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Structural, physicochemical, and emulsifying properties of pectin obtained by aqueous extraction from red pitaya (*Hylocereus polyrhizus*) peel

Propiedades estructurales, fisicoquímicas y emulsionantes de la pectina obtenida por extracción acuosa de la cascara de la pitaya roja (*Hylocereus polyrhizus*)

E. Alpizar-Reyes[1,6], J. Cruz-Olivares[1], S. Cortés-Camargo[2], M.E. Rodríguez-Huezo[3], J.O. Macías-Mendoza[4], J. Alvarez-Ramírez[5], C. Pérez-Alonso[1]*

[1]*Departamento de Ingeniería Química, Facultad de Química, Universidad Autónoma del Estado de México, Paseo Colón esq. Paseo Tollocan s/n, Col. Residencial Colón, C.P. 50120, Toluca, Estado de México, México.*

[2]*Departamento de Nanotecnología, Universidad Tecnológica de Zinacantepec, Av. Libramiento Universidad 106, Col. San Bartolo el Llano, C.P. 51361, Zinacantepec, Estado de México, México.*

[3]*Departamento de Ingeniería Química y Bioquímica, Tecnológico de Estudios Superiores de Ecatepec, Av. Tecnológico s/n esq. Av. Central, Col. Valle de Anáhuac, Ecatepec, C.P. 55210, Estado de México, México.*

[4]*Facultad de Ingeniería, Subsede Reforma, Universidad de Ciencias y Artes de Chiapas, Carretera Reforma - Juárez km 6.5 R/a. Col. Santa Cruz, C.P. 29500, Reforma, Chiapas, México.*

[5]*Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco No. 186, Col. Vicentina, C.P. 09340, México City, México.*

[6]*Laboratorio de Materiales - LabMAT, Departamento de Ingeniería Civil y Ambiental, Universidad del Bío-Bío, Av. Collao 1202, C.P. 4081112, Concepción, Chile.*

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Abstract

This work aimed to carry out a straightforward aqueous extraction of pectin from red pitaya peels (RPP), in a drive to promote the circular economy of the settlements where the fruit of this endemic species grows. The average pectin yield was 11.59 g/100 g dry basis, the degree of esterification was 60.35±1.35% (classified as high methoxyl), the galacturonic acid content was 54.36±1.03%, and the protein content of 5.86 ± 0.25%. The RPP pectin was analyzed in terms of physicochemical, functional, and structural features. The FTIR spectrum confirmed that the unveiled pectin structure was consistent with that reported for commercial pectins from different botanical sources. Pectin aqueous dispersions exhibited power-law shear thinning behaviour. Corn oil (10 mL) in 150 mL aqueous pectin solutions (0.12, 0.15, 0.30, 0.60 and 0.90%, w/v) emulsions exhibited increasing emulsifying activity and emulsifying stability with increased pectin concentration. It was concluded that the RPP pectin is an alternative potential new source of pectin for use in the formation and stabilization of oil-in-water food emulsions.

Keywords: Red pitaya; Pectin; Aqueous extraction; Rheological properties; Functional properties.

Resumen

En este trabajo se buscó el llevar a cabo una extracción acuosa simple de pectina de la cáscara de la pitaya roja (RPP), en un intento para promover la economía circular de poblaciones regionales en donde el fruto de esta especie endémica crece. Se obtuvo un rendimiento de pectina de 11.59 g/100 g en base seca, con un grado de esterificación de 60.35±1.35%, un contenido de ácido galacturónico del 54.36±1.03%, y un contenido de proteína del 5.86 ± 0.25%. La pectina RPP fue analizada en términos de sus características fisicoquímicas, funcionales y estructurales. El espectro FTIR confirmo que la estructura desvelada de la pectina era consistente con aquella reportada para pectinas de otras fuentes botánicas distintas. Las dispersiones acuosas de pectina exhibieron un comportamiento reoadelgazante ley de potencia. Emulsiones de aceite de maíz (10 mL) en 150 mL de una dispersión acuosa de pectina (0.12,0.15,0.30, 0.60 y 0.90% p/v) exhibieron una actividad emulsionante y una estabilidad emulsionante crecientes conforme la concentración de la pectina aumentó. Se concluyó que la pectina RPP es una fuente alternativa potencial de pectina para su uso en la formación y estabilización de emulsiones aceite-en-agua utilizadas en la industria de los alimentos.

Palabras clave: Pitaya roja, Pectina; Extracción acuosa; Propiedades reológicas; Propiedades funcionales.

* *Corresponding author. E-mail*: cpereza@uaemex.mx
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1 Introduction

The global pectin market was estimated in 1 billion USD in 2019 and was expected to grow at a compound annual growth rate of 6.5%, to reach a value of 1.5 billion USD by 2025 (Markets and Markets, 2019). The main source for global pectin production is from citrus fruits, which coupled to the high great demand has caused a rise in the cost of this commodity in the recent years (Lai *et al*., 2022). On the other hand, fruit processing industries generate wastes comprising peels, seeds, liquid juice and wash water waste, among others, which can be converted into added value products (Lai *et al*., 2022, Gutiérrez-Antonio *et al*., 2022; Martínez-Preciado *et al*., 2021). That is, some kinds of fruit waste have significant valorization potential as a source of pectin for use in numerous applications (Dao *et al*., 2021). Thus, in the recent last years, a drive for obtaining pectin from fruit peel wastes has emerged. In this way, pectin has been extracted from banana unripe peels and mango fruit peels (Lai *et al*., 2022), wampee (*Clausena lansium* (Lour.) Skeels) fruit peel (Peng *et al*., 2022), purple passion fruit (*Passiflora edulia* Sims) peel (Teng *et al*., 2022), passion fruit peel (de Oliveira *et al*., 2016), red flesh dragon fruit peel (*Hylocereus polyrhizus*) (Nguyen *et al*., 2022), and white-flesh dragon fruit (*Hylocereus undatus*) peels (Dao *et al*., 2021), among other botanical species.

