Food Chemistry 141 (2013) 3752–3758

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effect of extraction conditions on the yield and chemical properties of pectin from cocoa husks

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ARTICLE INFO

Article history:
Received 27 April 2013
Received in revised form 19 June 2013
Accepted 24 June 2013
Available online 2 July 2013

Keywords:
Cocoa husks
Pectin
Uronic acid
Degree of methylation
Degree of acetylation

ABSTRACT

Different extraction conditions were applied to investigate the effect of temperature, extraction time and substrate–extractant ratio on pectin extraction from cocoa husks. Pectin was extracted from cocoa husks using water, citric acid at pH 2.5 or 4.0, or hydrochloric acid at pH 2.5 or 4.0. Temperature, extraction time and substrate–extractant ratio affected the yields, uronic acid contents, degrees of methylation (DM) and degrees of acetylation (DA) of the extracted pectins using the five extractants differently. The yields and uronic acid contents of the extracted pectins ranged from 3.38–7.62% to 31.19–65.20%, respectively. The DM and DA of the extracted pectins ranged from 7.17–57.86% to 1.01–3.48%, respectively. The highest yield of pectin (7.62%) was obtained using citric acid at pH 2.5 [1:25 (w/v)] at 95 °C for 3.0 h. The highest uronic acid content (65.20%) in the pectin was obtained using water [1:25 (w/v)] at 95 °C for 3.0 h.

1. Introduction

Theobroma cacao L. (Sterculiaceae) is an economically important crop and the cocoa beans are used primarily in chocolate manufacturing. However, cocoa production generates substantial quantities of waste (Vriesmann, de Mello Castanho Amboni, & de Oliveira Petkowicz, 2011b). The pod shells of the cocoa fruits, which are commonly known as cocoa husks account for 52–76% of the pod wet weight. The husks are usually left to decompose on the cocoa plantation, which generates foul odors and causes botanical disease inoculum like black pod rot (Donkoh, Atuahene, Wilson, & Adomako, 1991). The increasing demand for cocoa beans has led to accumulation of cocoa husks and this represents a serious disposal problem.

One way to utilise cocoa husks is that it could be used as a source of pectin (Blakemore, Dewar, & Hodge, 1966). Pectins are complex polysaccharides found naturally in higher plants, which consist of mainly galacturonic acid units being linked by α-(1 → 4) linkages. They are known for their gelling properties and being used widely in the food industry, pharmaceutical industry and the cosmetic industry. Commercial pectins are extracted mainly from by-products from the food industry such as citrus peel, apple pomace and to a smaller extent, sugar beet pulp (May, 1990; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). In commercial practices, there are two types of pectin: high methoxyl (HM) pectin with a degree of methylation (DM) >50% and low methoxyl (LM) pectins with a DM <50%. HM and LM pectins have different physicochemical characteristics and thus different applications. HM pectins form gel in acidic systems (pH 2.0–3.5) with the presence of large concentrations (55–75%) of co-solutes such as sucrose or sorbitol; while LM pectins can gel in the absence of co-solutes, particularly sucrose, with the addition of divalent ions such as calcium, over a wide range of pH [pH 2.0–6.0] (Lopes da Silva & Rao, 2006; Yapo et al., 2007).

The physicochemical properties of pectin depend mainly on the plant source and conditions selected for isolation and purification of pectin. Extraction is therefore an important step in the isolation and recovery of pectin (Vriesmann, Teófilo, & de Oliveira Petkowicz, 2011a). Mineral acids such as hydrochloric acid (Adomako, 1972; Mollea, Chiampo, & Conti, 2008) and nitric acid (Arlorio, Coisson, Restani, & Martelli, 2001; Vriesmann et al., 2011a) had been employed to extract pectin from cocoa husks. However, using mineral acids in pectin production generate effluents which lead to environmental problems and economical inconveniences. In addition, water (Mollea et al., 2008; Vriesmann et al., 2011b) and citric acid (Vriesmann, Teófilo, & de Oliveira Petkowicz, 2012) had been employed to extract pectin from cocoa husks. Particularly, information on the effect of processing methods on the yield and quality of pectin from cocoa husks (Malaysia) using various extractants is not available. The objective of the present study was to investigate the effect of extraction conditions including temperature, time and substrate–extractant ratio on the yields, uronic acid contents, degrees of methylation and degrees of acetylation of pectins obtained from cocoa husks using different extractants and pHs.

