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Optimization of nitric acid-mediated extraction of pectin from cacao pod husks (*Theobroma cacao* L.) using response surface methodology

Lúcia Cristina Vriesmann[a], Reinaldo Francisco Teófilo[b], Carmen Lúcia de Oliveira Petkowicz[a,*]

[a] Universidade Federal do Paraná, Departamento de Bioquímica e Biologia Molecular, CP 19046, 81531-980 Curitiba-PR, Brazil
[b] Universidade Federal de Viçosa, Departamento de Química, Viçosa-MG, Brazil

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ABSTRACT

This study examined variables that influence the nitric acid-extraction of pectins from cacao pod husks. An initial screening study tested the main parameters influencing yield and uronic acid content by a factorial fractional 3^{3-1} design. Additional studies used response surface methodology using a central composite design. Yield was optimized by increasing temperature. Uronic acid content was higher when pH is decreased and temperature is increased, or when pH is increased and temperature is decreased. To optimize both parameters, extraction conditions of pH 1.5 at 100 °C for 30 min provided a predicted yield and uronic acid content of approximately 9.5% and 80%, respectively. The obtained experimental value for the yield was 9.0 ± 0.4%, the pectin presenting 66.0% uronic acid. Chemical and spectroscopic analyses showed that this pectin is mainly a homogalacturonan highly esterified and acetylated (DE 56.6%; DA 17.1%) with some rhamnogalacturonan insertions.

1. Introduction

Pectins are a family of complex, acid-rich polysaccharides from plant cell walls. They are widely used as gelling and stabilizing agents in the food, pharmaceutical and cosmetic industries. In addition, pectin intake has several positive effects on human health, including reduced serum cholesterol, reduced serum glucose, reduced cancer incidence, and improved immune response, amongst other effects (May, 1990; Mohnen, 2008).

The main classes of pectins include homogalacturonan and rhamnogalacturonan. Homogalacturonan is a linear polymer of α-1,4-linked D-galacturonic acid units, partially methyl-esterified at the C-6 carboxyl, and sometimes O-acetylated at the O-2 or O-3 positions. The backbone of homogalacturonan may be interspersed with α-1,2-linked L-rhamnosyl units that may carry neutral sugar chains at the C4-position, mainly composed of arabinose and galactose that constitute the rhamnogalacturonan region (Mohnen, 2008; Rolin, 1993).

The composition of pectin depends on the plant source and conditions employed during pectin isolation and purification. As such, extraction is an important step in the recovery of pectins, and the extraction conditions chosen depend upon the raw material and the desired product (Rolin, 1993).

Commercial pectins usually come from citrus peels and apple pomace, both by-products from juice manufacturing (May, 1990). Additional agricultural by-products investigated as sources of pectin include beet pulp (Levigne, Ralet, & Thibault, 2002; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007), soy hull (Kalapathy & Proctor, 2001), sunflower residues (Iglesias & Lozano, 2004), ambarella peels (Koubala, Mbome, et al., 2008), cocoa bean husks (Arlorio, Coisson, Restani, & Martelli, 2001; Mollea, Chiampo, & Conti, 2008), banana peels (Happi Emaga, Robert, Ronkart, Wathelet, & Paquot, 2008; Happi Emaga, Ronkart, Robert, Wathelet, & Paquot, 2008), passion fruit peels (Yapo & Koffi, 2006) and mango peels (Koubala, Kansci, et al., 2008). However, none of these satisfy industrial requirements which include high yields, best quality gels and functionality (Daniells, 2008).

As such, alternative sources of commercial pectins are of considerable interest. In this current work, cacao pod husks (*Theobroma cacao* L.), the first by-product of the cocoa industry, were investigated as a potential source of pectin. In the cocoa crop, only the beans (~10% fresh weight of the cacao fruit) are commercially valuable. The mature fruits of cacao are harvested and opened to extract the wet beans. After fermentation of surrounding pulp, the dried beans are used as the commercial form of cocoa, employed mainly for the chocolate industry but also for the pharmaceutical and cosmetic industries (Fagbenro, 1988; ICCO, 2010; Kalvatchev, Garzaro, & Cedezo, 1998).

Cacao pod husks constitute the remnants following removal of the cocoa beans and pulp from the mature fruit. The husks account for 52–76% of the pod wet weight (Donkoh, Atuahene, Wilson, & Adomako, 1991; Fagbenro, 1988) and are considered an undesirable waste product of the cocoa industry. Frequently, the husks are left to decompose, sometimes on the cacao plantation, which

* Corresponding author. Tel.: +55 41 3361 1661; fax: +55 41 3266 2042.
E-mail address: clop@ufpr.br (C.L.d.O. Petkowicz).