Pectin due to its favorable thickening and gelling properties, ease of production, obtainability from several botanical sources and potential health benefits, has been well developed for a variety of applications (Cao *et al*., 2020). The fine structure of pectin depends on the botanical source, and although the underlying physicochemical properties of all the pectins exhibit some similarities, their physicochemical, structural, functional properties and yield vary with extraction method, solvent selection and operating conditions of the extraction method employed (Dranca and Oroian, 2018; Dranca *et al*., 2020). Pectin extraction methods include conventional solvent extraction such as hot water extraction, and acidified water with organic or inorganic acids (Gharibzahedi *et al*., 2019) or un-conventional methods such as ultrasound-assisted extraction, subcritical water extraction, microwave-assisted extraction and ultrasound/microwave-assisted extraction (Rodríguez Robledo and Castro Vázquez, 2019).

With regards to solvent extraction, the process parameters affecting pectin yield and quality are well understood. However, extraction based on solvents might leave residual contaminants in the extracted pectin. Thus, extraction without organic or mineral acid is highly desirable from both pectin quality and environmental restrictions. Aqueous extraction is traditionally used to obtain botanical polysaccharides (Hosseini *et al*, 2016), where temperature, contact time and liquid/solid ratio (LSR) are design parameters (Samavati and Manoochehrizade, 2013).

Red pitaya (*Hylocereus polyrhizus*) fruit grows in the tropical and semi-desertic regions of Mexico. Its juicy pulp has a pleasant sweet, fresh and delicate taste, and the juice combines the functional properties of a natural red-purple food colorant with high antioxidant potency (Vaillant *et al*., 2005). The juice pleasant has gained a reputation as an "exotic and gourmet" beverage (Quiroz-González *et al*., 2020). Most pitaya beverage processing facilities are of an artisanal character and are located in the regional sites where the cactus grows. Pitaya peels have been explored as a potential source of pectin, which might lead to improvements of the economic input in regions of relatively low economic income (Zaidel *et al*., 2017). Woo *et al*. (2010) extracted pectin from red dragon fruit (*Hylocereus polyrhizus*) with citric acid acidified distilled water to pH 3.0, 3.5 and 4.0, 75 °C and different time interval (30, 60 and 120 min). The highest yield achieved was of 14.86%, after 60 min extraction and pH 3.5, whilst highest degree of esterification was obtained from treatment at pH 4.0, 120 min. Ismail *et al*. (2012) extracted pectin from *Hylocereus polyrhizus* peels using three different extraction conditions: 0.25% ammonium oxalate/oxalic acid at a pH of 4.6 at 85°C; 0.03 M HCl at a pH of 1.5 at 85°C; and deionized water at 75°C. Pectin extracted with ammonium oxalate gave the highest yield of pectin, with high purity and low ash content. The pectin extracted by whatever conditions was categorized as low-methoxyl pectin. Thirugnanasambandham *et al*. (2014) performed the microwave-assisted extraction of pectin from dragon fruit peel. They found that the optimum extraction conditions for the maximum yield of pectin (7.5%) were power of 400 W, temperature of 45 °C, extracting time of 20 min and liquid solid ratio of 24 mL/g. Zaid *et al*. (2016) extracted pectin from *Hylocereus polyrhizus* peels using a combination of ultrasound, stirring and citric acid. The influence of several process parameters namely agitation, temperature, time, pH and liquid solid ratio were investigated. The highest yield of pectin 42.5% (w/w) was obtained

when the extraction was carried out at agitation, temperature, time, pH and solid liquid ratio of 250 rpm 70 °C, 120 min, pH of 1.5 and 1:10 (w/v), respectively. Zaidel *et al*. (2017) investigated the extraction of pectin from *Hylocereus polyrhizus* peels using distilled water at 80 °C with different extraction time (20, 40, 60 and 80 min). The yield of pectin decreased from 20.34 to 16.20 % with the increase of extraction time, and pectin obtained was categorized as low-methoxyl pectin. Hu *et al*. (2021) separated pectin from pitaya peel (*Hylocereus polyrhizus*) using an aqueous two-phase system (PEG/$(NH_4)_2SO_4$ ATPS) finding that pectin yield increased with an increasing weight fraction of PEG and when the ammonia phase ratio was close to 0.5. Nguyen *et al*. (2022) extracted pectin *Hylocereus polyrhizus* peels using as solvent hydrochloric acid assisted by microwave extraction finding that maximum pectin yield of 17.61% and degree of esterification index of 51.41% was achieved under the following conditions: HCl acid concentration 0.032 M, material size D ≤ 1 mm, material/solvent ratio was 1/20 (g/mL), microwave power 100 W, and heating time is 15 min. Despite all of the above studies, there is a lack of information concerning the functional properties and physicochemical properties, and the reported yields vary wildly (7.5-42.5%) for pectin extracted from *Hylocereus polyrhizus* peels. These facts pinpoint the necessity to conduct fresh extraction experiments by a specific method and conditions, in order to accurately determine the salient features of the extracted pectin and be able to determine its suitability for specific potential applications. With this in mind, the aim of this work was to design a simple hot water extraction of pectin from *Hylocereus polyrhizus* peels endemic from Mexico, and to determine the structural, physicochemical and emulsifying properties of the pectin.

