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ORIGINAL ARTICLE

Effect of extraction condition on properties of pectin from banana peels and its function as fat replacer in salad cream

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Abstract Banana peels are wasted from banana processing industry. Pectin is a soluble dietary fibre usually prepared from fruit and vegetable processing wastes. Pectin extraction from banana peels thus should be an effective way of waste utilization. This study aimed to determine the effect of extraction condition on the properties of pectin from peels of Nam Wa banana (*Musa* (ABB group) 'Kluai Nam Wa') and its role as fat replacer in salad cream. Banana peel pectin (BPP) was extracted with HCl (pH 1.5) and water (pH 6.0) for 30–120 min at 90 ± 5 °C. Acid extraction yielded 7–11% pectin on a dry basis with galacturonic acid content (GalA), degree of methylation (DM), and viscosity-average molecular weight (M_v) of 42–47, 57–61%, and 17–40 kDa, respectively; while water-extracted BPP contained lower DM but higher GalA and M_v. Prolonged extraction raised the pectin yield but lowered the M_v of BPP and the viscosity of their solutions. Incorporation of BPP obtained from 60 min acid- and water-extraction into salad cream at 30% oil substitution level resulted in the decreases in viscosity and lightness. All reduced-fat samples were stable to cream separation during 3-weeks storage although the formula containing water-extracted BPP had larger oil droplet size and greater extent of droplet flocculation. There was no difference in sensory scores rated by 50 panelists on thickness, smoothness, and overall acceptability of the full- and reduced-fat salad creams. Therefore, Nam Wa banana peels can be an alternative source of pectin with potential application as fat replacer in food products.

Keywords Banana peel · Chemical structure · Fat replacer · Pectin · Salad cream

Introduction

Banana is a tropical plant with the estimated world's total production of 102 million tonnes in 2012, of which 57 million tonnes was produced in Asia (FAO 2013). Due to its abundant supplies, banana is usually preserved and further processed into several value-added products, e.g., dried banana and banana chips. In Thailand, a large quantity of banana peels is wasted from banana processing industry due to the rising demands of processed banana products. It was estimated that over 200 tonnes of banana peels is generated each day and tends to continually increase (Pangnakorn 2006). Therefore, the uses of banana peels would be beneficial both in reducing the amount and adding the value of industrial waste (Padam et al. 2014).

Pectin is a polysaccharide rich in galacturonic acid molecules that are joined together by α-1,4-glycosidic bonds. Pectin contains three polysaccharide structures, namely homogalacturonan (HGA), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II). The RG-I and RG-II domains are HGA backbone interrupted by side chains of neutral sugars, e.g., arabinose, rhamnose, and galactose. Some carboxyl groups on galacturonic acid backbone can be esterified by methyl group. The percentage of methyl-esterified galacturonic acid units in the pectin chains or degree of methylation (DM) is used to classified pectin to be high- (HMP), and low-methoxyl pectins (LMP), of which the DM are ≥50 and <50%,

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respectively. HMP and LMP have different conditions and mechanisms of gelling, i.e., HMP forms gel in the presence of high amount of sugar (55–85%); while LMP requires divalent ions to form gel (Brejnholt 2009). Regarding to its viscosity and gel-forming ability, pectin is widely used as thickening, gelling, and stabilising agents, as well as a fat replacer in many food products, e.g., low-fat spreads, margarine, and mayonnaise (Brejnholt 2009).

Pectin is generally extracted using acid solution under controlled condition at high temperature. After extraction, pectin is separated from the residue by filtration or centrifugation prior to precipitation with alcohol, washed, and finally dried and ground to obtain powdered pectin. The source and condition of extraction greatly affects the yield, chemical structure and properties of the obtained pectin (Wang et al. 2002). Under acidic condition, acid hydrolysis occurs, resulting in the lowering in molecular weight of pectin (Diaz et al. 2007). Extraction under neutral and alkali conditions also cause de-polymerization of pectin chains via β-elimination at C-4 position of methylated galacturonic acid units (Joye and Luzio 2000; Renard and Thibault 1996). Extended extraction time tends to increase the yield of pectin, despite that the methoxyl content and equivalent weight of pectin are decreased due to partial degradation (Kulkarni and Vijayanand 2010). Commercially, pectin is extracted from apple pomace and citrus peels (Lopes-da-Silva and Rao 2006). Pectin can also be extracted from other fruit and vegetable processing wastes, e.g., mango peel (Koubala et al. 2008), and passion fruit rind (Kulkarni and Vijayanand 2010).

The characteristics of pectin extracted from banana peels have been previously reported to depend largely on the extraction parameters as well as the varieties and maturity of the banana (Emaga et al. 2008a, b). However, the information on properties and the food application of BPP from Nam Wa banana (*Musa* (ABB group) 'Kluai Nam Wa'), which is widely grown and consumed in Thailand and other Southeast Asian countries, is still limited. Therefore, this study aims to use Nam Wa banana peels as an alternative source for the local production of pectin with an emphasis on determinations of the chemical structure, thickening and gelling abilities of the pectins extracted under different conditions, as well as their potential application as fat replacer in salad cream.

Materials and methods

Materials

Peels of Nam Wa banana at the 5th stage of ripening, of which the colour is more of green than yellow (Emaga et al. 2007), were collected from the small scale processed banana producers in Nakhon Pathom, Thailand during September 2012 to January 2013. Such varieties was selected since it is the most commonly-grown and consumed in Thailand. After collection, the peels were washed twice in tap water prior to blanching in distilled water at 100 °C for 5 min. The peels were then be chopped into pieces of 1 × 1 cm^2, packed in sealed low-density polyethylene (LDPE) bags and stored in the freezer at −20 °C until being used for extraction. The blanched peels consisted of 9.5% protein, 13.3% fat, 11.4% soluble dietary fibre, and 59% insoluble dietary fibre on a dry weight basis, as analysed according to the AOAC Official Methods (AOAC 2000). Ingredients of salad cream, i.e., soybean oil, refined sugar, distilled vinegar, mustard powder, iodized table salt, hen's egg, and lime were purchased from a local supermarket. All chemicals, unless otherwise stated, were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Pectin extraction