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generates foul odors and becomes a significant potential source of botanical disease inoculum like black pod rot (Barazarte, Sangronis, & Unai, 2008; Donkoh et al., 1991; Figueira, Janick, & BeMiller, 1993; ICCO, 2010; Kalvatchev et al., 1998).

For each ton of dry beans produced, ten tons of cacao pod husks are generated, which represents a serious challenge for waste management (Figueira et al., 1993; Kalvatchev et al., 1998). World production of dry cocoa beans is projected to rise from approximately 4.1 million tons in 2009/2010 to 4.5 million tons in 2012/2013 (Executive Committee, 2008), so the burden of cacao pod husk waste will continue to rise.

Processing of this cacao waste may provide a saleable commodity and reduce damage to the environment. An alternative could be its use in pectin production. Although cacao pod husk was investigated as a source of pectins (Adomako, 1972; Barazarte et al., 2008; Blakemore, Dewar, & Hodge, 1966), pectin yields were generally low. Hence, it is necessary to optimize the extraction process to improve pectin yield and quality. We now apply the design and statistical approaches of response surface methodology to optimize the nitric acid-mediated extraction of pectins from cacao pod husks to improve pectin yield and uronic acid content. The pectin obtained in the satisfactory optimized condition was then characterized.

2. Materials and methods

2.1. Materials

Dry cacao pod husks were kindly supplied by CEPLAC (Executive Commission of the Plan of Cocoa Farm Work, Itabuna, Bahia, Brazil), a governmental organization for promotion of cocoa agriculture in Brazil. These husks were milled in a Wiley Mill 934 using sieves of 2 mm and 1 mm, successively. The final material which passed through the 1-mm sieve (<18 mesh) was thereafter referred to as cacao pod husks flour (CPHF) and employed for pectin extraction.

2.2. Extraction of pectins from CPHF

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

2.3. Determination of pectin yield and uronic acid content

The pectin yield was determined by the ratio of the weight of the extracted pectin dried under vacuum to the original weight of CPHF (%, w/w). The moisture of CPHF (8.5%, w/w) was not deducted in the determination of yield. Uronic acid (UA) was estimated by the sulfamate/3-phenylphenol colorimetric method (Filisetti-Cozzi & Carpita, 1991), using galacturonic acid (10–100 μg/mL) as standard.

2.4. Experimental design

Variables were screened using a fractional factorial 3^{3-1} design (Table 1). The variables used in this design were aqueous nitric acid pH (*pH*), extraction temperature (*T*) and extraction duration (*t*), and test values were selected based on the literature (Rolin, 1993; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Five repetitions were performed in the central point (*c*) to make the estimation of possible pure error and experimental treatments were varied randomly to detect the presence of possible systematic errors. The responses measured were the pectin yield (% of CPHF weight) and uronic acid content (%) of the extracted pectins.

Table 1
Results of factorial fractional 3^{3-1} design.

Assay	*pH*	*T* (°C)	*Time* (min)	Yield (% of CPHF)	Uronic acid (%)
7	3 (+1)	50 (−1)	60 (0)	4.9	56.2
2	1 (−1)	75 (0)	90 (+1)	5.2	75.1
13c	2 (0)	75 (0)	60 (0)	6.6	54.0
9	3 (+1)	100 (+1)	90 (+1)	11.2	56.9
5c	2 (0)	75 (0)	60 (0)	6.7	55.8
3	1 (−1)	100 (+1)	60 (0)	6.4	82.5
4	2 (0)	50 (−1)	90 (+1)	5.4	50.6
11c	2 (0)	75 (0)	60 (0)	6.9	55.4
10c	2 (0)	75 (0)	60 (0)	6.9	56.7
1	1 (−1)	50 (−1)	30 (−1)	3.3	65.0
12c	2 (0)	75 (0)	60 (0)	6.3	52.3
8	3 (+1)	75 (0)	30 (−1)	7.1	50.9
6	2 (0)	100 (+1)	30 (−1)	9.0	59.8

Afterwards, from the results of the fractional factorial design, a Central Composite Design (CCD) was built using the same variables as in the fractional factorial design, but excluding those with lacked significance. Statistical models were developed for yield and uronic acid content and they were validated using the analysis of variance (ANOVA) (Teófilo & Ferreira, 2006). Treatment effects upon both responses were then assessed by response surface methodology (RSM) and the optimized condition was chosen to obtain optimized pectin (OP).

All calculations and graphics in this work were performed using electronic worksheets from Microsoft® Excel 2003 in accordance with Teófilo and Ferreira (2006).

