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Comparative study on chemical and emulsion properties of 'Saba' banana [*Musa acuminata* x *balbisiana* (BBB group) 'Saba'] peel pectin from different extraction methods

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Abstract

This study was conducted to investigate the chemical characteristics and emulsion properties of pectin from 'Saba' banana peel extracted using three different methods, namely, acid extraction using citric acid, enzymatic extraction using cellulase, and microwave-assisted extraction. Per cent yield, moisture and ash contents, equivalent weights, methyl and anhydrouronic acid contents, and the degree of esterifications were determined for each pectin extracted using the three different methods. Characterization of the extracted pectin revealed that all 'Saba' peel pectins (SBPs) had a high degree of esterification. Furthermore, acid-extracted pectin (AP) had the highest yield of 20.02% and had the highest purity, as indicated by the lowest ash content and equivalent weight (784.57), and highest anhydrouronic acid content (50.85%) compared to enzymatic (EP) and microwave-assisted extraction (MP). Scanning Electron Microscopy revealed smoother images of AP, which indicated that AP was more degraded compared to milder extraction methods, such as microwave-assisted extraction which yielded the least degraded pectin. Furthermore, emulsion formation and stability using SBPs were determined by measuring cream fraction. Results showed that all SBPs had cream fractions comparable to the control, and there was no difference among extraction methods. Microscopic observations (10×) showed that oil droplet size decreases with increasing concentration. Further, AP had smaller droplets, indicating that AP is capable of forming more stable emulsions compared to EP and MP. Results of this investigation revealed that, although all SBPs were highly esterified, the three extraction methods yielded pectins with different characteristics and purity.

1. Introduction

Pectin is a soluble fibre with a wide function in food, including gelling agents for jams and jellies and stabilizers for dairy products. Several studies have also discovered pectin's health benefits in cancer prevention (Leclere *et al*., 2013). Currently, commercial pectin powders are sourced out from citrus and apple pomace. In the Philippines, one agricultural waste material that can be a potential pectin source is 'Saba' banana peel, which generally has a high pectin content (Castillo-Israel *et al*., 2015). The extraction process can be optimized to get pectin's maximum yield (Maneerat *et al*., 2017).

'Saba' banana is second in terms of varietal production in the country, contributing to 29% of total banana production during the fourth quarter of 2020 (Philippine Statistics Authority, 2020). Cavendish, the most produced banana variety, is commonly exported, while 'Saba' banana is utilized for domestic consumption and industrial banana processing, such as banana chip and banana catsup production (Rivera, 2004). Moreover, peels constitute around 40% of the banana fruit. However, they are readily discarded as waste in the banana processing industry, when in fact, this waste material still contains high-value components such as pectin.

Pectin can be extracted using various extraction methods. The three most common forms of extraction include acid extraction, enzymatic extraction, and microwave-assisted extraction. In the acid extraction

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method, acids are used to separate pectin from its plant source. This method usually produces the highest yield. Enzymatic extraction, on the other hand, utilizes enzymes that hydrolyze plant components except for pectin. Lastly, microwave-assisted extraction exposes the plant material to microwaves to facilitate faster extraction of pectin. This method is the fastest and produces the highest quality pectin at a low cost.

The chemical properties of pectin are important determinants of its quality, purity, and possible applications. In this study, moisture and ash content and equivalent weight, anhydrouronic acid, and ultrastructure analysis were measured to assess the quality and purity of extracted pectin. Meanwhile, methoxyl content and degree of esterification are pectin characteristics that determine the functionality of the extracted pectin.

The application of pectin as emulsifiers and emulsion stabilizers has been explored and studied (Ngouémazong *et al*., 2015). Many factors have been found to influence emulsifying and emulsion-stabilizing pectin action, including structure, identities of side chains attached, polymer concentration, ionic strength, and pH (Nakauma *et al*., 2008; Siew and Williams, 2008). In recent studies, pectin has shown activities on the surface of the oil-water interface, facilitating the formation and stabilization of oil droplets during and after emulsification (Rivadeneira *et al*., 2020). In food, emulsifiers are used to homogenize immiscible food components such as oil and water. It is used as a food additive to improve palatability, maximize volume and aeration of some food items, reduce stickiness, enhance food flavour, improve texture, and impart foam stability (Msagati, 2013). Some food products that use emulsifiers are mayonnaise, ice cream, bread, and noodles.

