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FULL PAPER

Physicochemical characteristics, rheology, and emulsifying properties of ultrasound-extracted pectin from 'saba' banana peel

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Abstract

Banana peel is an abundant agricultural waste in the Philippines. Valorization of the waste was done by extracting pectin from the 'saba' banana peel using ultrasound technology. The ultrasound-extracted pectin (UEP) was characterized physicochemically. UEP was also compared with commercial low-methoxy pectin (LMP) in terms of rheology and emulsification ability. Results showed that UEP has higher protein and ash content, and lower methoxyl, total pectic content, and interfacial tension. During the emulsion preparation, increasing the concentration of both UEP and LMP resulted in an increased production of stable emulsion droplets. Also, UEP-stabilized emulsion had improved stability when higher oil fraction and ionic strength were used. In terms of pH, higher volumes of stable emulsion were produced by UEP and LMP at conditions close to pectin's isoelectric pH. Lastly, UEP and LMP produced emulsions that were stable in all stages of in vitro digestion.

1. Introduction

Banana is one of the major crops in the Philippines. In 2020, the Philippines produced around 9.05 million metric tons of bananas. Of this volume, about 2.5 million metric tons is of the 'saba' variety (Figure 1). According to the Philippine Statistics Authority (PSA) (PSA, 2020a; PSA, 2020b; PSA, 2020c; PSA, 2020d), 25% or more than 600,000 metric tons ‘saba’ is utilized for food processing. Of this, around 66% is composed of peel waste (Guerrero *et al*., 2016); hence, it is safe to say that the country produced more than 400,000 metric tons of waste in 2020. These wastes are often thrown away and end up in dumpsites. The accumulation of waste banana peel could pose environmental problems as it generates methane and causes pollution.

Figure 1. 'Saba' banana

Waste banana peels are resources with low commercial value. However, it can be converted to highly commercial outputs that can improve the overall economics of processing units, particularly for the small to medium-scale banana industry (Padam *et al*., 2014). There is increasing scientific evidence showing banana peels' physical properties, chemical compositions, and nutritive potentials can be utilized to produce value-added products for special food applications and nutraceuticals (Pathak *et al*., 2016). Analysis of the chemical composition of 'saba' banana peels has shown that a relatively high amount of pectic content is present in the peels, which can be extracted as pectin (Rivadeneira *et al.*, 2020). Between the unripe and ripe banana peels, the peels generated by the earlier are 39% cruder (Castillo-Israel *et al.,* 2015).

The market for pectin on a global scale is estimated to account for a value of USD 1.0 billion in 2019. It is expected to reach USD 1.9 billion by 2025 due to the increasing demand for natural and organic products (Markets and Markets, 2019). In the Philippines, data from the Department of Trade and Industry showed that about 95,000 kilograms were imported in 2011, valued at around $52.4 million (Philippine News Agency, 2015). Currently, the market price of commercial pectin depends on the brand, but the lowest price is PhP 5000. It is mostly (85%) produced from citrus peels (56% from lemons, 30% from limes, and 13% from oranges), and apple pomace (14%), with a minor fraction being

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obtained from sugar beet. The world's largest producers of dried citrus peel are in Argentina and Mexico, but producers are also located in Peru, Spain, and Bolivia. In South America and Sicily, pectin is often extracted directly from the wet citrus peel after juicing. Brazil and Mexico use orange and lime peel as the main pectin's raw materials, while current production in Germany mainly relies on apple pomace (Ciriminna *et al*., 2016).

The extraction of pectin from plant sources involves using water, acid, base, or enzyme in combination with heat, microwave, ultrasound, or pulse electric field. Among these methods, ultrasound technology was chosen for this study because of the simplicity of operation and extraction efficiency. In terms of intrinsic benefits, ultrasound has shown more effective mixing, faster energy and mass transfer, lower thermal and concentration gradient, lower extraction temperature, reduced equipment size, faster response to process extraction control, faster start-up, increased production, and elimination of process steps (Chemat *et al*., 2008). Studies on the use of ultrasound for pectin extraction have already been done for orange peel (Yousuf *et al*., 2018), dragon fruit peel (Lin *et al*., 2018), tomato waste (Grassino *et al*., 2015), jujube waste (Bai *et al*., 2015), grape pomace (Minjares-Fuentes *et al*., 2014), grapefruit peel (Bagherian *et al*., 2011; Wang, Ma, Xu *et al*., 2015), and sisal waste (Maran and Priya, 2015).

In the food industry, pectin is mainly used as a gelling agent in products like jams and jellies. Due to its structural characteristics, pectin is also used as an emulsifier or a stabilizer for emulsion products (Freitas *et al*., 2021). Emulsifiers are surface-active materials that can interact with the oil and water phases (Dickinson, 2009). The hydrophilic portion of the emulsifier is exposed to the aqueous phase, while the hydrophobic part is in the non-polar phase. The most common emulsifiers are low-molecular-weight compounds, typically consisting of a small hydrophilic head group and one or more hydrocarbon chains. The other type of emulsifier is the macromolecular polymer interacting with the protein component of the food (Dickinson, 2009). The polymers, in sufficient concentration, form a network that can effectively trap the emulsion droplets to prevent them from flocculating. Meanwhile, the protein component of the emulsion is adsorbed at the interphase, creating a highly viscoelastic interfacial film that stabilizes the emulsions (Corredig, 2009). As a result, the emulsion can maintain its homogeneous appearance and consistency of texture. Emulsions can also be stabilized by particles such as silica, natural clays, and polymers. These are called Pickering emulsions, whose primary characteristic is the particle's affinity for both oil and water. Pickering emulsion was named after Percival Spencer Umfreville Pickering, who discovered the said phenomenon (Bon, 2014).