It is expected that in this way the knowledge generated will be the basis that contributes to improving the economic activity of regions with relatively low-income conditions, promoting the recycling of the generated peel wastes discarded by artisanal pitaya juice producers.

2 Materials and methods

2.1 Materials

Fresh red pitaya (*Hylocereus polyrhizus*) fruits were purchased from a local market (Oaxaca City, Oaxaca, Mexico). The fruits were selected based on the following criteria: (i) should be free of wounds, stains, and defects; (ii) Uniformity in size, weight and color; (iii) the size selected was 6-8 cm in diameter, 200-400 g weight. The fruits were manually peeled, and the peels were carefully washed with distilled water so that no impurities such as dirt remained. Excess water was removed from the peels surface with absorbent paper. Then the peels were cut into small pieces of 0.5 cm^2 using a stainless-steel knife. The peels obtained were dried in a hot air oven (60 °C, 12 h). Afterwards the dried peels were ground into powder using a common grinder (100 Mini 2HP, Pulvex, Mexico City, Mexico) and the powder was passed through 40-mesh (420 μm) sieve. Finally, the red pitaya peel (RPP) powder was stored in black sealable bags to avoid moisture adsorption until they were used for the different analyses. All chemical reagents used in the experimental runs were reactive grade (Sigma Aldrich, Toluca, State of Mexico, Mexico). All the water used in the experiment was deionized.

2.2 Pectin extraction

A complete factorial design with replication was conducted to establish the highest pectin yield's processing conditions. The factors were: Liquid (deionized water)-Solid (RPP powder)-Ratio (LSR), the extraction temperature (°C), and the extraction time (min). Two levels of LSR (10:1 and 20:1), and extraction temperature (70 and 80 °C), and three levels of extraction time (60, 120, and 180 min) were selected. These extraction conditions were selected on base of the studies reported in the introduction. The totality of the experiments that were performed with replication are shown in the Appendix A (Table A1). Contour plots of the RPP pectin % yield as a function of temperature-time, LSR-time, and LSR-temperature were obtained with the help of a Minitab version 18.0 software (Minitab Inc., State College, Pa., USA) (see Appendix A Fig. A1 (a-c)). Extracted grouts were filtered with two layers of gauze and cooled down to achieve room temperature (about 20 °C). The filtrate was mixed with ethanol (95% purity) in a ratio of 2:1 v/v to precipitate the polysaccharides that constitute pectin and was stored (4 °C, 24 h). Subsequently, the polysaccharides were separated using centrifugation (Hermle Z323K, Hermle, Labortechnic, Germany) for 12 min at 524 × g (1700 rpm). The precipitate was washed twice with ethanol. The pectin was separated using filtration, and dried at 60 °C in a convective oven (HCX II model, San-son plus, Toluca, Mexico) under

circulating air for 24 h. The final dried pectin was obtained by homogenizing the size with a 40-mesh screen (420 μm).

2.2.1 Extraction yield

The percentage of yield was calculated as the percentage of the amount of RPP pectin used before the extraction process and the amount of RPP pectin obtained after the extraction (Ke *et al*., 2020):

$$Yield(\%) = \frac{\text{weight of red pitaya peel pectin obtained}}{\text{weight of red pitaya peel used}} \times 100 \quad (1)$$

2.3 Pectin characterization

2.3.1 Determination of moisture, ash, lipid and protein content of RPP pectin

Moisture, lipid, and ash contents of RPP pectin was determined according to the AOAC standard methods, 925.10, 920.85 and 923.03, respectively (Santos *et al*., 2013). The total protein content of the RPP pectin was estimated by Kjeldahl procedure (N×6.25) as described in AOAC official method 981.10.

2.3.2 Content of galacturonic acid (Gal A)

The Gal A content was determined involving carbazole with slight modifications (Ke *et al*., 2020). D-galacturonic acid was weighed as a standard parameter and formulated into a series of standard concentrations (0-300 μL/mL). Afterwards, 1.5 mL of sodium sulfate tetraborate solution were incorporated and the mixture was heated at 70 °C for 10 min. After cooling at room temperature for 1 h, about 50 μL of a carbazole solution was added and the resulting mixture was heated at 70 °C for 5 min. The absorbance was measured at 530 nm (Genesys 10 UV, Waltham, MA, USA).

2.3.3 Fourier Transform Infrared Spectroscopy (FT-IR) and determination of degree of esterification (DE)

Functional groups of RPP pectin powder were determined using Fourier transform infrared spectroscopy (FT-IR) using a spectrophotometer FT-IR GX System (Perkin-Elmer, Shelton, CT, USA) coupled to an ATR Dura Sample II accessory. All the spectra were an average of 16 scans from 3500 to 600 cm^{-1} at a resolution of 2 cm^{-1}.

Using the wavenumbers of the major peaks as a guide of the spectra in the region 1800-1500 cm^{-1}, the spectral region was deconvoluted by the curve-fitting method with the Levenberg-Marquadt algorithm and the peaks at 1725 cm^{-1} (esterified carboxyl, -COOR) and 1550 cm^{-1} (ionized carboxyl, -COO2) were adjusted and the area measured with the Lorentzian function to estimate the degree of esterification (DE) by following equations (Pappas *et al*., 2004):

$$DE = 147.7 \times R + 2.3013 \quad (2)$$

where

$$R = \frac{A_{1725}}{A_{1725} + A_{1550}} \times 100 \quad (3)$$

Here, A_{1725} and A_{1550} are the absorbance densities at 1725 cm^{-1} and 1550 cm^{-1}, respectively.