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http://dx.doi.org/10.1016/j.foodchem.2013.06.097

2. Materials and methods

2.1. Materials

Minced cocoa husks were kindly supplied by Guan Chong Cocoa Manufacturer Sdn. Bhd., Johor, Malaysia.

2.2. Chemicals and solvents

All chemicals and solvents used were of analytical reagent grade.

2.3. Pectin extraction

Pectin extraction from minced cocoa husks was carried out according to the method of Min et al. (2011) with some modifications. The minced cocoa husks was treated with 85% ethanol [substrate:ethanol, 1:4 (w/v)] for four times at 70 °C for 20 min. The mixture was then filtered with a microcloth (60 μm) to obtain the residues. An extractant (water, citric acid at pH 2.5, citric acid at pH 4.0, hydrochloric acid at pH 2.5 or hydrochloric acid at pH 4.0) with a specific substrate–extractant ratio [1:25 (w/v) or 1:10 (w/v)] was added to the residues and incubated at a specific temperature (95or 50 °C) and time (1.5 or 3.0 h). The slurry was then filtered with a microcloth. The filtrate was mixed with three volumes of 95% ethanol and centrifuged at 14,500*g* for 10 min. The precipitate obtained was washed with 70% ethanol and subsequently with 95% ethanol. The precipitate was then dried using a vacuum oven at 50 °C.

2.4. Pectin yield

The yield of pectin obtained was determined according to the method of Seggiani, Puccini, Pierini, Giovando, and Forneris (2009) with some modifications:

$$\text{Yield}(\%) = \frac{\text{Pure pectin(g)}}{\text{Initial dry cocoa husks(g)}} \times 100\%$$

where the word "pure pectin" stands for the pectin obtained on moisture and ash free basis.

Dry matter content of pectin was determined by drying the samples in an air-circulated oven at 105 °C for 6 h. The total ash content of pectin was determined by measuring the residue obtained after incineration in a muffle furnace at 550 °C for 12 h.

2.5. Uronic acid content

Uronic acid content of pectin was determined spectrophotometrically by the meta-hydroxydiphenyl method according to Blumenkratz and Asboe-Hansen (1973) with some modifications. Uronic acid content was calculated as the percentage of extracted pectin weight on moisture and ash free basis. Pectin in distiled water [0.1% (w/v), 0.4 mL] was mixed with 4 M sulphamic acid–potassium sulphamate (pH 1.6, 40 μL) and was agitated with a vortex mixer. After H_2SO_4 containing 0.0125 M sodium tetraborate (2.5 mL) was added, the mixture was agitated with a vortex mixer again, cooled in an ice bath, and brought to a boil for 20 min. After cooling in an ice bath, meta-hydroxydiphenol reagent (80 μL) was added and the absorbance was read at 520 nm with D-galacturonic acid as standard after an incubation time of 20 min.

2.6. Degree of methylation (DM) and degree of acetylation (DA)

The DM and DA of pectin were determined using a HPLC method according to Levigne, Thomas, Ralet, Quemener, and Thibault (2002b) with some modifications. Pectin (5 mg) was suspended in 0.5 mL of a solution containing 10 mM copper sulphate and 25 mM isopropanol as an internal standard; 0.5 mL of 1 M sodium hydroxide was added to achieve saponification. The reaction mixture was left at 4 °C for 1.5 h. Reaction mixtures were centrifuged for 10 min at 8000*g*. Supernatants were neutralised through a 2 mL syringe equipped with a Maxi-clean IC-H device (Alltech, USA) prior to injection on a Superspher 100 RP-18 end capped LiChroCART® 250–4 column (Merck, Germany). Elution was carried out with 4 mM sulphuric acid at 0.7 mL/min and 25 °C, with refractometric detection.

2.7. Statistical analysis

Data was interpreted by one-way analysis of variance (ANOVA) with SPSS 16 software. The statistical significance was evaluated at $p < 0.05$ level.

