Utilization of by-products of *Hibiscus sabdariffa* L. as alternative sources for the extraction of high-quality pectin

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Abstract The by-products of *Hibiscus sabdariffa* L. (HsL), obtained after soaking or decoction of the calyces of Colima and Sudan cultivars, were used for pectin extraction. After soaking, the wastes of both cultivars gave higher yields of pectin than those obtained by decoction. The pectin of the wastes by soaking presented high methoxyl groups, galacturonic acid content, viscosity and gelling capacity. Pectin of this treatment also exhibited bands in the regions of 1750 cm⁻¹ and 1630 cm⁻¹ that represents the C=O stretching vibrations of methyl ester and the

amounts and degree of esterification of the HsL pectin. Interestingly, the pectin retained the typical red color of fresh HsL calyces. The amounts of anthocyanins and ascorbic acid of pectin did not show effects against pathogenic microorganisms. Nonetheless, pectin of the Sudan HsL wastes obtained by soaking, exhibited higher properties than those of the citric pectin, thus, demonstrating its potential for industrial applications.

Keywords *Hibiscus sabdariffa* L. · By-products · Colima and Sudan cultivars · Pectin properties

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Introduction

Hibiscus sabdariffa L. (HsL) is a species that belongs to the *Malvaceae* family and that usually grows in tropical and subtropical world areas. The native plant of HsL originated in West of Africa; however, it is also known that it could be a native of India and other Asian countries (Cid-Ortega and Guerrero-Beltrán, 2015; Da-Costa-Rocha et al., 2014). Of the more than 100 HsL cultivars or seed varieties, the main commercial cultivars are produced in China, Thailand, Mexico and Africa (Plotto et al., 2004); all of which are distinguished by color, shape, appearance, and weight, with common names that are related to the region where they are grown. Some regions of Mexico, particularly the states of Guerrero and Oaxaca are the leading producers, with 85% of the domestic production. In 2016, the production of calyces of HsL in Mexico reached 7000 m/t, where the main producer was Guerrero state (SAGARPA, 2018). The major Mexican cultivars are Chinese, Sudan, and Creole or *Criolla* (also known as Colima), also, mutations of the native *Criolla* cultivar are being produced (Ariza-Flores et al., 2014; Salinas-Moreno et al., 2012).

Worldwide, HsL leaves, calyces, seeds, and roots are used in the preparing of local food and in traditional medicine (Da-Costa-Rocha et al., 2014; Villani et al., 2013). The calyces are mainly used for the preparation of soft drinks, jellies, jams, cookies, and spirits. Some of these products are industrialized, while others are produced only at small scale using artisanal practices (Ariza-Flores et al., 2014; Cid-Ortega and Guerrero-Beltrán, 2015; Nnam and Onyeke, 2003; Plotto et al., 2004). In certain, either artisanal or industrial manufacturing processes, the decoction of dry calyces for juice extraction generates large amounts of residues with few uses; these wastes are considered an economic and environmental issue by the manufacturers. In regards to the use of HsL residues, scarce data has been published, such as the decoction of the calyces to obtain polyphenols and dietary fiber from wastes (Sáyago-Ayerdi et al., 2013).

Pectins are polysaccharides that exist in the cell wall and in the non-woody parts of plants, mainly composed of units of D-galacturonic acid linked by α 1-4 glucosidic bonds (Nazaruddin et al., 2013). In the food industry, the main applications of pectin are as gelling, stabilizing, and thickening agents (Willats et al., 2006). Based on the degree of esterification (DE), pectins are known as high methoxyl (HM) if the DE is $> 50\%$ or as low methoxyl (LM) if the DE is $< 50\%$ (Arellanes et al., 2011; Nazaruddin et al., 2013). Pectins are commonly obtained under acid extraction or by means of using chelating agents; besides, the yield depends on multiple factors, such as the source, pH of the extraction medium, temperature, and extraction time (Arellanes et al., 2011; Canteri-Schemin et al., 2005; Happi-Emaga et al., 2012; Nazaruddin et al., 2013). Various types of acids have been tested for pectin extraction, such being that citric and malic acid produce the highest yields, while the former is preferred because it is economical and environmentally friendly (Canteri-Schemin et al., 2005). Apple bagasse and citrus peels are considered the major sources of commercial pectin; recently, other potential sources are being explored, such as peels of dragon fruit, passion fruit, and bananas, or sunflower wastes (Liew et al., 2014; Muhammad et al., 2014; Oliveira et al., 2016; Sahari et al., 2003). Regardless the diversity of organic materials, not all of these are suitable for the extraction of high-quality pectin (Arellanes et al., 2011). For HsL, few studies report the use of whole calyces as sources of pectin (Morton, 1974; Nazaruddin et al., 2013); however, there have not been investigations published about the use of HsL by-products for the obtaining of value-added food ingredients such as pectin.