2.3.4 Thermal characteristics

The thermal characteristics of the RPP pectin powder were studied using differential scanning calorimetry (DSC) and thermogravimetric analyses (TGA). Both, TGA and DSC studies were carried out using a thermal analyzer Netzsch STA 449 F3 (Jupiter®, Selb, Germany) with a heating ramp of 10 °C/min, in a nitrogen atmosphere at a flow rate of 20 mL/min. Samples were heated from 25 °C to 500 °C; aluminum crucibles of 5 mm diameter were used. Savitzky-Golay smoothing algorithm was employed for TGA curves.

2.3.5 Scanning electron microscopy analysis

Scanning electron microscopy (SEM) images were used to assess the particle morphology. The runs were conducted in a JSM-6510 model equipment (Jeol Co. LTd., Tokyo, Japan) under standard operating conditions. Micrographs at selected magnifications (100×, 300×, 1000× and 1700×) were used for illustration.

2.3.6 Apparent viscosity of aqueous pectin solutions

The apparent viscosity behavior over a shear rate range of 0.1-100 s^{-1} of RPP pectin aqueous solutions (2.0, 5.0, and 8.0 g/100 g) was determined with a Physica MCR 300 rheometer (Physica Messtechnik GmbH, Stuttgart, Germany) coupled to a 50 mm, 2° cone-plate geometry, whose edges were covered with silicon oil to avoid water evaporation. The experimental data were fitted to the following power-law:

$$\tau = k\dot{\gamma}^n \quad (4)$$

Here, τ represents the shear stress (Pa), $\dot{\gamma}$ denotes the shear rate (s^{-1}), k is the consistency coefficient (Pa.s^n) and n becomes the flow behavior index (dimensionless). The effect of temperature was assessed for temperatures of 20, 40 and 60 °C. Water evaporation minimization was obtained by using a solvent trap. The following Arrhenius model was used (Haminiuk *et al*., 2006)

$$k = k_0 e^{(Ea/RT)} \tag{5}$$

where k_0 is consistency coefficient (Pa.s^n), Ea is the activation energy (J/mol), R=8.314 J/mol.K. On the other hand, the dependence of viscoelasticity on pectin concentration was assessed for dispersions of 2.0, 5.0, and 8.0 g/100 g at 20 °C.

2.4 Functional properties

Oil (OHC) and water (WHC) holding capacities of pectin powder were estimated as described by Alpizar-Reyes *et al*. (2017) and García-Sánchez *et al*. (2020). Tests were carried out at three different temperatures (25, 45 and 65 ºC). For emulsifying properties, emulsions were obtained by homogenization of 150 mL of 0.12, 0.15, 0.3, 0.6 and 0.9% (w/v) pectin dispersion with 10 mL of corn oil (Cristal®) at 6400 rpm for 8 min. The emulsifying ability (EA) and stability (ES) were obtained by the procedure described previously (Alpizar-Reyes *et al*., 2017).

2.5 Statistical analysis

Tukey's test for a statistical significance ($p \leq 0.05$) and one-way analysis of variance (ANOVA) were conducted using Minitab version 17.0 software (Minitab Inc., State College, Pa., USA). All experiments were done in triplicate.

3 Results and discussion

3.1 Yield, proximate composition, degree of esterification (DE) and Gal A content

Commonly, temperature, extraction time, pH, and solvent-to-sample ratio have a determinant effect on the yield of extracted pectin. In this work, the best extraction conditions were: LSR of 20:1 v/w, the temperature of 80 °C, and extraction time of 120 min. The RPP pectin yield was 11.59/100 g dry basis (d.b.) (see Appendix A - Fig. A1). Zaidel *et al*. (2017) obtained pectin of dragon fruit peel from Malaysia by aqueous extraction with deionized water at 75 °C and 1 h of agitation, reporting a yield of 15.37 g/100 g dry basis. The difference between the two yields under similar extraction methods can be explained from differences in the operating conditions, as well as by the region and season of harvesting. Tang *et al*. (2011) extracted pectin from red pitaya peel from Malaysia with citric acid, for pH values between 2 and 5, and reported yields of about 9.83-16.76 g/100 g dry basis. The decrease in pH in the extraction process allows improving the yield percentages of pectin. However, the increased pH may accelerate the degradation of pectin and the increase in the esterification of the biopolymer (Yapo *et al*., 2007).

The analysis of RPP pectin showed the following results per 100 g: 2.15 ± 0.21 g moisture, 0.09 ± 0.01 g lipids, 1.22 ± 0.12 g ashes and 5.86 ± 0.25 g proteins. The moisture and ash content in the extracted RPP pectin were relatively low. Acceptable values for pectin powder should be less than 10 g/100 g for both properties in order to guarantee storage stability, purity of pectin and gel formation (Zaidel *et al*., 2017). On the other hand, the relatively low content of lipids and proteins are responsible for the emulsifying and stabilizing ability of pectin (Ke *et al*., 2020). Furthermore, Food and Agriculture Organization of the United Nations (FAO) recommends protein contents in pectin not exceeding 15.6 g/100 g (FAO/World Health Organization WHO, 2010).

