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Developing precipitation modes for preventing the calcium-oxalate contamination of sugar beet pectins

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ABSTRACT

Effects of precipitation modes on the co-precipitation of insoluble oxalates particles during the purification of sugar beet pectins (SBP) from the extract were investigated. It was observed that soluble oxalate ions formed insoluble oxalate salts with calcium and precipitated with pectins during ethanol precipitation as pH of the medium increased and the solvent changed from water to ethanol–water mixture. Comparison among the employed precipitation methods revealed that both the dialysis–ethanol-precipitation and metal precipitation effectively prevented the calcium-oxalate contamination of SBP. Emulsifying properties of DEPP, EPP and MPP were also studied. It was observed that DEPP performed better than the remainder with respect to emulsifying ability. Based on these results, we concluded that the dialysis–ethanolic-precipitation can be a suitable method for improving the purity as well as emulsifying properties of the resulting pectins.

1. Introduction

Pectic substances are a family of heterogeneous polysaccharides with complex structures, fulfilling textural and mechanical properties of primary plant cell wall (Agoda-Tandjawa, Durand, Gaillard, Garnier, & Doublier, 2012). It is widely believed that pectins are primarily comprised of homogalacturonan (HG) and secondarily rhamnogalacturonan-I (RG-I) blocks (Yapo, 2011). Some of the galacturonic acid residues among the HG backbone are methyl-esterified at C-6, whereas the hydroxyl groups are acetyl-esterified at O-2 or O-3 (Levigne, Thomas, et al., 2002; Ralet et al., 2005). Structural characteristics of the extracted pectins are actually different, depending on the source of material (May, 1990) and the extracting conditions used (Levigne, Ralet, et al., 2002; Yapo, Robert, et al., 2007).

Generally, commercial pectins are produced from citrus peel and apple pomace which are by-products from juice industry (May, 1990). More recently, pectins have been produced from alternative plant materials such as sugar beet pulp (Li, Fang, Phillips, & Al-Assaf, 2013; Ralet, Crépeau, Buchholt, & Thibault, 2003; Yapo, Robert, et al., 2007), chicory root (Robert, Emaga, Wathelet, & Paquot, 2008), yellow passion fruit (Yapo, 2009a), etc. Among them, sugar beet pulp is considered as a promising source for pectin manufacturing due to its high pectin content (Fishman, Chau, Cooke, & Hotchkiss, 2008). However, pectins extracted from sugar beet pectins are characterized by a lower molecular molar mass (Arslan, 1995), a higher neutral sugar and a higher degree of acetylation (Rombouts & Thibault, 1986) as compared to those of commercial citrus or apple pectins, which limits their application as gelling agents in food (Oosterveld, Beldman, Searle-van Leeuwen, & Voragen, 2000). Fortunately, sugar beet pectins have been proved to be good emulsifying agents in food systems (Williams et al., 2005; Yapo, Robert, et al., 2007) due to its distinguished structural components (i.e., proteinaceous moieties, acetyl groups and ferulic acids) that enable pectins to active the oil–water interface (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Siew & Williams, 2008).

The chemical composition of sugar beet pulp is rather complex. Reports revealed that sugar beet pulp contains not only pectic saccharides, but also non-pectic compounds such as ash and oxalate salts (predominantly calcium and secondary magnesium oxalates) (Joy, 1964). Pectins along with some of the non-pectic compounds may be solubilized by the extractant under acidic extraction conditions (pH 1.3–3.0, 60–100 °C) (Yapo, 2009b). Since oxalate salts, especially calcium and magnesium oxalates, have pH-dependent solubility (McComas & Rieman, 1942), they will change being totally soluble to insoluble when pH of the extract increases up to a certain range. Moreover, the pH of the oxalates containing

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medium will not remain the same but increase when the medium is subsequently mixed with alcohol for the precipitation of pectins (Faravash & Ashtiani, 2007). It is therefore likely that oxalate will bind with calcium to form insoluble salts and precipitate with pectins, thereby contaminating the obtained pectin. From the view point of food safety, the oral intake of insoluble oxalate salts is risk to human healthy as these compounds may gradually accumulate in the human body, and then cause damage to the kidney (Mulay et al., 2013). In view of that, it is essential to develop methods for purifying pectin from these non-pectic contaminants to improve the purity and thus the quality of the final extracted products.

Previous works showed that the purifying modes effected not only on the chemical characterization (Garna et al., 2007; Yapo, Wathelet, et al., 2007) but also on the gelling properties of pectins (Garna et al., 2007; Yapo, 2009b). However, knowledge on how precipitation methods co-precipitate the insoluble oxalate particles during the precipitation of pectins is still rare. Moreover, no precipitation methods are by far available for the purification of sugar beet pectins from calcium oxalate particles.

In our previous work, we reported the formation of insoluble calcium oxalate (dihydrate) particles during the extraction of sugar beet pectins (Guo, Zhu, Tang, & Yu, 2014). In this work, effects of different purifying methods on the co-precipitation of calcium oxalate particles and emulsifying properties of the obtained sugar beet pectins were further investigated. We believe that the results obtained in this work can be used to improve the purity and quality of sugar beet pectins.

2. Materials and methods

2.1. Materials

Fresh sugar beet pulp (*Beta vulgaris*) with sugar removed was a gift from Lvxiang beet sugar company (Xinjiang, China). Sugar beet pulp was thoroughly washed with running tap water to exclude soil, air-dried at 45 °C for 48 h, and then ground to approximately 20 mesh. It was stored at −20 °C until prepared for extraction. Unless otherwise stated, all reagents used were of analytical grade.

2.2. Acidic extraction

Dried sugar beet pulp was dispersed in water (solid–liquid radio 1:20, w/v) and set to pH 1.5 with 6 M HCl. Extractions were performed by heating the suspension at 85 °C under stirring (150 rpm) for 1 h. Extractions were stopped by cooling to room temperature with running tap water. Then the resulting slurries were filtered through nylon cloth, and centrifuged (10,000$g \times$ 20 min) to remove the residue. After centrifugation, the supernatant was pooled, prepared for purification.