3. Results and discussion

3.1. Effect of temperature on pectin yield

The effect of temperature of extraction on the yield of pectin obtained using various extractants and pHs with a substrate–extractant ratio of 1:25 (w/v) for 1.5 h is shown in Table 1. Increasing extraction temperature from 50 to 95 °C using citric acid at pH 2.5 or 4.0 and hydrochloric acid at pH 2.5 or 4.0 significantly increased ($p < 0.05$) the yield of pectin (Table 1). These were also demonstrated by Vriesmann et al. (2012), and Aravantinos-Zafiris and Oreopoulou (1992) that increasing temperature significantly increased the yield of pectin from cocoa husks by citric acid and orange peels by nitric acid, respectively. The heated acid helped to solubilise pectin and other pectic components held in the cell wall (protopectin), thereby increased the yield of pectin (Greve, McArdle, Gohlke, & Labavitch, 1994). A low temperature may be insufficient to permit the hydrolysis of protopectin (the insoluble form of pectin) by acids, thus obtaining lower yield of pectin (El-Nawawi & Shehata, 1987). Increasing temperature from 50 to 95 °C, however, showed no significant effect on the yield of pectin extracted by water (Table 1). Vriesmann et al. (2011b) showed a different trend whereby increasing temperature from 50 to 100 °C for extraction of pectin from cocoa husks using water increased the yield from 7.5% to 12.6%. A possible explanation for such a difference is that a different origin, variety and environmental growth conditions of the cocoa husks were used. The yield of extracted pectin in the present study was lower than that obtained in the study of Vriesmann et al. (2011b). Besides the reasons mentioned above, another reason for the difference is due to the method to quantify the yield of extracted pectin. The yield of extracted pectin in this study was determined on ash-free and moisture-free basis as compared to using moisture-free basis only in the study of Vriesmann et al. (2011b).

Extraction using citric acid at pH 2.5 or 4.0 obtained similar pectin yield as hydrochloric acid at pH 2.5 or 4.0 when an extraction temperature of 95 °C was used (Table 1). These were also demonstrated by Canteri-Schemin, Fertonani, Waszczynskyj, and Wosiacki (2005), Virk and Sogi (2004), and Kliemann et al. (2009) who compared the use of hydrochloric acid and citric acid on the extraction of pectin from apple pomace, apple peels and passion fruit peels, respectively. It was deduced by Joslyn (1962) that citric acid can extract the types of pectin being extracted by hydrochloric acid, particularly the protopectins. These results indicate that citric acid can be an alternative to using hydrochloric acid in pectin extraction of cocoa husks.

Table 1
Effect of temperature (°C), time (h) and substrate–solvent ratio (w/v) on the yield (%) of pectins extracted from cocoa husks using various extractants and pHs.

		Water		CA pH 2.5		CA pH 4.0		HCl pH 2.5		HCl pH 4.0	
Yield (%)	Temperature (°C)	50	95	50	95	50	95	50	95	50	95
		3.38 ± 0.23^{a}	3.98 ± 0.52^{ab}	3.58 ± 0.08^{a}	5.66 ± 0.64^{c}	3.72 ± 0.17^{a}	4.72 ± 0.07^{bc}	3.64 ± 0.13^{a}	5.13 ± 0.25^{c}	3.62 ± 0.14^{a}	5.23 ± 0.62^{c}
	Time (h)	1.5	3.0	1.5	3.0	1.5	3.0	1.5	3.0	1.5	3.0
		3.98 ± 0.52^{a}	4.78 ± 0.32^{ab}	5.66 ± 0.64^{bc}	7.62 ± 0.11^{d}	4.72 ± 0.07^{ab}	5.55 ± 0.44^{bc}	5.13 ± 0.25^{bc}	6.01 ± 0.36^{bc}	5.23 ± 0.62^{bc}	5.76 ± 0.14^{bc}
	Substrate:extractant (w/v)	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25
		3.92 ± 0.24^{a}	3.98 ± 0.52^{a}	7.00 ± 0.26^{c}	5.66 ± 0.64^{b}	4.79 ± 0.26^{ab}	4.72 ± 0.07^{ab}	5.56 ± 0.39^{b}	5.13 ± 0.25^{b}	4.97 ± 0.42^{ab}	5.23 ± 0.62^{b}

Values are means (n = 3) ± standard deviations.
^{abc}Values within the row with different superscript letters are significantly different (p < 0.05).

