LWT - Food Science and Technology 49 (2012) 108–116

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

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

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

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ARTICLE INFO

Article history:
Received 17 November 2011
Received in revised form
4 April 2012
Accepted 18 April 2012

Keywords:
Cacao pod husks
Citric acid
Response surface methodology
LM pectin
Acetyl
Rheology

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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0023-6438
doi:10.1016/j.lwt.2012.04.018

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).

High pressure size exclusion chromatography (HPSEC) was carried out using a multidetection equipment previously described (Vriesmann, Teófilo, et al., 2011), where CA-HYP (filtered at 0.22 μm; Millipore) was analyzed at 1.4 mg/mL in 0.1 M $NaNO_2$ solution containing 0.5 g/L NaN_3. The data were collected and processed by a Wyatt Technology ASTRA program.

Rheological properties of CA-HYP were first studied in aqueous solution at 5 g/100 g. CA-HYP was solubilized in deionized water with stirring for 16 h at 25 °C and then rested for 4 h before rheological analyses.

In order to form gels, CA-HYP was solubilized at 1.0–1.6 g GalA/100 g final mixture in both deionized water and 0.1 mol/L NaCl at pH 5. The mixtures were heated and when they reached 60 °C, a pre-heated calcium solution (60 °C) was dropped into the mixtures under continuous stirring, in a concentration to reach $R = 0.5$ in the final gel, according to the stoichiometric ratio $R = 2$ $[Ca^{+2}]/[COO^{-}]$, which relates the concentration of Ca^{+2} to the amount of non-esterified GalA residues (Fraeye, Duvetter, Doungla, Van Loey, & Hendrickx, 2010). The mixtures were then boiled, cooled and kept under refrigeration. Tests with increasing pH and decreasing calcium content (until $R = 0.2$) were also carried out.

Alternatively, CA-HYP at 0.99 g GalA/100 g pectin fraction was prepared under acidic pH (2.5–3.0) and high sucrose content (60 g/100 g).CA-HYP was solubilized in aqueous citric-acid solutions with stirring for 16 h at 25 °C, followed by the addition of sucrose during the heating of the mixtures. After boiling for 15 min with continuous stirring, sample was cooled to room temperature, pH was measured and it was stored under refrigeration for 16 h.

Rheological measurements were conducted in a Haake MARS rheometer coupled with a thermostatized bath HAAKE K15 and a DC5 heating circulator. The temperature of all analysis (25 °C) was controlled with a Peltier system (TC 81) and a Thermo Haake UTM. C60/2Ti or PP 35 Ti L spindles were employed in the analysis. Frequency sweeps were obtained in the range of 0.01–10 Hz within the linear viscoelastic region (obtained by strain sweep tests at 1 Hz). Flow curves were collected in the CR (controlled rate) mode, from 0.1 to 300 s^{-1} during 360 s. The software RheoWin 4.0 Data Manager was used to obtain the rheological and statistical parameters. All experiments were performed at least in duplicate and the results are the average values.

3. Results and discussion

In our previous study, nitric acid was employed for pectin extraction from cacao pod husks following an experimental design in conjunction with response surface methodology (Vriesmann, Teófilo, et al., 2011). Although the pectin yield obtained in the previous study was significant (11.5 g/100 g dry weight), in this study, we wanted to test an organic acid in an attempt to improve the extraction yield using an environmentally friendly extraction procedure. Apart to environmental benefits, citric acid was chosen based on reports that demonstrated that citric acid was more effective for pectin extraction than mineral acids in terms of yield and physicochemical properties (Klieman et al., 2009; Virk & Sogi, 2004; Yapo, 2009a).

3.1. Experimental design

Initially, a fractional factorial 3^{3-1} design was performed to investigate the influence of the extraction pH, extraction temperature and extraction duration on the pectin yield and the uronic acid content. The experimental design, factors, levels (coded and decoded) and responses are shown in Table 1.

The pectin yield ranged from 3.7 to 10.6 g/100 g CPHF. The highest yield was obtained when the CPHF extraction conditions were pH 1.0/60 min/100 °C. The uronic acid content ranged from 54.4 to 68.9 g/100 g of pectin, with the highest percent of uronic acid obtained when the cacao pod husks were treated at pH 3.0/90 min/100 °C.

Table 2 shows the estimated effects for the factorial design. The results indicate that the linear effect of temperature and the quadratic effect of time are significant with respect to pectin yield, while only the linear effect of temperature is significant with respect to uronic acid content. The yield increased significantly ($p < 0.05$) with increasing time and temperature of the extraction, and the uronic acid content increased significantly with increasing temperature. The pH of the extraction did not have a significant effect on either pectin yield or uronic acid content.

In contrast, when nitric acid was used in the extraction of pectins from cacao pod husks (Vriesmann, Teófilo, et al., 2011) at the same levels of as those used in the current work, the extraction time did not influence pectin yield or the uronic acid content. The extraction yield increased with increasing pH and temperature, whereas the uronic acid content increased with decreasing pH and increasing temperature (Vriesmann, Teófilo, et al., 2011).