2.3. Turbidity

2.3.1. Turbidity of the extract

Turbidity of the extract was determined using a 2100AN turbidimeter (HACH company, USA) established in a previous report (Guo et al., 2014). 50 mL acidic extract with an initial pH of around 1.5 was adjusted to desired values using 1 M NaOH, and made up to 100 mL with distilled water. Aliquots were taken for turbidity measurement. Measurements were carried out in triplicate.

2.3.2. Turbidity index of pectins

1 g pectins were dissolved in 100 mL citrate buffer (pH 3.5, 20 mM) at ambient temperature with mechanical stirring (250 rpm, 24 h). The pectin solution was centrifuged at 5000g for 15 min. The supernatant was discarded, and the residual pectins were washed with 200 mL distilled water. The suspension was centrifuged at 5000g for 15 min. After discarding the supernatant, the precipitate at the bottom was washed with 100 mL distilled water (twice), centrifuged and carefully washed into a 100 mL volumetric flask with distilled water. The resulting mixture was subjected to the turbidity test as described above. Measurements were carried out in triplicate.

2.4. Pectin purification

Purifying procedures were carried out as described in Fig. 1.

2.4.1. Ethanolic precipitation

1L clarified extract was divided into four equal parts. One quarter of the extract was adjusted to pH 4.5, and subsequently centrifuged for the removal of insoluble turbid substances (Section 2.3). This pH-adjusted extract was mixed with 3 volumes of 95% ethanol. The ethanolic precipitation medium was allowed to stand for 4 h at 25 °C to ensure the complete precipitation of pectins. The precipitated pectins were recovered through centrifugation and filtration, washed with 75% ethanol (twice) to remove free sugars. The remaining ethanol-insoluble fractions were recovered in distilled water, freeze-dried. Pectins obtained from the pH-adjusted extract were referred to as 4.5EPP. The second part of the mother extract was directly precipitated with ethanol, recovered, freeze-dried, and referred to as ethanolic precipitated pectins (EPP).

2.4.2. Dialysis–ethanolic-precipitation

250 mL of the mother extract was dialyzed against distilled water through a membrane with a 14,000 g/mol molecular weight cut off. This treatment was performed with gentle stirring at room temperature. During the dialysis, distilled water was changed every 4 h. The dialyzed-extract was centrifuged, vacuum-evaporated at 45 °C to reduce the whole volume to the initial level. After concentration, pectins in the extract were precipitated with ethanol, separated, freeze-dried as above, and referred to as dialysis–ethanol-precipitation-pectins (DEPP).

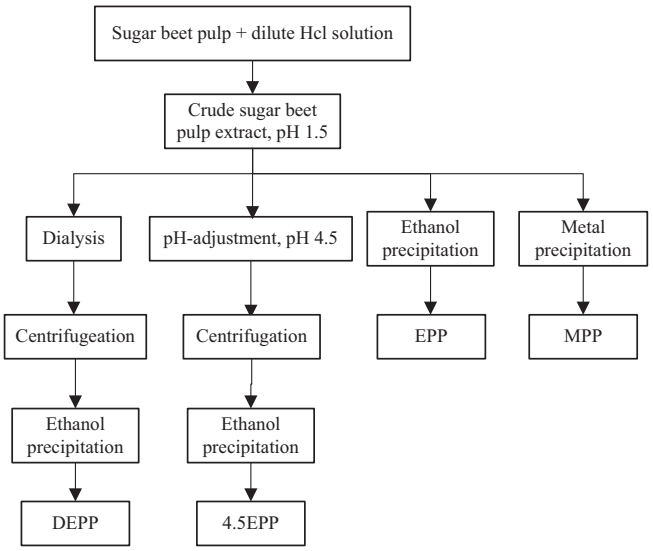

Fig. 1. Flow chart of the pectin preparation.

2.4.3. Metal-precipitation

The last part of mother extract was mixed with 25 mL 70 g L^{-1} solution of $CuSO_4$ (10:1, v/v) (Yapo, 2009b), the precipitation medium was stirred (200 rpm) at 4 °C. The Cu^{2+}–pectin complexes were recovered through centrifugation and filtration, dispersed in 1% (v/v) HCl – 60% (v/v) ethanol solution for 30 min (Hwang, Roshdy, Kontominas, & Kokini, 1992). The acidic-ethanol washing was repeated until the filtrate gave a negative response for the colorimetric cuprizon test (Keijbets & Pilnik, 1974). The pectin precipitates were washed by 75% (v/v) ethanol washing until the filtrate gave a negative response with silver nitrate. After evaporating ethanol, the remaining pectins were dissolved in distill water, freeze-dried, and referred to as metal-precipitated pectins (MPP).

2.5. Soluble oxalate

Soluble oxalate content of the extract was quantified colorimetrically (Allan, Band, & Rubio, 1986). 0.8 mL zirconium sulfate solution (0.57 mM), 0.69 mL quercetin solution (0.204 mg/mL) and 0.5 mL concentrate HCl were added to a 10 mL volumetric flask to form the quercetin–zirconium complex with a maximum absorbance at 430 nm. Different amounts of oxalate acid were added to cover the working range between 2.5 and 50 μg mL^{-1}. The mixture was diluted with distilled water to 10 mL and measured for absorbance at 430 nm using a UV-1901 spectrophotometer (Pgeneral Inc., Beijing, China). The oxalate content was in directly proportional to the decrease in absorbance at 430 nm, which corresponded to the degree of decomposition of quercetin–zirconium complex. Prior to the measurements, the tested extract was appropriately diluted to the concentration between 2.5 and 50 μg mL^{-1}. Then 0.7 mL was taken for determination according to the above procedures. Oxalate content was thereby calculated from a standard curve constructed using oxalate acid as a standard substance.

