycocholate, with more affinity of binding to taurocholate.

DISCUSSION

The pectins extracted using different methods were compared in terms of yield and lipid-lowering parameters. Previous research on the extraction of pectin from banana peel using HCl showed 16.54% (Castillo-Israel *et al.,* 2015) and 11% yields (Maneerat *et al.,* 2017). Meanwhile, using citric acid had pectin yields of 14.23% (Oliviera *et al.,* 2015) and 24.08% (Khamsucharit *et al.*, 2018). Microwave-assisted extraction of pectin from banana had previously produced pectin with a 2.58% yield (Swamy & Muthukumarappan, 2017), whereas an enzymatic extraction generated 9.33% (Hui *et al.,* 2013). Thus, acid extraction of pectin from banana peels produced the highest yield compared to other extraction methods. As expected, among the pectins, AP had the highest yield. This is due to the harsh conditions of acid extraction and temperature, which involves hydrolysis of protopectin to pectin and subsequent precipitation by ethanol, that were able to destroy the cell walls and expose the pectins. The milder extraction methods, namely enzyme and microwave-assisted extractions,

were less efficient as shown by the lower yields. These milder extractions require penetration of plant cell walls via microwave radiation or swelling by hydration (Sandarani, 2017). Swelling by water to rupture plant cell walls may not be efficiently completed considering the mild conditions of these extraction methods.

Pancreatic lipase inhibition by all pectins were relatively low compared with commercial pectins and the positive control Orlistat, and was much lesser in this study compared to previous studies. Isaksson *et al.* (1982) reported 65% and 90% lipase inhibition for low and high methoxyl pectin, where lipase inhibition seemed to be less pronounced in buffer systems than when performed in duodenal juice. Tsujita *et al.* (2003) also reported 40%–50% of lipase activity reduction at 1–5 mg/mL level of citrus pectin. A more recent study reported low methoxyl pectin extracted from apple pomace using citric acid to have 94.30% inhibition of lipase activity *in-vitro*. The lipase activity inhibition of pectin was attributed to a competitive mechanism where pectin forms a complex with lipase. The –COOH groups of pectin protonates histidine and possibly the hydroxyl group of serine at the active serine-histidine-aspartic/glutamic acid triad of lipase (Kumar & Chauhan, 2010). Furthermore, the action of pectin on lipase activity inhibition could be enhanced by high molecular weight, viscosity, and purity (Edashige, Murakami & Tsujita, 2008).

Inhibition of lipase activity by pectin is concentration-dependent. However, this study observed no trend in terms of the concentration of pectin and pancreatic lipase inhibition. Pectin levels that are commonly explored are at 0.1 to 5.0 mg/mL (Isaksson *et al.*, 1982; Tsujita *et al.*, 2003; Edashige *et al.*, 2008). Meanwhile, pectin concentrations in this study were only at 20–60 μg/mL, as permitted by the assay (Cheddah *et al.*, 2016). As the concentration used in this study was much lower, this could also be a possible reason for the observed results. This may imply that the mechanism for the possible lipid-lowering action of pectin is not by pancreatic lipase inhibition. Since the extracted SBPs were crude, the actual concentrations of pectin used in the reaction could be lower. Other components in the extracted SBPs could also interfere with the action of pectin as a lipase inhibitor.

Cholesterol-binding capacity results varied with different pectin concentrations. AP had the highest binding with cholesterol, which was comparable with commercial pectins and the positive control cholestyramine. Cholesterol-binding activity of pectin is affected mainly by pectin source and type, which includes degree of esterification and molecular weight. All the pectins in this study had high degree of esterification (>50%), which could be the reason for its high binding with cholesterol, thereby reducing cholesterol absorption by the body. Moreover, the viscosity of pectin also indirectly influences cholesterol binding (Brouns *et al.*, 2012). The concentration of pectin affects its viscosity in solution (Kar & Arslan, 1999), which suggests that increased concentration should correspond to increased viscosity. In this study, concentration effects were not apparent as shown by varying effects among samples. Furthermore, differences in cholesterol-binding capacity between concentrations were minimal. In EP and MP, concentrations as low as 20 μg/mL showed similar binding with the highest concentration, parallel to positive controls AP and CH. AP, on the other hand, had slightly lower binding at 20 and 100 μg/mL. Nevertheless, these slight differences

were still considered as significant binding of pectins to cholesterol, implying that the concentrations utilised in this study were not a substantial factor in the samples' cholesterol-binding capacity.

Studies on cholesterol-lowering capacity of pectin usually investigate the effect on mice, rats, and humans fed with high-cholesterol diets and either taking pectin alone or incorporating it in the subjects' diets. In a study by Soh, Kim & Lee (2003), an *in-vitro* experiment on pectin at a concentration of 0.1% showed that it had the highest absorbance with 2.88 mg/dL capacity (90% of total cholesterol). More recent studies on the cholesterol-lowering action of pectin that focused on *in-vivo* set-ups had proposed possible mechanisms on pectin's cholesterol-lowering action. The action of pectin on cholesterol *in-vivo* is influenced by the degree of esterification, molecular weight, and viscosity. Pectins with a high degree of esterification have shown more reduction of total cholesterol in humans (Brouns *et al.*, 2012). In animal models, highly esterified pectins also showed considerable total cholesterol reduction (Trautwein, Kunath-Rau & Erbersdobler, 1998; Dongowski & Lorenz, 2004). Moreover, pectins with low molecular weight, low viscosity, and high solubility were found to be less effective in lowering total cholesterol (Yamaguchi *et al.*, 1995). However, in this study, AP, which has a relatively intermediate degree of esterification of 51.09% and lower equivalent weight showed greater cholesterol reduction than pectins with high degree of esterification and equivalent weight - EP and MP. This effect could be attributed to the viscosity of AP. Upon dissolving the pectins in a buffer, the AP solution was more viscous than EP and MP. Furthermore, AP has an anhydrourinic acid content of 50.85%, indicating that it had much higher purity than EP and MP. The presence of other molecules could affect the action of pectin on cholesterol binding.