BPP were prepared as described by Kulkarni and Vijayanand (2010) with some modifications. The frozen banana peels were thawed prior to mixing with 0.05 M HCl or deionized (DI) water at a solid-to-liquid ratio of 1:2 (w/v). The pH of the resultant mixtures were adjusted to be 1.5 and 6.0 in order to prepare acid- and water-extracted BPP, respectively. The extraction was performed at 90 ± 5 °C with agitation on a hot plate for 30, 60, or 120 min. After extraction, the mixture was filtered through double-layer nylon cloth. The filtrate was mixed with 95% ethanol (Government Pharmaceutical Organization, Bangkok, Thailand) at a filtrate-to-ethanol volume ratio of 1:2 and left undisturbed at room temperature (28 °C) for 12 h. The precipitated pectin was harvested, washed twice with 95% ethanol at a volume ratio of 1:1, and dried at 50 °C in a hot air oven until dry (<10% moisture). The dried precipitate was ground, packed in sealed LDPE bags and kept in the desiccator at room temperature until being analysed.

Determination of BPP properties

Extraction yield

The yield of BPP obtained from each extraction condition was calculated on a dry-weight basis as follows:

$$\text{Extraction yield } (\%) = \frac{\text{Weight of dried pectin } (g) \times 100}{\text{Weight of dried peel taken for extraction } (g)}$$

Galacturonic acid content

Galacturonic acid content (GalA) was determined by the colourimetric assay using *m*-hydroxydiphenyl (Blumenkrantz

and Asboe-Hansen 1973). One millilitre of BPP solution (0.005% w/v in DI water) was mixed with 6 mL of 0.0125 M sodium tetraborate in H_2SO_4, heated in water bath at 95 °C for 5 min, and immediately cooled in an ice bath. Then, 100 µL of 0.15% (w/v) *m*-hydroxydiphenyl in 0.5% (w/v) NaOH was added and mixed thoroughly. The mixture was measured for absorbance at 520 nm by a UV–visible spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). GalA of BPP was determined against the standard curve prepared with galacturonic acid monohydrate (≥97% purity).

Monosaccharide composition

Analysis of monosaccharide composition was performed using HPLC according to Methacanon et al. (2014) with slight modifications. Pectin hydrolysis was carried out by adding 20 drops of 2 N trifluoroacetic acid into 1% (w/v) BPP solution in DI water and heated at 95 °C with shaking at 95 rpm in a shaking water bath for 6 h. The hydrolysates were filtered through 0.2 µL filter and injected (25 µL) to HPLC system (Agilent 1200 LC Series, Agilent Technologies, Santa Clara, CA, USA) equipped with liquid chromatograph pumps (Waters 515 HPLC Pump, Waters, Milford, MA, USA), and Shodex column (250 mm length, 4.6 mm internal diameter, 5 µm pore size; Asahipak NH2P-50 4E, Showa Denko, Tokyo, Japan) and electron light scattering detector (ELSD 800, Alltech, Nicholasville, Kentucky, USA). The mobile phase was acetonitrile:water (8:2) at a flow rate of 1 mL/min. The detector temperature was set at 80 °C and N_2 gas was set at 2 MPa. Data were collected and processed by Clarity software (DataApex, Prague, Czech Republic). Identification of sugars in BPP samples was performed by comparing with retention times of individual sugar standards including galactose, arabinose, fructose, maltose, and sucrose. Monosaccharide composition of BPP sample was reported as the proportion of peak area of each individual sugar to total peak area.

Degree of methylation

Degree of methylation (DM) was determined by Fourier-transform infrared (FTIR) spectroscopy (Singthong et al. 2004). All BPP powder were desiccated in a desiccator before analysis by placing on an attenuated total reflectance sampling accessory (Smart iTR, Thermo Fisher Scientific, Waltham, MA, USA) of an FT-IR spectrometer (Nicolet 6700, Thermo Fisher Scientific) equipped with a single bounce diamond crystal. FTIR spectra of the sample were obtained by co-adding 64 scans at the resolution of 4 cm^{-1} in mid-infrared region (4000–400 cm^{-1}). The obtained spectra of each sample were subtracted with a blank spectrum collected from atmosphere. The areas of peak at 1760–1745 and 1640–1620 cm^{-1}, which represent esterified carbonyl group (C=O) and free carboxylic group (COO−), respectively were integrated using a computer software (OMNIC version 8.1, Thermo Electron, Madison, WI, USA). DM was calculated using the following linear correlation:

$$\mathrm{DM}\ (\%) = 87.609\left(\frac{\mathrm{Area}_1}{\mathrm{Area}_1 + \mathrm{Area}_2}\right) + 25.768$$

where $Area_1$ and $Area_2$ are areas of the peaks appeared between 1760–1745, and between 1640–1620 cm^{-1}, respectively.

Viscosity-average molecular weight

The intrinsic viscosity of dilute solutions of BPP (0.025–0.2% w/v in 0.1 M NaCl) was measured using a Cannon–Fenske viscometer (Capillary No. 50, internal diameter 0.44 mm; Schott-Geräte, Hofheim, Germany). The temperature was controlled at 30 °C by placing the viscometer in a temperature-controlled water bath. The intrinsic viscosity of pectin was determined graphically by extrapolation of Huggins, and Kraemer plots to zero concentration. Viscosity-average molecular weight (M_v) of BPP was estimated from their averaged intrinsic viscosities according to Mark–Houwink–Sakurada equation:

$$[\eta] = K(M_v)^{\alpha}$$

where [η] is intrinsic viscosity (cm^3/g), and *K* and α are temperature-depending parameter constants, which are 0.0436 and 0.78, respectively for pectin dissolved in 0.1 M NaCl pH 7.0 (Lopes-da-Silva and Rao 2006).

Thickening ability

Apparent viscosity of BPP solution (2.5% w/v in DI water) was measured at a shear rate of 122 s^{-1} using a Brookfield viscometer (RVTDV-II, Brookfield Engineering Laboratories, Middleboro, Massachusetts, USA) fitted with a UL adapter 304 s/s (Part Number: ULA-Y). All measurements were carried out at room temperature.