It was reported that pectin extracted from citrus reduced the interfacial tension of oil droplets, allowing the formation of oil/water emulsion (Verkempinck *et al.*, 2018). Short-term stability due to bridging flocculation was also reported. Similarly, pectin from tomato and broccoli extract was investigated for emulsifying and stabilizing properties (Santiago *et al*., 2018). The pectin extracted from broccoli was observed to have better emulsification capacity. They attributed it to its higher protein content and possibly high amount of acetyl groups that can provide better steric stabilization. Additionally, sizes of emulsion droplets at various pH were monitored for 30 days, and they concluded that the stability of pectin-stabilized emulsion was highest at the isoelectric pH of pectin. They furthered that lower dissociation of the pectin carboxylic groups at this pH led to less intramolecular repulsions, more compact conformation of the adsorbed species at the oil-water interface, and better electrostatic interactions with positively charged proteins.

This study aimed to increase the value of bananas by extracting the pectin through ultrasonication and investigating the properties of banana pectin as an emulsifier. This study valorized 'saba' banana peel by extracting pectin, a high-value food ingredient, using ultrasound technology. Furthermore, physicochemical characterization of ultrasound-extracted pectin was performed, and a rheology study was conducted to evaluate UEP's effect on emulsions.

2. Materials and methods

2.1 Raw materials

Peels from unripe matured 'saba' bananas were obtained from a banana chips processing plant in Santa Cruz, Marinduque, Philippines. It was sliced into small pieces (approximately 2×2 cm^2) and then oven-dried at 50°C until constant weight. The dried banana peel was powdered using a countertop blender (Oster®, Classic Series Accurate Blend™, Boca Raton, Florida) and stored in a desiccator at room temperature. Chemicals in analytical reagent grade were purchased from Fisher Scientific, USA.

2.2 Ultrasound-extraction of pectin

The protocol for the extraction of pectin from 'saba' banana peel using ultrasound technology was adapted from Maran and Priya (2015) with slight modifications. Approximately 10% (w/v) of powdered banana peel in 0.003 M sulfuric acid was sonicated (Sonics, VibraCell™, Newton, Connecticut) at a 60% pressure

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amplitude for 20 mins in a cold-water bath (10-15°C). The sonicated mixture was filtered with a Miracloth (Calbiochem®, Millipore Corp., Billerica, Massachusetts), and the resulting filtrate was centrifuged (Thermo Fisher Scientific, Sorvall Lynx 6000, Osterode, Germany) at 15,000×*g* for 15 mins. The supernatant was filtered with a Miracloth to remove any remaining residue. The pooled filtrate volume was measured, and the pectin was precipitated by adding an equal volume of 95% ethanol. The mixture was centrifuged, and the precipitate was collected by filtration using Miracloth. Pectin was subjected to freeze-drying at −80°C and 0.004 mBar for 48 hrs (Labconco, FreeZone™, Kansas City, Missouri).

2.3 Physicochemical characterization of ultrasound-extracted pectin and low-methoxy pectin

The pectin samples were characterized physicochemically to obtain the equivalent weight, methoxyl content, total pectic content, protein, ash, and interfacial tension. In addition, the samples were subjected to Fourier-transform infrared (FTIR) spectroscopy to identify the major functional groups and to atomic force microscopy (AFM) to visualize the topography of the structures. The analyses were done in triplicates, and for the quantitative analyses, the average values were reported and compared.

2.3.1 Equivalent weight

The equivalent weight was measured using Rangana's method (Rangana, 1995). A 0.5 g sample was weighed into a 250-mL conical flask and mixed with 5 mL 95% ethanol. Approximately 1 g of NaCl, 100 mL of deionized H_2O, and six drops of phenol red were added to the mixture. The mixture was titrated with 0.1 N NaOH to the purple endpoint. Equivalent weight was calculated using the formula:

$$\text{Equivalent weight} = \frac{\text{weight of sample (g)}}{\text{volume of NaOH (mL)} \times \text{concentration of NaOH (N)}} \times 1000 \quad (1)$$

2.3.2 Methoxyl content

The methoxyl content was also measured by Rangana's method (Rangana, 1995) with modification. To the neutral solution (which contained 0.5 g of pectic substance), after the determination of equivalent weight, 25 mL of 0.25 N NaOH was added. The solution was shaken manually for 30 s and set for 30 mins at room temperature in a flask with a stopper. A 25 mL portion of 0.25 N HCl was added, and the resulting solution was titrated with 0.1 N NaOH until the purple endpoint. The methoxyl content was calculated using the formula:

$$\text{Methoxyl content (\%)} = \frac{\text{mL alkali x N alkali x 3.1}}{\text{weight of sample, g}} \quad (2)$$

Factor 3.1 is the converted equivalent weight of the methoxyl group multiplied by 100.

2.3.3 Total pectic content

The total pectic content was measured following the method described by Blumenkrantz and Asboe-Hansen (1973). A 7.5 mg sample was weighed into a test tube containing a magnetic stir bar. While in an ice bath (10-15°C), 5 mL cold H_2SO_4 was added, and the mixture was stirred to allow complete solubilization of the material. A total of 1.25 mL of deionized water was then added (dropwise), followed by continuous stirring for 5 mins. Another 1.25 mL of deionized H_2O was added (dropwise), followed by constant stirring for 5 mins. The sample was filtered with a 0.45 µm syringe filter. The filtered sample was diluted to 1:10 using deionized water.

A 1 mL of the diluted sample/standard was drawn out and transferred to a test tube in an ice bath (10-15° C). Approximately 6 mL of cold H_2SO_4/sodium tetraborate (4.767 g sodium tetraborate in 1 L of H_2SO_4) was added to the sample. It was mixed thoroughly and was allowed to cool in an ice bath (10-15°C). Tubes were boiled for 5 mins at 100°C, then placed immediately in an ice bath (10-15°C). One-tenths millilitres of 0.0125 M m-hydroxydiphenyl (0.15% in 0.5% NaOH - stored at 4°C in a container wrapped with aluminium foil) was added. The sample was repeatedly mixed in a vortex until it reached room temperature. The absorbance of the sample was read (Thermo Scientific, Evolution 201, Madison, Wisconsin) at 520 nm against a blank after 20 mins. For the standard calibration curve, 0 to 100 µg/mL of galacturonic acid in deionized H_2O was used. For blank, 0.1 mL of 0.5% NaOH, instead of m-hydroxydiphenyl, was added. The concentration of the total pectic substance, in terms of galacturonic acid, was derived from the standard curve.

