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Comparative Extraction of Pectic and Polyphenols from Mexican Lime Pomace and Bagasse

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ABSTRACT

Mexican lime bagasse and pomace are rich in pectin and they also represent an important source of value-added compounds such as polyphenols. Two different options for the combined recovery of pectin and phenolic compounds from Mexican lime bagasse and pomace, two byproducts of industrial lime processing, were developed. Conventional and microwave-assisted extraction methods were used. All pectic extracts presented a degree of esterification in the range of 70%. Pomace extracts had the higher pectin yield and the lower polyphenol content. Among the bioactive compounds identified by HPLC were two flavonones, naringin and hesperidin, all compounds were present in low concentration in the pectic extracts. Microwave pectic extracts presented depolymerization, as observed by molecular weight determination (12 KDa) and compared against conventional pectic extracts which presented a molecular weight of 670 KDa. The film forming capacity of pectic extracts was also evaluated. Antioxidant activity of pectic extracts was also assessed by three different methods; all extracts showed a better activity in Fe^{2+} chelating assay (62.85-73.32%) and lipid oxidation inhibition (63.07-72.28%) than in DPPH radical inhibition (5.32-6.65%). These findings indicate a correlation between the amount of phenolic compounds and the antioxidative capacity. Conventional pectic extracts from Mexican lime bagasse and pomace presented bioactive compounds with potential application for edible films and coatings in food industry.

Keywords: Pectin, Conventional Extraction, Microwave Extraction, Mexican Lime

1. INTRODUCTION

Citric fruits are consumed worldwide. In Mexico, they are one of the most important industrial crops and they account for 40% of the fruit-related cultivable surface, mainly in the Mexican South Pacific Coast, from Colima to Oaxaca. Mexican lime (*Citrus aurantifolia* Swingle) has an enormous economic potential, since Mexico produces 22% of fruit production worldwide (Recent reports of the Mexican Association for Agricultural Development). This fruit is used mainly in the beverage industry and for essential oil extraction. However, a considerable quantity of waste from bagasse is generated during citrus juice processing, closely to 45% of the fruit total weight (Ahmad *et al*., 2006; Ma *et al*., 2009). Although these wastes are used as animal feed and biofuel, the majority is discarded, causing environmental pollution. Therefore, citrus processing industries have been searching for applications for these wastes which are a rich source of natural chemical components, including phenolic compounds (mainly flavonoids) and other nutrients (vitamins, minerals, dietary fiber, essential oils, carotenoids and pectin) (Li *et al*., 2006; Masmoudi *et al*., 2008; Gonzalez-Molina *et al*., 2010).

Pectin is a complex vegetal polysaccharide found in the intercellular regions of the primary cell wall of