Gelling ability

The gelling ability of BPP was determined qualitatively by observing its ability to form HMP and LMP gels. HMP gel mixtures contained 1% (w/v) pectin and 60% (w/w) sugar at pH 3; while LMP gel mixtures contained 1% (w/v) pectin and 3 mM $CaCl_2$ at pH 6 (Zykwinska et al. 2009). The mixtures were heated at 70 °C for 15 min and left undisturbed in beakers at room temperature for 20 h to allow gel setting. The mixture was justified as gel if no flow was observed when the beaker was turned upside down.

Preparation of salad cream

In order to determine for the potential application of BPP as fat-replacer, salad cream containing BPP was prepared. The control formula (CS) contained 50% soybean oil, 18.5% sugar, 10.1% egg yolk, 8.5% vinegar, 7.2% distilled water, 3.5% lime juice, 1.6% salt, and 0.6% mustard powder. In order to prepare CS, egg yolk was whisked before mixing with other ingredients, except soybean oil. Soybean oil was then gradually added and continuously stirred until homogeneous. For reduced-fat formulas (RS-A and RS-W), 30% of soybean oil in CS was substituted with 2% (w/v) solution of acid- or water-extracted BPP, which was lastly incorporated into the emulsion. The sample was pasteurised by heating at 90 °C for 10 min, hot-filled into glass jars, and immediately cooled in ice bath for 30 min.

Quality determination of salad cream

Colour

The colour values (L*, a*, b*) were measured using spectrophotometer (ColorFlex EZ, Hunter Associates Laboratory, Reston, VA, USA). The L*, a* and b* values represent the lightness, redness and yellowness, respectively. Colour difference (ΔE*) of each RS and CS was calculated according to the following equation:

$$\Delta E^* = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2}$$

where L^*_0, a^*_0 and b^*_0 are colour values of CS.

Rheological properties

Rheological properties were determined at room temperature using a Brookfield viscometer (RVTDV-II) fitted with a small sample adapter (part number: SC4-28). The apparent viscosity and shear stress were recorded every 2 min while the shear rate was increased from 0.14 to 28 s^{-1} over 16 min. Yield stress was determined by extrapolating the plot between shear rate and shear stress to the intercept on the stress axis. Flow behaviour and consistency indices were determined by fitting the obtained flow curves using the Herschel–Bulkley Model:

$$\sigma - \sigma_0 = K\gamma^n$$

where σ is shear stress (Pa), σ_0 is yield stress (Pa), γ is shear rate (s^{-1}), *K* is consistency index (Pa s^n) and n is flow behaviour index (Rao 2014).

Droplet size and morphology

Particle size distribution was measured using a laser diffraction particle analyser (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). The samples were diluted in DI water to obtain 0.001% (v/v) fat content to avoid the multiple scattering effects. A relative refractive index of 1.10, which is the ratio of refractive index of soybean oil (1.4743) and water (1.3330), were used to calculate surface-weighed (d_{32}) and volume-weighed mean diameters (d_{43}):

$$d_{32} = \Sigma n_i d_i^3 / \Sigma n_i d_i^2$$

$$d_{43} = \Sigma n_i d_i^4 / \Sigma n_i d_i^3$$

where n_i is the number of droplets of diameter d_i.

Droplet morphology was observed under 400× magnification using an inverted microscope (Eclipse Ti-S, Nikon, Tokyo, Japan). Samples were diluted with DI water before placing on glass slides covered by cover slips. Pictures were taken from 3 different fields on each slide.

Phase separation

Stability of salad cream was evaluated by the extent of separation of cream layer or oil from the samples during 3 weeks of storage in screw-capped test tubes at room temperature, and 4 °C under refrigeration.

$$\text{Phase separation } (\%) = \frac{\text{Height of oil or cream layer (mm)}}{\text{Total height (mm)}} \times 100$$

Sensory quality

Sensory evaluation was conducted to test the acceptability of sample by 50 untrained panellists (24% male, 76% female, aged 22–58 years). Samples coded with 3-digit random numbers were served one at a time at room temperature in a random order. Panellists were asked to test samples along with lettuce and rinse their mouths with drinking water between samples. General appearance, colour, thickness, smoothness, taste, and overall acceptability of samples were evaluated using a nine-point hedonic scale, where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.

Statistical analysis

All experiments, except the sensory evaluation, were conducted in three replications of separate sets of experiment. Data were analysed using a computer statistics program (SPSS 17.0, SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to assess the mean difference among treatments. All statistical analyses were determined at 5%

level of probability ($p \leq 0.05$). Only significantly different results are discussed in the text. Data are presented as the mean and standard deviation.

Results and discussion

Effect of extraction condition on BPP properties

Typical FTIR spectra of acid- and water-extracted BPP (Fig. 1) consisted of characteristic peaks of polysaccharides from plant cell walls which corresponded to their major chemical groups. The peak occurring in a broad region between 3600 and 2900 cm^{-1} represents O–H stretching. The peak at 2930 cm^{-1} corresponds to O–CH_3 stretching from methyl ester of galacturonic acid. The peaks at 1760–1745 and 1640–1620 cm^{-1} are from esterified and free carboxylic groups, of which intensity ratio was used to determine DM of BPP. Finally, the peaks observed in 1400–950 cm^{-1} region corresponded to the typical profile of polygalacturonic acid (Kacuráková et al. 2000; Singthong et al. 2004). Extraction yield of BPP varied from 4.8 to 11% on a dry weight basis depending on the condition used. The higher yield was obtained when BPP were extracted under acidic conditions (Fig. 2a), possibly because the acid enhanced cell wall disruption and hence increase pectin release (Kirtchev et al. 1989).

