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Extraction and Characterization of Gelling and Emulsifying Pectin Fractions from Cacao Pod Husk

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Abstract With an annual production around 1.6 million tons, Côte d'Ivoire (Ivory Coast) is still today the world's largest producer of cocoa beans. This co-generates about 16 million tons of cacao pod husks which are usually left unutilized in plantations to rot, thereby becoming a significant source of disease inoculums. As a way of solving the environmental problem posed by this agrowaste and adding value to it, the pectic substances of cacao pod husk were investigated under different extraction conditions (pH 1.0, 2.0 and 3.0). The results obtained, with respect to yield, chemical, and macromolecular characteristics, showed that 3.7-8.6% cacao pod husk pectin (CPHP), with 50.9-74.8% galacturonic acid (GalA) content; 36.7-52.4% methylation degree (DM); 3.2-9.8% acetylation degree (DAc); 162-304 mL/g intrinsic viscosity [η], and 43-82 kDa viscosity-average molecular weight ($\overline{Mv}$), could be produced. Functional properties assays revealed that CPHP is a moderately efficacious gelling agent in sugar-acid-gels with 65-75% sucrose content. By contrast, CPHP appeared to be as effective as sugar-beet pulp pectin (SBPP) when assayed for the first time as an oil-in-water emulsifier and emulsion-stabilizer without the need for depolymerization. Cacao pod husk therefore stands as a new source of possible production of non-structurally modified pectin-derived emulsifier.

Keywords: *cacao pod husk, pectin, purification, gelation, emulsification*

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1. Introduction

Pectic substances are complex polysaccharides present in the cell wall of higher plants. Analysis of the glycosyl residue composition shows that pectins are basically composed of α-D-galactopyranosyluronic acid (α-D-Gal*p*A) and various neutral sugars (NS), three of which, viz. α-L-rhamnopyranose (α-L-Rha*p*), α-L-arabinofuranose (α-L-Ara*f*), and β-D-galactopyranose(β-D-Gal*p*) are commonly detected. Structural studies, using highly purified enzymatic preparations, also revealed that the glycosyl residues are not randomly distributed in pectin macromolecules, but are rather concentrated in different regions [1] that generally give rise to two building block copolymers, namely homogalacturonan (HG) and rhamnogalacturonan-I (RG-I). HG is an unbranched 1,4-α-D-Gal*p*A polymer partially methyl-esterified at *C*-6 position and sometimes acetyl-esterified at *O*-2 and/or *O*-3 positions. RG-I is a [1,4)-α-D-Gal*p*A-1,2-α-L-Rha*p*-(1,4] polymer partly branched with different kinds of NS side chains. Common branches of RG-I are 1,5-α-L-arabinan, 1,4-β-D-galactan, and arabinogalactan-I. They may, however, be ramified with more complex polysaccharide moieties, such as arabinogalactan-II and unusual galactoarabinan [2].

Commercial pectins are food additives mainly intended for the formulation of diverse gelling (food) products such as marmalades, jams, and preserves, and low calorie jellies to name a few. Usually, citrus peel and apple pomace represent the two main sources of industrial high (HMP) and low (LMP) methoxy pectins from Europe and the United States of America. However, these raw materials are not available throughout the year, and therefore attempt has been made to partially (or totally) substitute sugar beet pulp, which is cheaper and available in large quantities year-wide in western countries, for citrus peel and apple pomace. Unfortunately, pectin from sugar beet pulp (SBPP) exhibits poor gelling properties, mainly caused by high acetyl content (or acetylesterification degree [DAc >10-40]) and NS content (20-40%) and a rather low molecular size [3]. Nevertheless, these chemical and physiochemical features are likely to confer to SBPP efficacious oil-in-water (O/W) emulsifying and emulsion-stabilizing properties [4,5], which are comparable to those of gum Arabic [6], the benchmark polysaccharide-derived emulsifier.

Import of commercial citrus and apple (gelling) pectins and gum Arabic-emulsifier in emerging and developing

countries, such as Côte d'Ivoire and neighboring countries, to satisfy their demands represents expensive undertakings with low added values to domestically manufactured gelling and emulsifying products. To partially remedy to this problem, various agrowastes, which are locally available in large quantities, are screened for the production of pectins with good functional (gelling or emulsifying) properties. Studies on acid-extracted pectin from cacao pod husk suggested that it may structurally resemble commercial apple pectin [7]. However, pectin from cacao pod husk generally appears to exhibit lower gelling capability in sugar-acid-mediated HMP gels (HMP-SAG) than do citrus and apple pectins [8,9], probably due to lower methyl-esterification degree (DM) and relatively high DAc. However, the molecular characteristics of various pectins isolated from cacao pod husk appeared to be affected by the extraction conditions [10,11,12,13].

