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Cacao pod husks (*Theobroma cacao* L.): Composition and hot-water-soluble pectins

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ABSTRACT

The composition of cacao pod husks (CPHs), the main waste product of cocoa production, and some of the characteristics of their water-soluble pectins were investigated. Milled and dried CPHs were submitted to hot aqueous extractions (50 and 100 °C, 90 min, 1:25, w/v). The obtained fractions (labeled 50W and BW, respectively) yielded 7.5 and 12.6% pectins, respectively. The monosaccharide composition revealed the predominance of uronic acid, followed by galactose, rhamnose and arabinose, characteristic of pectins. Chemical and spectroscopic analyses (FT-IR and ^{13}C NMR) showed that both fractions contained high acetyl contents (DA 29.0 and 19.2%, respectively) and are composed of low methoxyl homogalacturonans (DE 37.0 and 42.3%, respectively) with rhamnogalacturonan insertions carrying side chains containing mainly galactose. Rheological analyses were carried out with 50W and BW. Solutions of both fractions at 5% (w/v) had a non-Newtonian shear-thinning behavior, however that of 50W showed higher apparent viscosity than that of BW. Dynamic oscillatory analyses showed that 5% (w/w) 50W pectin had weak gel behavior whereas at the same concentration, BW pectin behaved as a concentrated solution. Although further investigations are required, CPH seems be a potential source of both pectins and other natural agents such as fibers and phenolics, and its use is particularly justified by growing environmental awareness.

1. Introduction

Theobroma cacao L. (Sterculiaceae) is an economically important crop in several tropical countries. Its commercially valuable beans constitute about 10% of the cacao fruit's fresh weight. Cocoa beans are used primarily in chocolate manufacturing, but they also have pharmaceutical and cosmetic importance (Kalvatchev et al., 1998).

In the most recent cocoa harvest (from October 2008 to September 2009), worldwide production was greater than 3.5 million ton of dry beans (ICCO, 2009), 170,530 ton of which were produced in Brazil (CEPLAC, 2009).

During this period, the International Cocoa Organization (ICCO) daily price averaged US $2599/ton (ICCO, 2010), and the mean annual world billing was more than $9 trillion, indicating the cocoa agro-industrial sector's great importance around the world.

However, along with its great economic importance, cocoa production generates substantial quantities of waste. In this study, particular attention is given to cacao pod husks, which are generated after cocoa beans are extracted from the mature cacao fruit and are the main byproduct of the cocoa/chocolate industry.

For each ton of dry beans produced, 10 ton of wet cacao pod husks are generated, representing a serious disposal problem (Figueira et al., 1993; Kalvatchev et al., 1998). In most cases, these husks are underexploited and considered an undesirable waste of the cocoa/chocolate industry. Normally, they are left to rot on the cacao plantation, which can cause environmental problems. Besides producing foul odors, rotting cacao pod husks can propagate diseases, such as black pod rot, when left on the cacao plantations (Donkoh et al., 1991; Barazarte et al., 2008; Figueira et al., 1993; Kalvatchev et al., 1998).

Currently, increases in the production and processing of cocoa beans have generated increasing waste, resulting in million tons of cacao pod husks being disposed of every year. In Brazil and other cocoa-producing countries, processing this cacao waste could provide economic advantages and decrease some of the environmental problems. Because cacao pod husks are readily available, they could be used to recover value-added compounds such as pectins.

Pectins extracted from several plant byproducts are widely used as gelling, thickening and stabilizing agents and have several positive effects on human health, including lowering cholesterol and

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serum glucose levels, reducing cancer and stimulating the immune response (May, 1990; Mohnen, 2008).

The complex structures of these acid-rich polysaccharides from plant cell walls consist mainly of homogalacturonan (HG; ~65%) and rhamnogalacturonan-I (RG-I; 20–35%) (Mohnen, 2008).

HG is a linear homopolymer of α-1,4-linked D-galacturonic acid (GalA) units, partially methyl-esterified at the C-6 carboxyl and sometimes O-acetylated at O-2 or O-3. The backbone of HG is covalently linked to RG-I, which has repeating units of [-α-D-GalA-1,2-α-L-Rha-1,4-]$_n$. Between 20 and 80% of the rhamnosyl units of RG-I carry neutral sugar chains, primarily arabinans, galactans or arabinogalactans linked at O-4 (Mohnen, 2008).

Commercial pectins normally come from citrus peels and apple pomace, both of which are byproducts of juice manufacturing (May, 1990). The increasing demand for pectins with different properties indicates the need to find alternative pectin sources, and cacao pod husks are a potential, inexpensive candidate.

Previous studies have indicated the presence of pectins in CPHs (Adomako, 1972; Barazarte et al., 2008; Blakemore et al., 1966), but more detailed information is still necessary. With more information about CPHs, their use could be diversified, adding greater value to the pod and helping to solve the environmental problem presented by the discarded CPHs. The objective of this study was to describe the chemical composition of CPHs and investigate their utilization as a raw material in the isolation of hot-water-soluble pectins.