At constant extracting temperature and time, pH of extraction were the major influencing parameters for pectin isolation (Koubala et al. 2008; Levigne et al. 2002). The yield of acid-extracted BPP significantly increased ($p \leq 0.05$) when extraction was extended from 30 to 60 min, and remained unchanged after that; while that of water-extracted BPP was not affected by the length of extraction. The effect of length of extraction on pectin yield from different sources seems to be varied. Longer extraction time was reported to increase the yield of pectins from banana peel (Emaga et al. 2008b), but had no effect on apple pomace pectin (Garna et al. 2007). Extractions of BPP using different extractants and durations did not significantly affect GalA (Fig. 2b), which implies the purity of the obtained pectin (Brejnholt 2009). According to FAO and EU specifications, all BPP in this study should be classified as pectin-enriched materials since their GalA were less than 65% (Willats et al. 2006). The large standard deviations suggested the variations in the component of banana peels from different batches. GalA of BPP have been reported to depend largely on the severity of extracting medium and condition in other previous studies. BPP extracted under resembling condition to the present study contained comparable GalA (40–46%) (Emaga et al. 2008b); while natural weak acid extractants yielded BPP with higher GalA (68.4%) (Emaga et al. 2008a). It is noteworthy that the techniques used for determination of GalA in this study (Blumenkrantz and Asboe-Hansen 1973) also gives the same positive reaction with glucuronic acid, which can also be a component of pectins.

Results from neutral sugar analysis revealed that BPP extracted with water for 60 min contained 6.7% fructose, 4.8% arabinose, and 3.1% other sugars (data not presented). Greater amount of neutral sugars was observed in BPP extracted under similar condition at pH 1.5, i.e., 13.0% glucose, 6.1% maltose, 3.4% fructose, 2.3% arabinose, and 10.9% other sugars, which was consistent with its lower GalA. It could be implied from its higher sugar content that acid-extracted BPP contained longer side chains of neutral sugars and/or more hairy regions on its polygalacturonic acid backbone. A plausible explanation for the higher glucose content in acid-extracted BPP is that the strong acidity enhanced the hydrolysis of non-pectic substances in banana peels, especially starch, into glucose and degraded polysaccharides that could co-precipitate with pectin in ethanol, which also observed as its higher pectin yield (Fig. 2a). Although some monosaccharides, e.g., rhamnose, xylose, mannose, which are usually present in pectin, was not included in monosaccharide standards used in this study, our results suggested that such monosaccharides would account only for less than 10% of the total sugar. It has been reported that banana peel pectin extracted with water at 60 °C for 2 h contained 11% glucose, 2.5% galactose, and 2.2% arabinose with only 2.7% mannose, 0.8% xylose and 0.5% rhamnose (Emaga et al. 2008a).

The DM of BPP, calculated from the ratio of certain FTIR peaks (Fig. 1), was strongly associated with the pH of extraction (Fig. 2c). Acid-extracted BPP was HMP with ~60% DM while water-extracted BPP was LMP (~40% DM). The longer extraction time resulted in a slight increase in the DM of acid-extracted BPP ($p \leq 0.05$); while that of water-extracted BPP was unaffected. Acid extraction usually yields pectin with higher DM than that extracted at higher pH values. The similar DM (49–80%) was also reported for banana (*Musa* AAA) peel pectin extracted using 1 M H_2SO_4 solution (Emaga et al. 2008b). The slight increase in the DM of acid-extracted BPP at longer extraction time is surprising because harsher extraction conditions, e.g., time and temperature, are known to cause the de-esterification of polygalaturonic acid chains. However, the increase in DM has also been reported in the isolated pectin from mesocarp of citrus fruit when it was extracted using acid solution for longer period of time (Lima et al. 2010).

Acid-extracted BPP had lower M_v than water-extracted BPP (17–29 and 21–40 kDa, respectively; Fig. 2d) because it was depolymerized by acid hydrolysis into shorter polygalacturonic acid chains (Diaz et al. 2007). The longer

