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Extraction and characterization of pectin from cacao pod husks (*Theobroma cacao* L.) with citric acid

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ABSTRACT

Variables that influence the citric-acid extraction of pectins from cacao pod husk were examined. A screening study tested the main parameters influencing pectin yield and uronic acid content by a factorial fractional 3^{3-1} design. Further, response surface methodology was applied using a central composite design to examine the effect of a greater region of variable values on pectin yield and uronic acid content. The yield was optimized by increasing the temperature and time. None of the variables had a significant effect on the uronic acid content, and there was lack of fit of the model to the uronic acid content. From the fitted model, extraction conditions with aqueous citric acid at pH 3.0 for 95 min at 95 °C provided a predicted yield of approximately 9.0 g/100 g dry cacao pod husks. The obtained experimental value for the yield was 10.1 ± 0.3 g/100 g dry cacao pod husks, with the pectins containing 65.1 ± 0.8 g uronic acid/100 g fraction, DE 40.3% and DA 15.9%. At 5 g/100 g aqueous solution, the fraction behaved as a concentrated solution and presented a non-Newtonian shear-thinning behavior, well described by Cross Model. Additionally, the fraction formed gels at acidic pH and high sucrose content.

1. Introduction

Theobroma cacao L. (Sterculiaceae) is an important crop of several tropical countries. When ripe, pods are harvested from the trees and opened to extract the wet beans (~10% fresh weight of the cacao fruit). After fermentation of surrounding pulp, the beans are dried and bagged, constituting the cocoa of commerce, employed mainly in chocolate manufacturing (ICCO, 2011a; Kalvatchev, Garzaro, & Cedezo, 1998).

During the extraction of cocoa beans, pod husks, accounting for approximately 52–76% of the weight of the cacao fruit (Donkoh, Atuahene, Wilson, & Adomako, 1991; Fagbenro, 1988), are thrown away and may cause an environmental problem when dumped around the processing plants. In addition to foul odors due to decomposition, cacao pod husks may be a significant source of disease inocula, such as black pod rot (Barazarte, Sangronis, & Unai, 2008; Donkoh et al., 1991; Figueira, Janick, & BeMiller, 1993; Kalvatchev et al., 1998).

Because each ton of dry beans produced generates approximately ten tons of cacao pod husks (Figueira et al., 1993; Kalvatchev et al., 1998) and because the world production of dry cocoa beans is projected to rise from approximately 3.6 million tons in 2009/2010 (from October to September) to 3.9 million tons in 2010/2011 (ICCO, 2011b), the burden of cacao pod husk waste continues to increase and represents a serious challenge for waste management.

In cocoa producer countries, the processing of this cacao waste may offer economic advantages and decrease the extent of the associated environmental problems. An alternative method of processing cacao pod husks could be their use in pectin production, polysaccharides widely used as gelling and stabilizer agents in a variety of food, cosmetic and pharmaceutical products (Rolin, 1993; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995).

Nowadays, commercial pectins come from citrus peel and apple pomace, both by-products of juice production and are generally, extracted with hot, diluted mineral acid (Rolin, 1993; Voragen et al., 1995). The increasing industrial demand for pectins with varying ability to gel or stabilize products increases the need for pectins of different types or derivatives with tailored properties (Rosenbohm, Lundt, Christensen, & Young, 2003).

Previously, the extraction of pectins from cacao pod husks with a mineral acid – nitric acid – was optimized using response surface methodology, reaching maximum yields of approximately 11.5 g/100 g (dry weight) (Vriesmann, Teófilo, & Petkowicz, 2011).

Recent studies (Canteri-Schemin, Fertonani, Waszczynskyj, & Wosiacki, 2005; Klieman et al., 2009; Pinheiro et al., 2008; Virk &

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Sogi, 2004; Yapo, 2009a, 2009b) have shown that citric acid, an organic acid, is effective in pectin extraction in terms of yield and physicochemical properties. In addition, citric acid is a natural and safe food additive and is thus more attractive than commonly used strong mineral acids (nitric, hydrochloric or sulfuric acid) for the extraction of commercial pectins (Yapo, 2009b). Citric acid is also advantageous from an economic as well as an environmental point of view (Canteri-Schemin et al., 2005; Klieman et al., 2009; Pinheiro et al., 2008).

The use of an organic acid for the extraction of pectins from cacao pod husks would not only manage the disposal of this cocoa industry waste product but would also reduce the environmental impact from the corrosive effluents generated by conventional acids used for pectin extraction. In this study, we applied experimental design approaches to optimize the citric-acid-mediated extraction of pectins from cacao pod husks. The selected high-yield pectin was then characterized.

2. Experimental

2.1. Raw material

Dry cacao pod husks (*T. cacao*) were kindly supplied by CEPLAC (Executive Commission of the Plan of Cocoa Farm Work, Itabuna, Bahia, Brazil), a governmental organization for the promotion of cocoa agriculture in Brazil. These husks were milled in a Wiley Mill 934 miller using sieves of 2 mm and 1 mm, successively. The final material that passed through the 1-mm sieve is hereafter referred to as cacao pod husk flour (CPHF). CPHF was previously characterized (Vriesmann, Amboni, & Petkowicz, 2011) and was used in this work for pectin extraction with citric acid according to an experimental design.