2.6. Neutral sugars

Neutral sugars (NS) were determined by high-performance anion-exchange chromatography (HPAEC) with a pulsed amperometric detector according to Garna, Mabon, Wathelet, and Paquot (2004) with slight modification. Pectins were decomposed by 0.2 M trifluoroacetic acid, followed by an enzymatic hydrolysis by viscozyme L (Novozymes, Tianjin, China). The hydrolysate was heated at 100 °C for 3 min to stop the enzymolysis. After filtered through a membrane filter of 0.22 μm pore size, 25 μL of the hydrolysate was injected into the HPAEC system (ICS-5000, Dionex Corp., USA) equipped with a CarboPac PA1 column (4 × 250 mm) with a guard CarboPac PA1 column (4 × 50 mm). The neutral monosaccharides were eluted using 10 mM NaOH for 25 min. Uronic acids were eluted with a gradient of 170 mM CH_3COONa and 100 mM NaOH and kept for 10 min. All the samples were analyzed at 30 °C. Total neutral sugars were calculated by the sum of rhamnose (Rha), arabinose (Ara), galactose (Gal), glucose (Glc) and xylose (Xyl). Data were presented as means ± SD of triplicate.

2.7. Scanning electron microscope (SEM)

SEM measurement was performed using a TM-3000 tabletop microscope (Hitachi, Japan). Prior to the observation, samples were stuck on stubs with double-face tape and then coated with a gold–palladium layer in an ion sputter (E-1010, Hitachi, Japan). Observations were operated at an accelerating voltage of 15 kV.

2.8. Emulsifying properties

2.8.1. Preparation of emulsions

Compositions of the O/W emulsions were 0.1–2% SBP, 20% w/w medium-chain triglyceride (MCT) and 0.1% sodium benzoate. 0.1–2 g pectin powders were added to a solution containing citrate buffer (pH 3.5, 20 mM) and 0.1% sodium benzoate under magnetic stirring at 500 rpm for 12 h. 20 g MCT were added to the above solution, and then sufficient citrate buffer was added to make 100 g total solution. The two phases of the solution were subject to pre-homogenization using a Silentcrusher (Heidolph, Germany) at 20,000 rpm for 2 min immediately prior to the ultrasonic emulsification. The ultrasonic emulsification was conducted using an ultrasonic equipment (JY98-111DN, SCIENTZ technology Ltd., China), and operated at 300 W for different radiation time. In order to prevent the temperature effect on the emulsification, the maximum temperature of the emulsion during emulsification was set to below 30 °C by cooling the emulsions with circulated cold water.

2.8.2. Determination of droplet size

The droplet size of the emulsions prepared with different pectin samples was immediately analyzed by the light scattering method using a Malvern Mastersizer 2000 (Malvern, UK). Average droplet diameters of the emulsions were determined as $d_{4,3}$, characterized by the volume-moment mean diameter. Deionized water was used for dispersant of emulsions. The refractive indexes for the MCT and water were 1.45 and 1.33, respectively. All experiments were performed in duplicate.

2.9. Analytical

Ash content of pectin was measured after incineration in a muffle furnace at 550 °C for 6 h. Galacturonic acid (GalA) content was quantified colorimetrically by the automated m-phenylphenol (Blumenkrantz & Asboe-Hansen, 1973). Degree of methylation and acetylation was determined according to the HPLC method (Levigne, Thomas, et al., 2002). Protein content (N × 6.25) was determined by the Kjeldahl procedure. Ferulic acid (FA) was determined according to the spectrophotometry method (Alexander Oosterveld, Beldman, Schols, & Voragen, 1996). FTIR analysis was carried out as previously described (Guo et al., 2014).

2.10. Statistics analysis

Data obtained were statistically evaluated by the one-way analysis of variance (ANOVA). Prior to the ANOVA test, the Levene's test for equality of variances was performed. If the Levene's test was negative ($p > 0.05$), data were then evaluated by the Scheffe's posthoc comparison. Means were considered to be significantly different at p-value < 0.05.

3. Results and discussion

3.1. Effect of pH and duration of dialysis

Fig. 2a clearly shows both turbidity and soluble oxalate content of the extract were sensitive to pH of the extract. The starting extract contained considerable amounts of oxalate ions (249.1 μg mL^{-1}), confirming the acidic extraction of oxalates from the material. Turbidity of the extract increased as pH increased from 1.5 to 4.0, thereafter, it tended to be constant. In contrast, the soluble oxalate level decreased as pH increased from 1.5 to 4.5, and it leveled off despite further increased the pH. Moreover, the lowest oxalate level was obtained at pH 4.5, which

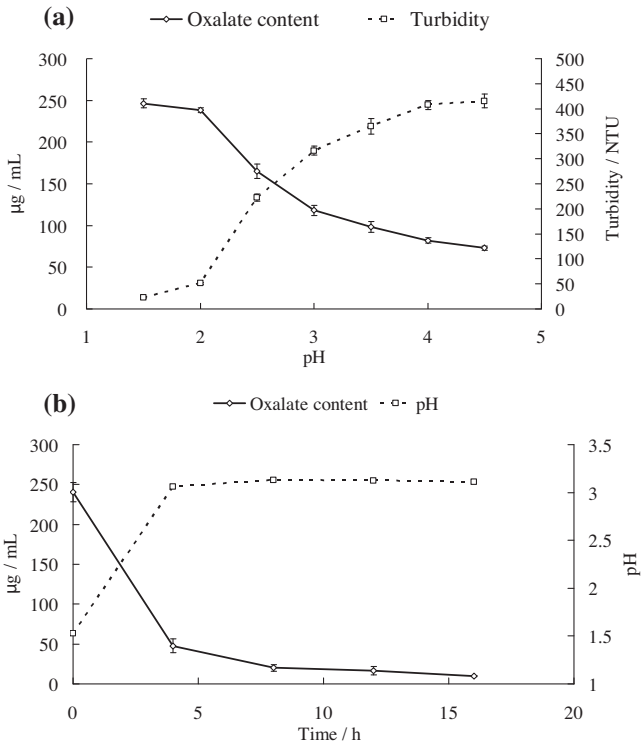

Fig. 2. Change in oxalate content as a function of (a) pH and (b) dialysis time. Turbidity of the pH-adjusted extract was measured immediately using a turbidimeter after the pH adjustment. pH of the dialysis-treated extract was subjected to the determination every 4 h during dialysis.

corresponded to the highest turbidity. These results suggested the increase in turbidity of the extract was related to the amount of insoluble oxalate salts formed with increasing pH. It was observed that both the turbidity and oxalate level (72.9 μg mL^{-1}) remained constant when pH was greater than 4.0, an indicative of the maximum formation of insoluble oxalates. The residue oxalates of the pH-adjusted extract suggested that the pH-adjustment was unable to completely eliminate the presented oxalates.