Marcon, Vriesmann, Wosiacki, Beleski-Carneiro, and Petkowicz (2005) extracted pectins from apple pomace with 5% (w/v) citric acid using a 2^2 factorial design with different times and temperatures. The obtained yield ranged from 5.7 to 16.8 g/100 g, and the increase in the yield was directly correlated with the increases in time and temperature of extraction, as observed for pectins extracted from CPHF with citric acid. The galacturonic acid content of their fractions (33.4–42.5 g/100 g) was not related to the extraction yield.

Canteri-Schemin et al. (2005) extracted pectins from apple pomace with citric, phosphoric, malic, tartaric, hydrochloric, sulfuric and nitric acids. Citric and nitric acids showed the highest yields among the organic and mineral acids tested. The highest average yield was found for citric-acid-extracted pectin (13.75 g/100 g yield). In the present work, citric-acid extraction reach 10.6 g/100 g yield.

Yapo (2009a) investigated the effects of acid type on the yield and characteristics of pectin from yellow passion fruit rind. Citric, nitric, and sulfuric acids were used, and the results showed that not only the acid type but also the acid concentration influenced the extracted pectin yields (3–14 g/100 g). The pectin amounts were significantly higher at lower extracting solvent pH, regardless of the acid type. Similar amounts of pectins were extracted with nitric and sulfuric acids. The yields of pectins extracted with citric acid were lower (2.8 and 5.1 g/100 g), differently of pectins from apple pomace (Canteri-Schemin et al., 2005) and similarly with our results. In addition, the acid citric extracted pectins from yellow

Table 2
Effect estimates for 3^{3-1} design on pectin yield and uronic acid content.

	Yield (g/100 g CPHF)				Uronic acid (g/100 g fraction)			
	Effect[a]	Std. err.	$t(6)$	p	Effect[a]	Std. err.	$t(4)$	p
Mean	***6.93***	0.16	44.24	0.00	***60.35***	0.65	92.33	0.00
pH	−0.53	0.41	−1.31	0.24	3.27	1.70	1.93	0.13
pH^2	−0.72	0.31	−2.32	0.06	0.97	1.30	0.74	0.50
Temp.	***5.37***	0.41	13.21	0.00	***6.53***	1.70	3.85	0.02
$Temp.^2$	−0.47	0.31	−1.51	0.18	−0.74	1.30	−0.57	0.60
Time	0.97	0.41	2.38	0.05	1.30	1.70	0.77	0.48
$Time^2$	***1.03***	0.31	3.30	0.02	2.81	1.30	2.16	0.09

[a] Values in bold and italics are significant at $\alpha = 0.05$ with six degrees of freedom for pectin yield using mean square residual error and four degrees of freedom for uronic acid using pure error.

passion fruit rind were reported having better physicochemical properties (Yapo, 2009a).

Once the results of the screening design were obtained, RSM was then applied using a CCD with two independent variables (time and temperature) to shift the levels of the variables for the interested and higher region. As pH was found not to influence the yield and uronic acid content, the pH was fixed at 3.0 in these experiments. The data obtained from the thirteen experiments are shown in Table 3.

Table 3 shows that the yields varied between 6.6 and 9.0 g/100 g of CPHF. The pectin yields from cacao pod husks presented here are similar to those obtained by Vriesmann, Teófilo, et al. (2011) using nitric acid (6.8–9.2 g/100 g) and Adomako (1972) using hydrochloric or acetic acids (8–11 g/100 g) but are superior to those obtained by Barazarte et al. (2008) with EDTA at different pH values (<5 g/100 g yield).

Table 4 shows the regression coefficients of the model built. Yield was influenced by linear effects of temperature and time ($p < 0.05$) and by a quadratic effect of temperature. However, the interaction between the variables time and temperature was not significant ($p > 0.05$). The linear regression coefficients for temperature and time were positive, indicating higher pectin yield at higher temperatures and times. The extraction yield of pectin from cacao pod husks was not related to the content of uronic acid. Individual effects of time and temperature, at the levels studied, did not influence the uronic acid content of pectins ($p > 0.05$).

Note in Table 4 that temperature (linear and quadratic factors) and time (linear factor) were significant for yield. The linear coefficients for temperature and time indicate that increase in the temperature and/or time produce an increase in yield. The linear coefficient of temperature indicates that a decrease in the temperature produces a quadratic drop in the yield.

From Table 4, no variable was significant at the studied levels, with respect to uronic acid content. Note in Table 3 that the values for uronic acid presented slight variation (52–62 g/100 g fraction) and an average of 56.59 g/100 g. This indicate a little variation non significant in the studied levels. From results presented in Tables 1 and 3, the levels that produce a satisfactory result for uronic acid are a temperature of ~95 °C and a time of ~95 min.

Table 3
Experimental points of CCD and responses obtained.