The general objective of this study was to analyze and compare the chemical characteristics, and emulsion properties of pectin extracted from 'Saba' banana peel wastes extracted using three methods: acid extraction, enzymatic extraction using cellulase, and microwave-assisted extraction. This information will be relevant in developing 'Saba' banana peel pectin as an emulsifier in food applications.

2. Materials and methods

2.1 'Saba' banana peel pectin extraction and characterization

Pectin extraction was done three times for each extraction method.

2.1.1 Preparation of 'Saba' banana peel powder

The preparation of 'Saba' banana peel powder followed the methods of Castillo-Israel *et al.* (2015). Unripe mature banana peel wastes were sliced into approximately 2×2 cm pieces and soaked in 0.05% sodium metabisulfite for an hour. Then, the peels were oven-dried at 55°C for 24 hrs. The dried peels were then cooled at ambient temperature and ground into flour using a grinding mill until it can pass through no. 80 mesh. The powdered banana peels were stored in polyethylene bags and placed in a desiccator.

2.1.2 Acid extraction

A total of 10 g of the dried 'Saba' banana peels were mixed with 250 mL distilled water, and 0.50 N HCl was added until a pH of 1.5 was reached. The mixture was then heated with continuous stirring at 90±5°C in a stirring hot plate for 4 hours. After heating, the solution was filtered using a 1-mm mesh screen with two layers of cheesecloth. The filtrate was collected and added with absolute ethanol in the ratio of 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 to remove impurities. The pectin was then oven-dried for 5 hrs at 50°C. Pectin yield was calculated using Equation (1):

$$Pectin\ yield\ (\%), dry\ basis = \frac{P}{Bi} \times 100 \quad (1)$$

Where, P = extracted pectin in grams and Bi = weight of alcohol-insoluble-residue (AIR) in gram.

2.1.3 Enzymatic extraction

2.1.3.1 Hydrolysis of 'Saba' banana powder

'Saba' banana peels were taken out of the desiccator at least 24 hrs prior to extraction for swelling. The powder was swelled using distilled water (8.0 mL/g 'Saba' banana peel powder) at room temperature.

2.1.3.2 Preparation of enzyme solution

Cellulase with an initial concentration of 200 to 400 units/mL was used to extract pectin. Apporximately 100 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. The enzyme solution was stored in a refrigerator at a chilling temperature until use.

2.1.3.3 Extraction of pectin

Enzyme solution (8.0 mL per gram 'Saba' banana peel powder) was added to the hydrolyzed sample. Extraction was conducted at 41–50°C for 3 hrs. Then, the solution was filtered using an ordinary cheesecloth, and the filtrate was collected and weighed. The filtrate was then subjected to heat at 50°C for 1 hr to deactivate the enzyme. Precipitation of pectin was performed by slowly adding absolute ethanol to the filtrate at a ratio of

1:2 (v/v). The solution was stirred using a magnetic stirrer for 1 hr and allowed to stand at room temperature to precipitate the pectin. The precipitate was washed with 95% ethanol to remove impurities. Pectin was then collected and oven-dried at 50°C for 5 hrs. The weight of pectin was measured and calculated using Equation 1.

2.1.4 Microwave-assisted extraction

'Saba' banana peel powder was weighed and placed in a 250-mL beaker. Diluted HCl solution with pH 3 was added to the powder in a solid-liquid ratio of 8.0%. The mixture was then placed in a microwave digester rotating disc (Ethos UP, High-Performance Microwave Digestion System, Torre Boldone, Italy) and heated to 195°C for 60 s. Microwave power was set at 1,000 W. After extraction, the mixture was cooled to room temperature and filtered using 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 two hours at 4°C. The coagulated pectin mass was filtered from the mixture and washed twice with 95% (v/v) ethanol to remove the remaining sugars. The wet residue was oven-dried at 40°C until a constant weight was achieved. Pectin yield was calculated using Equation 1.