2.3.4 Protein

Protein was measured using the protocols described by Walker (2002). Solution A was prepared by dissolving 1 g sodium bicinchoninate (BCA), 2 g Na_2CO_3, 0.16 g sodium tartrate, 0.4 g NaOH, and 0.95 g $NaHCO_3$ in 80 mL deionized H_2O. The solution was adjusted to pH 11.25 using 10 M NaOH and then diluted to 100 mL using deionized H_2O. Solution B was prepared by dissolving 0.4 g $CuSO_4.5H_2O$ in 8 mL deionized water. The solution was then diluted to 10 mL. Solution C was made by combining solutions A and B at a 50:1 (v/v) ratio. Approximately 2 mL of solution C were added to a 0.1 ml sample, mixed using a vortex, and then incubated at 60°C using a water bath (SI Analytics, Weilheim, Germany) for 15 mins. It was then cooled in an ice bath (10-15°C) to reach room

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temperature. The absorbance was read against a blank (0.1 mL deionized water in place of the sample) at 562 nm. For the standard, 0 to 60 µg of bovine serum albumin (BSA) was used. The concentration of the protein, in terms of BSA, was derived from the standard curve.

2.3.5 Ash

The ash content was measured following the protocols described by the Association of Official Analytical Chemists (AOAC) (2000). The evaporating dish containing the sample was ignited in a furnace (Thermolyne, FB 1400, Dubuque, Iowa) for 5 hrs at 550° C. The ignited sample was removed from the furnace, cooled in a desiccator, and then weighed. The sample was re-ignited for 30 mins. This procedure was repeated until a constant weight was obtained. The ash content

$$\%\text{Ash} = \frac{\text{mass of ash}}{\text{mass of sample}} \times 100 \quad (3)$$

was calculated using the formula:

2.3.6 Interfacial tension

The interfacial tension was measured following the protocols described by Li *et al.* (2014). Approximately 2% (w/v) of pectin in deionized water was prepared. Using the Kruss FM40 Easy Drop (Hamburg, Germany), around 15-25 µL of the solution was dosed at 10 µL/min on a cuvette filled with 2.5 mL n-dodecane, which was pre-purified by passing through an alumina column and distilled three times. The maximum droplet formed after dosing was incubated in the n-dodecane solution at room temperature (25°C) for 60 mins. The droplet was then illuminated using the built-in illuminator, and the equipment measured the interfacial tension of the pectin solution.

In principle, the interfacial tension between the inner and outer phases of the pectin solution droplet resulted in increased pressure inside the drop. The interfacial tension was calculated using the software's built-in Laplace equation (Equation 4), which describes the relationship between the pressure difference and the

$$\Delta p = \sigma\left(\frac{1}{R_1}+\frac{1}{R_2}\right) \quad (4)$$

interfacial tension.

Where $\Delta p = p_{inner} - p_{outer}$, σ = interfacial tension, R_1, R_2 = radii of horizontal and vertical circles of curvature.

2.3.7 Fourier transform infrared spectroscopy of ultrasound-extracted pectin and low-methoxy pectin

The protocol for the FTIR spectroscopy as described by Rivadeneira *et al*. (2020) was adopted in this analysis. Around 12.5 mg of UEP was mixed with 250 mg fine potassium bromide. The mixture was pulverized manually using a mortar and pestle and put into a pellet-forming die. A force was applied manually to form transparent pellets. For background measurement, a pellet holder containing KBr (without pectin) was inserted into the sample chamber (Thermo Scientific, Nicolet NEXUS 670 FTIR, Madison, Wisconsin). Infrared radiation (400-4000 cm^{-1}) was bombarded to the sample at a resolution of 4 cm^{-1} with data spacing every 1.928 cm^{-1} for 64 scans. The same procedure was done for the potassium bromide with pectin. The resulting spectra were used to identify relevant peaks. LMP was also analyzed for comparison.

2.3.8 Atomic force microscopy of ultrasound-extracted pectin and low-methoxy pectin

The sample for the atomic force microscopy was prepared following the protocols described by Li *et al*. (2019). UEP and LMP solutions (0.1 and 1% w/v) with a final pH of 6 were prepared. The solutions were centrifuged at 2,000×*g* for 10 mins, and the supernatant was collected for analysis. A freshly peeled mice sheet attached to an AFM sample mounting disk (previously rinsed with 1.0 mL water at 2,000 rpm for 40 s in a P6700 spin coater (Specialty Coating Systems Inc., Indianapolis, IN) was coated with 20 µL of the supernatant at 2,000 rpm for 20 s. The surface was covered and dried at room temperature for two hrs. The samples were scanned under an AFM (Bruker Corp., Multimode VIII, Santa Barbara, California) using a ScanAsyst-AIR probe in the ScanAsyst-Air mode. The scanning area was 5 µm x 5 µm, and the scan rate was 1 Hz. The generated AFM images were flattened by the Nanoscope Analysis software (Bruker Corp., Santa Barbara, CA).

2.4 Preparation and inspection of emulsion ultrasound-extracted pectin and low-methoxy pectin as emulsifiers

The preparation of emulsion was adapted from the protocols described by Qi *et al.* (2020) with slight modifications. Four set-ups were prepared wherein one parameter was varied in each set-up. The parameters considered were pectin concentration, oil fraction, pH, and ionic strength.

2.4.1 Various pectin concentrations

UEP and LMP (0.5, 1, 2% w/v, based on the final emulsion volume) were dissolved separately, with continuous stirring, in 0.02 M NaCl solution. The final pH was adjusted to 6, using 0.1 M HCl or NaOH. The solution was added to soybean oil to form a 30:70 (v/v) oil/water mixture.