	Runs[a]	X_1	X_2	Yield (g/100 g CPHF)	Uronic acid (g/100 g fraction)
Factorial points	1	−1	−1	6.6	52.2
	2	−1	1	7.2	55.1
	10	1	1	8.8	62.2
	12	1	−1	8.3	55.8
Center points	4	0	0	8.4	58.5
	6	0	0	8.5	54.8
	7	0	0	8.6	55.2
	11	0	0	8.4	58.1
	13	0	0	8.6	52.0
Axial points	9	−1.414	0	7.0	54.2
$A = 2^{1/2} \approx 1.414$	8	1.414	0	8.9	55.0
	5	0	−1.414	8.0	61.7
	3	0	1.414	9.0	60.9
Experimental domain					
Variables	−1.414	−1	0	1	+1.414
X_1: Temperature (°C)	70.86	75.00	85.00	95.00	99.14
X_2: Time (min)	52.75	60.00	77.50	95.00	102.24

[a] Citric-acid pH fixed at 3.

Table 4
Coefficient estimates for the central composite design and statistical analyses for pectin yield and uronic acid content.

	Yield				Uronic acid			
	Coeff.[a]	Std. err.	$t(7)$	p	Coeff.[a]	Std. err.	$t(4)$	p
Mean	***8.50***	0.13	64.97	0.000	***55.72***	1.19	46.80	0.000
Temp. (L)	***0.75***	0.10	7.24	0.000	1.48	0.94	1.57	0.191
Temp. (Q)	***−0.40***	0.11	−3.61	0.009	−0.97	1.01	−0.96	0.392
Time (L)	***0.31***	0.10	3.04	0.019	1.02	0.94	1.08	0.338
Time (Q)	−0.13	0.11	−1.13	0.296	2.38	1.01	2.36	0.077
Temp. × time	−0.02	0.15	−0.17	0.869	0.87	1.33	0.65	0.546

[a] Values in bold and italics are significant at $\alpha = 0.05$ with seven degrees of freedom for pectin yield using mean square residual error and four degrees of freedom for uronic acid using pure error.

In this work, the model was built only for the yield of pectin from cacao pod husks. Equation (1) shows the model using the codified coefficients.

$$\text{Yield}(\%) = 8.5 + 0.75\text{Temp.} - 0.40\text{Temp.}^2 + 0.31\text{Time} - 0.13\text{Time}^2 - 0.02\text{Temp.} \times \text{Time} \quad (1)$$

The model was validated using the plot of the observed vs. predicted values and the plot of the observed vs. raw residuals (Teófilo & Ferreira, 2006); both are presented in Fig. 1. These plots show that the values predicted by the model present a low error, and thus, the model is able to prediction, i.e., the model is fitted.

The surface of this model (Fig. 2) was built based on decodified coefficients and reveals a significant increase in the pectin yield with simultaneous increases in temperature and time. Based upon the data, a possible condition to maximize pectin yield from cacao pod husks could be the use of aqueous citric acid at pH 3.0/95 °C/95 min to achieve approximately 9.0 g/100 g yield (within the levels studied). If the moisture content of CPHF is considered (8.5 g/100 g), this value is 9.8 g/100 g.

3.2. Extraction in the selected optimized conditions

Following the optimized conditions above cited (pH 3.0/95 °C/95 min) using citric acid, a fraction called CA-HYP (citric-acid high-yield pectin) was obtained from cacao pod husks in an experimental yield of 10.1 ± 0.3 g/100 g, which is even greater than the expected value (9.0 g/100 g). If the moisture of CPHF is considered (8.5 g/100 g), the yield increases to 11.0 g/100 g, reaching the amounts obtained with apple pectin (Rolin, 1993; 10–15 g/100 g). The experimental yield of CA-HYP was higher than those obtained for pectins extracted from yellow passion fruit rind with citric acid (3.5–8.4 g/100 g, Yapo, 2009a, 2009b), but lower than the mean yield for pectins extracted with citric acid from apple pomace (13.75 g/100 g, Canteri-Schemin et al., 2005).

In comparison with pectins previously isolated from cacao pod husks, CA-HYP was obtained in a yield similar to the highest value obtained by Adomako (1972) by mild acid extractions (0.2 N HOAc, 8–11 g/100 g yield) and superior than those obtained by Barazarte et al. (2008) with EDTA at acidic pH (2.6–4.7 g/100 g yield) or that of the pectin extracted with nitric acid under optimization for high uronic acid content (9.0 g/100 g, Vriesmann, Teófilo, et al., 2011).