3.2. Effect of extraction time on pectin yield

The effect of extraction time on the yield of pectin obtained using various extractants and pHs with a substrate–extractant ratio of 1:25 (w/v) at 95 °C is shown in Table 1. Increasing extraction time from 1.5 to 3.0 h using citric acid at pH 2.5 significantly increased (p < 0.05) the yield of pectins (Table 1). Vriesmann et al. (2012) and Canteri-Schemin et al. (2005) reported that the yield of pectin obtained was directly correlated with an increase in extraction time using citric acid extraction of pectin from cocoa husks and apple pomace, respectively. Furthermore, the highest yield of pectin was obtained using citric acid at pH 2.5 at 95 °C for 3.0 h (Table 1). This indicates that citric acid at pH 2.5 is the most efficacious matrix for pectin extraction from cocoa husks and is a good alternative to using hydrochloric acid for pectin extraction of cocoa husks.

There was no significant effect on increasing the extraction time on the yield of pectin extracted using water, citric acid at pH 4.0 or hydrochloric acid at pH 2.5 or 4.0 (Table 1). This is in accordance with the study of Kalapathy and Proctor (2001) who studied the extraction of soy hull pectin using 0.1 N hydrochloric acid and found that longer extraction time do not affect the pectin yield. In water extraction of pectin, the medium lacks of hydronium ion to help solubilising other pectic materials which might not be water-soluble. The limiting factor would be the amount of water-soluble pectic material extractable regardless of the extraction time. The difference effect of time observed in extracted pectin yield by citric acid pH 2.5 and pH 4.0 showed that the yield of pectin extracted by citric acid was dependent on the pH. This is in accordance with the study of Yapo (2009) that the yield of pectin from yellow passion fruit rind was greater when extracted at lower pH than at a higher pH using citric acid.

3.3. Effect of substrate–extractant ratio on pectin yield

The effect of substrate–extractant ratio on the yield of pectin obtained using various extractants and pHs with at 95 °C for 1.5 h is shown in Table 1. Decreasing substrate–extractant ratio from 1:25 to 1:10 (w/v) using citric acid at pH 2.5 significantly increased (p < 0.05) the yield of pectin. The highest yield of pectin was obtained using citric acid at pH 2.5 using a substrate–extractant ratio of 1:10 (w/v) [Table 1]. This confirms the efficiency of citric acid at pH 2.5 to extract pectin from cocoa husks. There was, however, no effect on increasing the substrate–extractant on the yield of pectin using water, citric acid at pH 4.0 or hydrochloric acid at pH 2.5 or 4.0 (Table 1). According to Kulkarni and Vijayanand (2010), when all the pectins were completely extracted, further increase in the volume of extractant did not show any significant increase on the pectin yield.

3.4. Effect of temperature on the uronic acid content of pectin

The effect of temperature of extraction on the uronic acid content of pectin obtained using various extractants and pHs with a substrate–extractant ratio of 1:25 (w/v) for 1.5 h is shown in Table 2. Increasing the extraction temperature from 50 to 95 °C using citric acid at pH 4.0 or hydrochloric acid at pH 2.5 produced pectin with a higher uronic acid content (Table 2). This is in accordance with the study of Garna et al. (2007) who reported that the galacturonic acid content obtained from apple pectin were always higher at 90 than at 80 °C when extracted using sulphuric acid at pH 1.5 or 2.0. This may be attributed to the accelerated acid hydrolysis of pectin sugar side chains with increased temperature (Fraeye et al., 2007). However, extraction of pectin using citric acid at pH 2.5 showed a reversed trend in which it obtained pectin with a higher uronic acid content when using a temperature of 50 °C as

Table 2
Effect of temperature (°C), time (h) and substrate–solvent ratio (w/v) on the uronic acid content of pectins extracted from cocoa husks using various extractants and pHs.