Cacao (*Theobroma cacao* L., Sterculiaceae) is indeed an economically important crop in Côte d'Ivoire (Ivory Coast), and is cultivated for its oil-rich beans to produce cocoa powder and butter, which are mainly used in the western chocolate industry. Over the 2008/2012 period, worldwide production of dried cacao beans was around 4.5 million tons, 40% of which was from Côte d'Ivoire, the world's leading cacao producer. Once the valuable beans are extracted from mature ripe cacao pods, the husks, which account for 52-76% the fruit fresh weight [13], are usually left to decompose, as a mulch, in cacao plantations, thereby producing foul odors and becoming an abundant source of plant disease inoculum like black pod rot [14]. Therefore, it is necessary to find a way of adding value to husks, in order to increase incomes to farmers and circumvent the ecological problems they cause.

The scope of this study was to examine the functional (gelling and emulsifying) capabilities of pectins extracted from cacao pod husk using the conventional acid extraction method with varying strength of the extractant for optimization.

2. Experimental

2.1. Alcohol Insoluble Material (AIM) Preparation

Fresh husks of ripe cacao pods were collected from a cooperative of local growers (Adzopé, Bacon, Côte d'Ivoire). The husks were minced in a Kenwood mincer and immediately treated in 3 volumes of boiling 80% (v/v) ethanol for 25 min and cooled to room temperature. Alcohol-insoluble material (AIM) was continuously washed with 70% (v/v) ethanol to remove free sugars, pigments (especially phenolics and tannins), and other impurities as much as possible. The residue was then dried by solvent exchange (95% ethanol and acetone), placed in a fume hood for 5 h for residual acetone evaporation and oven-dried overnight. Dried AIM was ground in a hammer mill (Model 912, Winona Attrition Mill Co., Winona, MN) to pass through a 12 mm size sieve and was kept under moisture-free conditions until use.

2.2. Production of Pectins

Pectins were extracted from the cacao pod husk AIM by water acidified with 1 N HNO_3 to three different extractant strengths (pH 1.0, 2.0, and 3.0), while the other extraction parameters, namely, solid to liquid extractant (S/L) ratio, temperature (T°C), and time (t), were invariably kept to 1:25 (w/v), 75°C, and 90 min, respectively. Two successive extractions were performed before discarding any remaining insoluble cell wall fraction. At the end of every extraction, slurry was clarified and pectin extract was rapidly brought to pH 4.0 for stability. The first and second extracts were pooled, concentrated to desired volume and precipitated in 3 volumes of 95% ethanol at 5°C for 2 h. Pectin precipitates were washed two-times with 70% ethanol, followed by 95% ethanol and acetone, and kept for a while under a fume extractor (for residual acetone evaporation), and finally oven-dried at 40-45°C overnight and weighed. Extraction of pectins was carried out in three independent runs for each selected pH value. Dried pectin flakes were finely ground to pass through 60-mesh (# 0.25 mm) size sifters. Cacao pod husk pectin (CPHP) flours were canned in plastic containers and stored at room temperature under airless and moisture-free conditions until use.

Sugar beet pulp pectin (SBPP; GalA = 54.7% (w/w); TNS = 23.7% (w/w); DM = 31; DAc = 17) extracted by water acidified with 6 M H_2SO_4 to pH 1.5, at 80°C, and for 60 min [5], was used for comparison regarding the emulsifying (EA) and emulsion-stabilizing activities.

2.3. Characterization of Pectins

Pectin samples were first treated with a mixture of 1% (v/v) HCl/60% (v/v) ethanol (three times), and insolubles were exhaustively washed with 60% (v/v) ethanol to totally remove free sugars and salts (as much as possible) and converting all the carboxyl groups of pectin macromolecules to the free acid (-COOH) form, prior to correctly titrating them by 1 N NaOH solution. Pectins were characterized for their glycosyl residue (sugar) composition, esterification degree, molecular weight, gel-forming capability and emulsifying activity.