2.5. Characterization of optimized pectin (OP)

Moisture was determined by oven-drying at 106 °C for 24 h. Total carbohydrate, monosaccharide composition and ^{13}C NMR analyses were performed as described (Vriesmann & Petkowicz, 2009). Protein was determined according to Bradford (1976) using BSA as standard. Phenolic content was determined using the Folin–Ciocalteau's reagent (Singleton & Rossi, 1965) and gallic acid as standard.

Degree of esterification (DE) was determined by Fourier transform-infrared (FT-IR) as described (Vriesmann & Petkowicz, 2009).

Degree of acetylation (DA) was determined after quantification of acetyl by Hestrin colorimetric method (1949) employing erythritol tetraacetate as standard.

High pressure size exclusion chromatography (HPSEC) was carried out using a multidetection equipment: a Waters 2410 differential refractometer (RI); a Pharmacia LKB Uvicord VW 2251 ultraviolet detector at 280 nm (UV) and a Wyatt Technology Dawn F multi-angle laser light scattering (MALLS) detector. Four Waters Ultrahydrogel 2000/500/250/120 columns were connected in series and coupled to the multidetection equipment. A 0.1 M $NaNO_2$ solution, containing NaN_3 (0.5 g/l), was used as eluent. Previously filtered OP (0.22 μm; Millipore) was analyzed at 1.5 mg/ml and the data were collected and processed by a Wyatt Technology ASTRA program.

3. Results and discussion

3.1. Experimental design

As little information is available on the recovery of pectins from cacao pod husk, a fractional factorial 3^{3-1} design was initially performed to investigate the influence of the variables of extraction pH, extraction temperature and extraction duration on pectin yield and

uronic acid content. The experimental design, factors, levels (coded and decoded) and responses are shown in Table 1.

The pectin yield ranged from 3.3% to 11.2% of CPHF. The highest yield was obtained when CPHF extraction conditions were pH 3.0/90 min/100 °C. The uronic acid ranged from 50.6% to 82.5% in the pectins. The highest percent of uronic acid was obtained when the cacao pod husks were treated at pH 1.0/60 min/100 °C.

Table 2 shows that the linear and quadratic effects of pH and the linear effect of temperature had the most effect on pectin yield and uronic acid content. That is, the extraction yield increased significantly with increased pH and temperature ($p < 0.05$). However, uronic acid content increases with decreasing extraction pH and increasing extraction temperature. It is notable that the effect of extraction pH varies for the two responses: higher pH improves pectin yield but reduces uronic acid content and vice versa. In contrast, the duration of extraction did not impact either yield or uronic acid content. Because extraction time was a non-influent variable, the time was fixed at 30 min for the subsequent experiments to minimize cost.

Having the results of the first design, we then applied RSM using a CCD with two independent variables (pH and temperature) to examine the effect of various combinations of pH and temperature on pectin extraction from cacao pod husks. The data obtained from the thirteen experiments are shown in Table 3.

The yields varied between 6.8 and 9.2% of CPHF (Table 3). The pectin yields presented here are in accordance with those of Adomako (1972), who obtained 8–11% yield (dry basis) employing hydrochloric or acetic acid as extractants, but were superior of those obtained with EDTA at different pHs by Barazarte et al. (2008; <5%), both from cacao pod husks.

Compared with other non-commercial sources of pectins, the yields were similar of sunflower head residues (Iglesias & Lozano, 2004), cocoa bean husks (Arlorio et al., 2001; Mollea et al., 2008) and beet pulp (Yapo, Robert, et al., 2007), but inferior of those from mango peels (Koubala, Kansci, et al., 2008), ambarella peels (Koubala, Mbome, et al., 2008), passion fruit rind (Yapo & Koffi, 2006) and soy hull (Kalapathy & Proctor, 2001).

Table 4 shows the regression coefficients of the response function. Yield was influenced by linear effect of temperature ($p < 0.05$). However, pH did not significantly influence pectin yield ($p > 0.05$), and the interaction between the variables pH and temperature was not significant ($p > 0.05$). The linear regression coefficient for temperature was positive, indicating higher pectin yield at higher temperatures. In summary, higher temperatures improved pectin yield, independent of pH.

Barazarte et al. (2008) employed a factorial 3^2 design to extract pectins with EDTA solution in different pHs and temperatures. The yields varied between 2.6% and 4.7%. Higher yields were obtained at pH 5.0. At constant pH, there was an increase in the yields of pectin with the increase in the temperature of extraction (Barazarte et al., 2008).

Concerning the uronic acid content, values from 45.7% to 70.8% were found (Table 3). These contents here obtained by extraction with nitric acid are in agreement with the levels from cacao pod husks reported by Barazarte et al. (2008; 49.8–64.1%) and Adomako (1972; 62%).