		Water		CA pH 2.5		CA pH 4.0		HCl pH 2.5		HCl pH 4.0	
Uronic acid (%)	Temperature (°C)	50	95	50	95	50	95	50	95	50	95
		56.16 ± 6.36^{d}	44.07 ± 2.79^{bcd}	59.36 ± 5.92^{d}	31.19 ± 7.37^{abc}	40.30 ± 4.44^{a}	55.03 ± 6.71^{d}	32.58 ± 2.04^{ab}	54.69 ± 0.99^{d}	51.34 ± 8.02^{cd}	49.87 ± 5.36^{cd}
	Time (h)	1.5	3.0	1.5	3.0	1.5	3.0	1.5	3.0	1.5	3.0
		44.07 ± 2.79^{ab}	65.20 ± 6.69^{c}	31.19 ± 7.37^{a}	45.37 ± 4.62^{ab}	55.03 ± 6.71^{bc}	43.89 ± 6.49^{ab}	54.69 ± 0.99^{bc}	48.82 ± 1.58^{bc}	49.87 ± 5.36^{bc}	50.40 ± 0.01^{bc}
	Substrate:extractant (w/v)	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25
		44.26 ± 6.75^{ab}	44.07 ± 2.79^{ab}	38.63 ± 1.97^{ab}	31.19 ± 7.37^{a}	53.34 ± 8.93^{b}	55.03 ± 6.71^{b}	43.30 ± 4.04^{ab}	54.69 ± 0.99^{b}	32.75 ± 3.67^{a}	49.87 ± 5.36^{b}

Values are means (n = 3) ± standard deviations.
^{abcd}Values within the row with different superscript letters are significantly different ($p < 0.05$).

compared to 95 °C (Table 2). Garna, Mabon, Wathelet, and Paquot (2004) deduced that chemical hydrolysis of pectin with acid (1 or 2 M sulphuric acid, hydrochloric acid or trifluoroacetic acid) at high temperature (100 °C) seems to combine two simultaneous phenomena: firstly, the release of sugars as a product of hydrolysis of the pectin, and secondly, their degradation under the action of the acid and the heat. They showed that a lower temperature of 80 °C caused less degradation to pectin sugar side chains as compared to a higher temperature of 100 °C. Using citric acid at pH 2.5 to extract pectin from cocoa husks at 50 °C in the present study most likely caused less degradation to the pectin structure which contributed to a higher uronic acid content as compared to that using 95 °C (Table 2).

When the extraction was carried out at 50 °C, water obtained higher uronic acid content in the pectin extracted as compared to citric acid at pH 4.0 or hydrochloric acid at pH 2.5 (Table 2). This was also demonstrated by Hwang, Kim, and Kim (1998) who reported that hot water extraction of apple pectin yielded more galacturonic acids as compared to hydrochloric acid extraction of apple pectin. Koubala et al. (2008) showed that water-extracted pectin was particularly rich in uronic acid content as compared to hydrochloric acid-extracted pectin from ambarella peels. The uronic acid content (56.16%) of water-extracted pectin at 50 °C with a substrate–extractant ratio of 1:25 (w/v) for 1.5 h in the present study (Table 2) is higher than that (45.10%) of Vriesmann et al. (2011b). A possible explanation for such a difference is that a different origin, variety and environmental growth conditions of the cocoa husks were used. Another reason for the difference is due to the method to quantify uronic acid content in the pectin. Sulphamate/3-phenylphenol colorimetric method was used by Vriesmann et al. (2011b) to quantify the uronic acid content.

3.5. Effect of extraction time on the uronic acid content of pectin

The effect of extraction time on the uronic acid content of pectin obtained using various extractants and pHs with a substrate–extractant ratio of 1:25 (w/v) at 95 °C is shown in Table 2. Increasing the extraction time using water from 1.5 to 3.0 h produced pectin with a higher uronic acid content (Table 2). Longer extraction time most probably contributed to a higher uronic acid content due to increased hydrolysis of neutral sugar side chains. There was, however, no significant effect on increasing extraction time on the uronic acid content of pectin using citric acid or hydrochloric acid extractions (Table 2).