2.3.1. Proximate Analyses

The protein content of samples was colorimetrically determined at 750 nm by a Folin-phenol reagent assay using bovin serum albumin standard [15]. Calcium element was analyzed as previously reported [16], by flame atomic absorption spectrometry at 422.7 nm, using an Aanalyst 300 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT).

2.3.2. Glycosyl Residue Composition Analyses

To quantify monosaccharide constituents, purified pectins were first hydrolyzed with 1 $mol.L^{-1}$ H_2SO_4 (100°C, 3h) as previously reported [17].

The GalA content of purified pectins was colorimetrically estimated at 525 nm by a modified sulfamate-meta-hydroxydiphenyl (MHDP) assay using monoGalA standard [18].

Liberated NS from purified pectins, especially galactose/arabinose and rhamnose [19], were spectrophotometrically quantified at 340 nm using Megazyme assay kits (Megazyme International Ireland Ldt., Bray, Co. Wicklow, Ireland). The NS assays were based on the quantitative oxidation of galactose/arabinose and rhamnose to corresponding lactonic derivatives (D-galactono-(1,4)-lactone for α-L-arabinose and β-D-

galactose and L-rhamno-(1,4)-lactone for α-L-rhamnose) in the presence of corresponding dehydrogenases [β-galactose dehydrogenase (β-GalDH) plus galactose mutarotase (GalM) for α-L-arabinose and β-D-galactose, and L-rhamnose dehydrogenases (RhaDH) for α-L-rhamnose] and the coenzyme NAD^+, which is stoichiometrically reduced to NADH with maximum absorbance at 340 nm. D-galactose was quantitatively differentiated from L-arabinose by reading absorbance at different reaction times, namely after 6 min- and 12 min-reaction at room temperature, respectively. L-rhamnose was quantitatively determined after 1 h-reaction at room temperature. Total neutral sugar (TNS) was calculated as the sum of individual amounts of typical NS of pectins (Ara, Gal, and Rha) estimated. Alternatively, TNS was measured by the tri-reagent (anthrone, orcinol, and MHDP) colorimetric-H_2SO_4 assay as previously reported [20].

The relative proportions of RG-I to HG block copolymers **were** roughly appraised by calculating the molar ratio of rhamnose to galacturonic acid, and the degree of branching (DBr) of pectins rhamnosyl residues with NS side chains was estimated by equation 1 [16].

$$DBr(\%) = 100 \times Rha(mol\%) \Big/ \begin{bmatrix} Ara(mol\%) \\ +Gal(mol\%) \end{bmatrix} \quad (1)$$

The lower the value of DBr, the higher the level of branching of the (RG-I domains of) pectins.

The overall esterification degree (DE) of pectin samples was potentiometrically determined as previously described [20]. The acetylesterification degree (DAc) was colorimetrically measured at 510 nm by the hydroxamic acid assay using glucose pentaacetate standard [21], and the methylesterification degree (DM) was differentially evaluated.

All the measurements were performed in triplicates.

2.3.3. Macromolecular Analysis

The molecular weight of pectin samples was analysed by gel-filtration chromatography (GFC) on a high resolution Superdex-200 HR 10/30 column (Amersham Biosciences Corp., NJ). A molecular weight kit of pullulan standards ($\overline{Mw}$ ~ 6.0, 10.0, 21.7, 48.8, 113.0, 210.0, 393.0, and 805.0 kDa; $\overline{Mw}$ / $\overline{Mn}$ ~ 1.0-1.2) from American Polymer Standards Corp. (Mentor, OH) and purified homogenous HG standards ($\overline{Mw}$ ~ 60 and 100 kDa, $\overline{Mw}$ / $\overline{Mn}$ ~ 1.0-1.2) [22], with known intrinsic viscosity ([η]) and $\overline{Mw}$ values, were used for calibration. To better estimate the $\overline{Mv}$ of pectins, the so-called universal calibration technique (UCT) was used by plotting log ([η] × $\overline{Mw}$) versus the elution volume (V_e) of standards. Analyses were done in triplicates.

2.4. Gelling Properties

The gelling capability of pectins was evaluated by the determination of the strength of gels prepared under the following conditions: 55-75% soluble solids (sucrose), 0.70 wt % pectin, and at pH 2.3 as previously described [22].