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fruits and vegetables (Sothornvit and Pitak, 2007). Pectin is heterogeneous, with hygroscopic properties, is acid and water soluble, it does have gelling properties and can be used as a stabilizer of emulsions; because of those characteristics, it is considered a polymer with applications in pharmaceutical and food industries (Zapata *et al*., 2009; Coma, 2010). Recently, there have been some investigations into the use of pectin as raw material for preparation of edible films and coatings as an alternative to non-biodegradable packaging, that are considered environmental pollutants (Shrestha *et al*., 2003; Fishman *et al*., 2004; Maftoonazad *et al*., 2007; Assifaoui *et al*., 2010; Coma, 2010). Pectin is extracted from by-products or wastes from the fruit juice industry, mainly from apple pomace and citrus bagasse (Bochek *et al*., 2001). The main property of pectin is the ability to form gels; in fact, pectin gelling properties depend on several factors (uronic acids content, pH, degree of esterification, presence of solutes, molecular size). The degree of esterification is a critical parameter that influences the ability of pectin to form a gel (Coma, 2010). Commercial pectin are extracted conventionally at high temperatures by hydrolyzing protopectin by the use of acids such as sulfuric, phosphoric, nitric, hydrochloric or citric acid. Microbiological and enzymatic extraction methods are also widely used to obtain pectic extracts (Bochek *et al*., 2001; Sothornvit and Pitak, 2007; Zapata *et al*., 2009; Coma, 2010). Conventional extraction is the simplest and oldest method for removing the pectic acids from plant tissues, but this procedure has several disadvantages, including longer preparation time and lowered pectin yield than the pectin obtained by other methods (Fishman *et al*., 2006; Liu *et al*., 2006; Wang *et al*., 2007; Yapo *et al*., 2007; Yeoh *et al*., 2008) Microwave assisted extraction is a potentially feasible method to obtain pectin and other value-added compounds such polyphenols from agroindustrial wastes (Wang *et al*., 2007). The main advantages of microwave pectic extraction includes a rapid heating time because of the closed vessel used, control of temperature and pressure, for higher temperatures, higher pressures and insignificant volatility of solvents (Fishman *et al*., 2006). There are some reports that demonstrate that microwave extraction can lead to a considerable increase in yield and quality of extracted pectin (Bagherian *et al*., 2011); also, it has been demonstrated that pH, temperature, extraction time, agitation and solid to liquid ratio had effects on pectin yield and quality (Yapo *et al*., 2007; Bagherian *et al*., 2011). El-Nawawi and Shehata (1996) studied the effects of conditions on the yield of pectin extracted from Egyptian orange; they found a higher yield at 90°C, pH of 1.7 and 2 h of extraction. Fishman *et al*. (2006) used microwave heating to extract pectin under different conditions from lime peel and studied their effect on the yield and characteristics of pectin. In contrast, Liu *et al*. (2006) studied different extraction techniques using water at different pH and solvent ratios and found a higher yields with Soxhlet extraction than with Microwave extraction. Masmoudi *et al*. (2008) used acidified date juice to extract pectin from lemon by-product and found optimal conditions at 84.3°C, 3 h 34 min of extraction time and pH 2.8. Yeoh *et al*. (2008) used microwave and conventional methods to obtain pectin from orange peel with different extraction periods, different solvent pH and different types of solvent systems and found that a fifteen-minute microwave heating period was enough to extract the same amount of pectin as obtained from the traditional three-hour Soxhlet extraction period.

Mexican lime bagasse and pomace have an important potential of industrial relevance, because these are by-products of the lime processing industry and are excellent sources to extract high amounts of value-added bioactive molecules. These applications can be promising outcomes from an environmental and economic point of view. In our preliminary studies lime residues were found to be a rich source of pectin and phenolic compounds. Therefore, two alternative processes for the combined recovery of pectin and polyphenols, which can easily be integrated in an existing pectin production process, were developed in the present study. Furthermore, these two Mexican Lime raw materials were physicochemically characterized.

2. MATERIALS AND METHODS

2.1. Raw Materials

Mexican lime (*C. aurantifolia* Swingle) bagasse and pomace samples were obtained from the States of Chihuahua and Guerrero, Mexico, respectively. Bagasse is the product obtained after juice extraction including seeds, while pomace is the bagasse that was subjected to washing steps and a blanching process. Both materials were dried at 40°C and milled with a food processor.

2.2. Preparation of Pectic Extracts from Lime Bagasse and Pomace

Four Pectic Extracts (PE) were obtained by two methods for both raw materials.

Conventional method: Dry mass (6 g) of Mexican lime bagasse or pomace was placed in an Erlenmeyer flask with 120 mL of 1% citric acid solution. The mixture was stirred during all the extraction at 90°C for 1 h. The mixture was then filtered through a cheesecloth funnel.

Microwave assisted method: Pectic extraction was carried out as described by Fishman *et al*. (2006). Dry mass (2 g) of bagasse or pomace were placed in a reactor with 60 mL of 1% citric acid solution. The mixture was stirred during the extraction at 120°C for 5 minutes at 800 watts in a Mars Xpress microwave (CEM, USA); the mixture was then filtered through a cheesecloth funnel.

2.3. Proximate Analyses

Raw materials were characterized by the determination of proteins, carbohydrates, lipids, crude fiber and ash according to methods AOAC, 1998.

2.4. Physicochemical Characterization of PE

Uronic acids concentration was determined spectrophotometrically according to the m-hydroxydiphenyl method developed by Blumenkrantz and Asboe-Hansen (1973). The Degree of Esterification (DE) was obtained according to Gee *et al*. (1959). Total sugars content was determinate spectrophotometrically according to the Dubois *et al*. (1956) method. Pectin yield was also determined as follows Equation (1):

$$\text{Yield of pectin}(\%) = (m_1 / m) \text{x } 100 \quad (1)$$

where, m_1 (g) is the dried product weight and m (g) is the dried raw material weight.