The focus of this research was to explore the potential use of the by-products of *H. sabdariffa* L. of Colima and Sudan cultivars, as alternative sources of high-quality

pectin, and to compare its properties with those of the commercial citric pectin.

Materials and methods

Raw materials and reagents

Calyces of HsL of the Sudan and Colima cultivars were purchased at a local food market (Guadalajara, Mexico). Citric pectin was purchased from Meyer Chemical Reagents (Mexico City, MEX), ascorbic acid, ethanol, HCl, NaOH, KCl, and oxalic acid from Golden Bell Products Inc. (Anaheim, CA, USA), and 2,6-dichloroindophenol sodium salt hydrate from MERCK KGaA (Darmstadt, GER).

Extraction and yield of pectin

The wastes were obtained after juice extraction of HsL calyces in distilled water, by two methods: 1) soaking for 1 h at room temperature, and 2) decoction for 12 min, with a ratio of 1:10 (calyces-to-water). These conditions were selected based on preliminary experiments, and on reported procedures for the inactivation of pectin degrading enzymes and for the obtaining of HsL fiber (Grunauer and Cornejo, 2009; Sáyago-Ayerdi et al., 2013). The wastes were manually pressed for greater water removal, and subsequently, they were lyophilized in a freeze-dryer (ULP-CI-06, CIATEJ A.C., Jalisco, MEX) ground, sieved (0.5-mm mesh size), and stored in glass jars. The pectin was extracted from the powdered wastes under reflux for 30 min at boiling temperature, using a citric acid solution of pH 2.5 (Canteri-Schemin et al., 2005). The pectin was precipitated with ethanol in a ratio of 1:2 (gel-to-ethanol); the solution was stirred for a few minutes on a hotplate stirrer (984DA0CH, Daigger Scientific Inc., Vernon Hills, IL, USA), afterwards, it was allowed to settle for 15 min. The gelatinous precipitated was filtered under vacuum, using a synthetic cloth through a porcelain Buchner filter funnel. The wet pectin was lyophilized to preserve its red color, then, it was ground in a laboratory mill (MF 10.2, IKA®-Works, Wilmington, NC, USA) and sieved by a 100-mesh for particle size homogenization. The yield (d.b.) of pectin was determined as follows:

$$\text{Yield (\%)} = (\text{g of pectin} / \text{g of dry sample}) \times 100$$

Physicochemical determinations

The amount of moisture and water activity (a_w) were determined by using a moisture analyzer (Series MB50,

Aczet Pvt. Ltd., Mumbai, India) and a water activity meter (AquaLab Series 4, Decagon Devices Inc., Pullman, WA, USA), respectively. The pectin samples were placed into the chambers, and the results were read directly on the screens of the equipment. The ash content was determined according to the AOAC (2000) method number 924.05. The pH of 4% pectin solutions in distilled water was measured by the immersion method using a pH meter (UltraBasic-UB-10, Denver Instrument, Arvada, CO, USA). Meanwhile, the acidity was measured as titratable acidity, and the results were expressed as a percentage of citric acid (AOAC, 2000). The color of the powdered pectin was measured with a colorimeter (Chroma meter CR410, Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) using the CIELAB color space. Color coordinates L^* (Lightness), a^* and b^* were determined by taking five readings at different positions on the samples. The color difference (ΔE) was expressed as: $\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$, where ΔL^* , Δa^* and Δb^* represent the differences between the samples and the control. Finally, the viscosity (mPa s) of 1% pectin solutions was analyzed using a digital viscometer (DV-I Prime, Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) at speeds of 100 rpm with the spindle No. 4.