In addition, uronic acid contents of pectins from cacao pod husks presented in this work were similar of those from other raw materials, such as soy hull (Kalapathy & Proctor, 2001), ambarella peels (Koubala, Mbome, et al., 2008), banana peels (Happi Emaga, Robert, et al., 2008; Happi Emaga, Ronkart, et al., 2008) and mango peels (Koubala, Kansci, et al., 2008).

Table 4 presents the regression coefficients and parameters from statistical analysis based on the uronic acid data from Table 3. The extraction yield of pectin from cacao pod husks was not related to the content in uronic acid. Individual effects of pH and temperature, at the levels studied, did not influence the uronic acid content of pectins ($p > 0.05$). However, the interaction between the variables pH and temperature was significant ($p < 0.05$) and negative to extract pectins richer in uronic acid by using nitric acid. This interaction suggests that a better response is obtained when the pH is decreased within the positive levels of temperature (higher temperature) or the inverse, i.e., the pH is increased within the negative levels of temperature (lower temperature).

Barazarte et al. (2008) obtained pectins from cacao pod husks with higher uronic acid content at higher temperatures. At constant temperature, the lower pH increased uronic acid content. The effect of pH appeared only at 75 °C; the authors suggested an interaction of pH and temperature at pH 4.0. Barazarte et al. (2008) found no significant change in the uronic acid content in response to pH.

Eqs. (1) and (2) show the statistical models for yield and uronic acid (UA) content:

$$yield\,(\%) = 7.46 + 0.01\,pH - 0.01\,pH^2 + 0.85\,Temp + 0.21\,Temp^2 + 0.10\,pH \times Temp \quad (1)$$

$$UA\,(\%) = 50.62 - 0.61\,pH - 2.62\,pH^2 + 2.20\,Temp + 1.22\,Temp^2 - 6.28 pH \times Temp \quad (2)$$

Table 5 shows ANOVA results for both models. It is observed that regression was significant for pectin yield but not for uronic acid. This indicates that for yield, the coefficients are statistically interpretable and the model can be used for prediction. However, for uronic acid, the coefficients are statistically interpretable and the model cannot be used for prediction. This occurs due to high error related to model fit. Notice that the variance of regression is very close to lack-of-fit, what indicate low quality of model for prediction. The surfaces of this model will be built only interpretation, based on coefficients weight.

Regarding pectin yield (Fig. 1A), the higher yield was 10.5% within the levels studied here. If the moisture of CPHF is considered (8.5%), the value of the higher yield reaches 11.5%, similar to levels of apple pectin (10–15%, Rolin, 1993).

The surface in Fig. 1A reveals a significant increase in pectin yield with increasing temperature, independent of pH. Based upon the data, a possible condition to maximize pectin yield could be nitric acid at pH 3.5/100 °C/30 min to achieve 10.5% yield. These conditions would likely yield both higher hydrolysis of protopectin and lower depolymerization of the newly liberated pectin.

Regarding uronic acid results, values above 70% can be reached within the conditions of the design. Fig. 1B shows a significant increase in uronic acid content when pH is decreased and temperature is increased, or when pH is increased and temperature is decreased. However, after ANOVA analysis (Table 5) the regression was significant for pectin yield model, but not for uronic acid content model, showing low quality of the model for prediction of uronic acid content. A possible condition to maximize uronic acid content (~80%) is the use of nitric acid at approximately pH 1.5/100 °C/30 min. These conditions favor some degradation of pectin neutral side chains, leading to a high content of galacturonic acid present in the backbone of pectins because different sensitivities to acid hydrolysis are found in pectins (Yapo, Robert, et al., 2007).

The extraction conditions which maximize uronic acid content would likely reduce extraction yield from 10.5% to about 9.5%. On the other hand, the optimal extraction conditions for yield will reduce uronic acid content to about 50%, inferior to the minimum value for commercial pectins. In this case, it is preferable to gain in quality (high uronic acid content) even at the expense

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

	Yield/% of CPHF				Uronic acid/%			
	Effect[a]	Std. err.	$t(6)$	p	Effect[a]	Std. err.	$t(6)$	p
Mean	***6.63***	0.16	41.57	0.00000	***61.06***	0.93	65.99	0.00000
pH	***2.77***	0.41	6.69	0.00054	−***19.53***	2.40	−8.14	0.00019
pH^2	***0.83***	0.32	2.63	0.03907	−***10.13***	1.84	−5.51	0.00151
Temp.	***4.33***	0.41	10.48	0.00004	***9.13***	2.40	3.81	0.00891
$Temp.^2$	−0.22	0.32	−0.68	0.52072	−2.33	1.84	−1.27	0.25191
Time	0.80	0.41	1.93	0.10121	2.30	2.40	0.96	0.37489
$Time^2$	−0.72	0.32	−2.26	0.06464	4.02	1.84	2.18	0.07172

[a] Values in bold and italics are significant at $\alpha = 0.05$ and 6 degree of freedom for yield and uronic acid using mean squared residual error.