2.5. Polyphenols Extraction

For polyphenols extraction, bagasse or pomace was suspended in 80% acetone solution (1:4 w/w) and homogenized for 5 min using a laboratory blender. The slurry was filtered through Whatman No. 1 filter paper in a Buchner funnel. The filter cake was washed twice with the same solution and the filtrate was recovered and analyzed. The recovered filtrates for both bagasse and pomace were evaporated using a rotary evaporator (Büchi Laboratoriums-Technik AG-CH 9230 Flawil, Switzerland) at 40°C until less than 10% of the initial volume remained. The concentrated extracts were recovered with a final volume of 5 mL of methanol-1% acetic acid (1:1) and were kept frozen at -18±1°C until total phenolic content and HPLC analyses were done.

2.6. Total Phenolic Content

Total phenolic concentration in PE was determined using the Folin-Ciocalteu colorimetric method, as described by Singleton *et al*. (1999) with some modifications. As reference for the standard curve, gallic acid was used, with deionized water as solvent. Lime bagasse or pomace extracts (0.05 mL) were mixed with 3 mL deionized water and 0.2 mL Folin-Ciocalteu's phenol reagent (Sigma-Aldrich, USA). After 10 min, 0.6 mL 7% sodium carbonate solution was added and mixed. The mixture was incubated for 30 min at 37°C and then icecooled before absorptions were measured at 755 nm using a Perkin Elmer spectrophotometer (Lambda 25, USA). Absorbance values were compared with the standard curve to determine the equivalents of Gallic acid (mg Gallic acid equivalents/g dried extract).

2.7. HPLC Analyses

HPLC analyses were carried out using a Varian Pro-Star equipment (Palo Alto, CA, USA). A Varian Pro-Star 330 Photodiode Array (PDA) detector with detection at 280 nm was used. Fractionation of the injected material was carried out on a Varian C18 ODS column (5 µm, 250×4.6 mm) at 30°C, 3% acetic acid. Total run time was 25 min, equilibrium time 15 min and pressure ranged from 6 to 400 atm. Flow rate was 1 mL min^{-1} and the injection volume was 10 µL. Phenolic compounds in the sample were identified by the retention time using the following Sigma standards: hesperidin (USA) and naringin (China). For quantification and identification purposes, external calibration curves were prepared from standards. Other compounds were identified comparing with a database. Solutions were filtered through a 0.45 µm membrane filter (Millipore, Bedford, MA, USA) prior to injection. Peak integrated areas obtained from the chromatogram were plotted against known standard concentrations (naringin and hesperidin). Equations generated by linear regression were used to establish the phenolic compound concentrations in the samples.

2.8. Fourier Transform Infrared (FTIR) Spectroscopy Analyses

Mexican lime bagasse and pomace PE and commercial citrus pectin (Fagalab, Mexico, 70% GE) were investigated by Fourier Transformed Infrared Spectroscopy with Attenuated Total Reflectance (FTIR/ATR), which was done in a Perkin Elmer, Spectrum GX (USA) equipment operating at 4 cm^{-1} resolution. The mirror velocity was 0.08 cm^{-1} and 50 interferograms were co-added before Fourier transformation. Spectra were collected from 4000 to 650 cm^{-1}.

2.9. Gel Filtration Chromatography

The analyses were carried out on a Sephacryl S500 column. Pectic extracts (12 mg mL^{-1}) was filtered through 0.7 µm celullose microfilters and loaded (500 µL) to fractionate on the Fast Performance Liquid Chromatography system (Akta Prime, Amersham Buisciences). Column was equilibrated with NH_4Cl 0.1 M buffer (pH 4) and sample filtered (180 mL) with with 0.1 M NH_4Cl buffer, at a flow rate of 1 mL min^{-1}; 4 mL fractions were colected. Void and dead volume were determinated using Dextran blue 2000 and cobalt chloride, respectively. The obtained fractions were analyzed for uronic acids by the colorimetric method described above.