Extraction of pectin using water obtained a similar uronic acid content as citric acid and hydrochloric acid using an extraction time of 1.5 h (Table 2). Likewise, when the extraction was carried out at 3.0 h, water obtained higher uronic acid content in the pectin as compared to that of using citric acid but similar uronic acid content with the pectin extracted by hydrochloric acid (Table 2). These are in accordance with the study of Koubala et al. (2008) who reported similar uronic acid content in water-extracted and hydrochloric acid-extracted pectins from ambarella peels.

3.6. Effect of substrate–extractant ratio on the uronic acid content of pectin

The effect of substrate–extractant ratio on the uronic acid content of pectin obtained using various extractants and pHs at 95 °C for 1.5 h is shown in Table 2. Increasing the substrate–extractant ratio from 1:10 to 1:25 (w/v) increased the uronic acid content in the pectin extracted by hydrochloric acid at pH 4.0 (Table 2). This is similar to the study of Liu, Cao, Huang, Cai, and Yao (2010) in which the galacturonic acid content in pectin extracted from mulberry branch bark increased with an increase in substrate–extractant ratio using 0.1 M of HCl at 90 °C for 80 min.

Table 3
Effect of temperature (°C), time (h) and substrate–solvent ratio (w/v) on the degree of methylation (DM) (%) of pectins extracted from cocoa husks using various extractants and pHs.

		Water		CA pH 2.5		CA pH 4.0		HCl pH 2.5		HCl pH 4.0	
DM (%)	Temperature (°C)	50	95	50	95	50	95	50	95	50	95
		27.53 ± 5.53^{bcd}	39.26 ± 7.70^{d}	33.18 ± 8.84^{bcd}	57.86 ± 10.57^{e}	22.74 ± 3.58^{abc}	20.47 ± 0.76^{ab}	37.39 ± 1.28^{cd}	23.23 ± 4.27^{abcd}	9.60 ± 0.78^{a}	10.24 ± 0.60^{a}
	Time (h)	1.5	3.0	1.5	3.0	1.5	3.0	1.5	3.0	1.5	3.0
		39.26 ± 7.70^{c}	24.54 ± 2.43^{b}	57.86 ± 10.57^{d}	37.76 ± 1.96^{c}	20.47 ± 0.76^{b}	22.67 ± 1.61^{b}	23.23 ± 4.27^{b}	21.29 ± 1.72^{b}	10.24 ± 0.60^{a}	7.17 ± 1.35^{a}
	Substrate: extractant (w/v)	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25
		34.63 ± 1.82^{de}	39.26 ± 7.70^{e}	43.35 ± 7.07^{ef}	57.86 ± 10.57 ^{f}	15.06 ± 3.52^{abc}	20.47 ± 0.76^{bcd}	34.47 ± 4.83^{de}	23.23 ± 4.27^{cd}	7.50 ± 1.37^{a}	10.24 ± 0.60^{ab}

Values are means (n = 3) ± standard deviations.
^{abcdef}Values within the row with different superscript letters are significantly different ($p < 0.05$).

There was, however, no significant effect on increasing the substrate–extractant ratio on the uronic acid contents of pectin extracted by other extractants (Table 2).

3.7. Effect of temperature on degree of methylation (DM) and degree of acetylation (DA) of pectin

The effect of temperature of extraction on the DM and DA of pectin obtained using various extractants and pHs with a substrate–extractant ratio of 1:25 (w/v) for 1.5 h is shown in Tables 3 and 4. An increase in the extraction temperature from 50 to 95 °C using citric acid at pH 2.5 produced pectin with a higher DM (Table 3). There was, however, no significant effect on increasing the extraction temperature on the DM of pectin extracted by other extractants (Table 3).