2.1.5 Characterization of extracted pectin

Extracted pectins were pooled based on the extraction method used prior to characterization. All chemical characteristic measurements were done in triplicates.

2.1.5.1 Moisture content (MC)

Moisture content determination was done based on the Official Methods of the Association of Official Analytical Chemists (AOAC International, 2002). Three trials, each with 0.5 g of the extracted pectin from each extraction method, were weighed and ground to pass through an 80-mesh screen. The samples were dried in an oven for five hours at 105°C and then cooled in a desiccator. The dried samples were then weighed. Drying was repeated until a constant weight was obtained. Moisture content was determined using the equation:

$$MC\ (\%) = \frac{weight\ of\ sample\ -\ weight\ of\ residue}{weight\ of\ sample} \times 100$$

2.1.5.2 Ash content (AC)

Ash content determination was done based on official methods of AOAC International (2002). Approximately 0.5 g of the extracted pectin were weighed and ground to pass through an 80-mesh screen and placed in a tared crucible. The samples were ignited in a furnace for 5 hrs at 550°C. After ignition, the samples were removed from the furnace, cooled in a desiccator, and weighed. The samples were then reignited for 30 mins until a constant weight was obtained. Ash content was calculated using the formula:

$$AC(\%) = \frac{weight\ of\ residue}{weight\ of\ sample} \times 100$$

For the subsequent analyses, methods by Owens *et al*. (1952) were followed.

2.1.5.3 Equivalent weight (EW)

About 0.5 g of the dried pectin sample were placed in a 250-mL Erlenmeyer flask, and 5 mL of absolute ethanol was added. Next, 1.0 g of NaCl, 100 mL of CO_2-free distilled water, and 6 drops of phenol red indicator were added. The solution was stirred for 10 mins until the pectin samples dissolved. The solution was then titrated with 0.1 N standard NaOH until a pH of 7.5 was reached (Titration A). The equivalent weight was calculated using the formula:

$$EW\ (eq/L) = \frac{weight\ of\ sample\ (g)\ \times 100}{Vol.og\ NaOH\ (mL) \times N\ NaOH}$$

2.1.5.4 Methoxyl content (MeC)

Approximately 0.5 g of the dried pectin were dissolved in 25 mL of 0.25 N NaOH in a 250-mL Erlenmeyer flask. The solution was covered and allowed to stand for 30 mins at room temperature. A volume of 25 mL of 0.25 N HCl was then added. The solution was titrated with 0.1 N NaOH until it reached a pH of 7.5 (Titration B). MeC was then calculated using the formula:

$$MeC\ (\%) = \frac{Vol.NaOH\ (mL) \times\ N\ NaOH\ \times\ 3.1}{weight\ of\ sample}$$

2.1.5.5 Anhydrouronic acid (AUA)

This parameter was calculated using the data collected from EW and MeC using the formula:

$$AUA\ (\%) = \frac{176\ (y+z)}{wt.of\ sample\ (mg)} \times\ 100$$

where y = meq (Titration A) = 0.1 N NaOH x vol. of NaOH (mL) used and z = meq (Titration B) = 0.1 N NaOH × vol. of NaOH (mL) used.

2.1.5.6 Degree of esterification (DE)

DE was determined using the calculated MeC and AUA using the equation below (Shaha *et al*., 2013).

$$DE\ (\%) = \frac{176\ \times MeC\ (\%)}{31\ \times AUA\ (\%)} \times\ 100$$

2.1.5.7 Ultrastructure analysis

The extracted pectins using different methods were

sent to BIOTECH Electron Microscopy Service Laboratory in the National Institute of Molecular Biology and Biotechnology, UPLB, College, Laguna. Scanning Electron Microscope (SEM) images were obtained. The surface morphology of the pectin powder particles was examined using a Tabletop Scanning Electron Microscope (Phenom XL, Thermo Scientific). Powder particles were first fixed onto double-sided adhesive carbon tape mounted on an aluminium stub and then sputter-coated with gold-palladium at a current intensity of 5 mA for 2 mins using fine coat ion sputter (JFC-1100, JEOL, Japan). The sputter-coated particles were then examined with an accelerating voltage of 15 kV.