2.10. Antioxidant Activity

The antioxidant activity of PE was tested by three different methods, an oxidation inhibition assay using linoleic acid as the lipid source, a free radical scavenging assay 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) and a Fe^{2+} chelating assay.

Lipid oxidation inhibition assay: The lipid oxidation inhibition was performed using linoleic acid as the lipid source according to the method described by Starzynska-Janiszewska *et al*. (2008) with slight modifications. The linoleic acid solution was prepared by adding 0.56 g of linoleic acid and 1.5 g of Tween 20 to 8.0 mL of 96% ethanol. Each PE (50 µL) was mixed with 100 µL linoleic acid solution and 1.5 mL of 0.02 M acetate buffer (pH 4.0). Controls contained 50 µL of distilled water. All samples were mixed in a vortex and emulsions were incubated at 37°C; after 1 min, Then, 750 µL of 50 µM $FeCl_2$ solution (0.0994 g $FeCl_2$ and 0.168 g EDTA diluted to 1 L with distilled water) were added to induce the oxidation of linoleic acid. After the chosen incubation times (1 and 24 h), 1 mL of 0.1 M NaOH in 10% ethanol was added to 250 µL of the mixture to stop the oxidation process. After mixing, 2.5 mL of 10% ethanol was added and the absorbance measured at 232 nm against a 10% ethanol blank. The percentage of antioxidant activity was calculated according to Equation (2):

$$\text{Inhibition Percentage} = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right) / \text{abs}_{\text{control}}\right] \times 100 \quad (2)$$

DPPH scavenging capacity assay: The DPPH assay was carried out according to the methodology proposed by Molyneux (2004) 2.90 mL of 60 µM DPPH was added to 100 µL of each PE. The samples were placed in the dark and after 30 min the absorbance (Abs) was determined at 517 nm. Controls contained 100 µL of distilled water. PE, naringin, citric acid and gallic acid samples were prepared at 1 mg mL^{-1}. The radical scavenging capacity of the extracts was expressed as the DPPH percentage of inhibition using the same equation as used for the lipid oxidation inhibition assay.

Fe^{2+} chelating assay: The chelating activity of samples on Fe^{2+} was measured according to Heimler *et al.* (2007) PE (100 µL) of plant extracts or standards (1 mg mL^{-1}) were diluted up to 1.5 mL with 0.25 M acetate buffer (pH 4.75) and 25 µL of 2 mM $FeCl_2$ and 1 mL of the same solvent in which plant extracts or standards were dissolved, were added. The solution was incubated at room temperature for 20 min. After incubation, 100 µL of 5 mM ferrozine was added, the mixture was shaken and the absorbance was measured after 5 min at 562 nm; the same mixture but without the sample was used as blank. The ability of chelating ferrous ions was calculated according to Equation (3):

$$\text{Chelating activity \%} = \left[\left(A_0 - A_s\right) / A_0\right] \text{x} 100 \quad (3)$$

where, A_0 is the absorbance of the blank and A_s is the absorbance of the sample.

2.11. Film Forming Capacity Assay

The film forming capacity of pectic extracts was evaluated by preparation of thin films formed by evaporation of the casting solution. Crude pectic extracts (30 mL) were homogenized with 0.5, 1.0 or 1.5% glycerol and then were casted in a 150 mm Petri dish to let dry at 45°C for 24 h. The obtained films were peeled from the Petri dishes and stored at 20°C in a desiccator (50% RH). The dried films were visually inspected for appearance, homogeneity and integrity.

2.12. Statistical Analyses

All determinations were carried out in quintuplicate. Results are expressed as mean values ± standard deviation. Data were statistically analyzed using an ANOVA with a 95% of confidence. Data analysis was carried out using Minitab Statistical Software Version 16 (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSION

3.1. Mexican Lime Bagasse and Pomace Composition

Proximate composition of Mexican lime bagasse and pomace showed that there were significant differences among the two raw materials. The term “pomace” is applied to citrus peel which has been carefully dried after leaching with water at 50-60°C to lower the concentration of soluble sugars and acids (May, 1990; El-Nawawi and Heikal, 1996). As observed in **Table 1**, the composition of Mexican lime bagasse and pomace show significant differences on the different components analyzed; in most of the components, pomace has the lower values and lipid content is almost half of what is found in bagasse.

