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Complexation with whey protein hydrolysate improves cacao pods husk pectin surface active and emulsifying properties

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ABSTRACT

Pectin was extracted from cacao pod husks (CPHP) wastes, having a methylesterification degree of 83.22%, acetylation degree of 10.20%, and protein content of 3.68%. The adsorption dynamics of CPHP at the canola oil-water interface were studied, and diffusion (k_{diff}), penetration (k_{pen}) and rearrangement (k_{rea}) rate constants were determined. Canola oil-in-water (O/W) emulsions (E_{CPHP}) were stabilized which CPHP, and exhibited an initial area-volume mean diameter ($d_{3,2}$) of 113.60 nm, which increased to 162.0 nm during 28 days of storage. CPHP was electrostatically complexed with whey protein hydrolysate (WPH) in a weight ratio 5:1 and pH 3.25, in order to improve the surface active and emulsifying activities. The soluble complex ($SC_{WPH\text{-}CPHP}$) decreased the interfacial tension faster and to a lower value, and displayed higher k_{diff} and k_{pen} than CPHP. $SC_{WPH\text{-}CPHP}$ yielded O/W emulsions with $d_{3,2} < 90$ nm, which did not suffer significant changes in oil droplet size during storage. The apparent viscosity of $SC_{WPH\text{-}CPHP}$ aqueous dispersion was much lower than that of CPHP dispersion. The zeta potential of $E_{WPH\text{-}CPHP}$ was lower than for E_{CPHP}. Thus, it was concluded that the greater stability of $E_{WPH\text{-}CPHP}$ was due mainly to steric repulsion originated by the soluble complex adsorption layers around the oil droplets.

1. Introduction

Pectin is a heteropolysaccharide exhibiting a wide diversity in its molecular structure. Basically it is a group of polysaccharides rich in galacturonic acid comprising homogalacturonan (HG; homopolymer mainly composed of ~65% of α-D-galactopyranosyluronic acid units), rhamnogalacturonan-I (RG-I, heteropolymer of units repeating α-L-rhamnosyl) and rhamnogalacturonan-II (branched pectic domain containing a HG backbone). The galacturonic acid residues may be partially methyl-esterified on the carboxyl group at C-6 position and *O*-acetylated at C-2 or C-3 depending on plant species [1–3]. The molecular weight of pectin (Mw), viscosity, the protein content, the degree of acetylation (DAc), amidation (DAmd) and methylesterification (DM) are the main factors which dictate the functional properties of pectin [4,5].

Up to the last decade, most pectin applications stemmed from its gel-forming ability, rendering it as suitable to use as gelling, thickening, or stabilizing agent in a wide number of industrial domains, including the food, pharmaceutical and chemistry industries [6–8]. Nowadays, this hydrocolloid is gradually gaining acceptance as an effective emulsifier and the emulsifying properties are being increasingly assessed in numerous industrial applications [9]. Some pectins from different botanical sources have been tagged as showing surface-activity and to be capable of forming fine oil droplets, including citrus peel pectin [10–12], sugar beet pectin [10,13–15], and hawthorn pectin [16]. However, new sources of pectin from regional botanical sources and agroindustrial wastes continue to be explored.

The cacao (*Theobroma cacao* L.) fruit is an important crop of several tropical and sub-tropical countries, and cacao beans are the main ingredient of the multi-billion worldwide chocolate industry. After the beans extraction, the pod husks (accounting for approx. 52–76% of the weight of the cacao fruit) are discarded and represent a serious environmental problem [17]. Bazarte et al. [18] reported that for each ton of dry cacao beans, 10 tons of wet pod cacao husks were generated. The production of dry cacao beans for the period 2015/2016 was estimated as 3660 metric tons (36,600 metric tons of wet cacao pod husks) [19].

Recently, Yapo and Koffi [20] reported that cacao pod husks pectin exhibited emulsifying activity and emulsion-stabilizing activity,

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without providing information regarding the initial droplet size and the variation in droplet size of the oil-in-water emulsions with storage time.