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2.4.2 Various oil fractions

UEP and LMP (2% w/v) were dissolved separately, with continuous stirring, in 0.02 M NaCl solution. The final pH was adjusted to 6, using 0.1 M HCl or NaOH. The solution was mixed with soybean oil such that 30, 40, and 50% of oil fractions were achieved.

2.4.3 Various pH

UEP and LMP (2% w/v) were dissolved separately, with continuous stirring, in 0.02 M NaCl solution. The final pH was adjusted to 3, 4, 5, 6, and 7 using 0.1 M HCl or NaOH. The solution was added to soybean oil to form a 30:70 (v/v) oil/water mixture.

2.4.4 Various ionic strengths

UEP and LMP (2% w/v) were dissolved separately, with continuous stirring, in 0, 0.02, 0.05, 0.1, and 0.2 M NaCl solution. The final pH was adjusted to 6, using 0.1 M HCl or NaOH. It was added to soybean oil to form a 30:70 (v/v) oil/water mixture.

2.4.5 Homogenization and ultrasonication

All oil-water mixtures were homogenized with a dispenser (Kinematica, Polytron 10-35 GT, Luzern, Switzerland) at 10,000 rpm for 2 mins. Then, the resulting emulsion was subjected to ultrasonication (VCX-750, Sonics & Materials, Newton, CT) following the protocol described by Wang, Ma, Xu *et al*. (2015) with slight modifications – exposing the homogenized samples to ultrasound at a pressure amplitude of 60% for one minute.

2.4.6 Visual inspection of emulsion stability

The phase separation was promoted following the protocol described by Ercelebi and Ibanoğlu (2007), with slight modifications. The emulsion was transferred to a 25-mL plastic centrifuge and centrifuged at 4000×*g* for 5 mins. The thickness of the formed cream layer, measured manually using a calliper, was used to calculate the cream fraction (thickness of the cream layer/thickness of the whole sample). The stabilized emulsion was also viewed under a 40× magnification of the polarized microscope (Olympus®, BX51, Tokyo, Japan) with a built-in digital camera (Olympus®, DP70, Tokyo, Japan).

2.5 Rheology of emulsion

The rheological analysis of emulsion adapted the protocol by Qi *et al.* (2020). UEP and LMP (2% w/v) were dissolved separately, with continuous stirring, in 0.02 NaCl solution. The solution's pH was adjusted to 6, then added to soybean oil to form a 30:70 (v/v) oil/water mixture. The apparent viscosity profile was recorded from shear rates: 2 to 200 s^{-1} (TA Instruments Rheometer, AR 2000, New Castle, Delaware). Storage modulus (G') was also measured from 0.1 to 100 Hz at 25°C and 0.1% strain.

2.6 Emulsifying property of the emulsion

2.6.1 In vitro digestion of emulsion (oral phase)

A 30:70 (v/v) emulsion was prepared following the procedure in 2.4.2. Approximately 14 mL of this sample was gently mixed with 0.30 mL of fresh artificial saliva (Pickering Laboratories, Mountain View, California), 3 mM $CaCl_2$, 6 mM $K_2HPO_4{\cdot}3H_2O$, 6.4 mM KCl, 15 mM NaCl, 62 mM $NaHCO_3$, 0.005% (w/v) mucin-type II from the porcine stomach, and 0.1% (w/v) α-amylase type VI−B for 10 seconds (Borreani *et al*., 2017). The emulsion was viewed under a 40×objective.

2.6.2 In vitro digestion of emulsion (gastric and intestinal phase)

The protocols for the gastric and intestinal in vitro digestion of emulsion adapted to the protocol described by Borreani *et al.* (2017) with slight modifications. Four and four-tenths millilitres of simulated gastric fluid (SGF) consisting of 53 mM NaCl, 1 mM $CaCl_2$, 5.7 mM Na_2CO_3, and 14.8 mM KCl (preincubated at 37°C for 10 mins) were mixed with the sample from the oral phase. The solution was adjusted to pH 2, added with 0.70 mg of pepsin , and then stirred at 37°C for 120 mins (Liang *et al.*, 2016). The emulsion was viewed under a 40× objective.

For the intestinal phase, 3 mL of bile extract solution (4 g bile for every 100 mL of 0.5 M, pH 7 phosphate buffer) and 1.2 mL of pH 7 electrolyte mixture (0.5 mM $CaCl_2$, 2 mM KCl, 21 mM NaCl) were added to the sample from the gastric phase. The solution was adjusted to pH 7 and then combined with 0.50 g of pancreatin and 5.6 g of lipase from the porcine pancreas. The final mixture was maintained at 37°C and pH 7 with continuous stirring for 120 mins. The emulsion was viewed under a 40× objective.

2.6.3 Fatty acid analysis

Approximately 0.5 g of the sample from the intestinal digestion was added to a 50 mL ethanol/diethyl ether (1:1) mixture. Five drops of phenolphthalein were added as an indicator. The sample was then titrated with 0.1M KOH in ethanol until the pink endpoint (which lasted for at least 10 s) (AOCS, 2003). Acid number, which refers to the milligrams of KOH needed to react with the acid in a gram of sample, was calculated using the following formula:

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$$Acid\ number = \frac{56.1 \times C \times V}{M} \quad (5)$$

Where C = Molarity of KOH (mmol/mL), V = Volume of KOH used (mL), and M = Mass of the sample (g).

3. Results and discussion

3.1 Characteristics of ultrasound-extracted pectin and commercial low-methoxy pectin

The physicochemical characteristics of UEP and LMP are presented in Table 1. The equivalent weight, or the free galacturonic acid in the molecular chain of pectin (Rangana, 1995), was close to that of the LMP. High equivalent weight indicates the high gel-forming activity of the two pectins (Salma *et al*., 2012). The methoxyl content for UEP was within the typical values for LMP, which ranges from 0.1 to 7% (Featherstone, 2015). This characteristic, referring to the methyl ester group attached to the galacturonic acid chain, determines the appropriate protocol for the gelation of pectin. The purity of UEP from 'saba' waste banana peel, as described by the total pectic content, was within the acceptable limit (>65%) for food use set by the Joint FAO/WHO Expert Committee on Food Additives and the European Commission (Müller-Maatsch *et al*., 2016). Results also showed that UEP has a relatively higher protein content, providing a potential synergistic effect on some food applications, especially for emulsion stabilization. The mineral content (ash) of UEP was also higher than LMP. This result was expected since the latter has higher purity. Lastly, the interfacial tension of UEP was lower than LMP, which translates to higher interfacial activities and higher stability of emulsion (Kyomugasho, 2015).