Approximately 20-30% of the total dry peel solids must be removed after leaching in order to produce the light-coloured pomace (Braddock and Crandall, 1978), therefore and due to the washing process, a reduction of soluble solids such as sugars, minerals, protein denaturalization (inactive enzyme) and lipid extraction may occur (El-Nawawi, 1995). El-Nawawi and Heikal

(1996) analyzed the properties of the leached liquid from orange pomace and found soluble solids such sugars, essential oil, flavonoids, denatured proteins and organic acids. Based on these reports, it is suggests that Mexican lime pomace probably contains more extractable pectin than bagasse. The flavedo (coloured-portion) of citrus fruits contains a considerable proportion of oil, which is usually removed by a physical method before the original shape of the citrus fruit is destroyed or after the juice has been extracted. Most methods cause oil cells to burst in a gentle manner to prevent absorption of the oil by the albedo (spongy and uncoloured-portion). The oil is washed off, usually with an excess of water and the oil is usually recovered by centrifugation before juice processing (May, 1990).

3.2. Physicochemical Characterization of Pectic Extracts (PE)

A total of 2 L of pectic extract was obtained for each raw material (bagasse or pomace) and each extraction method (conventional or microwave) and this extract was used to determine total sugar and uronic acid concentration, degree of esterification and pectin yield. Results are shown in **Table 2**.

Bagasse Microwave extract (BM) and Bagasse Conventional extract (BC) were not significantly different in total sugar content. However, both pomace extracts (PM and PC) were significantly different in total sugar, uronic acids and pectin yield. Pomace Microwave extract (PM) contained the highest total sugars and the highest uronic acids content, as well as the highest pectin yield (**Table 2**). Microwave radiation liberates the cell wall matrix (Kratchanova *et al*., 2004) and thereby the peel tissues are rapidly and extensively opened by the microwave treatment. This lead to an increased interaction between extracting solution and raw material in the extraction process; therefore, it leads to an effective increase in pectic extraction yield (Bagherian *et al*., 2011). As such, pectin yield of PM extract was twice the one obtained from BM extract. Due to the solids removed from lime bagasse in the production of lime pomace, the content of pectin increased and has an impact in pectin yield from this material.

According to the statistical analysis, the degree of esterification was not related to the extraction method or the raw material used, since all results were on the range of 65 to 73% degree of esterification. With this result, the pectin extracted is considered as a material with a high degree of esterification and is similar to the results reported by Fishman *et al*. (2006) who found pectin from several lime parts (albedo, flavedo and pulp) with values of DE from 59 to 75%. In our work, bagasse raw material consisted of flavedo, albedo and pulp including seeds.

On the other hand, total phenolic content, expressed as gallic acid equivalents is shown in **Table 2**. The concentration of phenolic compounds was significantly different among the four treatments. Bagasse microwave extract showed the highest total polyphenol concentration (41.43±0.04 mg g^{-1} extract [gallic acid equivalents dry basis]) followed by bagasse conventional extract (37.67±0.05 mg g^{-1} extract [gallic acid equivalents dry basis]). The lowest total phenolic content was in pomace conventional extract (34.00±0.02 mg g^{-1} raw material [gallic acid equivalents dry basis]). Wang *et al*. (2007) analyzed eight different citrus fruits and found total polyphonic contents that ranged from 37.3±1.5 to 75.9±3.8 mg g^{-1} (Gallic acid equivalents dry basis), the highest value was from the edible portion of lemon (*C. limon*). Guimaraes *et al*. (2010) found 124.63±0.52 in lime (*C. aurantifolia*) peels. In our study, the total polyphenol was in the range of results reported by Wang *et al*. (2007), but values were lower than those reported by Guimaraes *et al*. (2010). These differences are mainly due to the specific method used by the authors, to recover the polyphenolic fraction.