2.5. Emulsifying Properties

Emulsifying and emulsion-stabilizing activities were appraised as fully described previously, except that paraffin oil was substituted for *n*-dodecane which was not available [5]. Before use, it was checked that paraffin oil per se possessed no emulsifying activity. Briefly, oil-in-water (O/W) pre-emulsions containing 0.5% (w/w) pectin dispersions (and 0.02% sodium azide as preservative) were prepared and sonicated for 1 min, at room temperature. The pre-emulsions were vigorously vortex-mixed for 1 min, at room temperature, and centrifuged at 527*g* to achieve good emulsions. The whole volume (Wv) of the emulsified system and the emulsified layer volume (ELV) were measured and emulsifying activity (EA) was calculated according to equation 2.

$$EA(\%) = (ELV/Wv) \times 100 \quad (2)$$

To study the emulsion-stabilizing activity (ESA), another set of emulsions were prepared fourfold for each sample in transparent graduated tubes as above. Two tubes out of the four were cooled to 4°C, and centrifuged, for 5 min, at 4°C after which the initial emulsified layer volumes (ELV_i) were measured, and the tubes were stored at 4°C. The other 2 tubes were treated the same way, but at room temperature and stored at that temperature. After 1 and 30 day(s) of storage, the remaining emulsified layer volumes (ELV_r) were measured, after centrifugation, and ESA was calculated using equation 3.

$$ESA(\%) = (ELV_r / ELV_i) \times 100 \quad (3)$$

2.6. Statistical Analysis

All the data were statistically appraised by a single-factor analysis of variance (ANOVA), followed by the Bonferroni's posthoc test for multiple comparisons, whenever applicable, using a GraphPad Prism V.3 software (GraphPad software Inc., San Diego, CA). Means of different treatments were considered to be significantly different at *p*-value < 0.05.

3. Results & Discussion

3.1. The Extraction Yield of CPHP

Table 1. Glycosyl residue composition, macromolecular features, and gelling capability of acid-extracted pectins from cacao pod husk

	CPHP		
	pH 1.0	pH 2.0	pH 3.0
Yield (% DRM)	5.4 ± 0.4a	8.6 ± 1.1b	3.7 ± 0.9a
GalA (% w/w)	74.8 ± 1.5a	64.7 ± 2.7b	50.9 ± 2.4c
Rha (% w/w)	1.5 ± 0.2a	3.7 ± 0.4b	5.9 ± 1.1c
Ara (% w/w)	1.2 ± 0.1a	2.6 ± 0.2b	4.5 ± 0.7c
Gal (% w/w)	5.2 ± 0.7a	8.4 ± 1.2b	13.9 ± 2.1c
TNS (% w/w)	7.9 ± 0.3a	14.7 ± 0.9b	24.3 ± 1.7b
Rha/GalA	2.4: 100a	6.8:100b	13.9:100c
DBr (%)	24.8 ± 1.8a	35.2 ± 4.1b	33.5 ± 2.1b
DM	36.7 ± 3.5a	52.4 ± 5.1b	44.3 ± 6.1ab
DAc	3.2 ± 0.6a	5.7 ± 0.9ab	9.8 ± 1.2b
Protein	2.6 ± 0.4a	3.8 ± 0.7ab	4.5 ± 0.9b
Calcium (µmol/g)	155.7 ± 4.8a	89.4 ± 2.5b	48.6 ± 3.1c
[η] (mL/g)	162 ± 7a	258 ± 5b	304 ± 9c
$\overline{Mv}$ (kDa)	43 ± 6a	82 ± 7b	71 ± 5c
Gel strength (° SAG)	Non gelling	108 ± 2b	Non gelling

Data are expressed as mean ± SD (n = 3). Mean values in the same line with different letters are significantly different (p <0.05).
CPHP: Cacao pod husk pectin
DRM: Dried raw material

The yield of CPHP extracted under different acid strengths ranged from 3.7 to 8.6% (Table 1).