Fig. 2b depicts the reduction in oxalate content during the dialysis. The oxalate content was found to be dramatically decreased when dialyzed for the first 4 h. Meanwhile, some insoluble oxalate salts formed and precipitated during dialysis as pH of the extract increased from 1.5 to 3.1 due to the removal of H^+ ions through the membrane pore. Hence, in this case, the decrease in soluble oxalate content was ascribed to the effects of dialysis treatment, as well as the precipitation of calcium oxalates. The oxalate content proceeded to decrease slowly with prolonged dialysis, while the pH of the solution remained around 3.0 probably because carboxyl groups of pectin backbone had reached the equilibrium of dissociation. After dialysis for 16 h, the soluble oxalate content decreased to an extremely low level (<2.5 μg mL^{-1}), therefore 16 h was selected for the optimal condition.

3.2. Turbidity index of pectins

Pectins recovered via four different precipitation methods were tested for turbidity index (Fig. 3) which to some extent was proportional to the amount of turbid substances. The higher the turbidity index, the more the presented turbid substances. EPP exhibited a significant higher turbidity when compared to the remainder, indicating insoluble oxalate salts were formed during the ethanol precipitation. It was noted that a slight increase in

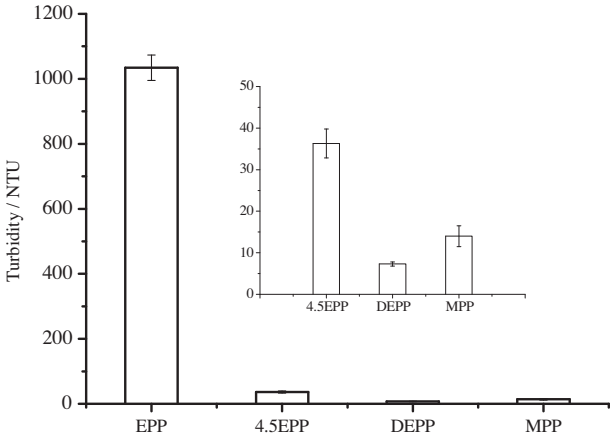

Fig. 3. Effects of precipitation mode on the turbidity index of the resulting pectins.

pH was also observed (increased from pH 1.5 to 2.0) when the extract was mixed with ethanol. Therefore, the change in solvent character along with the increase in pH of the medium was assumed for the formation of oxalate salts. Turbidity of 4.5EPP was 36.3 NTU, suggesting smaller quantities of insoluble oxalate salts. This further indicates that some of oxalates of the extract can be removed by pre-precipitating oxalate salts at higher pH. However, still a small amount of insoluble oxalate particles were present in 4.5EPP (see Fig. 4), which could be due to the lower solubility of oxalates in 75% ethanol solution than in water. There results observed with 4.5EPP indicate that the oxalates cannot be thoroughly pre-precipitated within the extract at pH 4.5. The turbidity index of DEPP was lower than that of 4.5EPP, indicating the better effects with the dialysis method against turbid substances.

The turbidity index of MPP was slightly lower than that of 4.5EPP. It has been reported that Cu^{2+} can form insoluble oxalate copper with ionized oxalate under suitable conditions (McAuley & Nancollas, 1960). However, in the present work, the pH of the medium during metal precipitation only increased from pH 1.5 to around 1.6, at which oxalate primarily presented as the form of $H_2C_2O_4$ and $HC_2O_4^{-1}$ (Ferguson, 2002). Hence, copper ions could not bind with oxalates to form copper oxalate during metal precipitation. The turbidity index of MPP was therefore ascribed to some insoluble pectic substances. Similar results were reported by Hwang et al. (1992) who reported the insolubilization of the final pectins caused by applied pressure during the filtration of pectins.

3.3. SEM observation

Insoluble substances isolated from various pectin samples were scanned using SEM in order to understand their morphology features. Fig. 4a–c shows the SEM pictures of IS presented in EPP. At magnification × 1000, the IS appeared to be fragments of different shapes and sizes (Fig. 4a). Although a small amount of pectins might present in the IS because of the incomplete washing, these fragments were primarily comprised of small particles in a bi-pyramidal shape (Fig. 4b and c) which is a typical feature of calcium oxalate dihydrate (Akhtar & Haq, 2013). The morphology of the IS with 4.5EPP (Fig. 4d and e) resembled those of EPP. However, the IS of DEPP and MPP showed a compact rough surface and showed an irregular appearance in size and form, which was distinguishing from those of EPP and 3.5EPP. On the basis of the SEM analysis, the IS presented in EPP and 3.5EPP was therefore