Table 1. Physicochemical characteristics of ultrasound-extracted pectin (UEP) and commercial low-methoxy pectin (LMP).

Characteristics (dry basis)	UEP	LMP
Equivalent weight, g/eq	500	400
Methoxyl content, %	3.18±0.1	7.61±0.1
Total pectic content, %	69.23±0.6	93.71±1.0
Total protein, %	8.47±0.2	1.94±0.2
Ash, %	5.32±0.2	1.90±0.2
Interfacial tension, $mN \cdot m^{-1}$	35.40±0.5	43.59±0.2

Values are presented as mean±SD of triplicates.

Relative to an acid-extraction method (Castillo-Israel *et al.*, 2015), the use of ultrasound technology in extracting pectin from 'saba' banana peel resulted in a higher-purity product. A similar trend was observed upon comparison with microwave-assisted extraction (Rivadeneira *et al.,* 2020) of pectin from the same substrate. Hence, ultrasound is the most effective method for extracting pectin from the 'saba' banana peel in terms of the purity of the product.

Ultrasound extraction of pectin from other substrates used the same parameters as 'saba' banana peel. Orange peel (Yousuf *et al*., 2018), dragon fruit peel (Lin *et al*., 2018), tomato waste (Grassino *et al*., 2015), jujube waste (Bai *et al*., 2015), grape pomace (Minjares-Fuentes *et al*., 2014), grapefruit peel (Bagherian *et al*., 2011; Wang, Ma, Xu *et al*., 2015), and sisal waste (Maran and Priya, 2015 all used 20-30 mins exposure to ultrasonic waves. While all these substrates used the same technology, the studies focused on the optimization of the ultrasound-extraction method and the yield of pectin. Other substrates, except jujube waste, produced a higher yield than 'saba' banana peel. However, the characteristics of pectin were not studied.

One of the proposed mechanisms for ultrasound-assisted extraction is the disruption of the cell structure of the plant tissue upon exposure to ultrasound irradiation. The said effect on plant tissue increases the accessibility of the solvent to the internal plant structure, thereby initiating the release of the cell contents (Li *et al*., 2004; Zhao *et al*., 2007; Supardan *et al*., 2012; Anese *et al*., 2013; Wang, Guo, Zhang *et al*., 2015; Raji *et al*., 2017). The other proposed mechanism is the series of expansion and compression in the medium brought about by the ultrasound (Adetunji *et al*., 2017). The expansion pulls the molecules apart while the compression puts them together. The series of activities creates a bubble that grows and, subsequently, experiences collapse as the negative pressure exerted exceeds the local tensile strength of the liquid (Luque-García and Luque De Castro, 2003).

For the FTIR (Figure 2), bands from 1800 to 1500 cm^{-1} contain the relevant functional groups for characterizing pectin (Manrique and Lajolo, 2002). The spectra for UEP and LMP exhibited absorption peaks at around 1630 - 1600 cm^{-1}, which accounts for the stretching of the carboxylic acid group of the ester carbonyl group. A peak at around 1740 cm^{-1}, which describes the stretching of the C=O bond of the ester carbonyl group, was also present in LMP but not in the UEP. These findings confirmed the result of the characterization where higher methoxyl content was observed for LMP.

AFM can be used to picture an individual pectin molecule and its aggregation. Figure 3 shows that both UEP and LMP, measured at 5µm × 5µm, are combinations of elongated single polymers and aggregates. For higher concentrations (1%), UEP's maximum height was higher than that of LMP, while the opposite trend was observed at a lower concentration (0.1%). The height difference was minimal for the latter concentration. At high concentrations (1%), it can be

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said that some chains of the UEP overlapped, as shown by the differences in the heights within the scanned area (Adams *et al*., 2003).

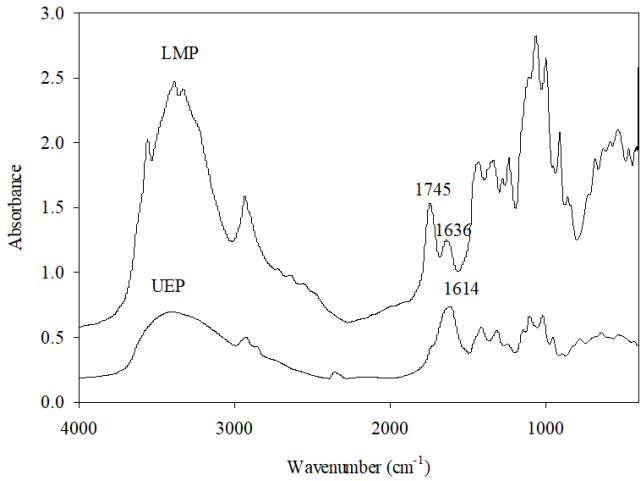

Figure 2. FTIR spectra of ultrasound-extracted pectin (UEP) from 'saba' waste banana peel and commercial low-methoxy pectin (LMP).