Table 1. Mexican lime Bagasse and pomace proximal composition on dry basis (g 100 g^{-1} material)

Composition	Bagasse [a]	Pomace [b]
Ash	0.30±0.00	0.20±0.00
Protein	7.20±0.04	4.30±0.02
Lipid	1.10±0.02	0.50±0.01
Crude fiber	15.6±1.50	24.1±1.00
Carbohydrate	75.8±2.11	70.9±1.13

Values followed by different lower-case letters are significantly different at $p<0.05$

Table 2. Pectic extracts characterization

PE	Total sugars (mg g^{-1} extract)	Uronic acids (mg g^{-1} extract)	Degree of esterification (%)	Pectin Yield (g 100 g^{-1})	Total phenolic* (mg g^{-1} extract)
PM	290.06±9.06 [a]	314.53±0.74 [a]	69.11±2.38 [a]	16.9±0.03 [a]	38.00±0.02 [a]
PC	234.94±4.56 [b]	230.84±38.70 [b]	72.79±2.70 [a]	15.1±0.02 [b]	34.00±0.04 [b]
BM	207.79±3.86 [c]	91.37±21.59 [c]	65.46±5.35 [a]	8.40±0.02 [c]	41.43±0.08 [c]
BC	209.30±2.21 [c]	131.37±6.70 [d]	68.44±3.24 [a]	13.3±0.03 [d]	37.67±0.05 [d]

PM = Pomace microwave extract; PC = Pomace conventional extract; BM = Bagasse microwave extract; BC = Bagasse conventional extract

Values followed by different lower-case letters are significantly different at $P<0.05$

* Total phenolic content expressed as gallic acid equivalents in dry basis

In our study, microwaved PE presented a higher polyphenol content than the PE obtained by the conventional method. Microwave energy can potentiate the bioavailability of free pharmacologically active natural compounds by preventing the binding of polyphenols to the plant matrix, because of the heating process in microwave that allows a homogeneous and instantaneous heating of all material (Robinson *et al*., 2009). Hayat *et al*. (2010a) found the highest amounts of total phenolic compounds in mandarin peels using a microwave method. They found that microwave extraction of phenolic compounds, increased by modifying the potency of the process, from 50.37±0.01 mg g^{-1} extract (250 Watts) to 58.08±0.01 mg g^{-1} extract (500 Watts).

3.3. Phenolic Compounds Present in Mexican Lime Bagasse and Pomace

The presence of different flavonoids in raw materials and PE are shown in **Table 3** and the respective flavonoids structures are given in **Fig. 1**. Only two flavanones were quantified using standards (hesperidin and naringin) for quantification. The presence of other flavonoids included coumaric acid in pomace raw material, catechin, resorcinol and methyl gallate in both raw materials (bagasse and pomace) were identified comparing with spectros obtained previously. A high intensity of pyrogallol signal was found in all PE. Ellagic acid and quercetin were found in both, raw materials as well as in PE. Ellagic acid is reported in literature for citric peels (Wang *et al*., 2007). Bocco *et al*. (1998) found only naringin and hesperidin in lime peels and several reports point out that hesperidin is the most abundant flavonoid in citric fruits (Rapisarda *et al*., 1998). The highest amount of flavonoids occurs in the peel and the main flavonoids glucosides found in citrus peel are naringin (Hayat *et al*., 2010b) and hesperidin (Londono-Londono *et al*., 2010). Poore (1934) reported that flavonoids glucosides are not leached from the peel, suggesting that they may be destroyed by the heat and mineral acids used during pectin extraction. However, El-Nawawi (1995) recovered naringin and pectin successively from grapefruit peel in a hot water leach at 88-90°C for 5 min. All samples except PM presented a higher naringin as compared to hesperidin; Wang *et al*. (2007) found naringin, hesperidin and neohesperidin as the main flavanones in eight different citric fruits, being hesperidin the most abundant. They also found quercetin as a flavonol found in high concentration in lemon (*C. limon*); in our study, we detected a peak corresponding to quercetin which remains in PE after treatment for pectin extraction.