Degree of esterification, methoxyl groups and galacturonic acid

Five grams of pectin were mixed with a solution containing 100 mL of 60% ethanol and 5 mL of concentrated HCl; afterwards, the mixture was homogenized at room temperature for 10 min (Liu et al., 2010). The residues were filtered in Gooch crucible glasses, washed six times with 15 mL of the ethanol: HCl solution, and then with 20 mL of ethanol each time. Finally, the residues were dried for 1 h at 40 °C. One-tenth of the dry sample (representing 500 mg of the original unwashed sample) was transferred into a 250-mL beaker with 2 mL of ethanol and 100 mL of distilled water. The mixture was then stirred until the pectin was completely dissolved. The volume was titrated with 0.5N NaOH and phenolphthalein as an indicator (termed V1). Later, 20 mL of 0.5N NaOH was added and allowed to stand for 15 min, 20 mL of 0.5N HCl was added, and the mixture was stirred until the pink color disappeared. The final volume was titrated with 0.5N NaOH and phenolphthalein as indicator (termed V2). The degree of esterification (DE) was determined as follows:

$$\text{Degree of esterification (\%)} = [V2/(V1 + V2)] \times 100$$

The percentages of methoxyl groups and galacturonic acid were determined as follows:

$$\text{Methoxyl groups (\%)} = V1 \times N \times \text{meq methoxyl} \times 100/w$$

$$\text{Galacturonic acid (\%)} = (V2 \times N \times \text{meq galacturonic acid} \times 100)/w$$

where N is the normality of NaOH, meq is the milliequivalents of methoxyl (0.031) and galacturonic acid (0.097), and w is the weight of the sample (g).

Functional groups by FT-IR

The chemical functional groups of pectin were identified by Fourier transform infrared spectroscopy (FT-IR), using a FT-IR spectrometer (Nicolet iS50, Thermo Electron Scientific Instruments LLC, Madison, WI, USA). The transmission method (laminated samples) was applied and the infrared spectra of the samples were measured in the region of 400–4000 cm^{-1} (Gnanasambandam and Proctor, 2000; Liu et al., 2010; Manrique and Lajolo, 2002; McCann et al., 1997). Although the titrimetric method can be used to characterize the identity of pectin, the instrumental method is one of the most sensitive to detect small differences in molecular structures, when working with materials of the same origin (Gnanasambandam and Proctor, 2000).

Total anthocyanins and ascorbic acid

One g of pectin was dissolved in 10 mL of acidified ethanol with 0.1 M HCl and stirred for 24 h, in the dark at room temperature (Camelo-Méndez et al., 2013). The extracts were filtered through Whatman filter paper No. 42, and then, two aliquots of 2 mL each, were mixed with potassium chloride (pH 1) and sodium acetate (pH 4.5) buffer solutions. The absorbance of each buffer was measured in a spectrophotometer (UNICO SpectroQuest 2800, United Products & Instruments Inc., Dayton, NJ, USA) at 510 and 700 nm, respectively. Total anthocyanins (Ta) were expressed as mg of cyanidin-3-glucoside/100 g of dry weight, as follows:

$$\text{Ta} = (A \times \text{MW} \times \text{Df} \times V \times 1000)/(\epsilon \times 1 \times w)$$

where, $A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$, MW is the molecular weight of cyanidin-3-glucoside (449.2 g/mol), Df is the dilution factor, V is the volume of the extract (mL), ϵ is the molar extinction coefficient of cyanidin-3-glucoside (26,900), and w is the weight of pectin (g). For the ascorbic acid determination, the pectin was macerated with 0.4% of oxalic acid in a proportion of 1:10 (w/v), maintained in darkness for 20 min at room temperature, and centrifuged at 8000×g. The supernatant was mixed with a sodium-acetate buffer solution (300 g of anhydrous sodium acetate, 700 mL of distilled water, and