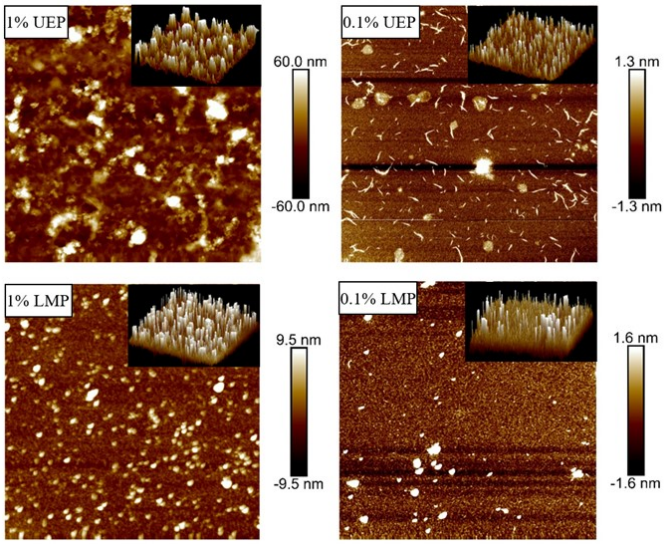

Figure 3. Atomic force microscopic images of ultrasound-extracted pectin (UEP) from 'saba' waste banana peel and commercial low-methoxy pectin (LMP) at concentrations of 0.1% and 1% (w/v). The size of the images is 5 µm × 5µm.

3.2 Phase separation of the emulsion

Based on Figure 4, both UEP and LMP were able to produce a stable emulsion. This was displayed by the formed cream layer on top of the mixture after centrifugation, which is used to accelerate the destabilization of the emulsion. Results (Figure 5) also showed that increasing the concentration of both UEP and LMP increased the volume of the stabilized emulsion. A significant difference ($P<0.05$) was observed between the two pectins at 0.5%. The emulsifying property of pectin can be attributed to its covalently bound proteins and carbohydrate moiety (Funami *et al*., 2007; Schmidt *et al.*, 2015). In this study, higher protein content means maximum exposure of the said functional groups for interfacial interaction. A consensus is that protein acts as an anchor between pectin and the formed oil droplet (Mengual, 1999; Funami *et al*., 2007).

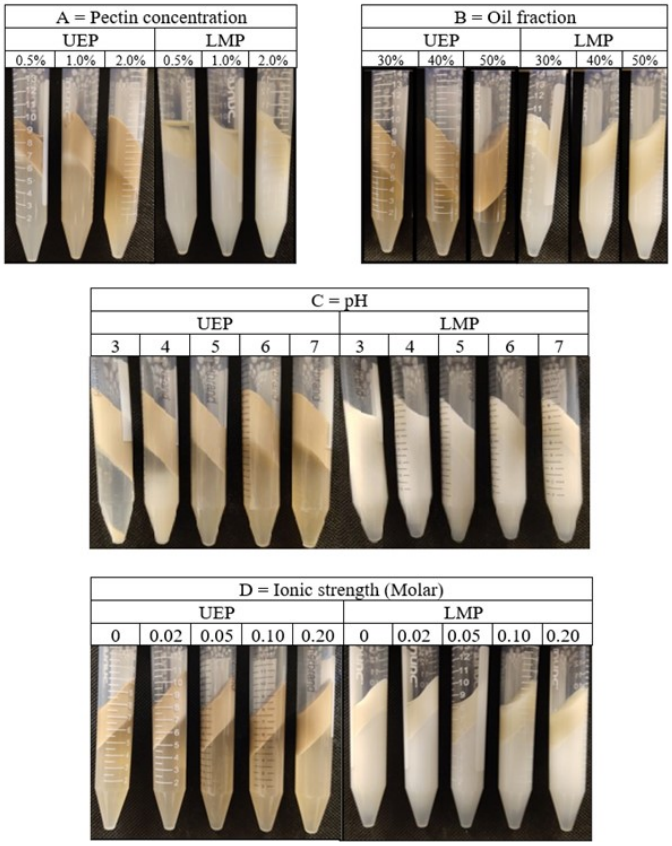

Figure 4. Appearance of stabilized oil/water emulsion using ultrasound-extracted pectin (UEP) from 'saba' waste banana peel and commercial low-methoxy pectin (LMP) at different conditions.

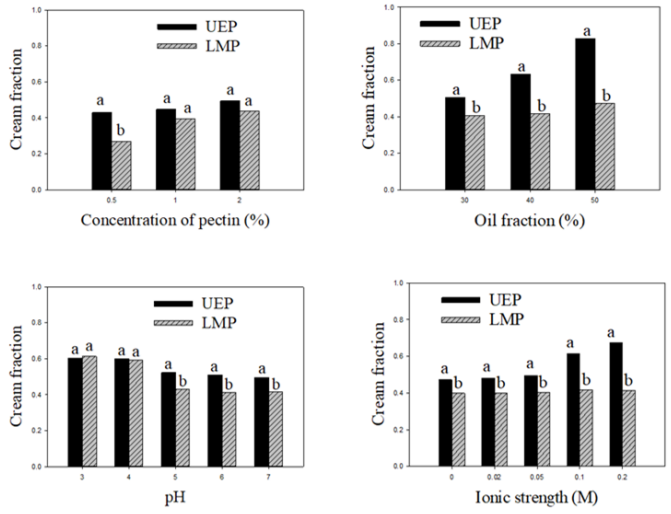

Figure 5. Effect of pectin concentration, oil fraction, pH, and ionic strength on the cream fraction of oil/water emulsion stabilized by ultrasound-extracted pectin from ‘saba’ waste banana peel (UEP) and commercial low-methoxy pectin (LMP) (A: 30% oil, pH 6, 0.02 M NaCl; B: 2% pectin, pH 6, 0.02 M NaCl; C: 2% pectin, 30% oil, 0.02M NaCl; D: 2% pectin, 30% oil, pH 6). (Grouping information based on Tukey's Method at 95% confidence level).

For emulsions with UEP as an emulsifier, increasing

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the ratio of the oil component resulted in a significantly higher ($P<0.05$) volume of the stable emulsion. This trend can be validated by the size of the emulsion droplets, as seen in Figure 6. Smaller droplets were formed at higher oil fractions; hence, the higher stability. The same trend was also observed in a recent study involving multiple lights scattering to measure emulsion stability. They found out that the stability of emulsions increases as the oil content of emulsions increases (Mengual, 1999).