Attri and Maini (1996) extracted pectins from galgal peels (an indigenous variety of lemon) with different mineral and organic acids and observed that mineral acids gave higher yields than did the organic acids. In contrast, Klieman et al. (2009) extracted pectin from passion fruit peel using citric, hydrochloric or nitric acid, and they observed that citric acid was the best acid for pectin extraction. Virk and Sogi (2004) extracted pectins from apple peel using

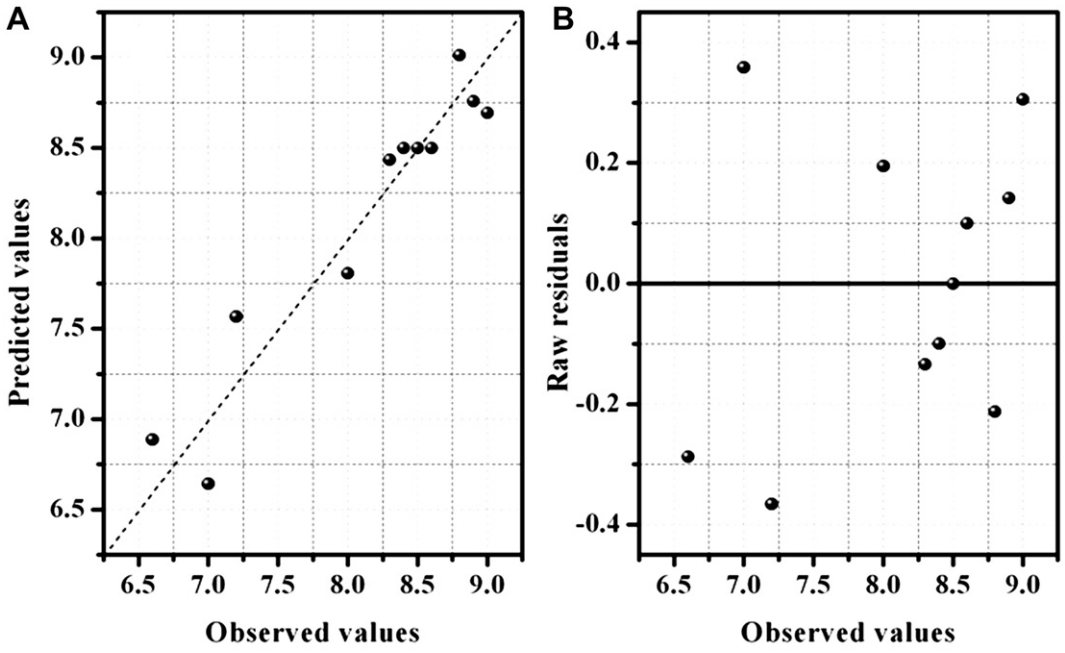

Fig. 1. Validation model for the yield of pectin. (A) Observed vs. predicted values; (B) observed vs. raw residuals.

HCl and citric acid and also observed that citric acid was more effective than HCl in terms of yield.

3.3. Characterization of pectin obtained in the optimized conditions

As showed in Table 5, CA-HYP fraction presented low moisture content (2.7 g/100 g) with high carbohydrate content (64.0 g/100 g CA-HYP), followed by proteins and phenolics (13.8 and 9.4 g/100 g, respectively). Monosaccharide composition showed that CA-HYP contains mainly uronic acid (65.1 g/100 g fraction). Rhamnose and galactose were found in higher proportions than the other monosaccharides. Similar monosaccharide composition was found for pectins from sugar beet pulp (Morris & Ralet, 2011), *Améliorée* mango peels (Koubala et al., 2008), okra (Sengkhamparn, Verhoef, Schols, Sajjanantakul, & Voragen, 2009) and optimized cacao pod husks pectin obtained with nitric acid (Vriesmann, Teófilo, et al., 2011).

The proportion of GalA units methyl-esterified at C-6 in relation to the total GalA units defines the degree of methyl-esterification (DE), which classifies pectins as high-methoxyl (HM pectins, DE $>$ 50%) and low-methoxyl (LM pectins, DE $<$ 50%). Degree of acetylation (DA) is the proportion of acetyl groups in relation to the total GalA units of the pectin. Both the DE and DA have a significant impact on pectin functional properties, influencing solubilization and gelation properties (Rolin, 1993). In contrast to native pectins (very often HM with low acetyl content) (Voragen et al., 1995), CA-HYP contained low-methoxyl pectins with high acetyl content (DE: 40.3%; DA: 15.9%; Table 5). LM pectins highly acetylated were also obtained from sugar beet pulp (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007) and okra (Sengkhamparn et al., 2009).

^{13}C NMR spectroscopy of CA-HYP (Fig. 3) allowed the investigation of its chemical structure. Signals of esterified and unesterified units of α-D-GalA from homogalacturonans were identified at δ 100.0 and 99.3, respectively, with their respective C-6 signals at δ 170.6 and 173.5, from methyl ester carbonyl carbons and carboxyl carbons, respectively. Signals of methyl carbons of esterified carbonyls in GalA units appeared at δ 52.8, whereas those of acetyl groups appeared at δ 20.4. Rhamnogalacturonans were also