The acetyl and methyl functional groups that are linked to the galacturonic acids are reflected in the degree of esterification (DE). In this way, high (DE > 50%) and low (DE ≤ 50%) methoxyl pectin are commonly found in botanical sources. The DE value of the RPP pectin was 60.35±1.35 %, indicating that this biopolymer can be considered as high methoxyl pectin. Methods based on water or mineral acids are commonly used for extraction of high methoxyl pectins. In contrast, hydrochloric, nitric, sulphuric and phosphoric acids are commonly used for low methoxyl pectins (Zhang and Mu, 2011). The DE value obtained in this work was higher than that of pectin of dragon fruit (*Hylocereus polyrhizus*) peels from Malaysia by aqueous extraction process, which was 46.96 ± 0.8 % (Zaidel *et al*., 2017). However, the DE value was lower than that obtained by Tang *et al*. (2011) for the pectin from red pitaya peel from Malaysia with citric acid (pH 2-5, DE 81.78-92.10 %).

The content of galacturonic acid (Gal A) is generally used to quantify the purity of the

extracted pectin. FAO and European Union (EU) have suggested that the Gal A should be higher than 65% for commercial pectin (FAO/WHO, 2010). The galacturonic acid content of RPP pectin polysaccharide was 54.36 ± 1.03%, which is in line with reports of anhydrouronic acid (AUA) content in red pitaya pectin, purple pitaya pericarp pectin and Gal A chayote pectin content of 45.25-52.45% (Zaidel *et al*., 2017), 46.62% (Montoya-Arroyo *et al*., 2014) and 57.25% (Ke *et al*., 2020), respectively. Low values of Gal A indicates a high fraction of proteins in the pectin extract (Zaidel *et al*., 2017).

3.2 Fourier Transform Infrared Spectroscopy (FTIR)

Figure 1 presents the FTIR spectrum in the regions 3500-2700 cm^{-1} and 1800-800 cm^{-1}. The broad band in the range 3300-3500 cm^{-1} can be ascribed to OH stretching. The peak located at about 2850-3000 cm^{-1} is linked to CH vibrations modes. In this case, CH, CH_2, and CH_3 bending and stretching vibration of galacturonic acid methyl esters are the main contributions (Güzel and Akpinar, 2019). The duplet 1614 cm^{-1} and 1722 cm^{-1} is characteristic of CO stretching vibration of methyl esterified carboxyl and non-esterified groups of pectin (Pereira *et al*., 2016; Mort *et al*., 1993). The fingerprint of aliphatic carboxylic acids (anionic form) is commonly linked to the bands at 1610-1550 and 1410-1300 cm^{-1}, which are linked to carboxylate group asymmetric and symmetric stretching, respectively (Manrique and Lajolo, 2002). These bands correspond to the characteristic wavelengths of polygalacturonic acid (Santos *et al*., 2013). Furthermore, the band at about 1439 cm^{-1} is ascribed to the deformation OCH_3 group, and the single peak at 1340 cm^{-1} is linked to OH tensile vibration band (Pereira *et al*., 2016).

The high absorbance at the band 1200-950 cm^{-1} is a fingerprint of polysaccharides. Marked absorbance at about 1100 cm^{-1} is typical of β-glycosidic linkages between sugar units (Santos *et al*., 2013). Meanwhile, the single peak located at 1225 cm^{-1} is attributed to CH_3CO stretching). The soft band located at 1020 cm^{-1} denotes COH deformation. Moreover, the small peak at 970 cm^{-1} is attributable to CO bending, and the peak at 884 cm^{-1} is associated to CCH and COH bending at the C-6 position (Pereira *et al*., 2016). The FTIR spectrum of RPP pectin is consistent with previously published results in terms

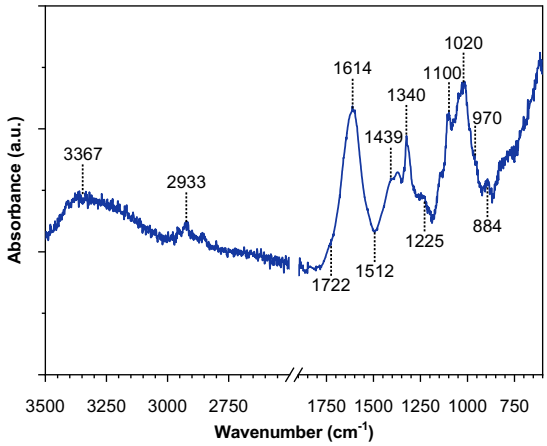
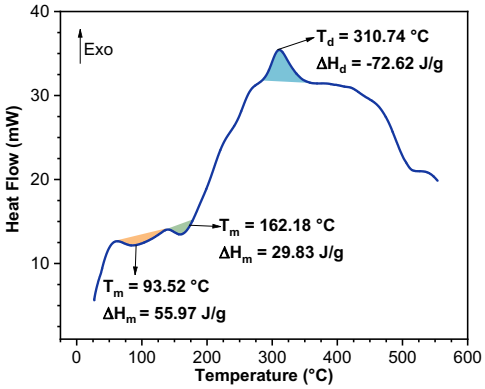


Figure 1. Fourier transform infrared spectroscopy (FTIR) of RPP pectin.

of band intensity and wavenumbers for commercial apple pectin, pomegranate peel powder, orange peel pectin, pectin from sisal waste (Hosseini *et al*., 2016; Pereira *et al*., 2016; Santos *et al*., 2013). On the other hand, the degree of esterification of the RPP pectin quantified in terms of the peak at 1614 cm^{-1} showed values of about 62.98±0.67 %, which is in agreement with previous reports (Güzel and Akpinar, 2019). It has been shown that pectin obtained with water as solvent has high esterification degrees (DE > 50%) (Güzel and Akpinar, 2019; Pappas *et al*., 2004).