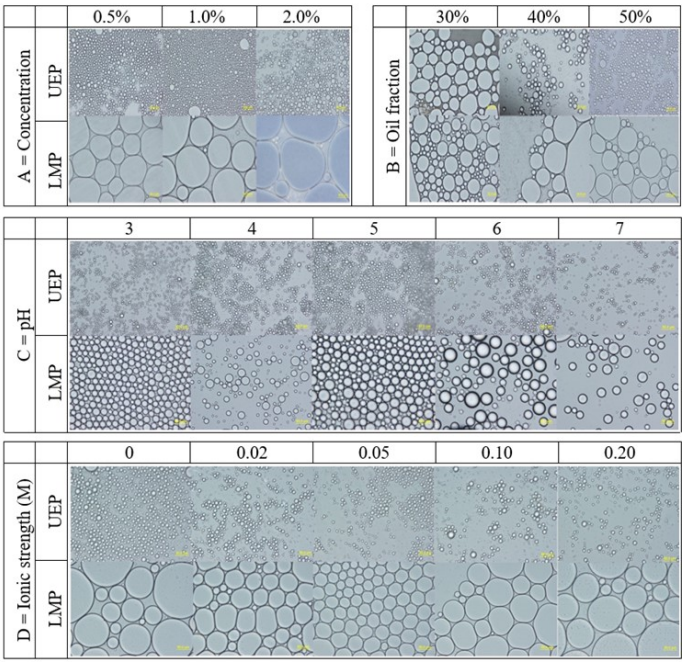

Figure 6. Effect of pectin concentration, oil fraction, pH, and ionic strength on the microscopic appearance of oil/water emulsion stabilized by ultrasound-extracted pectin from 'saba' waste banana peel (UEP) and a commercial low-methoxy pectin (LMP) (A: 30% oil, pH 6, 0.02 M NaCl; B:2% pectin, pH 6, 0.02 M NaCl; C: 2% pectin, 30% oil, 0.02M NaCl; D: 2% pectin, 30% oil, pH 6).

Varied pH also resulted in differences in the cream fraction. The volume of the stabilized emulsion produced by both UEP and LMP at pH 3 and 4 were higher than at pH 5 to 7. In a related study, Akhtar *et al*. (2001) compared citrus pectin-stabilized emulsions at pH 4.7 and 7 and observed that emulsions were more stable at the lower pH. According to Nakauma *et al.* (2008), emulsions flocculate at the isoelectric point of the stabilizer's carbohydrate moiety due to reduced repulsion. Their study utilizing sugar beet pectin decreased emulsion stability at pH above the pectin's isoelectric point. The same trend was also observed in the sizes of the emulsion droplets, which are smaller at the isoelectric pH of the pectin.

The presence of NaCl also affected the stabilization of the emulsion. While the amount of NaCl resulted in a non-significant ($p<0.05$) change in the volume of stabilized emulsion when LMP was used as an emulsifier, an increase in the volume of the stabilized emulsion was observed for UEP at higher NaCl concentrations. The increase in ionic strength increases the electrostatic shielding effect of Na^+, resulting in the reduction of both the charge around the emulsion droplet and the repulsion between pectin molecules (Zhuang *et al*., 2019). Like the effect of pH near the isoelectric pH, the pectin molecules can pack on the surface droplets with more coverage, hence more stability. A similar effect of ionic strength on stability was reported using pomegranate peel pectin (Zhuang *et al*., 2019).

In general, UEP produced a higher volume of stabilized emulsion than the LMP. The presence of a higher concentration of protein in UEP may be responsible for improved emulsion stability. The adsorption of protein at the oil/water interface is followed by protein unfolding, which promotes the interactions and surface tension reduction (Dickinson and McClements, 1995). Studies by Youssef and Barbut (2009) on the effect of protein level on the stability of the emulsion and that of Loi *et al*. (2019) presented the same trend. That is, protein contributes to the stability of an emulsion. They furthered that increasing the protein composition in an emulsion improves the latter's stability.

The appearance of droplets is presented in Figure 5 to explain the behaviour of the emulsions further. Emulsions are considered unstable when the droplets can fuse to form bigger and bigger droplets. In contrast, small droplet sizes indicate more stability (Oyeneyin, 2015). Minimal changes in the size of small droplets suggest that the interfacial attraction between the oil droplets is strong due to the pectin polymer forming a fine film coating around individual oil droplets (Maphosa and Jideani, 2018). Upon centrifugation, the emulsions' microscopic appearance showed that smaller droplets were formed for all parameters when UEP was used as an emulsifier; hence, a more stable emulsion. This finding supports the calculated cream fractions of the emulsions, where UEP has higher values than LMP. It was also observed that changes in the size of droplets were most visible for various oil fractions in UEP emulsion. As the oil fraction increased, smaller droplets were formed, and a higher cream fraction was recorded, hence, increasing stability.

3.3 Rheological property of the emulsion

Using UEP and LMP as emulsifiers, both emulsions exhibited a non-ideal liquid with shear-thinning behaviour wherein the apparent viscosity decreases with increasing shear rate (Figure 7). Between the two, UEP produced an emulsion with higher viscosity. This finding can be explained by the difference in the stabilizers' level

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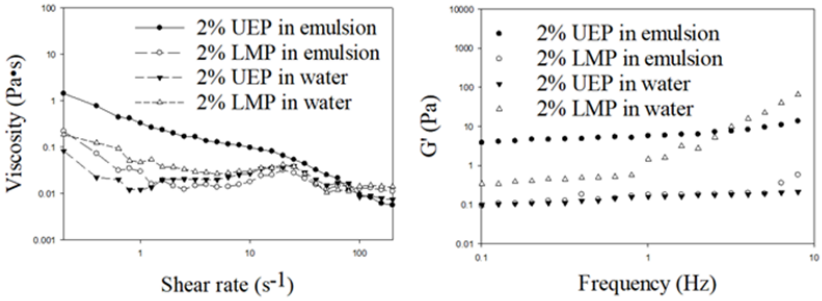

Figure 7. Rheology of oil/water emulsion stabilized by ultrasound-extracted pectin (UEP) from 'saba' waste banana peel (UEP) and commercial low-methoxy pectin (LMP) (2% pectin, 30% oil, pH 6, 0.02M NaCl).

of esterification, with LMP doubling the methoxyl content of UEP. A study comparing the effect of the citrus pectin's degree of esterification (DE) on the intrinsic viscosity showed that increasing the DE decreases the latter (Morris *et al*., 2000). They furthered that pectin with lower DE is more rigid. It may also be attributed to the charge distribution along the molecule (Yoo *et al*., 2006). Lower DE translates to lower surface charge, less repulsion, and, thus, higher viscosity of the continuous phase. Lastly, based on the microscopic appearances of the respective emulsions, UEP produced smaller droplets, which decreased the average distance between droplets, leading to an increase in both the hydrodynamic interaction and the viscosity (Pal, 1996).