Citric acid at pH 2.5 obtained the highest DM in the pectin extracted when a temperature of 95 °C was used (Table 3). This was also demonstrated by Yapo (2009) that citric acid-extracted pectin from yellow passion fruit rind had a higher degree of esterification as compared to those of nitric acid- and sulphuric acid-extracted pectins. Water-extracted pectin had a higher DM as compared to that of hydrochloric acid-extracted pectin at pH 4.0 (Table 3). Mollea et al. (2008) and Koubala et al. (2008) also showed that water-extracted cocoa pectin and ambarella peels pectin were more methylated than that of hydrochloric-extracted pectin, respectively. The pectins extracted using water and hydrochloric acid were all LM pectins ranging from DM 7.17–39.26% (Table 3). In commercial applications, LM pectins are less commonly produced as compared to HM pectins. However, in recent times, there is more interest in the manufacturing of LM pectins due to its gelling characteristic which is suitable for the production of low-calorie and dietetics foods. This is in accordance to the increased health awareness among consumers nowadays.

Degrees of acetylation between 3% and 8% are considered as low in pectin (Koubala et al., 2008). Acetyl groups can cause steric hindrance of chain association and considerably reduce the binding strength of pectin with Ca^{2+} and contributes to poor gelling properties (Ralet, Crepeau, Buchholt, & Thibault, 2003). In the present study, the DAs in pectin from cocoa husks (Table 4) were low. An increase in the temperature of extraction of pectin using hydrochloric acid at pH 2.5 from 50 to 95 °C produced pectin with a lower DA (Table 4). This can be explained by the hydrolysis of acetic acid groups from galacturonic acids under drastic extraction conditions (Garna et al., 2007). Using water as an extractant, Vriesmann et al. (2011b) showed that an increase in the extraction temperature from 50 to 100 °C obtained a lower DA in the pectin from cocoa husks. This was not shown in this study as there was no significant effect on increasing the extraction temperature from 50 to 95 °C on the DA of the water-extracted pectin (Table 4). A possible explanation for such a difference is that a different origin, variety and environmental growth conditions of the cocoa husks were used. Another reason for the difference is due to the method to quantify DA in the pectin. Hestrin colorimetric method using erythritol tetraacetate as a standard was used by Vriesmann et al. (2011b) to quantify DA whereas saponification followed by separation of acetic acid and an internal standard on a C18 column and quantification using refractometry was used in the present study.

An increase in the temperature of extraction of pectin using hydrochloric acid at pH 4.0 from 50 to 95 °C produced pectin with a higher DA (Table 4). There was no effect on increasing the extraction temperature from 50 to 95 °C on the DA of the citric-extracted pectin (Table 4). These results indicate that the DA of pectin depends largely on the nature of extractant and a combination of factors such as extraction temperature and type of

Table 4
Effect of temperature (°C), time (h) and substrate–solvent ratio (w/v) on the degree of acetylation (DA) (%) of pectins extracted from cocoa husks using various extractants and pHs.

		Water		CA pH 2.5		CA pH 4.0		HCl pH 2.5		HCl pH 4.0	
DA (%)	Temperature (°C)	50	95	50	95	50	95	50	95	50	95
		1.31 ± 0.22^{ab}	1.98 ± 0.06^{bc}	1.29 ± 0.16^{ab}	2.05 ± 0.46^{bc}	2.38 ± 0.42^{c}	2.57 ± 0.25^{c}	1.75 ± 0.19^{bc}	1.01 ± 0.05^{a}	1.80 ± 0.35^{bc}	2.38 ± 0.52^{c}
	Time (h)	1.5	3.0	1.5	3.0	1.5	3.0	1.5	3.0	1.5	3.0
		1.98 ± 0.06^{cde}	1.51 ± 0.23^{abc}	2.05 ± 0.46^{cde}	1.70 ± 0.25^{bcd}	2.57 ± 0.25^{ef}	3.48 ± 0.31^{f}	1.01 ± 0.05^{a}	1.25 ± 0.13^{ab}	2.38 ± 0.52^{de}	2.64 ± 0.07^{ef}
	Substrate: extractant (w/v)	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25	1:10	1:25
		2.12 ± 0.34^{cd}	1.98 ± 0.06^{cd}	1.52 ± 0.18^{bc}	2.05 ± 0.46^{cd}	2.15 ± 0.42^{bcd}	2.57 ± 0.25^{cd}	1.58 ± 0.14^{bc}	1.01 ± 0.05^{a}	3.16 ± 0.44^{d}	2.38 ± 0.52^{bcd}

Values are means ($n = 3$) ± standard deviations.
^{abcde}Values within the row with different superscript letters are significantly different ($p < 0.05$).

extractant. In addition, Levigne, Ralet, and Thibault (2002a) reported that the pH of an extractant had more influence on the DA of sugar beet pectin than the extraction temperature.