Table 3
Experimental points of CCD and responses obtained.

Assay[a]	*pH*	*T* (°C)	Yield/% of CPHF	Uronic acid/%
1	2 (−1)	75 (−1)	6.8	45.7
3	2 (−1)	95 (+1)	8.8	70.8
8	2.5 (0)	99.14 (+α)	8.5	49.5
10c	2.5 (0)	85 (0)	7.8	49.4
7	2.5 (0)	70.86 (−α)	6.8	54.8
13c	2.5 (0)	85 (0)	7.6	48.4
12c	2.5 (0)	85 (0)	7.4	48.1
6	3.207 (+α)	85 (0)	7.1	57.3
5	1.793 (−α)	85 (0)	7.3	52.6
4	3 (+1)	95 (+1)	9.2	52.5
11c	2.5 (0)	85 (0)	7.4	57.1
2	3 (+1)	75 (−1)	6.8	52.5
9c	2.5 (0)	85 (0)	7.1	50.1

[a] Extraction time fixed at 30 min.

Table 4
Coefficient estimates for CCD and statistical analysis 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	***7.46***	0.19	39.02	0.00000	***50.62***	1.66	30.52	0.00001
pH (L)	0.01	0.15	0.10	0.92549	−0.61	1.31	−0.46	0.66760
pH (Q)	−0.01	0.16	−0.07	0.94675	2.62	1.41	1.86	0.13576
Temp (L)	***0.85***	0.15	5.63	0.00079	2.20	1.31	1.68	0.16858
Temp (Q)	0.21	0.16	1.32	0.22862	1.22	1.41	0.87	0.43416
pH × *Temp*	0.10	0.21	0.47	0.65409	−***6.28***	1.85	−3.38	0.02769

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

of yield. As such, the recommended extraction condition is pH 1.5/100 °C/30 min to provide a yield and uronic acid content of approximately 9.5% and 80%, respectively. This condition was used for extraction and the pectin obtained in the optimized condition (OP) was then characterized.

3.2. Characterization of pectin obtained in the optimized condition (OP)

Extraction of cacao pod husks in the selected satisfactory conditions (nitric acid pH 1.5/100 °C/30 min) gave rise to fraction

Table 5
ANOVA for pectin yield and uronic acid content.

Variation	Yield					Uronic acid				
	SS[a,f]	df[b]	MS[c]	F[d]	p[e]	SS[a]	df[b]	MS[c]	F[d]	p[e]
Regression	***6.157***	5	1.2315	6.74	0.013	252.46	5	50.49	1.50	0.301
Residues	1.279	7	0.1828			235.66	7	33.67		
Lack of Fit	1.007	3	0.3357	4.94	0.079	180.61	3	60.20	4.38	0.094
Pure Error	0.272	4	0.0680			55.03	4	13.76		
Total SS	7.437	12				488.12	12			

[a] SS: sums of squares.
[b] df: degree of freedom.
[c] MS: mean squares.
[d] *F*: *F* distribution.
[e] *p*: *p* value.
[f] The bold and italic values are significant at $\alpha = 0.05$.