2.2. Extraction of pectins from CPHF

Pectins were extracted from CPHF with aqueous citric acid (1:25 g:mL) in a Fisatom 557 bath under reflux, using a mechanical blender at 250 rpm and the extraction conditions established by the experimental design (Section 2.3). After centrifugation at 15,400 × *g* for 30 min, each extract obtained was filtered using a synthetic fabric and treated with ethanol (2:1 mL:mL) to precipitate the polysaccharides. After 16 h at 4 °C, the polysaccharides were washed three times with ethanol and dried under vacuum.

2.3. Experimental design

Initially, the variables aqueous citric-acid pH (pH), extraction temperature (Temp.) and extraction duration (time) were screened using a fractional factorial 3^{3-1} design (Table 1) to investigate the influence of these main extraction parameters on the pectin yield (g/100 g of CPHF weight) and the uronic acid content (g/100 g of the fraction). Test values were selected based on the literature (Rolin, 1993; Voragen et al., 1995). The level values for pH were 1.0, 2.0 and 3.0; for extraction temperature were 50, 75 and 100 °C; and for extraction duration were 30, 60 and 90 min. Experimental treatments were varied randomly to detect the presence of possible systematic errors. Five replicates were performed in central point to make the estimation of possible pure error.

The effects of the different variables on the pectin yield and the uronic acid content were then assessed by response surface methodology (RSM) using the central composite design (CCD) (Teófilo & Ferreira, 2006). CCD was built using the same variables as in the fractional factorial design, but excluding the variable pH because it lacked significance. Thus, the pH of citric acid employed in CCD extractions was kept constant (pH 3) and the dependent variables (responses) were pectin yield and uronic acid content of the extracted pectin.

Table 1
Factors coded (in bracket) and decoded levels used in the factorial fractional 3^{3-1} design and the obtained results.

Assay	pH	Temp. (°C)	Time (min)	Yield (g/100 g CPHF)	Uronic acid (g/100 g fraction)
7	3 (+1)	50 (−1)	60 (0)	5.6	59.8
8	3 (+1)	75 (0)	30 (−1)	5.4	56.3
13c	2 (0)	75 (0)	60 (0)	6.6	63.8
6	2 (0)	100 (+1)	30 (−1)	9.0	62.2
12c	2 (0)	75 (0)	60 (0)	6.8	65.2
10c	2 (0)	75 (0)	60 (0)	6.7	63.2
2	1 (−1)	75 (0)	90 (+1)	7.8	56.9
9	3 (+1)	100 (+1)	90 (+1)	9.7	68.9
1	1 (−1)	50 (−1)	30 (−1)	3.9	57.8
11c	2 (0)	75 (0)	60 (0)	7.1	64.0
3	1 (−1)	100 (+1)	60 (0)	10.6	60.5
5c	2 (0)	75 (0)	60 (0)	6.7	59.7
4	2 (0)	50 (−1)	90 (+1)	3.7	54.4

c: Central point.

The regression coefficients for the linear, quadratic and interaction terms were determined using multiple linear regression (MLR). The significance of each effect and regression coefficient was judged statistically by computing the *t*-value and associated errors. The regression coefficients were then used to generate response surfaces, and the model was validated using the plot of observed vs. predicted values and the plot of observed vs. raw residuals (Teófilo & Ferreira, 2006). All calculations and graphics in this work were performed using electronic worksheets from Microsoft® Excel 2003 in accordance with Teófilo and Ferreira (2006). A difference was considered statistically significant when $p < 0.05$.

2.4. Determination of yield and uronic acid content of pectins

The pectin yield was determined by the ratio of the weight of the extracted pectin dried under vacuum to the original weight of CPHF, in g/100 g. The moisture content of CPHF (8.5 g/100 g) was not deducted in the determination of yield. Uronic acid was estimated by the sulfamate/3-phenylphenol colorimetric method (Filisetti-Cozzi & Carpita, 1991) using galacturonic acid as standard.

2.5. Characterization of the optimized pectin (CA-HYP)

Moisture was determined after oven-drying at 105 °C for 24 h. Total carbohydrate was measured by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using glucose as standard. Protein was determined according to Bradford (1976) employing BSA as standard. Phenolic content was obtained using the Folin–Ciocalteu's reagent (Singleton & Rossi, 1965) and gallic acid as standard. Neutral monosaccharide composition was determined after hydrolysis with 2 M trifluoroacetic acid (5 h, 100 °C) and derivation to alditol acetates, followed by gas–liquid chromatography (GLC) analysis, as described by Vriesmann and Petkowicz (2009). Uronic acid was estimated as previously cited. Degree of methyl-esterification (DE) was determined by quantification of methyl-esterified and free uronic acid band areas using Fourier transform-infrared (FT-IR), as reported (Vriesmann & Petkowicz, 2009). Degree of acetylation (DA) was determined after quantification of acetyl by Hestrin colorimetric method (1949) employing erythritol tetraacetate as standard. ^{13}C NMR spectrum of fraction in D_2O (30 mg/mL) was obtained at 70 °C using a Bruker DRX 400 Avance spectrometer incorporating Fourier transform and chemical shifts are expressed in δ (ppm) relative to acetone (δ 30.2).