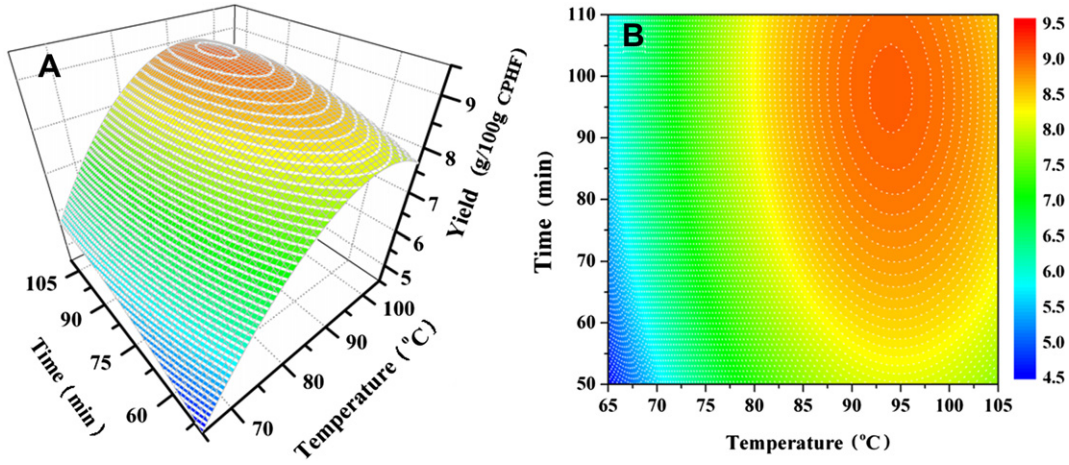

Fig. 2. Response surface of the pectin yield. (A) 3D surface and (B) 2D surface.

Table 5
Composition of CA-HYP fraction from cacao pod husks flour (CPHF).

Composition (g/100 g fraction)	CA-HYP[d]
Moisture	2.7 ± 0.3
Carbohydrate[a]	64.0 ± 0.6
Protein[a]	13.8 ± 1.4
Phenolic[a]	9.4 ± 0.05
Monosaccharides[b]	
Rha	8.2 ± 1.88
Ara	1.9 ± 0.83
Xyl	1.4 ± 1.56
Man	2.6 ± 1.42
Gal	16.7 ± 1.42
Glc	4.1 ± 0.48
Uronic acid[a]	65.1 ± 0.8
DE[c]	40.3 ± 0.7
DA[a]	15.9 ± 0.3

[a] Determined by colorimetric methods.
[b] Determined by GLC.
[c] Determined by FT-IR.
[d] Expressed on dry basis, except for moisture.

identified in CA-HYP. Characteristic signals of C-1 and CH_3-6 signals from Rha units appeared at δ 98.5 and 16.6, respectively. The anomeric region also showed signals at δ 103.3 and 102.4 from β-1,4-D-Gal units (substituted or not at O-6, respectively). In the aromatic carbons region, signals at δ 115.1, 116.2, 144.0 and 154.8 were identified, suggesting the presence of phenolic compounds. All assignments were based on literature values (Vriesmann, Amboni, et al., 2011; Vriesmann, Teófilo, et al., 2011; Westereng, Michaelsen, Samuelsen, & Knutsen, 2008).

The NMR data for CA-HYP fraction are in agreement with the presence of highly acetylated pectins containing LM homogalacturonans with RG-I insertions carrying galactans side chains.

Although CA-HYP presented a slightly lower yield and higher contents of total carbohydrate and uronic acid, their composition and ^{13}C NMR spectrum closely resembles the pectins obtained from cacao pod husks by boiling aqueous extractions (Vriesmann, Amboni, et al., 2011). It seems that, both citric acid and water, were able to remove LM pectins (DE ~40%) probably arising from the middle lamella.

Fig. 4 shows the HPSEC elution profile of fraction CA-HYP. Due to the high-molar mass (1.806×10^6 g/mol), the primary peak (~38 min) was detected by both, the differential refractometer (RI) detector and the multiangle laser light scattering (MALLS) detector. Another peak was observed at higher elution time (>40 min), with a less intense RI signal and no MALLS detection, indicating lower concentration and lower-molar mass (6.450×10^5 g/mol). Comparing to the pectins obtained from cacao pod husks with boiling water, CA-HYP had higher molar mass (Vriesmann, Amboni, et al., 2011).

Dynamic viscoelastic properties of solutions of CA-HYP at 5 g/100 g were studied by frequency sweeps obtained at 25 °C (Fig. 5). Both elastic (G') and viscous (G'') moduli increased with the frequency, being G' more dependent on frequency than G'', until reach a frequency of ~10 Hz, where the cross-over between the moduli occurs. Similar results were obtained by Vriesmann, Amboni, et al. (2011) for boiling-water extracted pectins from cacao pod husks and Min et al. (2011) for pectins from apple pomace obtained by chemical and combined physical/enzymatic treatments. However, the pectins from apple pomace at 5 g/100 g presented $G'' > G'$ over the range of frequency analyzed (Min et al., 2011). These authors observed that pectins with lower DE appeared to have more elastic properties than those with higher DE (Min et al., 2011). The results obtained for CA-HYP confirmed this trend. CA-HYP (40.3% DE) showed higher elastic properties than pectins from cacao pod husks extracted with boiling water (42.6% DE; Vriesmann, Amboni, et al., 2011) and apple pomace pectins (58 and 69% DE; Min et al., 2011).