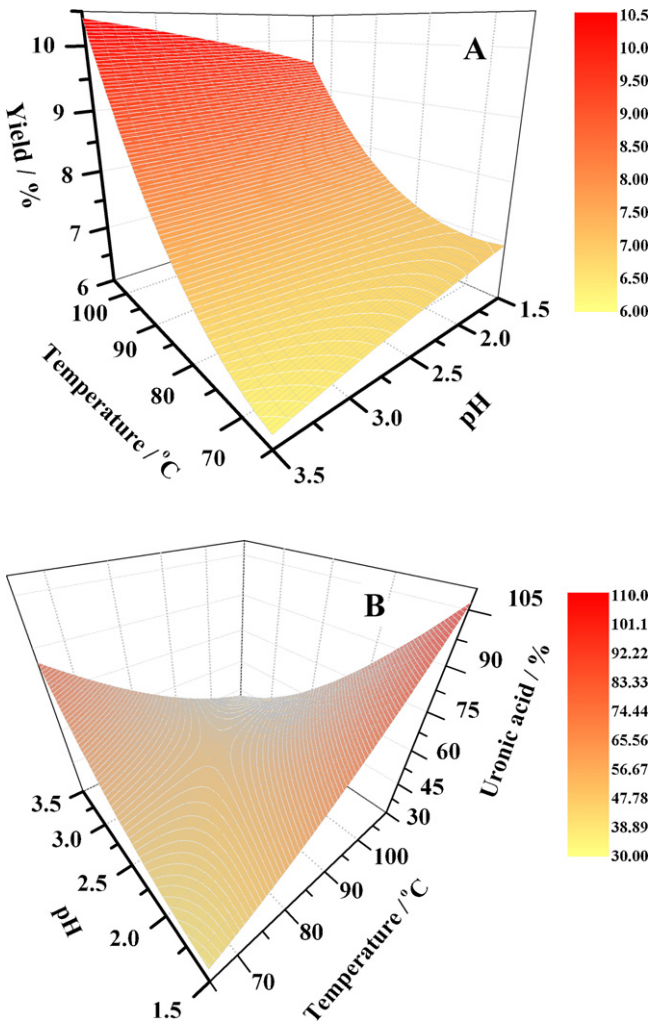

Fig. 1. Response surface of the pectin yield (A) and pectin uronic acid content (B).

OP (*o*ptimized *p*ectin), whose experimental yield (9.0 ± 0.4%, w/w CPHF) is in good agreement with the predicted one (9.5%). If the moisture of CPHF (8.5%) is considered, the yield of OP is 9.8% (w/w dry CPHF).

The yield of OP from cacao pod husks was superior than that obtained under optimizing conditions from passion fruit rind (7.5%; Yapo, 2009) and lower than those of acid extracted pectins from ambarella peels (>10%; Koubala, Mbome, et al., 2008) and mango peels (>10%; Koubala, Kansci, et al., 2008).

OP had 5.8% moisture (Table 6) and consisted of 69.9% carbohydrate, 3.6% protein and 3.9% phenolic compounds. Protein content is at the same levels of yellow passion fruit rind pectins obtained under optimizing conditions (Yapo, 2009) and citrus pectins (Kurita, Fujiwara, & Yamazaki, 2008), but moisture and carbohydrate are lower than those from citrus pectin (Kurita et al., 2008).

As the yield of pectins from CPHF was improved at higher temperatures, and UA content tended to increase at lower pH in higher temperatures, we chose a condition which could improve both yield and UA content. However, the experimental value of UA was inferior to the predicted one, and could be justified by the low quality of model for prediction, as previously discussed.

As shown in Table 6, the chemical composition of OP indicated that the fraction consists mainly by uronic acid (66.0%). Its level is comparable of yellow passion fruit rind pectin obtained under optimizing conditions (62.4%; Yapo, 2009).

Table 6
Composition of the pectin obtained in the optimized condition (OP) from cacao pod husks flour (CPHF).

Composition	(%, w/w)[e]
Moisture	5.8 ± 0.2
Carbohydrate[a]	69.9 ± 1.2
Protein[a]	3.6 ± 1.8
Phenolic[a]	3.9 ± 0.6
Monosaccharides[b]	
Rha	10.0 ± 1.0
Ara	2.7 ± 1.8
Xyl	0.7 ± 0.5
Man	1.0 ± 0.5
Gal	16.8 ± 0.9
Glc	2.8 ± 0.6
Uronic acid[a]	66.0 ± 2.2
DE[c]	56.6 ± 1.4
DA[d]	17.1 ± 0.5

[a] Determined by colorimetric method, mean of triplicate ± SD.
[b] Determined by GLC, mean of triplicate ± SD.
[c] Degree of esterification, determined by FT-IR, mean of duplicate ± SD.
[d] Degree of acetylation, determined by colorimetric method, mean of triplicate ± SD.
[e] Expressed on dry basis, except for moisture.

Galactose was the predominant neutral monosaccharide of OP (16.8%). Other monosaccharides typical of pectins were rhamnose (10.0%) and arabinose (2.7%). These constitute the rhamnogalacturonan region.

Besides the carbohydrates typical of pectins, OP also contains minor amounts of mannosyl, xylosyl and glucosyl units, which were considered contaminants. A lugol iodine test discarded the presence of starch. Thus, the presence of these sugars suggested the co-extraction of hemicelluloses (<5%).

Depending on species and tissues, native pectins are generally highly methylated and slightly acetylated (Voragen et al., 1995). OP from cacao pod husks presented degrees of methyl esterification (DE) of 56.6% and acetylation (DA) of 17.1%, characterizing a high-methoxyl (HM) pectin, highly acetylated. Its DE is comparable to that obtained for mango peel pectins (Koubala, Kansci, et al., 2008) and yellow passion fruit rind pectins (Yapo, 2009). However, although DA of OP is lower than that of sugar beet pectins (Levigne et al., 2002), its value is higher than those reported for pectins from other by-products (Happi Emaga, Robert, et al., 2008; Happi Emaga, Ronkart, et al., 2008; Koubala, Kansci, et al., 2008; Koubala, Mbome, et al., 2008; Yapo & Koffi, 2006; Yapo, Lerouge, Thibault, & Ralet, 2007).