1000 mL of glacial acetic acid) and 8 mL of 2,6-dichloroindophenol solution (12 mg of 2,6-dichloroindophenol sodium salt in 1000 mL of distilled water). The absorbance was read at a wavelength of 520 nm, and the ascorbic acid content was calculated by using a standard curve of ascorbic acid at different concentrations (0–50 mg/mL). The results were expressed as mg of ascorbic acid/100 g of dry weight (Lara-Cortés et al., 2014).

Antimicrobial capability

The antimicrobial capability of pectin was determined through the plate-diffusion method (CLSI, 2006). The pectin solutions of 1, 2, and 3% (w/v) were poured into sterile Petri dishes, dried at 40 °C for 18 h, and cut in small circles of 6 mm in diameter (sensi-disc). The antimicrobial activity was evaluated against *Escherichia coli* (ATCC 25922), *Salmonella enterica* (ATCC 14028), *Listeria monocytogenes* (ATCC 19111), *Staphylococcus aureus* (ATCC 29213), and *Pseudomonas aeruginosa* (ATCC 27853). The cultures were grown on trypticase soy agar at 35 ± 2 °C for 24 h. The bacterial suspensions were prepared in Müller–Hinton broth and the inoculum was spread on Petri dishes with Müller–Hinton agar. The pectin sensidiscs were used to test each microorganism and drops of lactic acid (10 uL of 50% lactic acid) were used as the control. After the described procedure, the Petri dishes were incubated at 35 ± 2 °C for 16 to 20 h. The diameter of the inhibition zones was reported in mm. The degree of sensitivity was reported as follows: susceptible, > 21 mm; intermediate, 17–20 mm, and resistant, < 17 mm.

Statistical analysis

The statistical analysis was performed with the use of a Statgraphics Centurion XV ver. 15.2.06[®] software. The data was presented as the mean value $\pm$ standard deviation of three replicates. All the analyses were carried out in triplicate, unless indicated. One-way ANOVA was conducted and the post hoc Duncan's test was selected to determine significant differences ($p < 0.05$) between treatments.

Results and discussion

Pectin yield

The by-products of the soaked calyces of both cultivars Colima and Sudan gave the highest yields of pectin, with 14.59 and 15.88%, respectively. Yet, the decoction of the calyces caused the loss of pectin, which provided lower

yields (10.48 and 12.20%, for Colima and Sudan wastes, respectively). Statistically, the main factors (type of treatment and cultivar) significantly ($p < 0.05$) influenced pectin yield. Even though heat causes the degradation of pectin, it is recommended to apply a thermal pretreatment (95 °C for 15 min) prior to the pectin extraction (Grunauer and Cornejo, 2009). The purpose of this thermal pretreatment was mainly to inactivate the enzymes that cause the hydrolysis of methyl groups, and which confer pectin's gelling capability, but also may promote the formation of LM pectin (Sahari et al., 2003). Although the heat treatment decreased pectin yields, there were still significant amounts of pectin that could be extracted. Similar results to these of HsL pectin were reported for sunflowers washed with hot water prior to the pectin extraction (10.56–11.42%) (Sahari et al., 2003).

To our knowledge, currently there is no data on the use of HsL by-products as sources of pectin. Whereas, for whole calyces (Arab, Terengganu, UKMR-1, UKMR-2, of either parental or mutant cultivars) without juice depletion, Nazaruddin et al. (2013) reported yields of 6.50–9.77 and 11.30–18.70%, using HCl and ammonium oxalate solutions, respectively; such results are comparable to our findings for the Sudan wastes.