3.8. Effect of extraction time on DM and DA of pectin

The effect of extraction time on the DM and DA of pectin obtained using various extractants and pHs with a substrate–extractant ratio of 1:25 (w/v) at 95 °C is shown in Tables 3 and 4. Increasing the extraction time from 1.5 to 3.0 h using water or citric acid at pH 2.5 produced pectin with a lower DM (Table 3). The phenomenon of demethylation in pectin is therefore observed using water extraction at 95 °C. Pinheiro et al. (2008) explained that in order to obtain a higher degree of esterification from passion fruit peel pectin with high-citric acid concentrations, a shorter extraction time is necessary. This is due to the combination reaction of a high acid concentration and a longer contact time during the pectin extraction process accelerated de-esterification (Joye & Luzio, 2000). There was no significant effect on increasing the extraction time from 1.5 to 3.0 h on the DM of pectin extracted using other extractants (Table 3). It is also observed that pH do not influence the DM of hydrochloric acid-extracted pectin regardless of the extraction time (Table 3). There was no significant effect on increasing extraction time on the DA of the pectin extracted by all extractants (Table 4), indicating extraction time did not influence the DA of the pectin extracted.

3.9. Effect of substrate–extractant ratio on DM and DA of pectin

The effect of substrate–extractant ratio on the DM and DA of pectin obtained using various extractants and pHs with at 95 °C for 1.5 h is shown in Tables 3 and 4. There was no significant effect on increasing the substrate–extractant ratio from 1:10 to 1:25 (w/v) in the DM of the pectin extracted by all extractants (Table 3). These results for the DM had the same tendency as those observed for the DA except for the hydrochloric acid-extracted pectin at pH 2.5 (Table 4). The highest DM was obtained using citric acid at pH 2.5 with a substrate–extractant ratio of 1:25 (w/v) for 1.5 h (Table 3) and a HM pectin (DM 57.86%) was produced. The lowest DM was obtained using HCl at pH 4.0 with a substrate–extractant ratio of 1:25 (w/v) for 3 h and a low methoxyl pectin (DM 7.17%) was produced (Table 3). The selection and control of the extraction conditions is therefore important to determine the degree of methylation of pectin. The highest DA (3.48%) was obtained using citric acid at pH 4.0 with a substrate–extractant ratio of 1:25 (w/v) for 3 h whereas the lowest DA (1.01%) was obtained using HCl at pH 2.5 with a substrate–extractant ratio of 1:25 (w/v) for 1.5 h (Table 4).

4. Conclusions

Various extraction conditions were investigated in this study to produce pectin from cocoa husks. The yields of pectin obtained from cocoa husks ranged from 3.38% to 7.62% whereas the uronic acid contents of pectin ranged from 31.19% to 65.20%. The DMs of pectin extracted using water, citric acid and hydrochloric acid ranged from 24.54% to 39.26%, 15.06% to 57.86% and 7.17% to 37.36%, respectively. The pectin obtained from cocoa husks was mainly LM pectin, depending on the nature and pH of extractant used. Water and hydrochloric acid produced pectin with a smaller DM range in which all were LM pectin. Conversely, citric acid produced pectin with a wider DM range in which it can be of LM or HM pectin, depending on the extraction conditions. The DAs of pectin extracted using water, citric acid and hydrochloric acid ranged 1.31–2.12%, 1.29–3.48%, 1.01–3.16%, respectively. The

highest pectin yield, 7.62%, was produced using citric acid at pH 2.5 [1:25 (w/v)], 95 °C for 3.0 h, comprised of 45.37% uronic acid content, 37.76% DM and 1.70% DA. The potential of using citric acid as an extractant in pectin extraction from cocoa husks was shown in this study.

Acknowledgement

The authors gratefully acknowledge the financial support of Monash University Sunway Campus Bioactive Compounds Research Strength Grant (Project No. BCHH-SS-6-01-2012).

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