2.2 Emulsifying property of pectin

The extracted pectin powders were dissolved in 2 mM NaCl solution with pH of 5.0 at three different concentrations (0.5%, 1.0%, and 1.5%) in triplicates. Pectin solutions and soybean oil were mixed in a ratio of 70:30. The mixtures were vortexed for 5 mins to promote emulsion formation. The emulsions were then centrifuged for 10 mins. The cream fraction was measured using the equation below. The emulsions were also observed under a microscope at a low power objective (10× magnification) to compare the appearance of oil droplets from the emulsions formed.

$$Cream\ fraction = \frac{thickness\ of\ cream\ layer}{thickness\ of\ the\ whole\ sample}$$

2.3 Statistical analyses

All data obtained were expressed in mean ± SD and were subjected to One-way Analysis of Variance (ANOVA) at $P \leq 0.05$. Significant differences among treatments observed in ANOVA were determined using 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).

3. Results and discussion

3.1 'Saba' banana peel pectin extraction and characterization

Pectin was extracted from 'Saba' banana peels using three different extraction methods. Among the extraction methods, acid extraction had the most extreme treatment conditions. It has the longest extraction time of 300 mins, heat application at 85°C, and 0.45 M citric acid, which gave the extraction solution a pH of 1.50. The method used was based on optimized conditions of acid-based extraction conducted by Castillo-Israel *et al.* (2015). Extreme treatment conditions were employed to ensure the degradation of cellulose, which is an insoluble fibre that is also present in the cell walls of 'Saba' banana peels. Meanwhile, enzymatic extraction was the mildest treatment due to a low temperature of 50°C and no acids. Microwave-assisted extraction was intermediary with the highest temperature of 195°C and has used acid, but extraction time was done rapidly for 1 min.

3.2 Pectin characterization

Pectins extracted using three extraction methods are shown in Figure 1. All pectins were fine powders and of the same brown colour with a distinct smell after drying and grinding, which is similar to pectin extracted using acids by Castillo-Israel *et al*. (2015) from 'Saba' banana peels. The difference in the chemical characteristics of pectin samples is shown in Table 1. Acid extraction had the highest yield (20.02%), followed by microwave-assisted extraction (14.22%), and enzymatic extraction had the lowest yield (6.18%). 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 and Muthukumarappan, 2017), whereas an enzymatic extraction generated 9.33% (Kumoro *et al*., 2020). Based on previous studies, acid extraction of pectin from banana peels produced the highest yield compared to other extraction methods. The harsh extraction conditions of acid extraction hydrolysis of protopectin to pectin and subsequent precipitation by ethanol, enabling it to have the highest yield among the three methods. Milder extraction conditions, the microwave-assisted and enzymatic extraction, showed a lower yield than acid-extraction. These milder extractions require penetration of plant cell walls via microwave radiation or swelling by hydration (Panouillé *et al*., 2006; Sandarani, 2017). Swelling by water to rupture plant cell walls may not be efficiently completed considering the mould conditions of these extraction methods.

Figure 1. Pectins extracted from 'Saba' banana peels using various extraction methods. (L-R: acid extraction, enzymatic extraction, microwave-assisted extraction).

Acid (AP) and microwave-assisted (MP) extractions produced pectins with higher moisture content (10.15 and 9.20%, respectively) than enzymatic extraction (EP; 7.46%). Furthermore, all pectins extracted had lower

Table 1. Yield and chemical characteristics of pectin extracted from 'Saba' banana peels using different extraction methods.