Physicochemical properties of pectin

Table 1 summarizes the results of the physicochemical properties of pectin extracted from the wastes of Colima and Sudan cultivars, compared with those of the commercial citric pectin. As shown, the moisture content (7.50–8.58%) of pectin wastes obtained through both treatments and cultivars was within the parameter ($< 12\%$) established by the Food and Agriculture Organization (FAO) (FAO, 2016). On the contrary, these values were significantly ($p < 0.05$) higher in comparison with those of the commercial citric pectin (4.03%) tested in this study. Higher moisture percentages (16.4–17.8%) have been reported for other sources of pectin (Arellanes et al., 2011; Oliveira et al., 2016); hence, these differences, attributed to the drying conditions of pectin, influence the final moisture content of the materials. It is well known, that low values of moisture and a_w contribute to food product's stability during storage. About the a_w of pectin, the values (0.32–0.39) exhibited did not show significant differences for both treatments and cultivars. Although, these values were slightly higher than those of citric pectin (0.22), HsL pectin had a sufficiently low water activity that ensures its stability.

The ash content is a parameter that influences the gelling properties of pectin, and, according to the FAO, a content of 3% ash is required for good-quality pectin. Nonetheless, the maximal limit reported for good-quality pectin gels is

Table 1 Physicochemical properties of pectin obtained from wastes of Colima and Sudan (*Hibiscus sabdariffa* L.) cultivars

Measurement	FAO ² /WHO ³ /JECFCA ⁴	Citric pectin	Colima		Sudan	
			Soaking	Boiling	Soaking	Boiling
Moisture ¹ (%)	Maximum 12	4.03 ± 0.06 ^a	7.50 ± 0.26 ^b	8.58 ± 0.37 ^c	7.63 ± 0.48 ^b	8.08 ± 0.7 ^c
Ash (%)	Maximum 3	1.69 ± 0.02 ^a	3.22 ± 0.01 ^b	7.72 ± 0.09 ^c	3.19 ± 0.02 ^b	6.85 ± 0.03 ^d
a _w	–	0.22 ± 0.00 ^a	0.32 ± 0.01 ^b	0.39 ± 0.04 ^b	0.36 ± 0.01 ^b	0.38 ± 0.08 ^b
pH	–	3.09 ± 0.01 ^a	3.00 ± 0.03 ^b	2.99 ± 0.01 ^{bc}	3.02 ± 0.02 ^b	2.95 ± 0.02 ^{bce}
Viscosity (mPa s)	–	528 ± 21 ^{ad}	995 ± 17 ^b	415 ± 24 ^c	1024 ± 123 ^b	600 ± 69 ^d
Titrateable acidity ² (%)	–	4.56 ± 0.00 ^a	11.06 ± 0.00 ^b	8.72 ± 0.19 ^c	14.36 ± 0.45 ^d	8.39 ± 0.50 ^{ce}
Methoxyl groups (%)	Minimum 6.7	16.25 ± 0.45 ^a	17.69 ± 2.49 ^a	3.97 ± 0.23 ^b	25.84 ± 0.07 ^c	3.83 ± 0.45 ^{be}
Galacturonic acid (%)	Minimum 65	57.86 ± 1.40 ^a	71.35 ± 9.22 ^b	26.36 ± 0.73 ^c	96.41 ± 2.39 ^d	18.00 ± 2.84 ^e
Degree of esterification (%)	Minimum 60	87.93 ± 0.29 ^a	77.56 ± 0.91 ^b	47.15 ± 1.41 ^c	83.96 ± 1.85 ^d	66.88 ± 2.65 ^e

The values are expressed as mean ± standard deviation ($n = 3$). Different letters indicate significant difference ($p < 0.05$) among column, for each measurement, according to post hoc Duncan's mean values comparison test