The capacity of SBPs to bind to cholesterol solubilised in cholesterol micellar solubility (CMS) is an important parameter in measuring the lipid-lowering capability of SBPs. CMS created for the assay mimics the micelle, which is composed of bile acids, fatty acids, and monoglycerides that are supposedly liberated from triacylglyceride hydrolysis by lipase and dietary cholesterol (Lehninger, Nelson & Cox, 2008). This study showed significant binding of cholesterol, which prevents it from being soluble in the micelle. These micelles are vital to lipid metabolism because they serve as carriers of both dietary cholesterol and fatty acids for absorption in the intestines. Once cholesterol is not solubilised in the micelle, it forms a separate oil phase in the intestinal lumen and becomes unavailable for absorption (Jesch & Carr, 2017). All extracted SBPs had high degree of esterification (>50%), which supports literatures' claims that they can reduce cholesterol absorption. Pectins with high degree of esterification are related to the chelation of bile acids and eventually increased lumen viscosity, which has also been associated with reducing cholesterol absorption (Brouns *et al.*, 2012).

In the digestive system, one possible mechanism for the cholesterol-lowering property of pectin is the increased lumen viscosity, leading to a reduced rate of glucose diffusion and absorption in the small intestine. This effect consequently causes a decrease in insulin production. Reduced insulin then decreases the activity of 3-hydroxy-3-methylglutaryl CoA enzyme, which is responsible for the synthesis of cholesterol (Celus *et al.*, 2018). However, *in-vitro* results from this study suggested that pectin itself binds to cholesterol by lowering the actual concentration of cholesterol in the micelle solution.

Bile acids are a group of amphiphilic molecules synthesised from cholesterol, which is naturally produced by the body for their role in digestion, transportation, and absorption of dietary lipids in the gastrointestinal tract (Lopez-Pena, Arroyo-Maya & McClements, 2019). Bile acids have a plate-like structure with polar and non-polar sides, which enable them to adsorb to oil-water interfaces (Cabral & Small, 2010). In lipid digestion, bile acids adsorb to the surfaces of lipids in gastrointestinal tract, breaking it down into smaller sizes with greater areas to act upon by lipase (Euston, 2017). Furthermore, bile acids are also an integral part of the micelle that solubilise and transport triglyceride hydrolysis products into the epithelium cells (Lopez-Pena *et al.*, 2019).

All samples had good binding with bile acids at the lowest concentration, 20 ug/mL. All pectins had better binding with taurocholate compared to glycocholate, with values statistically similar to commercial pectins and cholestyramine. Previous *in-vivo* studies confirmed the binding of pectin to cholesterol by the increase in bile acid excretion in the faeces (Dongowksi & Lorenz, 2004; Brouns *et al.*, 2012). An *in-vitro* study also showed the binding of pectin with high molecular weight and low degree of esterification to cholic acid (Rubio-Senent *et al.*, 2015). Binding to bile acids is one possible mechanism in the hypocholesterolemic property of pectins. Increased excretion of bile acid in faeces of subjects fed with pectin indicated that less bile acid was available, thus promoting the conversion of cholesterol to bile acid. This conversion eventually reduces cholesterol levels (Fang *et al.*, 2018).

Pectin molecules are negatively-charged in neutral pH due to their linear anionic regions of galacturonic acids. Similarly, bile acids are also negatively charged. Nevertheless, both molecules have non-polar groups that may interact with each other via hydrophobic forces. For instance, the non-polar region of bile acids may be bound to the non-polar methyl groups of pectin (Lopez-Pena *et al.*, 2019). Aside from hydrophobic interactions, increased bile acid excretion *in-vivo* was associated with gel formation and viscosity effects in the gastrointestinal tract (Dongowski, 2007). Since pectin was incubated with either sodium taurocholate or sodium glycocholate, the results of this study suggested that SBPs were able to bind to bile acid itself without the influence of environmental conditions in the gastrointestinal tract.

This study had demonstrated the possible lipid-lowering mechanisms of pectin and had also compared different extraction methods in terms of the properties of the pectins extracted from 'Saba' banana peels. The pectin samples tested were limited to the crude form, which have already shown positive responses to cholesterol and bile acid binding. Further purification of the samples can possibly show higher lipid-lowering capacities.

CONCLUSION

The possible mechanisms of lipid-lowering action by SBPs were cholesterol and bile acid binding. AP was the most effective in binding cholesterol and bile acids. MP and EP showed similar binding, but were slightly lower than AP. Acid extraction was observed to be the best method, as it resulted in pectin with the highest purity and best lipid-lowering activity, with significant binding to cholesterol, taurocholate, and glycocholate.

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Authors' contributions

EAGM, principal investigator, prepared the research design and methods for the *in vitro* experiment, performed the experiments, analysed the data, and prepared the draft of the manuscript; GPJV, extracted the pectin powder using acid extraction methods and helped in the *in vitro* experiments; RJP, optimised and performed the pectin extraction using microwave-assisted methods; VJC, assisted in the measurement of *in vitro* lipid-lowering activity using ultra high pressure liquid chromatography; TMAO, provided the laboratory space and equipment for the *in vitro* experimentation and reviewed the manuscript; CKAT, project leader, obtained the project funding, conceptualised the entire study, supervised all the activities and reviewed the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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