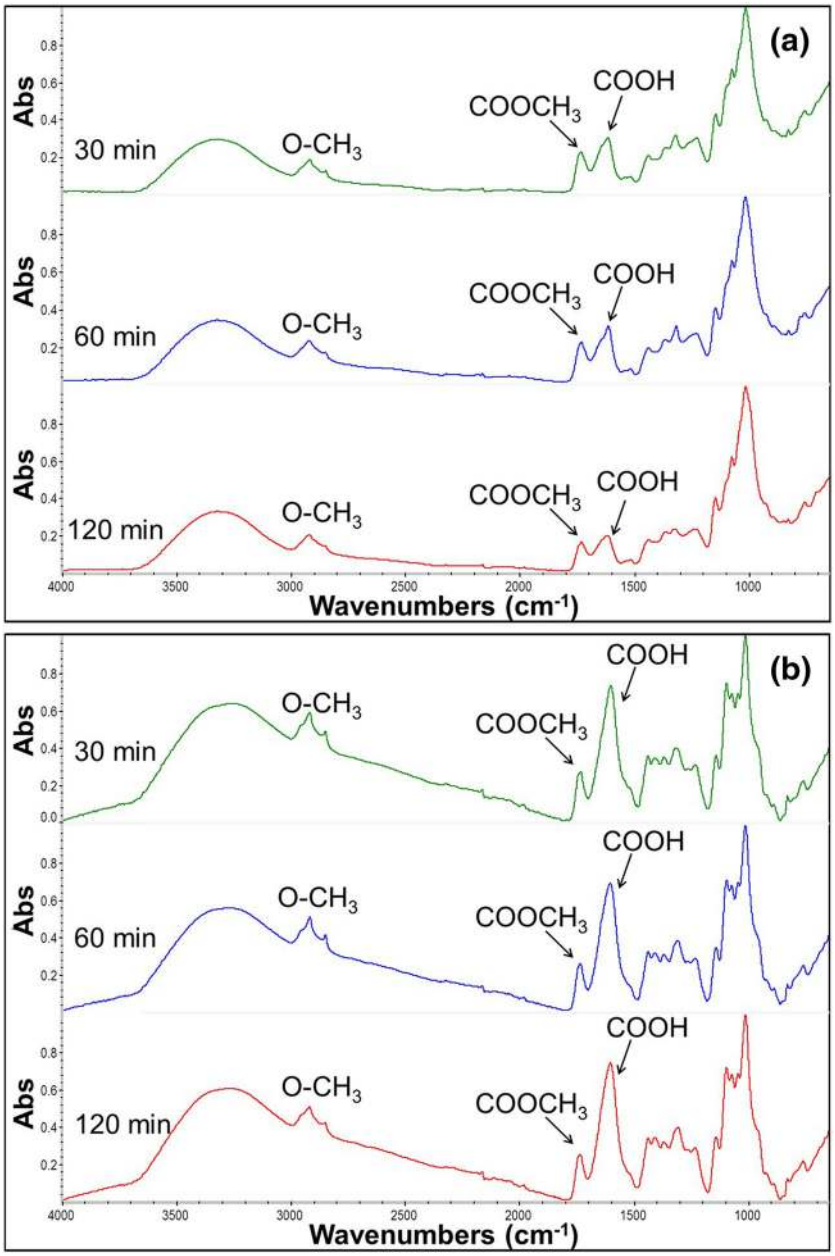

Fig. 1 Typical FTIR spectra of BPP extracted using acid (**a**) and water (**b**) at 90 ± 5 °C for 60 min

extraction time at any pH resulted in BPP with lower M_v since the hydrolysis proceeded longer. M_v of BPP from all extractions in this study were lower than that of pectin extracted from Cavendish banana cell wall material by using hot citric acid (pH 1.8) at 75 °C for 60 min (87.3 kDa) (Yapo 2009) and weight-average molar mass of banana (*Musa* AAA) peel pectin extracted using 1 M H_2SO_4 under various conditions (87–248 kDa) (Emaga et al. 2008b). This could be due to the variations in type, composition and characteristic of banana peel as well as the different determination techniques used in each study.

Thickening ability of BPP was determined from the viscosities of 2.5% BPP solutions measured at the shear rate of 122 s^{-1}. The results show that the apparent viscosity of BPP solutions varied from 8 to 15 mPa s and the solutions of water-extracted BPP were more viscous (Fig. 2e). The longer extraction time significantly decreased the thickening ability of BPP, resulting in the lower viscosity of its solution. Such change was more pronounced in acid-extracted BPP. The less ability to provide thickening effect of acid-extracted BPP corresponded well with its lower M_v (Fig. 2d) and higher DM (Fig. 2c). Similar to any other hydrocolloids, ability of pectin in providing the thickening effect depends largely on its molecular weight and molecular conformation. Pectin with higher molecular weight and more rigid structure and

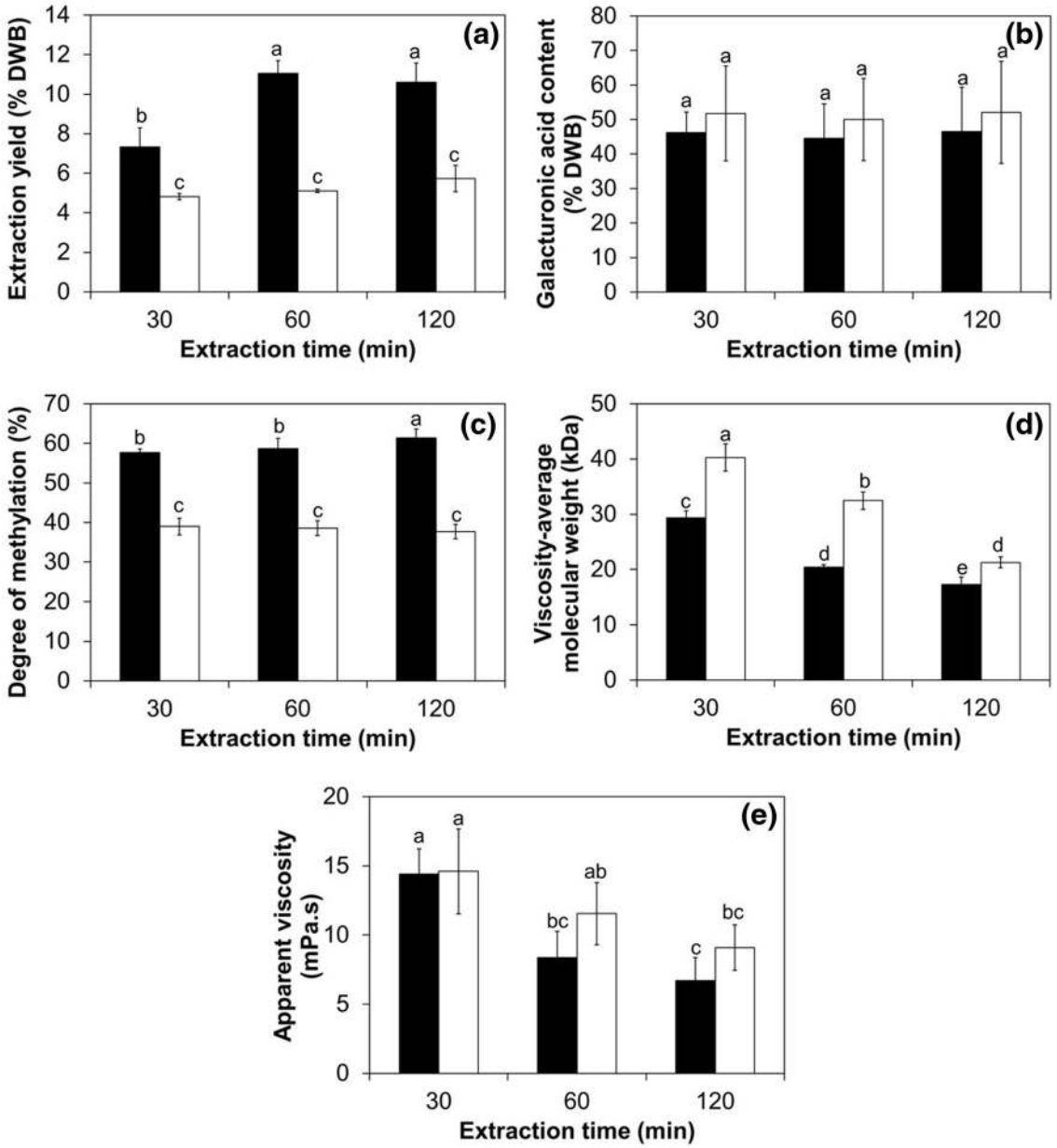

Fig. 2 Extraction yield (**a**), galacturonic acid content (**b**), degree of methylation (**c**), viscosity-average molecular weight (**d**), and apparent viscosity at 122 s^{-1} of 2.5% (w/v) solutions (**e**) of BPP extracted at 90 ± 5 °C for 30, 60 and 120 min. *Filled bars* represent acid-extracted BPP; *open bars* represent water-extracted BPP