¹g/100 g of sample, wet basis

²FAO Food and Agriculture Organization

³WHO World Health Organization

⁴JECFA Joint Expert Committee on Food Additives

10% (Norazalina and Nazarrudin, 2011). In this study, the pectin of wastes by soaking and citric pectin had the lowest ash percentages ($\leq 3.22\%$) as recommended by the FAO. These values showed significant differences ($p < 0.05$) in comparison with those of pectin from wastes by decoction, which contained twice the ash content (6.85 and 7.72%, for Sudan and Colima pectin, respectively). Nonetheless, these percentages remained within the maximal limit reported (Norazalina and Nazarrudin, 2011). In general, there is a great variation in the percentage of pectin ash from different sources, which content depends on the chemical composition of the material and its treatment conditions (Arellanes et al., 2011; Nazarrudin et al., 2013). For the pH values, also related to the gelling capacity of pectin, there were no significant differences between pectin of the wastes of both cultivars and treatments. In fact, the range of values obtained (2.95–3.02) was quite similar to that of citric pectin (3.09). Furthermore, the percentage of methoxyl groups, was significantly ($p < 0.05$) higher (25.84%) in Sudan pectin of the wastes by soaking than that of Colima (17.69%) under the same treatment, or that of citric (16.25%) pectin. Besides the methoxyl groups and the pH, the gelling capacity of pectin is also associated to the degree of esterification (DE), which was superior for both citric (87.93%) and Sudan pectin (83.96%) of the wastes by soaking. In contrast, boiling caused a decrease of methoxyl groups ($\leq 3.97\%$) and DE values ($\leq 66.88\%$) for both cultivars, as consequence of thermal degradation. Low percentages of methoxyl groups and DE values have been reported in pectin of “Manzano” banana peel, which are considered of LM (Arellanes et al., 2011); whereas other

authors have found that pectin of banana peels are of high methoxyl groups (Oliveira et al., 2016), similarly to the Sudan pectin in this study.

About the galacturonic acid content, the Sudan pectin of the wastes by soaking had the highest percentage (96.41%) compared to that of citric pectin (57.86%) ($p < 0.05$). Again, heat treatment induced to the losses of galacturonic acid in the pectin of both cultivars, since lower percentages were determined in pectin of the wastes by boiling, with 26.36 and 18.00%, for Colima and Sudan, respectively. These results contrast against those of a recent study, which reported that high temperatures, long extraction times, and low pH values might increase the galacturonic acid content (Oliveira et al., 2016). Whereas another study reported that long extraction times cause a rise to the release of high concentrations of uronic acid, and high temperatures decrease the galacturonic acid content (Chan and Choo, 2013). Based on these statements, we postulate that the composition of the material and the extraction conditions of pectin determine the resistance of the functional groups under the selected experimental conditions.

The viscosity of pectin of both cultivars, was significantly ($p < 0.05$) higher (995–1024 mPa s) in pectin extracted from the wastes after soaking than that by boiling (415–600 mPa s); where the latest exhibited a viscosity more similar to that of citric pectin (528 mPa s). Similarly, the pectin of the wastes after soaking, exhibited a high gelation degree of 100 (data not shown), indicating that 1 g of pectin can gel 100 g of sugar. This means that HM pectin may form gels in the presence of sugars at low pH values (2.0–3.5) and ash content ($\leq 3\%$) (Kulkarni and

Vijayanand, 2010; Nazaruiddin et al., 2013). These findings may be of great value to the food industry, since pectin extracted from wastes by soaking may be used as excellent thickening and gelling agents. Contrariwise, the pectin of the wastes by boiling did not exhibit a strong gel-forming capability, suggesting the loss of the functional groups, whose percentage was comparable to that of LM pectin.

According to the FAO recommendations, the pectin color should be clear; however, in this study, pectin presented a distinctive reddish color. Thus, the natural color of the calyces was maintained after the pectin extraction; this characteristic may be an advantage in the development of food products that present such tonalities. The pectin of HsL wastes exhibited lower values of lightness (L^* : black at $L^* = 0$ and white at $L^* = 100$) after soaking, in comparison with pectin of wastes by boiling, for both cultivars (Table 2). These findings suggest that the heat treatment degraded the red pigments to some extent, yet certain amount of pigments remained attached to the material. The color coordinates a^* (from red⁺ to green⁻) and b^* (from yellow⁺ to blue⁻), exhibited values in the positive region, which indicate the predominance for red and yellow, respectively. Furthermore, the color difference of pectin had values of $\Delta E > 2$ ($\Delta E: \Delta E < 1.0$, yet not noticeable to the naked eye, from 1–2 is hardly noticeable, and $\Delta E > 2.0$ is visually detectable), being visibly detectable for both treatments and cultivars. However, the Sudan pectin that resulted from wastes through the soaking process had the lowest ΔE values, indicating that this treatment had the highest retention of HsL pigments. Statistically, all color parameters were significantly different ($p < 0.05$) for both treatments (soaking and boiling), as well as the studied cultivars.