The pectin yield was significantly influenced by the acid-extractant strength (p <0.05). The highest yield (8.6%) was obtained at pH 2.0, which thus appeared to be the optimized (pH) condition. The latter is in agreement with pectin yields reported under various optimized conditions: 8-11% for acetic acid-pH 2.8/95-100°C/20 min [7]; 9.7% for SHMP-citric acid-pH 3.5-4.5/75°C/60 min [8]; 2.6-4.7% for EDTA-HCl-pH 3.0-5.0/60-90°C/60 min [10]; 8-9% for HCl-pH 2.5/95°C/60 min [11]; 8.0-9.8% for HNO_3-pH 1.5/100°C/30 min [12], and 7.6% for citric acid-pH 2.5/95°C/180 min [13]. However, this pectin yield range is lower than that (10.44-17.30%) reported elsewhere under conditions using ammonium oxalate-citric acid-pH 1.6-4.6/85°C/60-120 min [23], probably due to differences in extractants and/or in cacao origin (from Malaysia in their study).

The decrease in the CPHP yield from 8.6 to 5.4%, when the extractant strength was increased from pH 2.0 to pH 1.0, suggested that solubilized pectin polymers were partly degraded under more severe acid (pH) conditions. Moreover, the pectin isolated at pH 1.0 appeared to be brown-colored. This might pose a marketing problem, as color of pectin is an important factor affecting the appearance of the gel produced. Different workers [8,11] have also observed that pectins extracted from cacao pod husk were variably colored from light brown to red. This is thought to be caused by co-extraction and co-precipitation of phenolics and tannins, known to be abundant in cacao pod husk, under harsher extraction conditions; and variation in the final color of pectin product from light brown to red could be explained by pretreatment or not of the raw material by alcohol washings (producing AIM), as done in this study, before extracting pectins.

3.2. Chemical and Macromolecular Features of Pectins

3.2.1. Glycosyl Residue Composition

The glycosyl residue composition of purified pectins is shown in Table 1. The GalA content of extracted CPHP ranged from 50.9 to 74.8%. These quantities of GalA were significantly different from one another (p <0.05), indicating that the extractant strength influenced the amount of the basic constituent of pectins. The GalA content (64.7%) of CPHP isolated with an optimum yield (at pH 2.0) was comparable to those (62.1-66.0%) reported under various extraction conditions above-specified [7,10,12,13,23], except for the SHMP-extracted pectin where a much higher GalA content (77.2%), however similar to that found for the pH 1.0-CPHP isolate, has been reported [8], probably due to differences in extractant nature and/or in cacao (from Malaysia in their study). SHMP, as a Ca^{2+}-complexant, is indeed assumed to solubilize (Ca^{2+}-cross bridged) loosely-bound pectins (from the middle lamellae) which are rich in HG block copolymers [24], whereas acid-extractant usually hydrolyzes NS-rich "protopectins [5]. These results showed that pectins with high galacturonic acid contents (≥65%), and hence of a relatively high purity, could be produced from cacao pod husk under optimized conditions.

The three typical NS of pectic substances, viz. rhamnose (Rha: 1.5-5.9%), arabinose (Ara: 1.2-4.5%), and galactose (Gal: 5.2-13.9%) were detected in all the three extracted CPHP in significantly different amounts from one sample to another (p <0.05). Galactose was the most profuse NS in all the isolated CPHP, consistent with previous work [7,12]. This suggested that the RG-I domains of pectic substances within the cell wall of cacao pod husk were mainly branched with galactan and/or arabinogalactan side chains. The TNS content, determined as the sum of the three individual NS, was in the range of 7.9-24.3%, suggesting that CPHP might amply be ramified with NS side chains. The rather low TNS content (7.9%) of the pH 1.0-CPHP isolate, with a high GalA content (74.8%), indicated that pectin macromolecules within this sample were likely degraded mainly through the NS-branched RG-I regions. The TNS content of CPHP is within the range (9.25-29.5%) found in the literature [7,12].

Furthermore, the TNS content (11.5-26.8%) of CPHP, as appraised by the tri-reagent colorimetric method, was slightly higher, suggesting that isolates might contain NS other than those detected. Some other NS, notably xylose (0.7-1.2%) in addition to glucose (2.8%) and mannose (0.7%), have been detected in acid-extracted pectins from cocao pod husk [7,12]. In contrast to the latter workers [12], the former [7] did not detect glucose and mannose, especially glucose which was rather abundant in commercial apple pectin he used for comparison. In the case of apple pectin, glucose can be assumed to be contaminant from either xyloglucans or residual starchy polysaccharides, because the latter are profuse in apple fruit pomace [1]. As regards cacao pod husk pectin, one can assume that both xylose and glucose are likely from xyloglucan contaminants. However, the fact that only xylose was detected elsewhere [7] suggested that this NS type may also be from the lately purified pectic polysaccharide (xylogalacturonan) from (extracted pectins from) miscellaneous plant materials such as apple pomace and yellow passion fruit rind [1,2,22].