3.3 Thermal properties

The thermal behavior of RPP pectin between 25 °C and 500 °C is presented in Figure 2.a, where two endothermic peaks at 80.23 °C and 159.47 °C, and one exothermic peak at 310.74 °C, can be observed. The first endothermic peak is due to the desorption and vaporization of water physically absorbed in the pectin. The peak temperature was T_m = 80.23 °C and the enthalpy was ΔH_m = 38.70 J/g (Parikh and Madamwar, 2006). The second endothermic peak with peak temperature T_m = 159.47 °C and enthalpy ΔH_m = 27.42 J/g can be related to the elimination of structural water (dehydration reactions) (Moussout *et al*., 2016). It has been pointed out that this endothermic peak might be related to hydrogen bonding linking galacturonic acid units, as well as to conformational changes (Einhorn-Stoll and Kunzek, 2009). The third peak corresponds to an exothermic process at T_d = 310.74 °C and ΔH_d = -42.77 J/g, corresponding to thermal degradation of pectin.



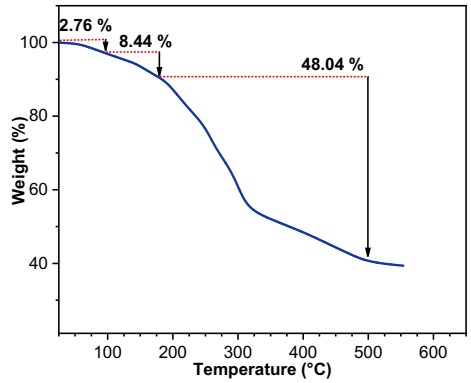


Figure 2. Thermal analysis of RPP pectin: a) DSC and b) TGA.

The TGA response (Figure 2.b) showed three phases at 25-80.23 °C, 80.23-160 °C and 160-325 °C. About 1.89 % weight loss was presented in the first phase, an effect that might be reflecting the evaporation of physically absorbed water in pectin. The weight loss in the second phase was about 5.34%, corresponding to the elimination of structural water as the temperature increased until 160 °C (Moussout *et al*., 2016; Parikh and Madamwar, 2006). The third phase displayed a fast weight loss (37.99 %) due to the polysaccharide decomposition. This phase is characterized by extensive thermal degradation of galacturonic acid chains, accompanied by the formation of solid char and the formation of various gaseous products (Combo *et al*., 2013; Einhorn-Stoll and Kunzek, 2009). The total weight loss at 500 °C was to 38%.

3.4 Surface morphology

Figure 3 displays images of RPP pectin powder at different magnifications (100×, 300×, 1000× and 1700×). Figure 3.a (100×) reveals a heterogeneous distribution of the shape and size of RPP pectin particles. Figure 3.b (300×) shows irregular RPP pectin particles formed by overlapped sheets. A closer view in figures 3.c (1000×) and 3.d (1700×) reveals irregular, rough surface with some micro-fractures and scattered particles. Dick *et al*. (2019) reported that the surface morphology and microstructure of a biopolymer is consequence of the extraction,

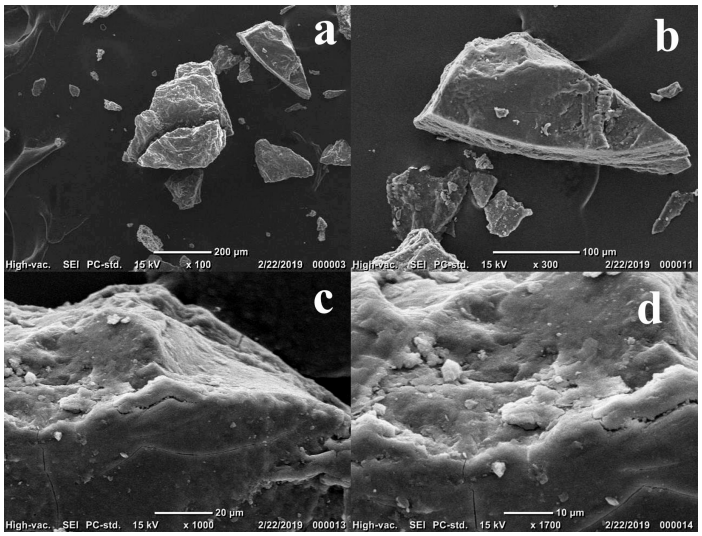


Figure 3. Morphology by SEM of RPP pectin at different magnifications.

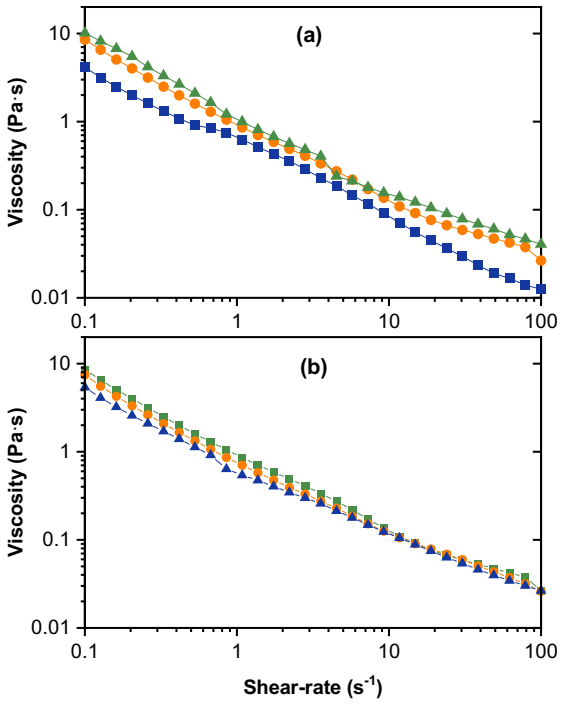


Figure 4. (a) Effect of concentration on the apparent viscosity of pectin dispersions at 20 °C. Symbols: blue square -2 g/100 g, orange circle -5 g/100 g, green triangle -8 g/100 g. (b) Effect of temperature on the apparent viscosity of pectin dispersions at 5 g/100 g. Symbols: blue square 20 ºC, orange circle -40 ºC, green triangle -60 ºC.

purification, drying and grinding method. Similar morphology were obtained for pistachio green hull pectin (Kazemi *et al*., 2019) and for kiwi pectin in contrast with apple and orange pectin that usually shows more compact and homogenous surfaces (Güzel and Akpinar, 2019).