3.4 In vitro digestion of emulsion

Digestion of lipids consists of an interfacial reaction since it depends on the lipase adsorption onto the oil droplet surface (Torcello-Gómez *et al.*, 2011). Therefore, incorporating it in an emulsion can improve digestion since a larger surface area will be available for lipase adsorption.

Results (Figure 8) showed that both emulsions using UEP and LMP were found to be stable in all stages of in vitro digestion. While UEP provided a more stable emulsion before digestion, subjecting both emulsions to oral, gastric, and intestinal digestion did not affect the original stability.

In the final phase of digestion, hydrolysis of the ingested lipid generates free fatty acid (FFA) and diacylglycerols (Pafumi *et al*., 2002). Figure 9 shows a drastic increase in fatty acid in the first hour of intestinal digestion and a gradual increase in the remaining hour. Between the two emulsifiers, UEP resulted in a lower concentration of fatty acid, indicating its ability to inhibit lipid digestion.

Overall, the observed stability of emulsion throughout the in vitro digestion and the lower fatty acid concentration in the intestinal phase suggest that the UEP can be used in inhibiting lipid digestibility and can potentially be used in the development of reduced-calorie emulsion-based functional food products.

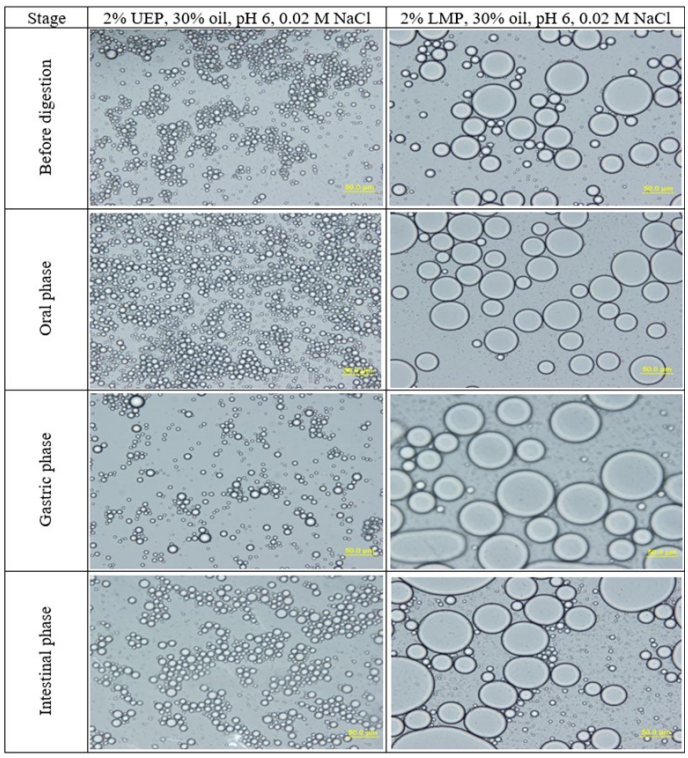

Figure 8. Appearance of oil/water emulsion stabilized by ultrasound-extracted pectin (UEP) from 'saba' waste banana peel (UEP) and commercial low-methoxy pectin (LMP) at different stages of digestion.

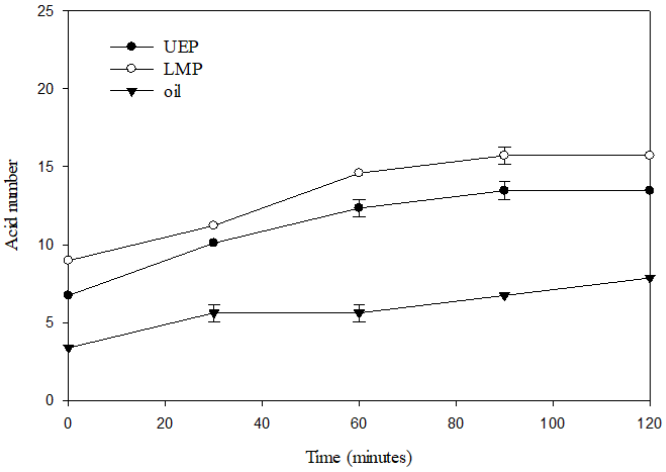

Figure 9. Free fatty acid concentration during the intestinal digestion of oil/water emulsion stabilized by ultrasound-extracted pectin (UEP) from 'saba' waste banana peel (UEP) and commercial low-methoxy pectin (LMP) (2% pectin, 30% oil, pH 6, 0.02 M NaCl).

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4. Conclusion

Using UEP from 'saba' waste banana peel as an emulsifier resulted in a more stable emulsion than an LMP. Despite having lower purity, the synergistic effects of the protein and pectin established a lower interfacial interaction with oil and water. Higher pectin and ion concentration, higher oil fraction, and pH close to pectin's isoelectric pH were the conditions that increased the volume of the stabilized emulsion. For both UEP and LMP, in vitro digestion did not affect the stability of the emulsion, with the former performing better in inhibiting lipid digestion. The production of UEP, considering the results of this study, has increased the value of the Philippine 'saba' banana. The utilization of waste banana peel as a source of high-value food ingredients is another leap towards zero-waste food production.

Conflict of interest

The authors declare no conflict of interest.

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