Apart from sugar constituents, proteinaceous compounds (2.6-4.5%) and calcium (48.6-155.7 µmol/g) were detected and their quantities in pectins seemed to be affected by the extraction conditions. Also, the observed light brown color of CPHP suggested that small quantities of phenolics might be present too. Relatively small fractions of proteins (1.10-3.6%), phenolics (3.9%), and ash (8.9%) of which Ca and K were the major elements have previously been quantified in pectins from (ripe) cacao pod husk [7,12].

The relative proportions of HG to RG-I block copolymers, as judged by the rhamnose to galacturonic acid molar ratio (2.4:100-13.9:100), suggested that the pectin HG building block copolymers were predominant over RG-I block copolymers in all the purified CPHP. Nevertheless, the pH 1.0-CPHP appeared to be by far richer in HG, thereby substantiating extensive degradation of NS-branched RG-I at this extraction pH. Degree of branching was only faintly affected, suggesting that rhamnosyl residues in all the pectin samples were still highly branched with NS side chains.

3.2.2 Degrees of Esterification

The degrees of methylesterification (DM) and acetylesterification (DAc) of extracted CPHP varied in the

range of 36.7-52.4 and 3.2-9.8, respectively (Table 1). Both DM and DAc were moderately influenced by the extraction conditions, suggesting that acetylated LMP macromolecules were likely to be dominant over acetylated HMP polymers within the cell wall of cacao pod husk. The DM values are consistent with those reported under various extraction conditions as afore-specified: 37.9-52.2% [10]; 25-31.1% [11]; 56.6% [12]; 7.17-57.86% [13]; and 45.26-55.31% [23].

The DAc value was similar to the DAc (4.0%) found by Mollea et al. [11]; higher than the DAc (1.01-3.48%) found by Chan and Choo [13], and lower than the one (17.1%) reported by Vriesmann et al. [12], probably due to some differences in acid extractions and or cacao pod origin (Brazil in their study). It has, for example, been shown that DAc (2.46%) of acid-extracted pectin from pumpkin pulp is usually lower than DA (7.06-10.68%) of enzymatically (glycosidases)-extracted pectins from the same raw material [25]. Compared with acetylated pectins from other sources, acetylation degree appeared to be lower in CPHP than in acid-extracted pectins from chicory roots (5-16%) [26] and sugar beet pulp (3.1-29.2%) [5].

3.2.3 Macromolecular Features

The intrinsic viscosity ([η]) and viscosity average-molecular weight ($\overline{Mv}$) of CPHP varied from 162 to 304 mL/g and from 43 to 82 kDa, respectively. The two macromolecular parameters of pectins were significantly influenced by the extraction conditions (p <0.05). The rather low values of [η] (and $\overline{Mv}$) of the pH 1.0-CPHP suggested that it might have an overall extended conformation as dominantly imposed by its rigid rod-like HG regions, whereas the other two pectins could have random coil conformation, as judged by the rather high values of [η] and $\overline{Mv}$ [17]. These results also substantiated that the pH 1.0-CPHP was likely to contain only short RG-I regions, as a result of extensive degradation, and might therefore resemble a NS-containing polygalacturonan.

3.5. Gelling Capability

The gelling capability of CPHP was examined at three different sucrose concentrations (55, 65 and 75 %wt sucrose content). Different gelling behaviors were then observed. At 55% sucrose content, none of the three purified CPHP was capable of forming sugar-acid gels (only highly viscous solution-like systems were obtained). At 65% sucrose content, as a standard gel should contain, only the pH 2.0-CPHP yielded a moderately strong gel (108) (Table 1). At 75% sucrose content, pH 1.0-CPHP was still non-gelling, pH 3.0-CPHP gave a tenuous gel-like system, while pH 2.0-CPHP yielded a firmer gel (142), which approached the standard value required for HMP-SAG (150). These results clearly demonstrated that acid-extracted pectin from cacao pod husk does not seem to be a strong gelling agent for preparing HMP-SAG. It may probably be suitable for the preparation of Ca^{2+}-induced LMP gels (LMP-CG) on the basis of its low-to-moderate DM (36-53%). The relatively moderate gelling capability of CPHP in sugar-acid gel formation could be accounted for by its chemical and macromolecular characteristics, viz. a generally moderate GalA content (~60%) along with moderate methylesterification (~50%) and viscosity-average molecular weight ($\overline{Mv}$: 80 kDa) and probably also because of appreciable acetylesterification (3.0-10.0%). The data obtained are consistent with previous findings of rather moderate gel grade (70-129) for pectins from cacao pod husk [8,9].