Characteristics	Pectin Sample*		
	AP	EP	MP
Pectin Yield, %	20.02±6.08[a]	6.18±0.20[b]	14.22±0.10[ab]
Moisture Content (%)	10.15±0.78[a]	7.46±0.03[b]	9.20±0.12[a]
Ash Content (%)	3.63±0.33[b]	9.56±1.04[a]	3.50±0.10[b]
Equivalent Weight	784.57±354.98[c]	5,842.27±407.34[b]	15,583.65±1597.15[a]
% Methyl Content	4.53±1.11[a]	2.47±0.25[b]	2.78±0.17[ab]
% Anhydrouronic acid	50.85±3.85[a]	17.07±1.48[b]	17.34±0.37[b]
% Degree of Esterification	51.09±15.52[b]	82.19±1.60[a]	90.94±3.55[a]

Values are presented as mean±SD. Values with the same superscript within the same row are not significantly different based on Tukey's HSD at 5% level of significance

AP = acid extraction, EP = enzymatic extraction, MP = microwave-assisted extraction

values for moisture content than commercial citrus pectin (12.03%; Castillo-Israel *et al*., 2015), which implies that in terms of moisture, all extracted pectins were within desirable results. Based on the Compendium of Food Additive Specifications (Joint FAO/WHO Expert Committee on Food Additives [JECFA] 2009), pectins used as food additives should have moisture loss upon drying (105°C for 2 hrs) of not more than 12%. Low moisture contents are desirable for pectin powders to inhibit the potential growth of microorganisms during storage. Pectin is a food additive; thus, its microbial quality as an ingredient is greatly considered. The stability of pectin is also affected by its moisture content. High moisture content encourages the growth of microorganisms that may produce pectinase enzymes that can hydrolyze the pectin (Norazelina *et al.*, 2011).

Moreover, EP had significantly higher ash content (9.56%, P≤0.05) than the other two extraction methods (3.50–3.63%). Ash content measures the inorganic residue that remains after ignition or complete ignition of organic matter in a foodstuff, and it represents the total mineral content of foods (Marshall, 2010). Therefore, low ash content indicates the purity of the pectin. AP and MP, therefore, had higher purity than EP. Commercial citrus pectin (CP) had an ash content of 1.76% (Castillo-Israel *et al*., 2015), while the standard for total insoluble pectin as a food additive should be not more than 3% (JECFA, 2009). The slightly higher ash content of pectins from 'Saba' banana peels compared to commercial pectin may be due to the impurity of the extracted samples. Further purification of the extracted pectins is necessary before food application. Nevertheless, good quality gels may still be formed by the extracted pectins from 'Saba' banana peels since all of them had an ash content of less than 10% (Norazelina *et al.*, 2011).

The equivalent weight of pectin extracted using the microwave-assisted method was highest at 15,538.65, followed by enzymatic extraction and acid-extraction, which have an equivalent weight of 5842.27 and 784.57, respectively. Highly pure pectins should have a lower equivalent weight. Commercial pectin from the study of Castillo-Israel *et al*. (2015) had an equivalent weight of 893, which is slightly higher than AP. In previous studies, apple pomace pectin has an equivalent weight of 833.33 to 1666.30 (Kumar and Chauhan, 2010), while mango peel pectin has an EW of 943 (Rehman *et al.*, 2003). Equivalent weight measures the total esterified galacturonic acid in the molecular chains of pectin. The low equivalent weight of the extracted pectins could be due to pectin degradation, where the esterified galacturonic acids became free acids. This further implies that EW is related to the degree of esterification and methoxyl content. The high degree of esterification causes a decrease in pectin's free acid content, thus rendering higher EW (Ramli and Amswati, 2011).

In terms of methoxyl content, AP (4.53%) has a higher value than EP (2.47%). Meanwhile, microwave-assisted pectin has a methoxyl content of 2.87%, which is not significantly different from AP and EP. Methoxyl content measures the moles of methyl alcohol in 100 g galacturonic acid. This parameter dictates the pectin's ability to form a gel (Constenla and Lozano, 2003). AP, which has the highest methoxyl content would have better gel-forming capabilities if applied to high-sugar gels, such as in jellies and jams. Pectins from different sources often have varying degrees of methylation. This is one reason why some pectin sources cannot be used to produce commercial pectin (Thakur *et al.*, 2009). In relation to methoxyl content, the degree of esterification measures the galacturonic acid residues that have been esterified with methoxyl (Morris *et al*., 2010). Microwave-assisted and enzymatic extraction produced pectins with a higher degree of esterification of 90.94% and 82.19%, respectively, compared to acid extraction (42.22%, P≤0.05). Pectin with a degree of esterification of more than 50% is considered high methoxyl pectin (Morris *et al*., 2010). Based on the results, all extracted pectins are high methoxyl, which is the same as CP (Castillo-Israel *et al*., 2015), indicating that they are capable of forming gels at higher pH due to the presence of fewer carboxylate anions at any given pH