provides more viscous solution (Brejnholt 2009). The longer extraction time and more acidic condition could enhance pectin hydrolysis and hence resulted in the more degraded, shorter pectin chain. Moreover, prolonged extraction at high temperature used in this study could also decrease the viscosity of BPP solution due to the accelerating effect of heat on de-polymerization reaction (Diaz et al. 2007). The fact that acid-extracted BPP contained more methylated carboxylic groups on their polygalacturonic acid backbone (Fig. 2c) may also affect their thickening ability (Fig. 2e). Due to the hydrophobicity of methoxyl groups, pectins with higher DM are less able to hydrate and provide viscosity to the solution. It should also be noted that the difference in pH of solutions of acid- and water-extracted BPP used for viscosity measurements might led to their different viscosity. Solutions of water-extracted BPP had pH values of 6.7 while those of acid-extracted BPP were about 2.9, which is lower than the pK_a of galacturonic acid ($pK_a = 3.5$). Therefore, in the solutions, the carboxylic groups on polygalacturonic chains of acid-extracted BPP did not dissociate, resulting in the limited ability to form hydrogen bonds with water

molecules, and hence the lower apparent viscosity of the solutions.

All acid-extracted BPP samples formed HMP gel in the presence of sugar under acidic condition, which corresponded to their DM (Fig. 2c). HMP gel is formed due to the mechanism of pectin–pectin interactions, which is promoted by high content of soluble solids, e.g., sugar and acid, that creates low water activity condition (Morris et al. 1980). In addition, the low pH leads to low ionization of carboxyl groups which also minimizes the electrostatic repulsive forces between pectin chains (Morris et al. 1982; Oakenfull and Scott 1984). Surprisingly, none of the water-extracted, low-methylated BPP formed LMP gel in the presence of Ca^{2+}. The LMP from Krueo Ma Noy (*Cissampelos pareira*) leaves were also reported not to form gel in the presence of Ca^{2+} at pH 5–8, while gelation was observed at lower pH, probably because the stronger acidity reduced the dissociation of carboxylic groups and charge density of pectin chain that diminished electrostatic repulsion and promoted interchain interaction (Singthong et al. 2005). The lack of gelation in LMP was also observed in water-extracted pectin from dragon fruit peel, which is reported to be due to the high ash content of the pectin (Nazaruddin et al. 2011).

Quality of salad cream containing BPP solution as a fat replacer

Based on their yield of the extraction and properties, BPP extracted with acid solution and with DI water for 60 min at 90 ± 5 °C were selected to evaluate their potential use as fat replacers in salad cream. From preliminary experiments, substituting 30% of oil with solution of 2% (w/v) BPP resulting in the appropriate thickness and appearance of salad cream, while higher levels of oil substitution seemed to give salad cream with very slimy gel, lack of body and dark colour. Therefore, such level of oil substitution was used to prepare RS-A and RS-W.

The colour of RS-A tended to be darker, redder and less yellow when compared to CS. A similar manner of significant differences was also observed in the colour values of CS and RS-W but to a greater extent, as indicated by the higher ΔE* value. The major reason for the changes in the colour of both BPP-containing samples is the reddish brown colour of BPP solutions, especially the higher a* value of acid-extracted BPP. The lower in lightness of water-extracted BPP solution led to the darker colour of its respective RS (Table 1).

Oil substitution with BPP solution resulted in the decrease in viscosity of salad cream. This was majorly

Table 1 Physico-chemical properties of control and reduced-fat salad creams containing 2% (w/v) BPP solutions as a fat replacer at 30% oil substitution level

Parameters[1]	Formula[2]		
	CS	RS-A	RS-W
pH	3.43 ± 0.01[b]	3.32 ± 0.01[c]	3.49 ± 0.01[a]
Colour values			
L*	81.2 ± 0.01[a]	77.7 ± 0.01[b]	72.3 ± 0.07[c]
a*	5.59 ± 0.01[c]	6.67 ± 0.01[a]	6.26 ± 0.03[b]
b*	40.5 ± 0.04[a]	31.8 ± 0.01[b]	30.5 ± 0.03[c]
ΔE*	N/A	9.42 ± 0.03[b]	13.4 ± 0.00[a]
Rheological properties			
Apparent viscosity at 28 s^{-1} (mPa s)	2377 ± 15.3[a]	1127 ± 11.6[c]	1413 ± 5.77[b]
Yield stress (Pa)	87.9 ± 3.87[a]	86.9 ± 5.58[b]	60.0 ± 2.24[c]
Flow behaviour index	0.67 ± 0.05[a]	0.64 ± 0.12[a]	0.62 ± 0.04[a]
Consistency index (Pa s^n)	83.50 ± 8.95[a]	42.44 ± 9.86[b]	56.36 ± 4.16[b]
Mean particle size[3]			
d_{32} (μm)	9.40	8.62	9.87
d_{43} (μm)	27.20	22.70	31.80

N/A not applicable

[1] Means ± standard deviation of triplicate measurements

[2] CS, full-fat salad cream (control); RS-A and RS-W, reduced-fat salad creams using 2% (w/v) acid-, and water-extracted BPP solutions as fat replacer, respectively