FT-IR spectral analysis

The main functional groups that identify pectin are OH^- , free and esterified carboxyl (COOH), and methoxyl groups ($-\text{O}-\text{CH}_3$) (Manrique and Lajolo, 2002; Liu et al., 2010). By the FT-IR technique, the pectin obtained from the wastes of both cultivars and treatments revealed the existence of vibrations detected in the regions close to

3400 cm^{-1} , 2900 cm^{-1} , 1750 cm^{-1} , which represent $\text{O}-\text{H}$ stretching of hydroxyl groups, $\text{C}-\text{H}$ stretching of the $-\text{CH}_2$ groups and $\text{C}=\text{O}$ stretching vibration of methyl ester that is utilized to prove the DE of pectin (Fig. 1A, B). Also, the intense bands appearing at 1630 cm^{-1} are related to the amounts and degree of esterification of the HsL pectin. It is noticeable that the pectin obtained from the wastes by soaking (Fig. 1B, C), exhibited a similar absorption pattern as the one identified in citric pectin used as our control (Fig. 1A). These results corroborate that pectin resulting from wastes of this treatment exhibits the main functional groups as those detected in citric pectin, which until now is one of the main types of commercial pectin. Additionally, carboxylic groups in the regions of $1330\text{--}1440\text{ cm}^{-1}$ and $875\text{--}960\text{ cm}^{-1}$ disappeared in the pectin of wastes of both cultivars treated with heat; again, indicating that heat affects the chemical structure of the material. It is known that peaks located in the region of 1200 cm^{-1} correspond to pectin with a high degree of esterification. In this study, pectin of the wastes by soaking, for both cultivars, showed near regions between 1230 and 1260 cm^{-1} ; these regions were similar to those detected in citric pectin, between 1228 and 1230 cm^{-1} . In the opposite manner, pectin of wastes by boiling exhibited a displacement of the peaks that was attributed to the loss of methoxyl groups induced by heat treatment (Sahari et al., 2003).

Total anthocyanins, ascorbic acid and antimicrobial capability

Compounds such as anthocyanins and ascorbic acid are related to the antioxidant capability of HsL. Although pectin retained the red color of the calyces, low anthocyanin contents were quantified, particularly, in Colima pectin obtained from wastes by boiling. In comparison, by soaking, the anthocyanin content of pectin of the Sudan wastes was significantly ($p < 0.05$) higher (Table 3). In accordance to the color parameters, the Sudan pectin of the wastes by soaking exhibited lower luminosity values than those of Colima, because in nature, the Sudan cultivar has strong reddish tones, which reduce L^* values; therefore, higher contents of anthocyanin are preserved in this

Table 2 Color parameters of pectin obtained from wastes of Colima and Sudan (*Hibiscus sabdariffa* L.) cultivars

Color parameter	Colima		Sudan	
	Soaking	Boiling	Soaking	Boiling
L^*	48.6 ± 0.9^a	57.4 ± 0.5^b	32.6 ± 0.3^c	56.8 ± 0.3^b
a^*	16.5 ± 0.2^a	13.9 ± 0.1^b	17.6 ± 0.2^c	18.1 ± 0.1^d
b^*	8.1 ± 0.1^a	6.7 ± 0.0^b	2.2 ± 0.1^c	5.0 ± 0.0^d
ΔE	13.2 ± 0.5^a	21.3 ± 0.4^b	11.8 ± 0.2^c	26.8 ± 0.3^d