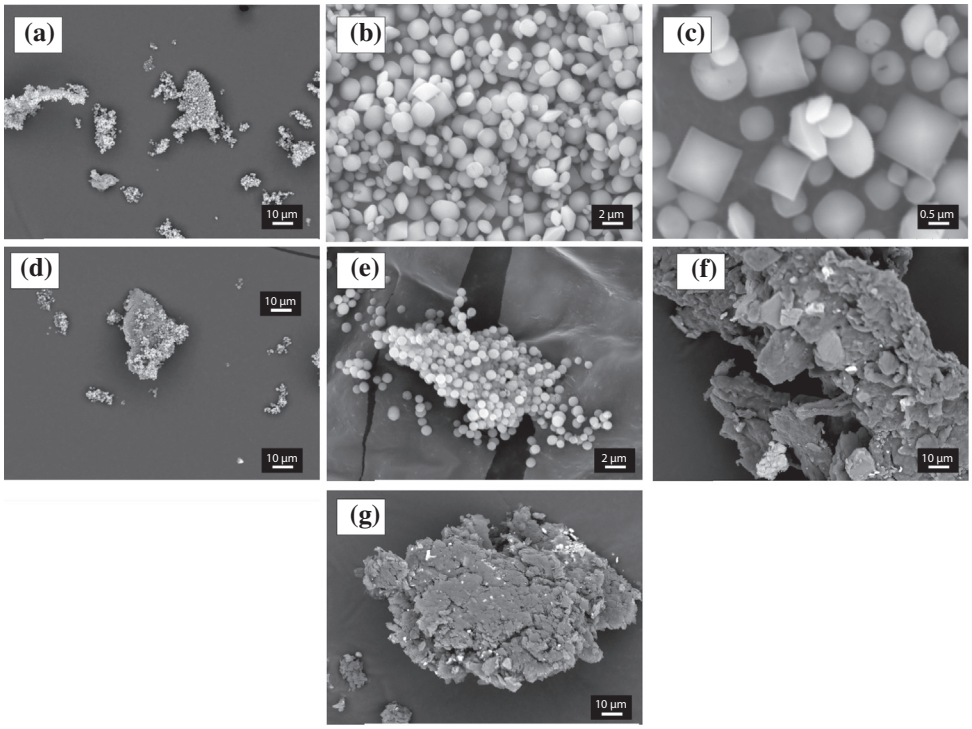

Fig. 4. SEM pictures of the insoluble substances obtained from (a–c) EPP, (d and e) 4.5EPP, (f) DEPP and (g) MPP.

considered as calcium oxalate particles (Guo et al., 2014), while the IS in DEPP and MPP were insoluble pectins.

3.4. Pectin yield

Pectin yield varied from 5.8% to 10.8% w/w of the starting material, dependant on the precipitation mode used. MPP yielded the lowest amount of pectins among three pectins. The lower yield with metal precipitation might partly due to the degradation of glycosidic linkages of side chains by the employed acidic washing during the sample preparation (Hwang et al., 1992). It could also be explained by the highly selective affinity of Cu^{2+} toward binding the homogalacturonan, but the hairy region. In contrast, addition of ethanol (3 volumes) effectively interrupted the interactions between pectin chain segments and the solvent molecules. Not only high molecular polysaccharides, but also oligosaccharides of low molar mass can be recovered via the ethanol precipitation (Yapo, 2009b). For this reason, the yield of MPP was much lower than that of EPP. The yield of DEPP was also lower than that of EPP, although both pectin samples were purified by using the same ethanol precipitation. This could be attributed to the removal some low molecular weight substances during dialysis as the pore size of the membrane was rather large.

3.5. Pectin characterization

Table 1 shows the chemical features of different pectins. GalA content of the pectins decreased in the order: MPP (63.8%) > EPP (56.6%) ≈ DEPP (56.1%). Hence, metal precipitation appeared to be an effective approach for enriching GalA. GalA contents of EPP and DEPP were relatively decreased by the high NS. This difference in GalA with precipitation modes was in agreement with results in previous reports (Hwang et al., 1992; Yapo, 2009b).

NS content ranged from 14.0% to 19.5%, according to the precipitation modes. The NS content of MPP (14.0%) was lower than that of EPP (19.5%), which was mainly deprived from significantly differences in the amounts of Rha, Ara and Gal. It should be noted that the dialysis may result in a decrease in NS content as oligosaccharides or some polysaccharides of relative low molar mass are easily to pass through the membrane pore (14,000 nominal MWCO). Expectedly, DEPP had a lower NS content than that of EPP. MPP had a significant lower rhamnose content among the pectins indicating Cu^{2+} might be less effectively in forming complexes with the rhamnogalacturonan-I (RGI) blocks. Glc (0.1–0.3%) and Xyl (0.3–0.4%) were only presented in small quantities in all pectins.

Protein content of the examined samples increases in the order: MPP (1.3%) < EPP (5.1%) ≈ DEPP (5.4%). Hence, the significant lower protein content of MPP revealed that metal precipitation is more selective than ethanol precipitation against proteinaceous moieties.

Table 1
Yield (% w/w), chemical composition (% w/w), degree of methylation (% mol), and degree of acetylation (% mol) of various pectin samples.[a]

	MPP	DEPP	EPP
Yield	5.8 ± 0.2^{b}	9.6 ± 0.5^{c}	10.8 ± 0.5^{d}
GalA	63.8 ± 1.0^{c}	56.1 ± 0.5^{b}	56.6 ± 1.2^{b}
Rha	4.3 ± 0.1^{b}	4.5 ± 0.0^{c}	5.1 ± 0.1^{c}
Ara	3.5 ± 0.2^{b}	4.2 ± 0.2^{c}	5.0 ± 0.1^{d}
Gal	5.8 ± 0.2^{b}	6.9 ± 0.2^{c}	8.8 ± 0.1^{d}
Glc	0.1 ± 0^{b}	0.2 ± 0.1^{c}	0.3 ± 0^{c}
Xly	0.3 ± 0.1^{b}	0.4 ± 0^{b}	0.3 ± 0.1^{b}
NS	14.0 ± 0.5^{b}	16.2 ± 0.5^{c}	19.5 ± 0.1^{d}
FA	0.38 ± 0.01^{b}	0.46 ± 0.02^{c}	0.54 ± 0.02^{d}
Protein	1.3 ± 0.2^{b}	5.4 ± 0.4^{c}	5.1 ± 0.4^{c}
Ash	0.2 ± 0.1^{d}	1.4 ± 0.2^{c}	3.8 ± 0.2^{b}
DM	43.2 ± 2.3^{b}	44.5 ± 1.2^{b}	42.5 ± 3.1^{b}
DA	23.2 ± 0.9^{b}	24.0 ± 1.6^{b}	22.8 ± 2.0^{b}

[a] Data are presented as means ± standard deviations (n = 3); Mean values in the same row with different letters are significantly different (p < 0.05).