(Sriamornsak, 2007). Similarly, pectins extracted from banana peels using acids were also high methoxyl pectins (Castillo-Israel *et al.*, 2015; Oliviera *et al.*, 2015; Maneerat *et al*., 2017; Khamsucharit *et al*., 2018). High-methoxyl pectins can form gels in an acidic medium with a pH range of 2.0–3.5 with 55% (or higher; w/w) sucrose. Low-methoxyl pectins, on the other hand, form gels at a wider pH range (2.0–6.0) in the presence of divalent ions such as calcium (Ca^{2+}) (Sandarani, 2017).

Another characteristic of pectin that was determined was anhydrouronic acid value, which measures the purity of the extracted pectin. According to the Food Chemical Codex (1996), pectins' anhydrouronic acid value should not be less than 65%. In this study, all extracted pectins were less than 65%, indicating that the pectins are not of high purity. Other molecules such as proteins, sugar, and starch may contribute to the extracted pectin's impurity, suggesting that further purification is needed to improve its quality for food applications. CP, which has undergone purification processes, has an anhydrouronic acid value of more than 65% (Castillo-Israel *et al*., 2015).

Scanning electron microscopy (SEM) images in Figure 2 show that the surface of AP was smoother while rough surfaces were observed in EP and MP. This result indicates that pectin from acid extraction was more degraded compared to the other two. The degradation of AP was also confirmed through its equivalent weight, which was the lowest among the three extraction methods. Low equivalent weight indicates pectin degradation (Ramli and Amswati, 2011). The microwave -extracted pectin had the roughest surface showing that the extracted pectin is in an undegraded polymer state. As this study shows, it has a higher molecular weight and degree of esterification. Rough surface for pectin has also been observed in previous studies (Jiang *et al*., 2012; Begum *et al*., 2017). During acid extraction, long-time exposure to acid and heat may have contributed to the degradation of pectin compared to the other two methods' milder conditions. In a previous study, Fracasso *et al*. (2018) reported an SEM image of citrus pectin described as fibrous or at least a rough structure with mean sizes between 5 and 100 μm.

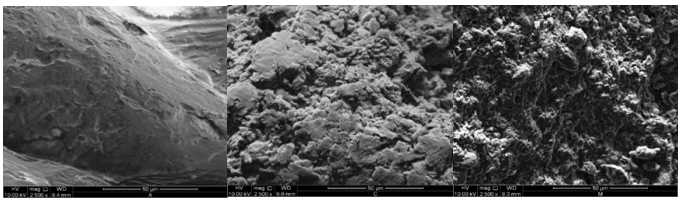

Figure 2. Scanning electron microscopy (SEM) images of pectins at 2500× magnification. (L-R: acid-extracted pectin, enzymatically extracted pectin, microwave-assisted extracted pectin).

3.3 Emulsifying properties of 'Saba' banana peel pectin

The extracted pectin was used to stabilize oil-in-water emulsion formed using 2 mM NaCl solution and soybean oil in the ratio of 70:30. The addition of NaCl, or any salt, allows adsorption of the negatively charged pectin at pH 5 (pKa = 3.5) at the surface of oil droplets formed in the emulsion (Surh *et al*., 2006). This interaction reduces electrostatic repulsion among pectin molecules and promotes cation-induced crosslinking of pectin chains (Ngouémazong *et al*., 2015).

The formation of cream after centrifugation of the emulsion was measured as a cream fraction. As shown in Table 2, cream fractions of emulsions formed using 'Saba' banana pectins (SBP) extracted using enzymatic and microwave methods were either not significantly different with or higher than CP at all concentration levels ($P \leq 0.05$). However, AP has a lesser cream fraction at 0.5%. Emulsion separation was characterized by an optically opaque cream layer at the top and transparent to turbid serum layer at the bottom (Surh *et al*., 2006).

Table 2. Cream fraction of pectins extracted using three different methods at three concentrations.