3.5 *Apparent viscosity of pectin dispersions*

The apparent viscosity as function of the shear rate for different RPP pectin concentrations (20 °C) is shown in Figure 4a. The apparent viscosity showed a decreasing behavior with the shear rate in the range 0.1-100 s^{-1}, indicating non-Newtonian pseudoplastic behavior (i.e., shear-thinning pattern). Also, the apparent viscosity showed a positive trend with the pectin concentration, which is an effect that was probably caused by entanglement of polymeric coils (Guimarães *et al*., 2009). The entanglement of polymer coils induces mutual interpenetration between pectin molecules, limiting their mobility and increasing viscosity (Ndjouenkeu *et al*., 1996). A positive correlation between concentration and viscosity of pectin solutions was previously reported (Cui *et al*., 2020; Deng *et al*., 2020).

The power-law model (R^2 ≥0.96) accurately described the behavior flow curves for RPP pectin at of different concentrations. The parameters of the power-law model for dispersions are shown in Table 1. As expected for shear-thinning fluids, the flow behavior index (n) is in range of $0 < n < 1$. Shear-thinning non-Newtonian behavior is considered to be strong for $n < 0.6$ (Chhinnan *et al*., 1985). The values of consistency coefficient (k) varied in the range 0.643 - 1.030 Pa.s^n, an effect that is commonly ascribed to an increase in water binding capacity (Gómez-Díaz and Navaza, 2003). Mandarin citrus peel (Zhang *et al*., 2018) and *Malus domestica Fălticeni* apple pomace (Dranca *et al*., 2020) are instances of pectin showing power-law function patterns.

Figure 4b shows that the apparent viscosity decreased with the temperature, which may be attributed to increased mobility of the pectin molecules, as well as to a reduction of the water-pectin interaction (Bohdanecky and Kovar, 1982). The behavior of viscosity with temperature is presented in Table 1. As expected, the parameter n approached the unity as the temperature was increased. Besides, the consistency coefficient (k) decreased with temperature. The temperature dependency of the viscosity was described by means of the Arrhenius function (Table 1). The estimated activation energy was 8.668 kJ/mol, which is an indicator of molecule movement to the activation state. In this way, the dispersion tend to an easy flow for higher activation energy values (Haminiuk *et al*., 2006).

3.6 *Functional properties of RPP pectin*

3.6.1 *Water holding capacity (WHC)*

Table 2 presents that the WHC of RPP increased with the temperature. Such increase can be explained from enhanced molecular mobility of pectin components, which in turn leads water absorption and improved degree of water held by the pectin. Similar results were obtained for melon, pomegranate and kiwi pectin with 1.61, 2.35 and 2.69 g water/g, respectively (Güzel and Akpinar, 2019), and for chayote pectin with 3.14 g of water/g (Ke *et al*., 2020). WHC values between 4.54 and 5.42 g water/g have been obtained in pectins from *Opuntia ficus* indica cladodes, apple, and orange peels.

Table 1. Power law parameters for RPP pectin.

	k	n	R^2
20 °C			
2.0 % (w/w)	0.643 ± 0.19 [a]	0.277 ± 0.011 [a]	0.979
5.0 % (w/w)	1.018 ± 0.11 [b]	0.217 ± 0.010 [b]	0.964
8.0 % (w/w)	1.030 ± 0.21 [c]	0.189 ± 0.006 [c]	0.961
5.0 % (w/w)			
20 °C	1.018 ± 0.018 [b]	0.098 ± 0.020 [b]	0.963
40 °C	0.789 ± 0.026 [a]	0.182 ± 0.005 [a]	0.962
60 °C	0.665 ± 0.043 [c]	0.189 ± 0.004 [c]	0.986
Arrhenius model			
k_0	0.029 ± 0.003 [a]		
E_a	8.668 ± 0.281 [a]		
R^2	0.994		

k: Consistency index (Pa s^n). *n:* Flow behavior index (dimensionless). k_0: Consistency index (Pa s^n) at a reference temperature. E_a: Activation energy (kJ/mol).
Data are presented as means ± SD (n=3). Values with different letters in the same column indicate significant difference ($p \leq 0.05$).

Table 2. Functional properties of RPP pectin.

Temperature	*WHC* (g of water / g of powder)	*OHC* (g of oil / g of powder)
25 °C	2.00 ± 0.20 [c]	0.78 ± 0.03 [c]
45 °C	6.27 ± 0.15 [b]	0.86 ± 0.02 [b]
65 °C	10.60 ± 0.20 [a]	0.96 ± 0.02 [a]

WHC: Water Holding Capacity. *OHC:* Oil Holding Capacity.
Data are presented as means ± SD (n=3). Values with different letters in the same column indicate significant difference ($p \leq 0.05$).