FA content of pectins ranged from 0.38% to 0.54%. Furthermore, FA content appeared to be correlated with the amounts of two glycosyl residue monosugars, Ara and Gal, suggesting that ferulate groups were attached to neutral sugars of the side chains (Saulnier & Thibault, 1999). EPP (0.54% w/w) appeared to be the most FA-rich sample, followed by DEPP (0.46% w/w). This result indicated the dialysis treatment might result in losing ferulate-oligosaccharides of low molar mass. MPP had the lowest FA content. This loss of FA could be explained by the degradation of arabinan and galactan side chains.

% DM of the different pectins ranged from 42.5 to 44.5, while % DA ranged from 22.8 to 24.0. DM and DA of the pectins were not especially influenced by the precipitation modes ($p > 0.05$).

EPP had the highest ash content (3.8%), which may be partly ascribed to the co-precipitation of calcium oxalates. The lower ash content observed with DEPP (1.4%) revealed the employed dialysis treatment effectively improved the pectin purity. However, MPP gave the best result in terms of ash content (0.2%), probably due to the higher selectivity of metal precipitation for pectins. Since ash content to some extent was in proportional to the amounts of inorganic salts, another explanation for the lower content of MPP could be the removal of acid-soluble ash during the post acidic ethanol washing.

3.6. FTIR spectroscopic analysis

FTIR spectra of EPP, DEPP and MPP are shown in Fig. 5. All three pectin samples had strong absorption bands at 3465, 2941, 1743, 1647 cm^{-1}, which were similar to those reported in previous work (Kalapathy & Proctor, 2001; Kamnev, Colina, Rodriguez, Ptitchkina, & Ignatov, 1998). By comparing the FTIR spectra, it was obvious that there were some structural differences between MPP and the remainder as evidenced by the relatively different intensities of absorption bands at 1743 and 1647 cm^{-1}. Comparison of relative intensities of absorption bands at 1743 and 1647 cm^{-1} may be used to reflect the degree of esterification of pectins as the former was due to esterified carboxyl groups, while the latter was partly assigned to free carboxyl groups (Gnanasambandam & Proctor, 2000). It should be noted that the ionization of free carboxyl (protonated) groups would weaken the absorption band at 1743 cm^{-1} but strengthen the band at 1647 cm^{-1} (Kamnev et al., 1998). Since no significant difference was observed in DM and DA (Table 1), in this section, the relative intensities of these two bands were correlated to the ash content that may include the bound cation ions. As for DEPP and EPP, the intensity of the absorption band at 1743 cm^{-1} was weaker than that at 1647 cm^{-1}, and no appreciable differences in the relative intensities of absorption bands at 1743 and 1647 cm^{-1} were observed although DEPP had a significantly lower ash content ($p < 0.001$) (Table 1). One possible reason could be that the dialysis treatment only removes free inorganic salts rather than cations bound with pectins. In the case of MPP, the intensity of the absorption band at 1743 cm^{-1} was stronger than at 1647 cm^{-1}. By considering its extremely low ash content, the differences in the relative intensities of absorption bands at 1743 cm^{-1} and 1647 cm^{-1} were resulted from the removal of bound cations and thus the decreased degree of ionization. This could be explained by the fact that MPP was subjected to repeatedly ethanol washing under acidic condition.

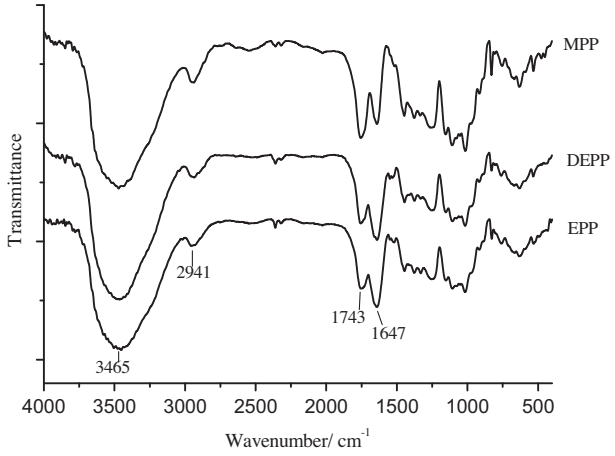

Fig. 5. FTIR spectra of pectins.

3.7. Emulsifying properties

Effect of ultrasonication time on the droplet size of 20% w/w oil (MCT) in water emulsions prepared using 1% pectins was shown in Fig. 6a. It was observed that the $d_{4,3}$ decreased from 9.7 μm to 2.0 μm as the ultrasonication time increased up to 90s and then remained constant. Consequently, this value was taken as the suitable ultrasonication time for obtaining a desired emulsification level.

Fig. 6b shows the droplet sizes of the emulsions prepared using varying concentrations of different pectin samples. It was found that the droplet sizes decreased as the polymer concentration increased up to 2% whatever kind of pectins. At any examined pectin concentration, the droplet sizes of the emulsions prepared using