Sample*	Cream Fraction (%)		
	0.05%	1.00%	1.50%
AP	9.80±3.90^{b}	41.20±1.50^{a}	42.50±1.50^{a}
EP	37.8±2.60^{a}	40.90±1.30^{ab}	38.20±1.20^{a}
MP	41.8±0.60^{a}	41.90±3.10^{a}	41.10±3.40^{a}
CP	39.4±2.40^{a}	34.80±1.50^{b}	38.50±3.30^{a}

Values are presented as mean±SD. Values with the same superscript within the same row are not significantly different based on Tukey's HSD at 5% level of significance

AP = acid extraction, EP = enzymatic extraction, MP = microwave-assisted extraction

Furthermore, the emulsions formed have started separating into cream and serum within five minutes after agitating with a vortex. This relatively fast separation suggests that all of the droplets were aggregated and had rapidly moved towards the top of the emulsion (Surh *et al*., 2006). Creaming is related to emulsion instability wherein the droplets, having a lower density than the continuous phase, move upward and separate from the continuous phase. Thicker cream formed indicates more phase separation was promoted; thus, the more unstable the emulsion is (Pathak, 2017). At 0.5%, AP was able to form more stable emulsions than the other two extraction methods and CP. Meanwhile, at 1.0%, CP yielded a more stable emulsion compared to that of extracted SBPs. Finally, at 1.5%, CP and SBPs had comparable emulsion stabilities (38.2–42.5%). The results at 1.5% pectin were similar to that of potato pulp pectin, which had emulsion stability of 36.54 –46.0% (Yang *et al*., 2018). In another previous study,

sugar beet pectin was found to have greater emulsion stability of 62–79.4% (Ma *et al*., 2013).

External factors that influence the formation of emulsions include pectin concentration, the addition of salts, and pH. With increasing pectin concentration, the concentration of adsorbed pectin increases and remains relatively constant from a critical or threshold pectin concentration. In contrast, emulsion droplets' size decreases, indicating that the surface of oil droplets is wholly covered with emulsifiers at those concentrations (Siew and Williams, 2008). Microscopic observations of emulsion formation at 10× magnification revealed that oil droplets formed by the extracted banana pectin were larger than that of CP (Figure 3A). Generally, as the concentration of pectin increases, oil droplets appear to be smaller. The sizes of oil droplets in the emulsions stabilized by AP (Figure 3B) decrease as the concentration of pectin increases. In Figure 3C and 3D for pectins extracted using enzymes and microwave-assisted methods, respectively, at 0.5% level of pectin, the oil droplets appear in remarkably larger sizes compared to that of 1.0% and 1.5%. However, oil droplets of emulsions formed by EP and MP at 1.0% and 1.5% appear similar. Furthermore, coalescence and flocculation are more pronounced in EP and MP. These observations indicate instability of the emulsion because the pectin could not maintain the encapsulation of oil droplets. Non-adsorption of pectin could have caused the flocculation of oil droplets (Surh *et al*., 2006). Flocculants could enhance creaming because they rise faster than droplets due to the large effective radius (Psillakis, 2019). Moreover, larger oil droplets or the presence of coalescence is a subsequent result of flocculation because the droplets with lesser pectin adsorption at the surface are forced closer together (McClements, 2015). Compared with CP (Figure 3A), oil droplets of emulsions formed by SBP were bigger but still decreasing in size with concentration. The emulsifying activity of CP was superior to the extracted SBP, as shown by smaller oil droplets. In a previous study, depolymerized CP with DE of 70% has shown effective emulsifying activity at pH 4.7, the concentration of 4 wt%, and molecular weight of 70 kg/mol on 20 vol% rapeseed oil (Akhtar *et al*., 2002).

SBPs and commercial CP are both high methoxyl pectins. Pectins with high DE and low molecular weight, such as citrus and apple pectins, are usually not good emulsifiers due to low protein content and low acetyl groups (Schmidt *et al*., 2015). Ngouémazong *et al*. (2015) further confirmed that the presence of methyl esters in the homogalacturonan component hinders emulsification. Furthermore, Leroux *et al*. (2003) further stated that the emulsifying activity of pectin from plant sources depends entirely on the source. The variations among the emulsifying activity of pectins from different sources can be attributed to the presence of acetyl groups and neutral sugars, protein content, degree of polymerization, and molecular size (Wu *et al*., 2015). Therefore, in emulsion studies, it is essential to determine the chemical composition of the pectin of interest.