High WHC values from different mango varieties pectins in a range from 9.5 to 13.65 g water/g (Nguyen *et al*., 2019), and in commercial citrus pectin values of 10.35 g water/g (Rubio-Senent *et al*., 2015). Several authors attribute the difference in the WHC values to intrinsic and extrinsic factors. For instance, the amount of free hydroxyl groups in chemical structure, porosity of particles, pH, and content of galacturonic acid are factor that have been demonstrated as central for water holding capacity of pectin (Kazemi *et al*., 2019; Bayar *et al*., 2018; Rubio-Senent *et al*., 2015).

3.6.2 Oil holding capacity (OHC)

Oil holding capacity reflects the absorption of oil through lateral nonpolar sites within protein molecules (Alpizar-Reyes *et al*., 2017). Table 2 shows the results of OHC at different temperatures. The OHC increased with the temperature, an effect that can be attributed to a higher molecular mobility that favors the bind of non-polar part of the pectin to the oil. In general, the OHC values were lower than those reported for commercial citrus pectin (0.93 ± 017 g oil/g) (Bayar *et al*., 2018), and sour orange peel pectin with 1.32 g oil/g (Hosseini *et al*., 2016). It has been reported that the hydrophilic nature of pectin components has a determinant effect in the OHC of pectin (Bayar *et al*., 2017).

3.6.3 Emulsifying properties

Pectin is formed by protein moieties and polysaccharides. The emulsifying activity (EA) of pectin is linked to protein moieties, whereas the emulsion-stabilizing ability can be related to structural characteristics and conformation of the carbohydrate fraction (Alpizar-Reyes *et al*., 2017). Table 3 shows that the emulsifying ability of RPP pectin increased with the concentration. Increased fractions of pectin lead to decreased oil fraction during emulsification.

Table 3. Emulsifying properties of RPP pectin.

w/v (%)	Emulsifying activity (%)	Emulsifying stability to heating (%)
0.12	65.18 ± 1.79 [c]	56.25 ± 1.79 [d]
0.15	69.64 ± 2.06 [c]	65.18 ± 1.79 [c]
0.3	84.82 ± 1.79 [b]	67.31 ± 2.22 [c]
0.6	89.29 ± 2.92 [a, b]	75.00 ± 2.22 [b]
0.9	90.18 ± 1.79 [a]	79.46 ± 1.79 [a]

Data are presented as means ± SD (n=3). Values with different letters in the same column indicate significant difference ($p \leq 0.05$).

The protein moiety attached to pectin molecules becomes adsorbed at the oil/water interface, increasing in this way the interfacial tension (Zhang *et al*., 2020). The tail of the hydrophilic carbohydrate chain is embedded into the aqueous phase, thereby which in turn incorporates steric barrier against flocculation and droplet coalescence. The obtained results of emulsion activity in this study were higher than the obtained data from citrus medica peel (46.5%) (Pasandide *et al*., 2017) and sugar beet pulp (~43-47%) (Yapo *et al*., 2007). Kazemi *et al*. (2019) had EA values between 40.7 and 53.4% for pectin extracted from sour orange peel, these values depending on the pectin extraction process. Lower results of EA were obtained using three cultivars of mango peel pectin in a range from 11.8% to 33.2%, at 0.5% w/v of pectin (Nguyen *et al*., 2019). On the other hand, the emulsion stability (ES) quantifies the breakdown of the emulsion under heating conditions. Proteins adsorbed on the surface of oil droplets unfold and non-polar amino acids are exposed when an emulsion is heated. Thus, oil droplets carry out hydrophobic attraction and flocculate (McClements, 2015). Table 3 shows the emulsifying stability (ES) of RPP pectin at different concentration of pectin solution. The ES increased as the pectin concentration increased in the emulsion. Pectin in the emulsion involves a greater number of oil drops with a thicker layer, which hampers the spread the pectin during heating, preventing the union of oil droplets, and therefore the instability of the emulsion is retarded (Dickinson, 2018). Emulsion stability is mainly affected by conformation and molecular weight of a pectin since these characteristics influence in the absorption to the oil droplet in an emulsion (Cui *et al*., 2020). Similar results of ES to heating were obtained using grapefruit peel pectin extracted at pH=3.0, with ES=75% at 1% w/v (Cui *et al*., 2020) and using three cultivars of mango peel pectin an ES mean of 65.1% for mature peels and ES mean of 34.5% for ripe peels at 0.5% w/v (Nguyen *et al*., 2019).

Conclusions

Pectin was obtained from red pitaya peels (RPP) by aqueous extraction. SEM analysis showed irregular and rough particles, with some micro-fractures surface. The yield (11.59/100 g d.b.) and Gal A content (54.36 ± 1.03%) were slightly lower than that reported for other conventional pectin sources. The RPP pectin presented high thermal stability (310.74 °C) and a high degree of esterification (60.35±1.35%) which classifies it as a high methoxyl pectin. Also, the emulsifying activity and emulsifying stability of RPP pectin were comparable or higher than that reported for other pectin sources and could be attributed to the small inherent protein content detected. The high RPP pectin/oil volume ratio increased both the emulsifying activity and emulsion stability, which is a desired property. Therefore, the results showed that the RPP pectin has an adequate balance between physicochemical characteristics and functional properties that make it a potentially suitable substitute of commercial pectins for applications in the industry, particularly in the formation and stabilization of emulsions. In addition, the results obtained in this work justify the fact of taking advantage of, maximizing, and giving an added value to the peel of the red pitaya fruit in order to develop a process to produce pectin. In this way, help the rural communities where this fruit is cultivated to improve their environment and quality of life.

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