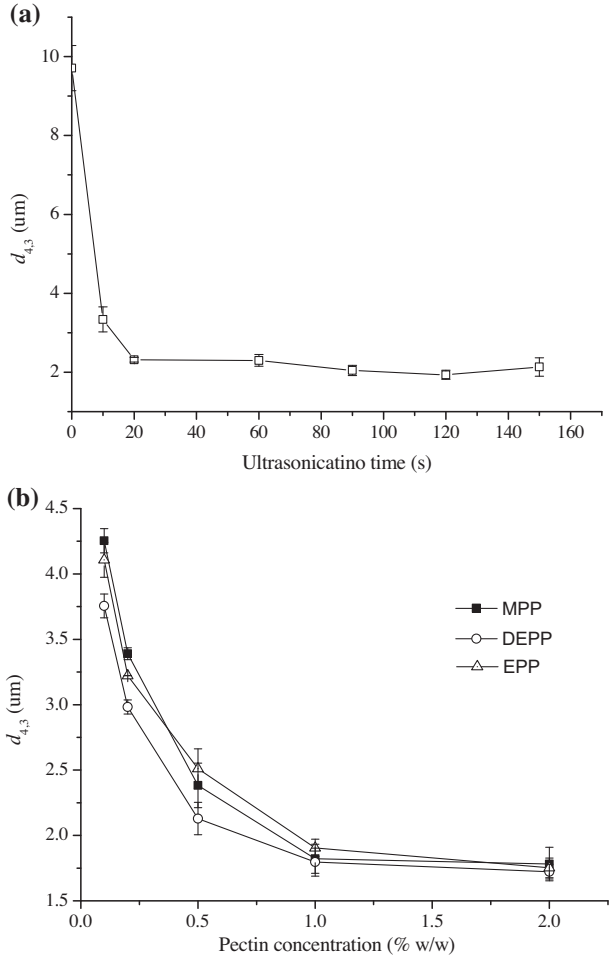

Fig. 6. Droplet size of the emulsions prepared with DEPP and EPP as a function of (a) ultrasonication time and (b) pectin concentration.

DEPP were smaller than those of EPP, confirming the higher emulsifying properties of the former. Comparison of emulsifying ability between DEPP and EPP indicates that the former may contain more molecules that are able to adsorb and activate the oil–water interface. It was evidenced by previous reports (Leroux et al., 2003; Williams et al., 2005) that only a small proportion of pectin molecules that were responsible for the emulsifying activity adsorbed in the surface of oil droplet. By relating the pectin purity to the emulsifying performance, we proposed that the removal of ash and low molecular weight polysaccharides/oligosaccharides during the dialysis might relatively enrich the concentration of interface-activated molecules of DEPP. It should be also mentioned that the droplet size analyzed by the laser diffraction technique may be overestimated due to the presence of calcium oxalate particles in particular those of a large size >1.5 μm (Fig. 4c). Therefore, the calcium-oxalates contamination might be a source of error in the droplet size measurement.

The emulsifying activity of MPP was comparable to that of EPP, but inferior to that of DEPP. In this section, the emulsifying ability of MPP was not specially compared with that of EPP because the latter contained calcium oxalate particles that may negatively disturb the $d_{4,3}$. Many reasons may account for the relative poor emulsifying properties of MPP. The first reason may be due to the fact that MPP contained a lower content of proteinaceous moieties (Table 1) that were proved to adsorb to oil droplets (Leroux et al., 2003; Siew & Williams, 2008) probably as an anchor (Funami et al., 2007). The second reason could be associated with the decrease in the content of FA that also contributes to the hydrophobic character of the whole pectins. The results suggest that metal precipitation is less efficacious than the dialysis–ethanol-precipitation one in terms of emulsifying ability.

4. Conclusions

Oxalates were extracted under acidic conditions, and then bound with calcium to form insoluble calcium oxalate particles during the conventional alcohol precipitation. Precipitation mode influenced the co-precipitation of oxalate calcium. One the one hand, metal precipitation was more selectively in precipitating pectins and would not provoke the co-precipitation of calcium oxalates. However, metal precipitation not only gave a lower yield, but also negatively influenced emulsifying properties of the obtained SBP. On the other hand, pectins precipitated from the dialysis-treated extract using the dialysis–ethanol-precipitation method were free of calcium oxalate contamination, and exhibited desired emulsifying properties. Results obtained in this work suggested the oxalates of sugar beet extract can be effectively removed by means of physical filtration. It would be feasible to use membrane filtration for the removal of oxalates, ash and some low molecular weight polysaccharides on a large scale, thereby improving the purity and quality of the extracted pectins.

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References

Agoda-Tandjawa, G., Durand, S., Gaillard, C., Garnier, C., & Doublier, J. L. (2012). Properties of cellulose/pectins composites: Implication for structural and mechanical properties of cell wall. *Carbohydrate Polymers, 90*(2), 1081–1091.

Akhtar, K., & Haq, I. U. (2013). Chemical modulation of crystalline state of calcium oxalate with nickel ions. *Clinica Chimica Acta, 418*, 12–16.

Allan, A. L., Band, B. S. F., & Rubio, E. (1986). Spectrophotometric determination of oxalate in aqueous solution. *Microchemical Journal, 34*(1), 51–55.

Arslan, N. (1995). Extraction of pectin from sugar-beet pulp and intrinsic viscosity-molecular weight relationship of pectin solutions. *Journal of Food Science and Technology, 32*, 381–385.

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*(2), 484–489.

Faravash, R. S., & Ashtiani, F. Z. (2007). The effect of pH, ethanol volume and acid washing time on the yield of pectin extraction from peach pomace. *International Journal of Food Science & Technology, 42*(10), 1177–1187.

Ferguson, R. J. (2002). Predicting calcium oxalate scale. *Corrosion*.

Fishman, M. L., Chau, H. K., Cooke, P. H., & Hotchkiss, A. T. (2008). Global structure of microwave-assisted flash-extracted sugar beet pectin. *Journal of Agricultural and Food Chemistry, 56*(4), 1471–1478.

Funami, T., Zhang, G. Y., Hiroe, M., Noda, S., Nakauma, M., Asai, I., et al. (2007). Effects of the proteinaceous moiety on the emulsifying properties of sugar beet pectin. *Food Hydrocolloids, 21*(8), 1319–1329.

Garna, H., Mabon, N., Robert, C., Cornet, C., Nott, K., Legros, H., et al. (2007). Effect of extraction conditions on the yield and purity of apple pomace pectin precipitated but not washed by alcohol. *Journal of Food Science, 72*(1), C001–C009.

Garna, H., Mabon, N., Wathelet, B., & Paquot, M. (2004). New method for a two-step hydrolysis and chromatographic analysis of pectin neutral sugar chains. *Journal of Agricultural and Food Chemistry, 52*(15), 4652–4659.