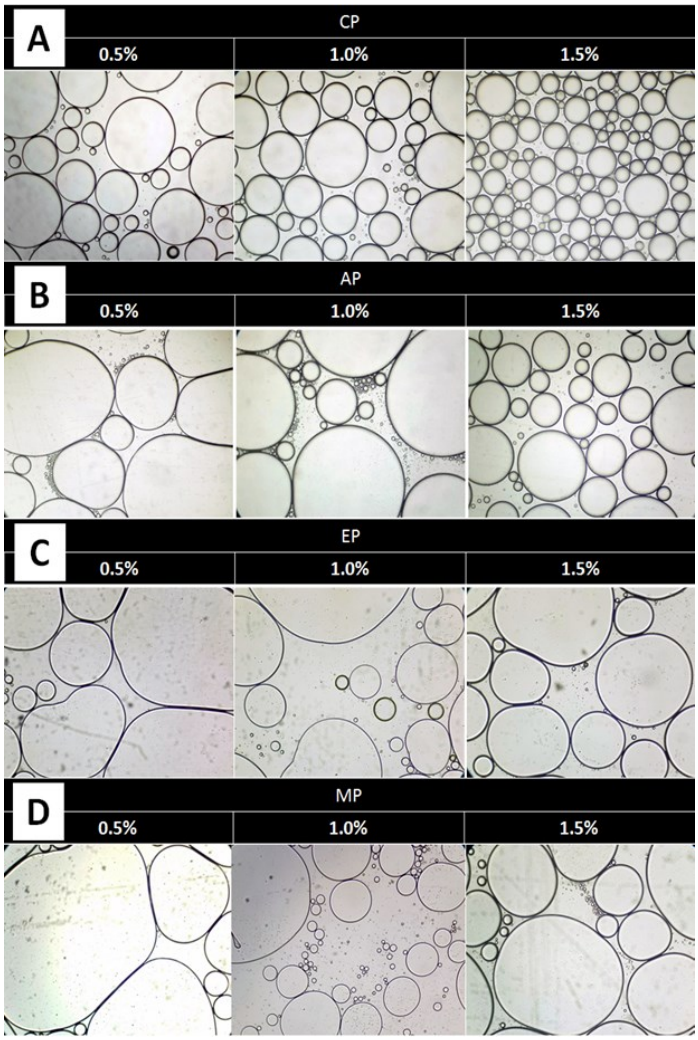

Figure 3. Oil droplets formed after pectin-assisted emulsion of soybean oil and 2 mM NaCl solution was centrifuged for accelerated phase separation (10× magnification). CP = Commercial citrus pectin, AP = acid extraction, EP = enzymatic extraction, MP = microwave-assisted extraction

4. Conclusion

Pectin generated from acid extraction had the highest yield and highest purity among the treatments but was in a degraded state; enzymatic extraction produced pectins with low purity and was quite undegraded, and microwave-assisted extraction yielded pectins with high purity and was undegraded. Furthermore, all extracted SBPs had high degree of esterification (DE > 50%), in the order AP < EP < MP. Also, all SBPs have comparable emulsifying activity with CP, but the emulsion formed was not stable at lower concentrations.

In conclusion, the extracted pectin from the three

different methods showed varying characteristics but were all considered to have a high degree of esterification. In terms of emulsifying capacity, SBPs have comparable emulsifying stability with CP; however, oil droplet sizes appear bigger than commercial CP. Oil droplets from emulsion formed by AP were smaller and more consistent than those of EP and MP. This study has shown the potential of SBP as an emulsifying agent, particularly the acid extracted pectin.

For future studies, it is recommended that rheological investigations, such as viscosity, will be explored. Refining the extracted pectin and application to food systems, such as jams and jellies, are also suggested.

Conflict of interest

The authors declare no conflict of interest.

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