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

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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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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.