Gnanasambandam, R., & Proctor, A. (2000). Determination of pectin degree of esterification by diffuse reflectance Fourier transform infrared spectroscopy. *Food Chemistry, 68*(3), 327–332.

Guo, X.-M., Zhu, S.-M., Tang, Q., & Yu, S.-J. (2014). Characterisation of the turbid particles in the extraction of sugar beet pectins. *Food Chemistry, 162*, 99–103.

Hwang, J., Roshdy, T. H., Kontominas, M., & Kokini, J. L. (1992). Comparison of dialysis and metal precipitation effects on apple pectins. *Journal of Food Science, 57*(5), 1180–1184.

Joy, K. W. (1964). Accumulation of oxalate in tissues of sugar-beet, and the effect of nitrogen supply. *Annals of Botany, 28*(4), 689–701.

Kalapathy, U., & Proctor, A. (2001). Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. *Food Chemistry, 73*(4), 393–396.

Kamnev, A. A., Colina, M., Rodriguez, J., Ptitchkina, N. M., & Ignatov, V. V. (1998). Comparative spectroscopic characterization of different pectins and their sources. *Food Hydrocolloids, 12*(3), 263–271.

Keijbets, M. J. H., & Pilnik, W. (1974). Some problems in the analysis of pectin in potato tuber tissue. *Potato Research, 17*(2), 169–177.

Leroux, J., Langendorff, V., Schick, G., Vaishnav, V., & Mazoyer, J. (2003). Emulsion stabilizing properties of pectin. *Food Hydrocolloids, 17*(4), 455–462.

Levigne, S., Ralet, M.-C., & Thibault, J.-F. (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. *Carbohydrate Polymers, 49*(2), 145–153.

Levigne, S., Thomas, M., Ralet, M. C., Quemener, B., & Thibault, J. F. (2002). Determination of the degrees of methylation and acetylation of pectins using a C18 column and internal standards. *Food Hydrocolloids, 16*(6), 547–550.

Li, X. Y., Fang, Y. P., Phillips, G. O., & Al-Assaf, S. (2013). Improved sugar beet pectin-stabilized emulsions through complexation with sodium caseinate. *Journal of Agricultural and Food Chemistry, 61*(6), 1388–1396.

May, C. D. (1990). Industrial pectins: Sources, production and applications. *Carbohydrate Polymers, 12*(1), 79–99.

McAuley, A., & Nancollas, G. (1960). Complex formation in solutions of copper oxalate. *Transactions of the Faraday Society, 56*, 1165–1171.

McComas, W. H., & Rieman, W. (1942). The effect of pH on the solubility of calcium oxalate. *Journal of the American Chemical Society, 64*(12), 2948–2949.

Mulay, S. R., Kulkarni, O. P., Rupanagudi, K. V., Migliorini, A., Darisipudi, M. N., Vilaysane, A., et al. (2013). Calcium oxalate crystals induce renal inflammation by NLRP3-mediated IL-1β secretion. *The Journal of Clinical Investigation, 123*(1), 236–246.

Oosterveld, A., Beldman, G., Schols, H. A., & Voragen, A. G. J. (1996). Arabinose and ferulic acid rich pectic polysaccharides extracted from sugar beet pulp. *Carbohydrate Research, 288*, 143–153.

Oosterveld, A., Beldman, G., Searle-van Leeuwen, M. J. F., & Voragen, A. G. J. (2000). Effect of enzymatic deacetylation on gelation of sugar beet pectin in the presence of calcium. *Carbohydrate Polymers, 43*(3), 249–256.

Ralet, M.-C., Cabrera, J. C., Bonnin, E., Quéméner, B., Hellìn, P., & Thibault, J.-F. (2005). Mapping sugar beet pectin acetylation pattern. *Phytochemistry, 66*(15), 1832–1843.

Ralet, M.-C., Crépeau, M.-J., Buchholt, H.-C., & Thibault, J.-F. (2003). Polyelectrolyte behaviour and calcium binding properties of sugar beet pectins differing in their degrees of methylation and acetylation. *Biochemical Engineering Journal, 16*(2), 191–201.

Robert, C., Emaga, T. H., Wathelet, B., & Paquot, M. (2008). Effect of variety and harvest date on pectin extracted from chicory roots (Cichorium intybus L.). *Food Chemistry, 108*(3), 1008–1018.

Rombouts, F. M., & Thibault, J.-F. (1986). Feruloylated pectic substances from sugar-beet pulp. *Carbohydrate Research, 154*(1), 177–187.

Saulnier, L., & Thibault, J.-F. (1999). Ferulic acid and diferulic acids as components of sugar-beet pectins and maize bran heteroxylans. *Journal of the Science of Food and Agriculture, 79*(3), 396–402.

Siew, C. K., & Williams, P. A. (2008). Role of protein and ferulic acid in the emulsification properties of sugar beet pectin. *Journal of Agricultural and Food Chemistry, 56*(11), 4164–4171.

Williams, P. A., Sayers, C., Viebke, C., Senan, C., Mazoyer, J., & Boulenguer, P. (2005). Elucidation of the emulsification properties of sugar beet pectin. *Journal of Agricultural and Food Chemistry, 53*(9), 3592–3597.

Yapo, B. M. (2009a). Biochemical characteristics and gelling capacity of pectin from yellow passion fruit rind as affected by acid extractant nature. *Journal of Agricultural and Food Chemistry, 57*(4), 1572–1578.

Yapo, B. M. (2009b). Pectin quantity, composition and physicochemical behaviour as influenced by the purification process. *Food Research International, 42*(8), 1197–1202.

Yapo, B. M. (2011). Pectic substances: From simple pectic polysaccharides to complex pectins – A new hypothetical model. *Carbohydrate Polymers, 86*(2), 373–385.

Yapo, B. M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chemistry, 100*(4), 1356–1364.

Yapo, B. M., Wathelet, B., & Paquot, M. (2007). Comparison of alcohol precipitation and membrane filtration effects on sugar beet pulp pectin chemical features and surface properties. *Food Hydrocolloids, 21*(2), 245–255.