The viscosity curve of 5 g/100 g CA-HYP aqueous solution at 25 °C (Fig. 6) showed a shear-thinning, pseudoplastic flow behavior as reported for other pectin solutions (Hwang & Kokini, 1992; Min et al., 2011; Vriesmann, Amboni, et al., 2011).

Cross equation, with four parameters, can describe the general flow curve of pseudoplastic fluids (Cross, 1965). Thus, it was

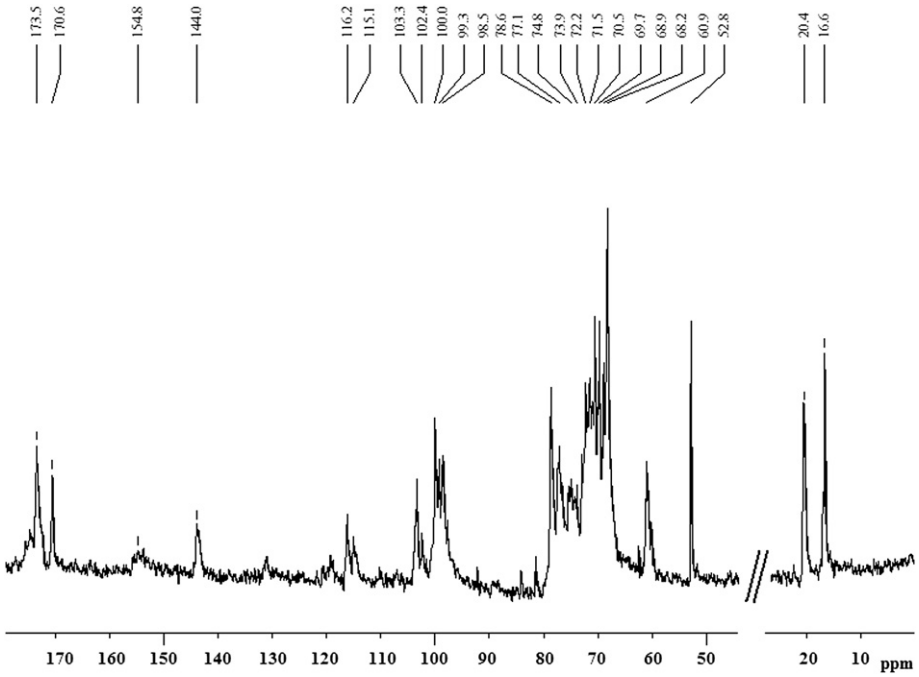

Fig. 3. ^{13}C NMR spectrum of CA-HYP fraction obtained at 70 °C in D_2O.

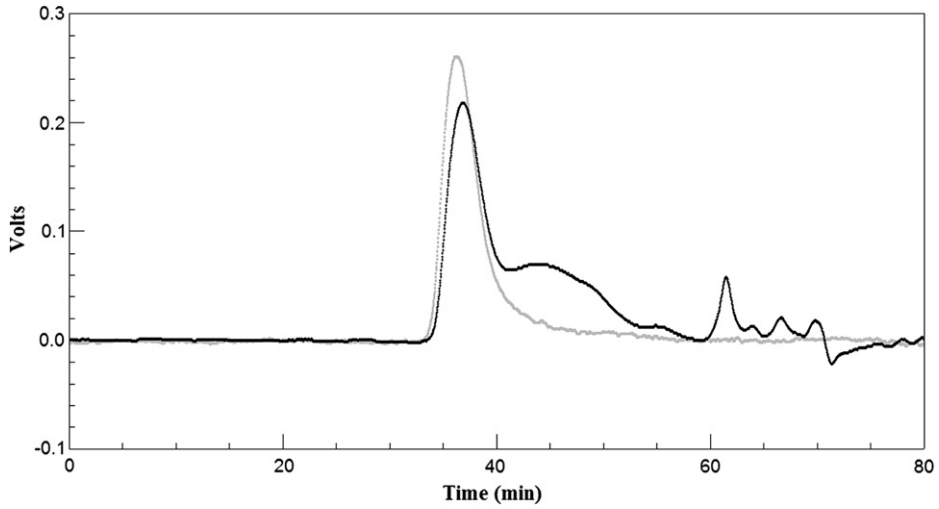

Fig. 4. Elution profile of CA-HYP fraction obtained by HPSEC-MALLS/RI (—— MALLS, 90°; —— RI).

employed to fit the experimental data of apparent viscosity, η (Pa s), vs. shear rate, $\dot{\gamma}$ (1/s) for CA-HYP, according to the equation: $\eta = \eta_\infty + (\eta_0 - \eta_\infty)/[1 + (\dot{\gamma}/\dot{\gamma}b)^n]$, where η_0 is the zero-shear rate viscosity (Pa s), η_∞ is the infinite-shear rate viscosity (Pa s), $\dot{\gamma}b$ is the shear rate at which the fluid changes from Newtonian to Power-law behavior (1/s) and n is the flow behavior index (−).