Pectin is a multifunctional ingredient susceptible to chemical and enzymatic conversions, and selective alterations of pectin molecular structure may render the polymer suitable for numerous applications. In this context the complexation between protein and polysaccharide molecules under special conditions of pH and weight ratio between both biopolymers can promote the formation of soluble complexes (SC), which may exhibit improved emulsifying properties than the individual biopolymers [21–24].

Thus, an ongoing research topic is the identification and characterization of natural emulsifiers which have the potential of emulsifying and stabilizing emulsion oil droplets [25,26] and that can also be successfully used in food matrices [27–29]. Therefore, the objectives of this research were to: (a) obtain and characterize the pectin extracted from cacao pod husks (CPHP); (b) establish the conditions for obtaining whey protein hydrolysate (WPH)-CPHP soluble complex ($SC_{WPH\text{-}CPHP}$); (c) evaluate the dynamic interfacial adsorption of $SC_{WPH\text{-}CPHP}$, and (d) and to evaluate the stability of oil-in-water emulsions emulsified with $SC_{WPH\text{-}CPHP}$ in comparison to CPHP.

2. Materials and methods

2.1. Materials

Cacao fruits (*Theobroma cacao* L., var. Trinitario) from the Chontalpa region of the State of Tabasco, Mexico, was kindly supplied by the Local Agricultural Association of Cacao Producers of Huimanguillo. Whey protein hydrolysate (WPH; Hilmar™ 8390, 78% protein on dry basis, 5.5% fat, moisture 3.5%, ash 4.5%, lactose 3.0% and molecular weight distribution of 70.8% < 1000 Da, 12.9% 1000–5000 Da, 6.6% 5000–20,000 Da and 9.5% > 20,000 Da) was supplied by Hilmar Cheese Company (Hilmar, CA, USA). Citric acid, hydrochloric acid (HCl), sodium hydroxide (NaOH), magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), sulfuric acid (H_2SO_4) and ethanol, were analytical grade and purchased from J.T. Baker (Xalostoc, State of Mexico, Mexico). Potassium sulfamate, sodium tetraborate, 3-phenylphenol. Other reagent-grade chemicals were purchased from Sigma-Aldrich Mexico (Toluca, State of Mexico, Mexico). Deionized water was used in all the experiments.

2.2. Extraction of cacao pod husks pectin (CPHP)

Cacao pod was cut lengthwise and seeds were removed manually from the husk. Husks were cut into small pieces, dehydrated in an oven with air circulation (Riossa Digital, HCF-62, Mexico City, Mexico) at 55 °C until achieving constant weight (~36 h). Dried pieces were milled (Nixtamatic^{MR}, Mexico City, Mexico) using successive sieves of 2 mm and 1 mm. Cacao pod husks flour (CPHF) that passed across the 1 mm sieve was used for the extraction of pectin.

CPHP was extracted by acid hydrolysis as proposed by Vriesmann et al. [17], with slight modifications. Briefly, 100 g of CPHF were mixed with 2.5 L of citric acid (1:25 w/v) and put in a water bath with constant stirring. Extraction conditions were: pH of 3.0, 85 °C and 90 min extraction time. The resulting extract was cooled to room temperature (23 ± 2 °C) and filtered using a synthetic cloth, then this extract was centrifuged at 8000 × g for 10 min (Centrifuge 5810 R, Eppendorf, AG, Hamburg, Germany) and the precipitate was discarded (impurities). Supernatant was added with 96% ethanol in a 2:1 volume ratio and maintained for 24 h at 4 ± 1 °C, to allow pectin precipitation [30]. Pectin was then separated by filtration through Whatman No. 1 filter paper and washed twice with 70% ethanol in a 1:1 volume ratio for removal of free sugars, pigments and other impurities. Excess alcohol was removed by centrifugation at 8000 × g for 25 min at 20 °C, and the supernatant was discarded. The pellet was dried at 55 °C in an oven with air circulation until constant weight was achieved. The dried pectin was ground in a mortar in order to obtain a fine powder. Extraction yield was estimated as (1):

$$\text{Yield (\%)} = (\text{pectin powder (g)}/\text{cacao pod husk flour (g)}) \times 100\% \quad (1)$$

Moisture content of CPHP was determined with a moisture analyzer (Ohaus, MB23, Parsippany, NJ, USA) by drying the sample at 105 °C to constant weight [31].