3.6. Emulsifying Properties

As CPHP appeared to be amply acetylated with mitigated gelling ability, the sample with high acetyl content (the pH 3.0-isolate), which hardly gave a tenuous gel-like system, was also assayed for EA and ESA. The results obtained are summarized in Table 2.

Table 2. Emulsifying (EA) and emulsion-stabilizing (ESA) activities of oil/0.5% (w/w) pectin solutions

	EA (%)	ESA (%)			
Storage time	0 day	1 day		30 days	
Storage temperature (°C)	23°C	4°C	23°C	4°C	23°C
CPHP[a]	35.9 ± 0.6	62.1 ± 2.5	83.4 ± 1.7	59.7 ± 2.2	75.6 ± 1.8
SBPP[b]	43.2	78.1	65.5	78.1	65.3

Data are expressed as mean ± SD (n = 3). Mean values in the same line with different letters are significantly different (p <0.05).
[a]CPHP: cacao pod husk pectin extracted at HNO_3-pH 3.0/75°C/90 min
[b]SBPP: sugar beet pulp pectin extracted at H_2SO_4-pH 1.5/80°C/60 min. Data are from ref [5].

It was observed that CPHP possessed effective oil-in-water EA (35.9%) and ESA at 4°C (59.7-62.1%) and 23°C (75.6-83.4%). However, emulsions appeared to be steadier at 23°C than at 4°C in contrast to what has previously been observed for SBPP [5]. Compared to the latter pectin, the oil-in-water EA of CPHP was slightly lower, but both pectins displayed similar ESA, indicating that CPHP could be another potential pectin-derived emulsifier.

To date, sugar beet pulp represented the most potential source of production of pectin-derived emulsifier, because SBPP exhibits interesting EA and ESA in addition for the raw material to being cheap enough and available in large quantities in Europe and USA [5,6]. Pectins from some other few sources such as citrus peel, apple pomace, apricot pulp, and cauliflower leaves and florets have also been reported to be potential oil-in-water emulsifiers [27,28]. However, to be effectively surface-active, these pectins which are primarily of very high molecular sizes (> 100-300 kDa), and are commonly used as gelling agents, need first to be (chemically or enzymatically) depolymerized to about 50-80 kDa, thereby engendering extensive structural modifications. SBPP, as well as CPHP, does not need such structural modifications to display efficacious EA and ESA. To the best of our knowledge, the present findings demonstrate, for the first time, that acid-extracted pectin from cacao pod husk, a largely available agrowaste in tropical regions, especially in West Africa, and singularly in Côte d'Ivoire, the world's top producer of cocoa, can stand as an efficient pectin-derived (oil-in-water) emulsifier.

4. Conclusions

With an annual production around 1.6 million tons, Côte d'Ivoire is hitherto the first producer of cocoa beans in the world. This co-generates about 16 million tons of cacao pod husks, which are usually left unutilized in cacao plantations to rot, thereby becoming a significant source of disease inoculums. As a way of remediating to ecological problems and adding value to them, the pectic substances of cacao pod husk were investigated and results interestingly showed that valuable pectin gelling agent and particularly pectin-emulsifier could be produced from this raw material without the need for structural modifications with chemicals or enzymes. Cacao pod husk therefore appears to be a new potential source of production of marketable natural pectin-derived emulsifier.

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Statement of competing interests

The authors have no competing interest.

Abbreviations:

AIM	alcohol insoluble material
CPHP	cacao pod husk pectin
Dac	degree of acetyl-esterification
DE	degree of esterification
DM	degree of methyl-esterification
DRM	dried raw material
EA	emulsifying activity
ESA	emulsion-stabilizing activity
HMP-SAG	high methoxy pectin sugar acid gel
LMP-CG	low methoxy pectin calcium gel
SBPP	sugar-beet pulp pectin
TNS	total neutral sugar

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