The distribution of acetylation between homogalacturonan (HG) and rhamnogalacturonan (RG) is not clearly established. Highly acetylated HG is known to be abundant in sugar beet and potato (Willats, McCartney, Mackie, & Knox, 2001). Acetyl groups were claimed by some authors to be highly present in the RG-I, such as in bamboo shoot, flax, potato, leek, apple or onion pectins (Bonnin et al., 2008). In okra pectins, the acetyl groups were not only attached to galacturonic acid units in HG and RG-I, but also to rhamnose and/or galactose units in side chains of RG-I (Sengkhamparn et al., 2009).

Although acetyl groups may be linked to the GalA (Caffall & Mohnen, 2009), Rha and/or Gal residues (Sengkhamparn et al., 2009), the degree of acetylation (DA) is calculated in relation to GalA units. In certain cases, up to 90% of GalA residues may be *O*-acetylated, and this is known to hinder enzymatic breakdown of HG and also alter solubility and gelation properties (Willats et al., 2001). However, high levels of acetyl in pectins may confer different properties to them. For example, highly acetylated beet pectins have surface-active and emulsifying properties (Yapo, Robert, et al., 2007).

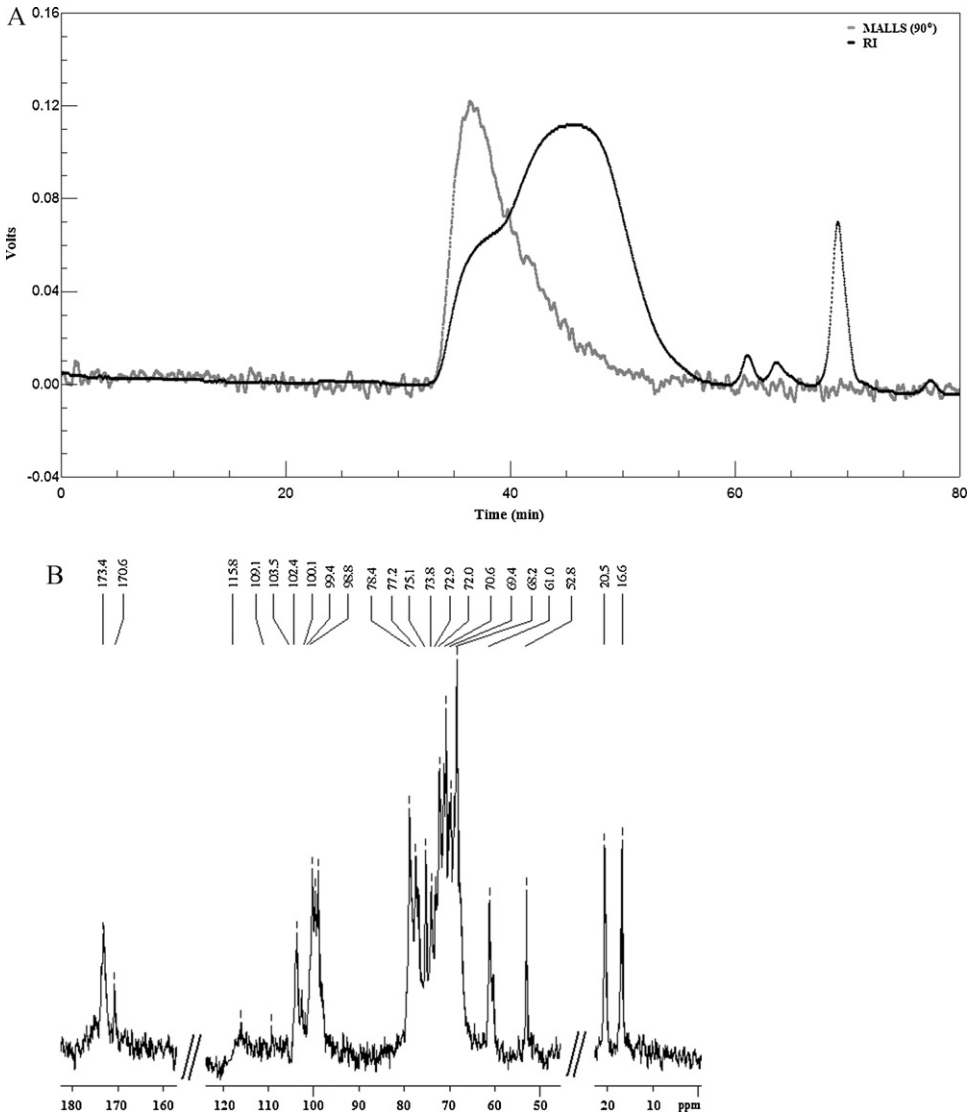

Fig. 2. (A) Elution profile of OP from cacao pod husks obtained by HPSEC-MALLS/RI and (B) ^{13}C NMR spectrum of OP from cacao pod husks: solvent D_2O at 70 °C.