Different letters indicate significant difference ($p < 0.05$) among column, for each color parameter, according to post hoc Duncan's mean values comparison test

Fig. 1 Infrared spectra of citric pectin (A) and pectin obtained from wastes of *Hibiscus sabdariffa* L. cultivars: (B) soaking Sudan, (C) soaking Colima, (D) boiling Sudan, and (E) boiling Colima

cultivar. In regard to ascorbic acid, the clear calyces are reported to contain higher amounts (Alma Blanca cultivar with 90.0 mg ascorbic acid/100 g of dry weight) than those namely as darker (Colima and Sudan cultivars with 33.2 and 72.0 mg ascorbic acid/100 g of dry weight, respectively) (Nnam and Onyeke, 2003; Salinas-Moreno et al., 2012). Pectin of both cultivars had a significantly ($p < 0.05$) less content of ascorbic acid, depending on the treatment of HsL calyces. The pectin of wastes, after soaking, contained twice the amount (0.15–0.20 mg/100 g of dry weight) of ascorbic acid, for both cultivars. Such low values, which are below those reported for fresh calyces are attributed to the known sensitivity to thermal treatment of this compound, during pectin extraction under reflux, and to the oxidation reactions of the ascorbic acid.

Recently, it has been reported the capability of anthocyanins and other phenol compounds to react as antagonistic of foodborne pathogens (Cisowska et al., 2011);

therefore, pectin of HsL wastes, which showed different contents of anthocyanins and ascorbic acid (the second historically used as preservative of foods), was tested in its antimicrobial capability. The antimicrobial capability was evaluated only for pectin of the wastes by soaking, since this treatment showed the highest anthocyanin content for both cultivars. Due to the presence of anthocyanins and the low pH of pectin, some microbial inhibition was expected; however, pectin solutions (1–3%) did not have the potential to inhibit the growth of the tested microorganisms. There was a null inhibition against *E. coli*, *S. enteritidis*, *S. aureus*, and *L. monocytogenes*, indicating that pectin by itself cannot inhibit the growth of these pathogenic bacterial strains. Only *P. aeruginosa* showed inhibition zones of 3 and 4 mm, when exposed to both Colima and Sudan pectin, respectively. The sensitivity of a microorganism is categorized as “resistant” when the diameter of inhibition around the microbial growth is < 17 mm (CLSI, 2006); hence, the zone of bacterial inhibition around the disks containing the pectic solutions could not be considered as significant.

It is concluded that the HsL by-products of the Colima and Sudan cultivars proved to be alternative sources of pectin. The highest yields of pectin were obtained from the wastes by soaking of the fresh calyces of the Sudan cultivar. Pectin of this cultivar and treatment also exhibited the highest quality (methoxyl groups, galacturonic acid, viscosity, and gelation degree), which is comparable or even superior to that of commercial citric pectin. A distinctive characteristic of pectin was its reddish color, attributed to the presence of HsL pigments, still attached to the material after the pectin extraction. Therefore, pectin of HsL by-products, particularly from the Sudan cultivar, may have a great potential for industrial applications.

Table 3 Total anthocyanins and ascorbic acid of pectin obtained from wastes of Colima and Sudan (*Hibiscus sabdariffa* L.) cultivars

	Citric pectin	Colima		Sudan	
		Soaking	Boiling	Soaking	Boiling
Total anthocyanins ¹	ND	75.00 ± 3.66 ^a	18.89 ± 0.95 ^b	242.00 ± 15.15 ^c	31.49 ± 0.71 ^d
Ascorbic acid ²	0.07 ± 0.002 ^a	0.15 ± 0.030 ^b	0.07 ± 0.001 ^a	0.20 ± 0.074 ^{bc}	0.10 ± 0.034 ^{ab}

Different letters indicate significant difference ($p < 0.05$) among column, for each compound, according to post hoc Duncan's mean values comparison test

ND not detected

¹mg of cyaniding-3-glucoside/100 g of dry weight

²mg of ascorbic acid/100 g of dry weight

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