The values found for the four parameters for the flow of CA-HYP were η_0: 7.993 Pa s; η_∞: 0.1189 Pa s; $\dot{\gamma}b$. 1.607 1/s and n: 0.6231. The obtained coefficient of determination (R^2) was 0.9988, indicating that Cross equation can be used to describe CA-HYP flow.

Thus, the results showed that CA-HYP fraction at 5 g/100 g solution presented zero-shear rate viscosity (η_0: 7.993 Pa s) higher than pectins from apple pomace in the same concentration which were extracted by chemical and physical/enzymatic treatments (η_0: 0.638 and 0.135 Pa s, respectively; Min et al., 2011). Moreover, the flow behavior index of the solution of CA-HYP (n: 0.6231) was lower than those of pectin samples from apple pomace in the same concentration ($n > 0.7$; Hwang & Kokini, 1992; Min et al., 2011), suggesting that CA-HYP pectins are more pseudoplastic.

Furthermore, the ability of CA-HYP to form gel was investigated. As CA-HYP contained LM pectins, initially gel formation in the presence of calcium ions was examined. Samples at 1.0–1.6 g GalA/100 g final mixture in both deionized water and 0.1 mol/L NaCl at pH 5 with calcium $R = 0.5$ did not form gel. R value of 0.5 was chose because theoretically up to this value, all calcium ions are bound in pectin egg-boxes to form strong gels (Fraeye et al., 2010). Tests with increasing pH and decreasing calcium content (until $R = 0.2$) were also carried out. However, again the gel formation did not take place and precipitation was observed.

The high DA of CA-HYP (15.9%) might be responsible by the absence of gelling properties in the presence of calcium. The high proportion of acetyl groups cause a steric hindrance of chain association and considerably reduce the binding strength of pectin with Ca^{+2} (Fraeye et al., 2010; Williamson et al., 1990). Also, the presence of side chains (RG-I) in CA-HYP, as demonstrated by the monosaccharide composition and ^{13}C NMR, could hamper the intermolecular interactions between pectin chains and consequently, the calcium gel formation (Fraeye et al., 2010).

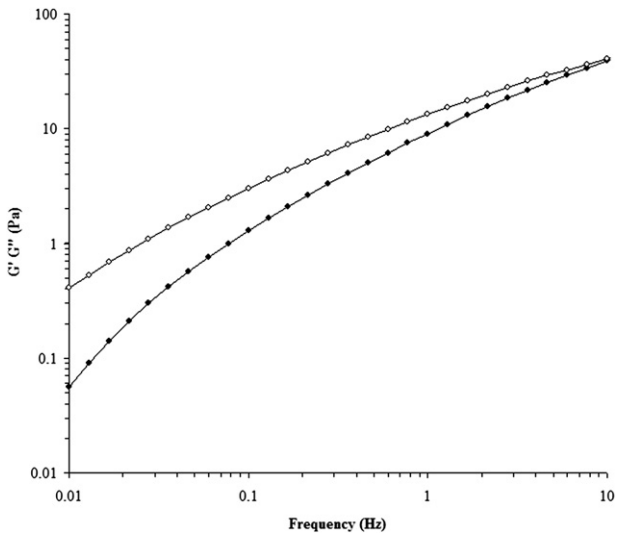

Fig. 5. Frequency sweep at 25 °C of CA-HYP fraction at 5 g/100 g solution (—●— G'; —○— G'').

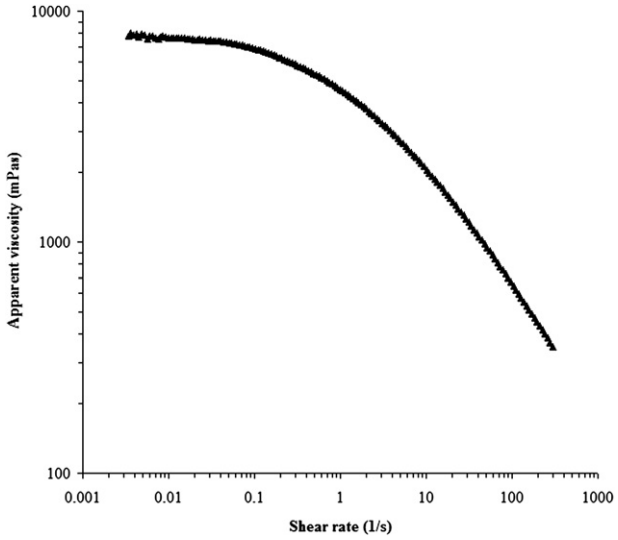

Fig. 6. Influence of shear rate on the apparent viscosity at 25 °C of CA-HYP solution at 5 g/100 g.