2.3. Physicochemical characterization of CPHP

2.3.1. Determination of the degree of methylesterification (DM), degree of amidation (DAmd) and degree of acetylation (DAc)

Degree of methylesterification (DM), degree of amidation (DAm) and degree of acetylation (DAc) of CPHP was determined as reported by [3], with slight modifications. Pectin solution (0.500 g/100 mL of deionized water) was titrated with 0.1 mol/L NaOH using phenolphthalein as the indicator and the volume consumed was recorded as V1. Afterwards, saponification of pectin was initiated by adding of 20 mL of 0.5 mol/L NaOH and allowed to react for 15 min under constant stirring. The reaction was stopped with the addition of 20 mL of 0.5 mol/L HCl. The excess HCl was neutralized by titration with 0.1 mol/L NaOH and the volume consumed was recorded as V2. Subsequently, the solution was saturated with 20 mL of 2.5 mol/L NaOH and mixed in a 1000 mL distillation flask and connected to a condenser, whose delivery line was submerged into the mixture of 150 mL of deionized water and 20 mL of 0.1 mol/L HCl. The mixture was distilled until ~100 mL of the distillate was collected. The excess HCl was then titrated with 0.1 mol/L NaOH using methyl red as the indicator and the consumed volume was recorded as S. A blank test was performed using 20 mL of 0.1 mol/L HCl and the volume consumed of HCl was recorded as B. The difference of B and S was assumed to be V3.

For determining the amount of acetyl, 0.500 g of the CPHP were dispersed in 25 mL of 0.125 mol/L NaOH, after stirring for 4 h, the solution was diluted in a 50 mL volumetric flask to volume. An aliquot of 20 mL of diluted solution and 20 mL of Clark's solution (containing 100 g $MgSO_4{\cdot}7H_2O$, 0.8 mL H_2SO_4 and 180 mL H_2O) was transferred to a 1000 mL distillation flask and the first 15 mL of the distillate were collected by separated in a graduated cylinder. Immediately the steam supply was started and the distillation was continued until 150 mL of the distillate was obtained. This distillate was titrated with 0.05 mol/L NaOH to a pH of 8.5, and the volume required, in mL, was recorded as A. Deionized water was used as a blank for titration and the volume consumed was recorded as A_0. The difference of A and A_0 was assumed to be V4. Eqs. (2)–(4) were used for estimating DM%, DAmd% and DAc %:

$$\text{DM\%} = \text{V2 (mL)}/(\text{V1 (mL)} + \text{V2(mL)} + \text{V3(mL)} - \text{V4(mL)}) \times 100 \quad (2)$$

$$\text{DAmd\%} = \text{V3 (mL)}/(\text{V1 (mL)} + \text{V2(mL)} + \text{V3(mL)} - \text{V4(mL)}) \times 100 \quad (3)$$

$$\text{DAc\%} = (\text{V4} \times 10^{-3}\text{(L)} \times 0.05\ \text{(mol/L)})/((0.500\ \text{(g)} \times 0.821)) \times 194.14\ \text{(g/mol)} \times 100 \quad (4)$$

2.3.2. Methoxyl percentage

Methoxyl percentage (MeO%) was determined according to Zouambia et al. [32]. MeO is related to DM because the amount of methoxyl groups in 100% of esterified pectin is 16.32%, the methoxyl percentage was calculated from the following Eq. (5).

$$\text{MeO\%} = (16.32/100) \times \text{DM} \quad (5)$$

2.3.3. Determination of galacturonic acid

Galacturonic acid content was estimated by the sulfamate/3-phenylphenol colorimetric assay, reported by Filisetti-Cozzi and Carpita