[3] Means from duplicate analysis

[a,b,c] Means with different superscripts within the same row are significantly different ($p \leq 0.05$)

caused by the decreased fat content, which conduces to the body, i.e., viscosity, thickness and cling, and texture, i.e., creamy and smooth mouthfeel, of salad cream (Ma and Boye 2013). The highest viscosity was observed in CS, followed by RS-W and RS-A, respectively (Table 1). The higher viscosity of RS-W than RS-A corresponded to the higher viscosity of water-extracted BPP solution (Fig. 2e). Acid-extracted BPP might form gel during the preparation because the vinegar and sugar in the recipe created the condition that is suitable for HMP gelation. The formed gel of acid-extracted BPP thus contributed to the stronger network structure that resulted in the higher viscosity of the respective RS-A at low shear rates, and sharply decreased with the increasing shear rate due to its lower flow behaviour index and more shear thinning behaviour. There might also be the interaction between the pectin and oil droplets in both RS formulas. It has been reported that the interaction between LMP weak-gel and oil droplets could provide the structure and fat-like texture to low-fat

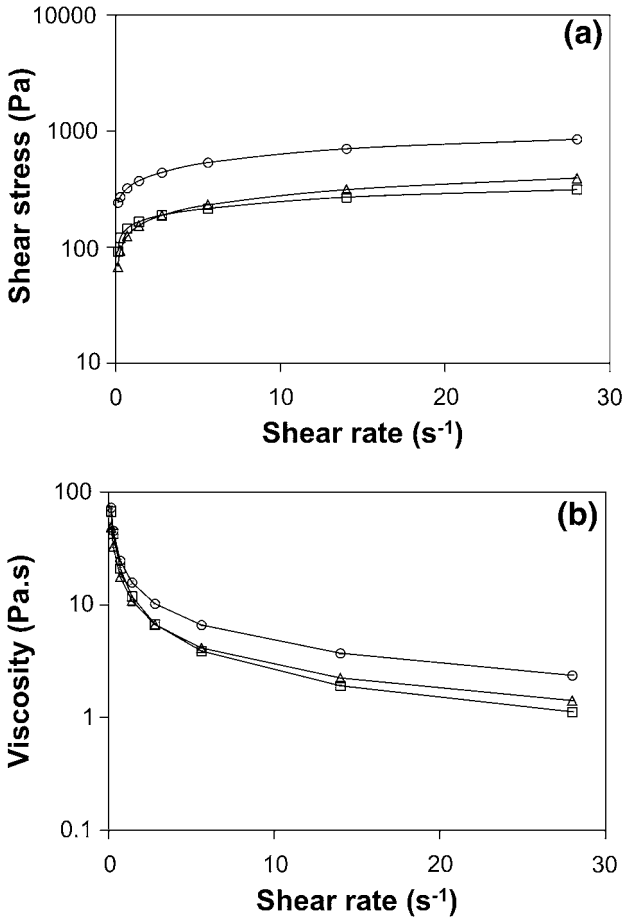

Fig. 3 Shear stress (**a**) and apparent viscosity (**b**) of (*circle*) control salad cream, and reduced-fat salad creams containing 2% (w/v) solution of (*square*) acid-extracted and (*triangle*) water-extracted BPP as fat replacer at 30% oil substitution level. Measurements were carried out at room temperature (28 °C)

mayonnaise (Liu et al. 2007). The incorporations of BPP resulted in RS with lower yield stress compared to CS, and RS-A exhibited higher yield stress than RS-W (Table 1; Fig. 3a). The yield stress implies the ability of salad cream to retain its adherence to the surface of salad and also the ease in being poured from its container (Ma and Barbosa-Cánovas 1995). Ma and Boye (2013) stated that the decrease in yield stress indicated the weakening of the gel structure of salad dressing, which makes the dressing be more easily to pour or flow. From the flow curves, all salad cream samples are non-Newtonian fluids with shear-thinning behaviour (Fig. 3b). Flow behaviour index of all salad cream samples were 0.6 (Table 1), indicating the more shear-thinning behaviour. Both RS-A and RS-W had lower consistency index than the control formula, which is consistent with their apparent viscosities (Table 1).

The particle size distributions of all samples were multimodal, of which the range and mode of oil droplet size were similar (Fig. 4a, c, e). The smallest particle sizes, i.e., $d_{32} \sim 9$ μm and $d_{43} \sim 23$ μm, were presented in RS-A (Table 1); whereas RS-W contained higher proportion of large oil droplets (Fig. 4e), of which the d_{32} and d_{43} of RS-W were 10 and 32 μm, respectively (Table 1). The d_{43} were about three times larger than d_{32} for all samples, suggesting that droplets were flocculated. Such differences in droplet size and morphology were confirmed by the micrographs taken from salad cream samples (Fig. 4b, d, f). CS contained more number of oil droplets than both RS samples (Fig. 3a, b). The largest oil droplets and the most extensive flocculation were observed in RS-W (Fig. 4e, f), which could be one of the reasons for the higher viscosity of RS-W (Table 1). Flocculation was known to increase the effective volume fraction of the emulsion, and thus increase the viscosity (McClements 2005). There was no observed separation of cream layer in CS during 3 weeks of the stability test. Slight creaming (<3% of the total height) was observed in both RS formulas at a similar extent (data not presented), even though RS-W was more flocculated (Fig. 4f). This is due to the fact that the viscosities of both RS samples were lower than CS (Table 1). Substitution of oil with BPP solution resulted in the decrease in amount, and hence the higher mobility, of oil droplets in the emulsions. However, both acid- and water-extracted BPP were able to provide sufficient viscosity to the aqueous phases to maintain the stability of RS. Mun et al. (2009) suggested that the addition of thickening agent such as gums or starch to the aqueous phase of low-fat product can slow down the droplet movement and prevent creaming of the emulsion.

Acceptability scores indicated that the full- and reduced-fat salad creams were significantly different ($p \leq 0.05$) only in appearance and colour (Table 2). Substituting 30% of soybean oil in salad cream with BPP solutions resulted in a slight decrease in the acceptability scores on appearance and colour from “like moderately” to “like slightly”.