Besides, other types of pectins from cacao pod husks could probably be obtained using different conditions during isolation and purification. Alternatively, chemical modification might be done with treatment in acidic methanol, which removes the acetyl groups and simultaneously increase the proportion of ester groups (May, 1990).

The elution profile of OP obtained by HPSEC-MALLS/RI as a function of elution time is shown in Fig. 2A. RI gives a signal proportional to the concentration, whereas the MALLS response increases with the molecular mass. OP displayed a multimodal profile, probably arising from a mixture of pectins with different molecular mass. It is possible to observe a peak eluted at ~38 min, detected with high intensity by light scattering, which coincides with RI. A more intense RI peak, eluted after 42 min, coincides with lower light scattering intensity. These data indicate the presence of a high molecular mass polymer (1.681×10^6 g/mol; d*n*/d*c* 0.128) in a lower concentration than the predominant polymer with lower molecular mass (3.498×10^5 g/mol; obtained using dextran standards—data not shown).

Heterogeneous elution profiles were also reported for pectins from banana peels (Happi Emaga, Robert, et al., 2008), sugar beet pulp (Yapo, Robert, et al., 2007), yellow passion fruit rind (Yapo, 2009) and cupuassu pulp (Vriesmann & Petkowicz, 2009).

UV detector at 280 nm was used to detect the presence of aromatic rings, as those found in phenolic compounds and proteins. It was detected two low peaks eluting after 55 min (data not showed) which coincide with minimal RI detection. These data suggest that phenolic compounds or proteins present in OP (Table 6) probably are the lowest molecular mass components, as they eluted after the other polymers.

The chemical structure of OP was investigated by ^{13}C NMR spectroscopy (Fig. 2B). Signals at δ 100.1 ppm and 99.4 ppm were assigned to C-1 of esterified and un-esterified units of α-galacturonic acid, respectively. The respective C-6 signals were observed at δ 170.6 ppm and 173.4 ppm, from methyl ester carbonyl carbons and carboxyl carbons, respectively (Tamaki, Konishi, Fukuta, & Tako, 2008; Vriesmann & Petkowicz, 2009; Westereng, Michaelsen, Samuelsen, & Knutsen, 2008; Yu et al., 2010).

Methyl carbons of acetyl groups appeared at δ 20.5 ppm. A signal was identified at 115.8 ppm. Since resonances from 114.4 to 118.6 ppm are characteristic of CH phenolic (Wawer, Wolniak, & Paradowska, 2006), this probably arises from phenolic compounds, present in low amount in OP.

Typical signals of rhamnogalacturonans were also identified in OP. α-L-Rha*p* showed C-1 and CH_3-6 signals at 98.8 ppm and 16.6 ppm, respectively. The C-1 NMR region also contained signals

at δ 109.1 from α-L-Ara*f*, and δ 103.5 and 102.4 from β-1,4-D-Gal units (substituted at O-6 and non-substituted, respectively). All above assignments were based on literature values (Petersen, Meier, Duus, & Clausen, 2008; Vriesmann & Petkowicz, 2009; Westereng et al., 2008; Yu et al., 2010).

The data are in agreement with the presence of pectins highly acetylated containing HM homogalacturonans with RG-I insertions carrying side chains mainly composed of galactans. Rheological properties of OP pectins from CPHF will be investigated in order to suggest their potential uses.

4. Conclusions

Hot nitric acid extraction appears suitable for the recovery of pectins from cacao pod husks. The best results for extraction yield are obtained with pH 3.5/100 °C, indifferent to extraction duration. When the pH of extraction is 1.5, the yield is reduced but pectins have higher content of uronic acid, which improves their quality as commercial polymers. The conditions chosen for cacao pod husks pectin extraction (pH 1.5/100 °C/30 min) provided an experimental yield of 9.0% with 66.0% uronic acid. The pectin was composed by a homogalacturonan highly methyl-esterified and acetylated, presenting some rhamnogalacturonan insertions carrying side chains containing mainly galactose.

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