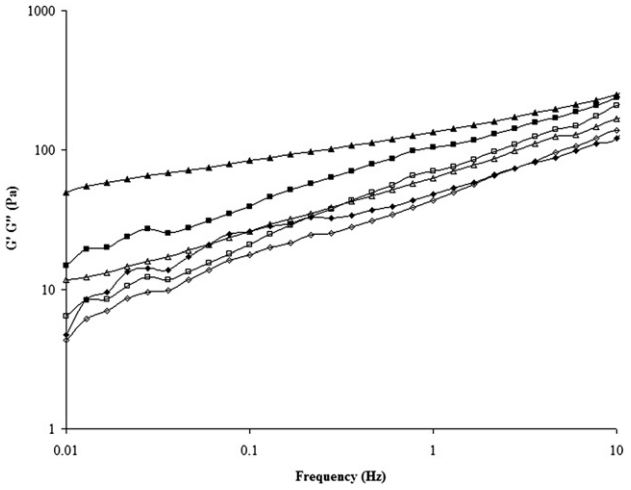

Fig. 7. Frequency sweeps at 25 °C of CA-HYP fraction at 0.99 g GalA/100 g sample containing 60 g sucrose/100 g final mixture at different pHs (—▲— G', pH 2.5; —△— G'', pH 2.5; —■— G', pH 2.7; —□— G'', pH 2.7; —◆— G', pH 3.0; —◇— G'', pH 3.0).

For sugar beet pectins, it has been proposed that high acetyl contents (Pippen, McCready, & Owens, 1950) and high proportion of side chains (Matthew, Howson, Keenan, & Belton, 1990) are responsible by their poor gelling properties in the presence of Ca^{+2}. It was observed that the reduction of these structural components improve the sugar beet pectin gelling ability (Matthew et al., 1990; Pippen et al., 1950).

Moreover, not only the amount of de-esterified GalA units (~60%) but also the distribution of esterified and non-esterified GalA units in the pectins from CA-HYP might influence the calcium gel formation. The formation of egg-box junction zones through Ca^{+2} only is possible when the pectin has sequences with a minimum number of non-esterified GalA (Fraeye et al., 2010).

LM pectin can also form gels in absence of Ca^{+2} if pH is lower than 3.5. In this condition, non-esterified carboxyl groups are protonated, reducing electrostatic chain repulsion and enabling the interaction between pectin chains through hydrogen bonding. If sucrose is present, the water activity is reduced and pectin–pectin interactions predominate under pectin–water interactions (Fraeye et al., 2010; Voragen et al., 1995). Thus, the rheological behavior of CA-HYP at 0.99 g GalA/100 g was evaluated at low pH (2.5–3.0) with addition of 60 g sucrose/100 g final mixture.

The variation of elastic (G') and viscous (G'') moduli with frequency (0.01–10 Hz) at 25 °C for CA-HYP at low pH and addition of sucrose is shown in Fig. 7. Samples at pH 2.7 and 2.5 showed elastic modulus higher than viscous modulus ($G' > G''$) over the frequency range analyzed and G' was less dependent of frequency than G'', especially at pH 2.5, characterizing a weak gel-like behavior. For the sample at pH 3.0, at lower frequencies $G' > G''$ and a cross-over between the moduli occurred at approximately 2.8 Hz.

Löfgren, Walkenström, and Hermansson (2002) also obtained a weak gel with LM pectins (DE 33.5%) at low pH/high sucrose concentration. At 0.75 g pectin concentration/100 g and pH 3.0 with 60% sucrose, in the absence of calcium ions, the gel shows a G' of 30 Pa and a G'' of 20 Pa at 1 Hz. For the same frequency, sucrose concentration and pH, CA-HYP at 0.99 GalA/100 g sample showed higher values of G' and G'', 48 Pa and 43 Pa, respectively.

4. Conclusions

Hot citric-acid extraction appears suitable for the recovery of pectins from cacao pod husks. Slight variation of the uronic acid content (52–62 g/100 g fraction) was observed at the studied levels. However, the extraction yield increased significantly with increasing temperature and time. The experimental yield of pectin in the selected satisfactory conditions (pH 3.0/95 °C/90 min) was found to be in good agreement with the predicted yield (10.1 g/100 g vs. 9.0 g/100 g, respectively).

The pectin obtained is an LM homogalacturonan highly acetylated (DE 40.3%; DA 15.9%) containing rhamnogalacturonan insertions with galactose-rich side chains and showed a non-Newtonian shear-thinning behavior, well fitted by Cross Model. Although gel formation with calcium ions was not observed, the pectin was able to form gels under low pH/high sucrose content, suggesting possible applications as additive in acidic products.

The citric-acid-mediated extraction of pectins from the main by-product of cocoa production would not only help to reduce the costs of the production of cocoa products but would also manage the disposal of this waste in an environmental friendly manner through the use of a natural and safe food additive.

Acknowledgments

The authors thank Adonias de Castro Virgens Filho, Miguel Moreno-Ruiz and CEPLAC/CEPEC for supplying the cacao pod husks and CNPq for financial support.

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