2. Material and methods

2.1. Cacao pod samples

Dry CPHs (*Theobroma cacao*) and entire pods were generously supplied by CEPLAC (Executive Commission of the Plan of Cocoa Farm Work, Itabuna, Bahia, Brazil), a government organization for the promotion of cocoa agriculture in Brazil. Entire pods were employed to obtain the fruit's weight and moisture content. Dry CPHs 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 (<18 mesh), which will hereafter be referred to as cacao pod husk flour (CPHF), was analyzed and used as the raw material for pectin extraction.

2.2. Extraction of phenolic compounds and low molecular mass carbohydrates

Phenolic compounds were extracted in quadruplicate from CPHF as described by Lecumberri et al. (2007) and analyzed spectrophotometrically as soluble polyphenols.

Low molecular mass components were extracted from the CPHF with 80% ethanol (1:30, w/v) at 60 °C for 5 h. After centrifugation, the supernatant was separated and the residue of the extraction was submitted twice to the same extraction procedure. The three supernatants were then grouped. This procedure was done in duplicate. Each obtained extract was concentrated to a known volume. The total carbohydrate content and reducing sugars were measured.

2.3. Extraction of pectins

Water-soluble pectins were extracted from the CPHF (1:25, w/v, 90 min) in a Fisatom 557 bath at 50 °C (fraction 50W) and 100 °C (fraction BW) under reflux, using a mechanical blender at 250 rpm. After centrifugation at 15,400 × *g* for 30 min, each extract was filtrated in synthetic cloth and treated with ethanol (2:1, v/v) to precipitate the polysaccharides. After 16 h at 4 °C, the polysaccharides were washed three times with ethanol and dried under vacuum. The pectin yield was determined by the ratio of the weight of the extracted pectin dried under vacuum to the original dry weight of the CPHF in % (w/w).

2.4. Analytical methods

Moisture, total ash, minerals, crude protein (N × 6.25), lipids, soluble, insoluble and total dietary fiber contents of CPHF were determined according to the Association of Official Analytical Chemists (AOAC) guidelines (2005). Lignin content was determined as the amount of acid-insoluble material remaining after a two-stage sulfuric acid hydrolysis (Adams, 1965). All analyses were carried out in triplicate ($n = 3$) with the exception of dietary fiber determination ($n = 2$). The results were expressed as g/100 g (dry basis) with the exception of minerals, which were expressed as mg/100 g or mg/1000 g.

Phenolic contents were analyzed using Folin-Ciocalteu's reagent (Singleton and Rossi, 1965) and gallic acid as the standard. Total carbohydrate contents were measured by the phenol-sulfuric acid method (Dubois et al., 1956), and reducing sugars were estimated by a modified Somogyi method (Nelson, 1994), using glucose as the standard for both measurements. Uronic acid contents were determined by the sulfamate/3-phenylphenol colorimetric method (Filisetti-Cozzi and Carpita, 1991), using galacturonic acid as standard. Protein content in pectic fractions was determined according to Bradford (1976), using BSA as the standard.

2.4.1. Hydrolysis

CPHF was solubilized and partially hydrolyzed with 72% (w/w) H_2SO_4 for 1 h at 0 to 4 °C, diluted to 8% and kept at 100 °C for 15 h. The hydrolysate was neutralized with $BaCO_3$, and the insoluble material was removed by filtration. The soluble part was divided in two aliquots: one for determining the neutral monosaccharide composition and the other for determining the total carbohydrate and uronic acid contents. In contrast, pectic fractions were hydrolyzed with 2 M trifluoroacetic acid (5 h, 100 °C), followed by concentration the hydrolysates to dryness.

After hydrolysis, monosaccharides were reduced with aqueous $NaBH_4$ and acetylated with pyridine-acetic anhydride (1:1, v/v, 16 h, at 25 °C). After extraction with chloroform and successive washing with $CuSO_4$ and water, the resulting alditol acetates were analyzed by gas-liquid chromatography (GLC) using a model 5890 S II Hewlett-Packard gas chromatograph at 220 °C (flame ionization detector and injector temperature, 250 °C), with a DB-210 capillary column (0.25 mm internal diameter × 30 m), a film thickness of 0.25 μm and nitrogen as the carrier gas (2.0 ml/min).

2.4.2. HPSEC analysis

High-pressure size-exclusion chromatography (HPSEC) was carried out using 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 an eluent. Previously filtered pectic fractions (0.22 μm; Millipore) were analyzed at 1.5 mg/ml and the data were collected and processed by a Wyatt Technology ASTRA program.

2.4.3. Determination of degrees of O-methyl esterification (DE) and acetylation (DA)

The DEs of the pectic fractions were determined in duplicate by Fourier transform-infrared (FT-IR) as described by Vriesmann and Petkowicz (2009). The DAs of the samples were determined in