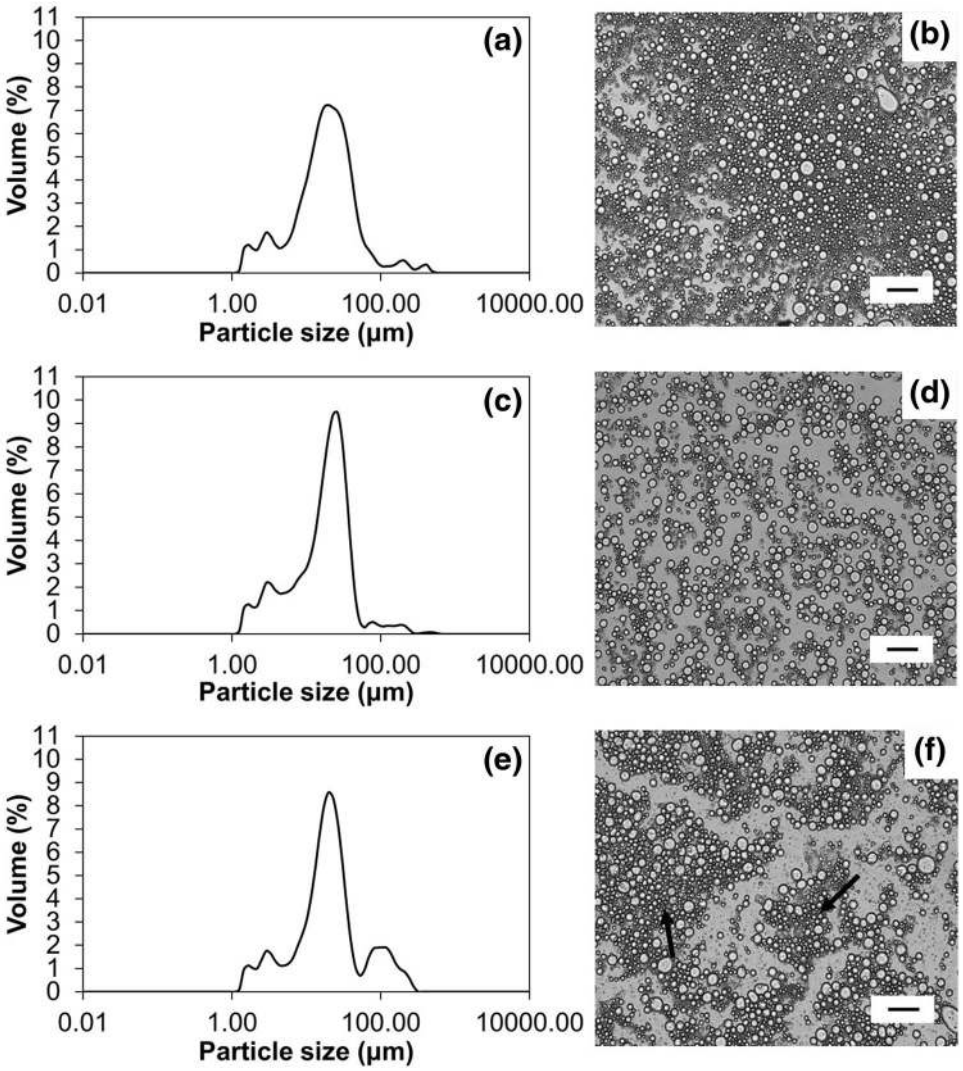

Fig. 4 Particle size distribution (**a**, **c**, **e**) and optical micrographs taken (**b**, **d**, **f**) of control salad cream (**a**, **b**), and reduced-fat salad creams containing 2% (w/v) solution of acid-extracted (**c**, **d**) and water-extracted BPP (**e**, **f**) as fat replacer at 30% oil substitution level. *Scale bar* represents 100 μm. The *arrow* points the flocculated droplets

Table 2 Sensory acceptability scores of control and reduced-fat salad creams containing 2% (w/v) BPP solutions as a fat replacer at 30% oil substitution level

Formula[1]	Sensory acceptability scores[2,3]					
	Appearance	Colour	Thickness	Smoothness	Taste	Overall acceptability
CS	6.56 ± 1.39^{a}	7.02 ± 1.10^{a}	5.44 ± 1.74^{a}	6.62 ± 1.35^{a}	6.60 ± 1.40^{a}	6.74 ± 1.23^{a}
RS-A	5.84 ± 1.58^{b}	5.74 ± 1.60^{b}	5.98 ± 1.41^{a}	6.66 ± 1.49^{a}	6.62 ± 1.61^{a}	6.56 ± 1.61^{a}
RS-W	5.86 ± 1.47^{b}	6.04 ± 1.35^{b}	5.82 ± 1.52^{a}	6.52 ± 1.34^{a}	6.46 ± 1.75^{a}	6.48 ± 1.62^{a}

[1] CS, full-fat salad cream (control); RS-A and RS-W, reduced-fat salad creams using 2% (w/v) acid-, and water-extracted BPP solutions as fat replacer, respectively

[2] Rated on 9-point hedonic scale: 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely

[3] Means ± standard deviations from 50 panelists

[a,b,c] Means with different superscripts within the same column are significantly different ($p \leq 0.05$)

The lower acceptability in the colour of both RS samples corresponded with the differences in their colour values from the control sample (Table 1). Although the rheological parameters, i.e., viscosity, yield stress and consistency index, of both RS samples were significantly lower than the control sample (Table 1), the scores on thickness, smoothness and overall acceptability were not significantly different (Table 2). It is likely that the panellists may not be able to detect such differences among the samples, or that the differences were detectable but the attributes of

both RS samples were still acceptable by the panellists. The results suggested that the incorporation of water- and acid-extracted BPP solutions did not affect the consumer's acceptability of salad cream. Therefore, both BPP have potential to use as fat replacers in salad cream at oil substitution level of up to 30%.

Conclusion

The yield and chemical properties, i.e., DM, M_v, and neutral sugar composition, of BPP depended largely on the severity of condition including pH and the length of extraction, which consequently affect the thickening and gelling abilities of BPP. Partial substitution of oil in salad cream by pectin from banana peels darkened the colour and lowered the viscosity and rheological properties of the product but did not affect the consumer acceptability. Therefore, peels of Nam Wa banana can be an alternative source for production of pectin with the potential application as fat replacer for high-fat food products.

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