Hesperidin content was higher in PE than Pomace raw material. Some polyphenols as hesperidin can react with other components of the vegetal tissue, interfering with pectin extraction, either by improving or decreasing pectin availability. It is well known that hesperidin can form a stable colloidal suspension with pectin; the specific interaction between those two molecules, it is possible because of the neutral sugars in the hesperidin molecule and in the polyuronide polymer of pectin (Rapisarda *et al*., 1998; Ben-Shalom and Pinto, 1999). Also, it has been demonstrated that the neutral sugars in pectin are involved in the specific interaction with hesperidin and this interaction can explain that hesperidin is recovered with pectin extraction from pomace (Cerna *et al*., 2003). There are many variables that can influence the behavior of polyphenols extracted along with pectin, but according to the conditions used, it was suggested that temperature and solvent type were the main factors related to polyphenol content in PE. In the case of microwave-assisted extraction, energy power and microwave application time influences are factors that affect the release of phenolic compounds from the raw material. Several studies has demonstrated that higher levels of these two factors can lead to degradation of flavonols, including naringin (Cerna *et al*., 2003; Fishman *et al*., 2006; Hayat *et al*., 2010a).

Table 3. Phenolic presence in raw materials and pectic extracts

Component	Pomace	Bagasse	PM	PC	BM	BC
Naringin	2.56 mAU	102.8 mAU	2.27 mAU	6.94 mAU	10.70 mAU	9.40 mAU
Hesperidin	1.92 mAU	55.2 mAU	7.12 mAU	6.77 mAU	8.19 mAU	8.01 mAU
Coumaric acid	*	ND	ND	ND	ND	ND
Catechin	*	*	ND	ND	ND	ND
Pyrogallol	*	*	**	**	**	**
Resorcinol	*	*	ND	ND	ND	ND
Methyl gallate	*	*	ND	ND	ND	ND
Quercetin	*	*	*	*	*	*
Ellagic acid	*	*	*	*	*	*

ND Not detected; * Presence; ** High intensity
PM = Pomace microwave extract; PC = Pomace conventional extract; BM = Bagasse microwave extract; BC = Bagasse conventional extrac

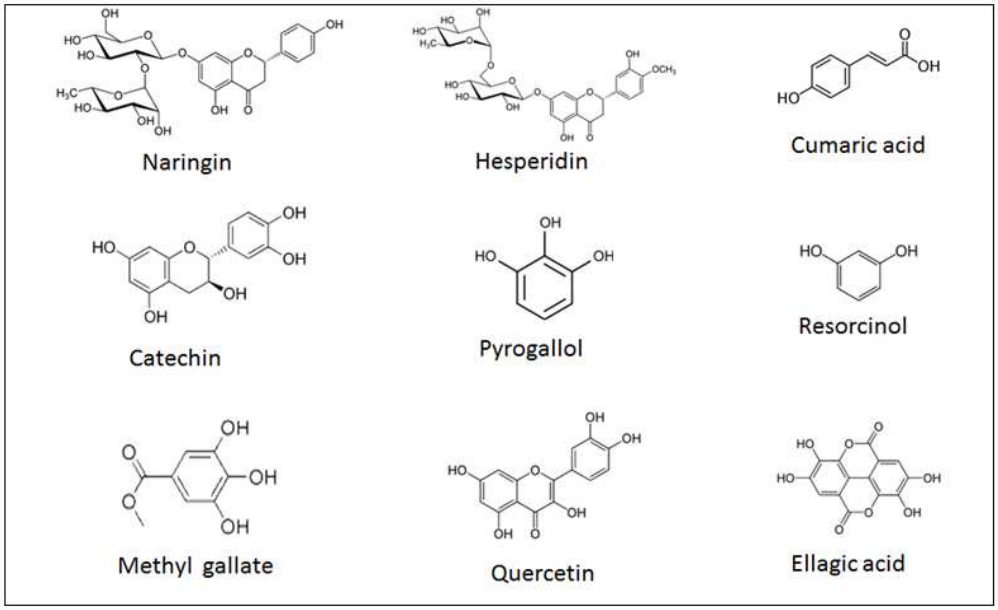

Fig. 1. Structures of flavonoids identified in pectic extracts from Mexican lime bagasse and pomace

3.4. Molecular Weight and Composition of the Polymers Present in the Pectic Extracts

In order to confirm the identity of the pectin from the extracts of Mexican lime, PE polysaccharides were analyzed by FT-IR and their spectra were compared against polygalacturonic acid and commercial pectin used as standard (**Fig. 2**). It was found that the FT-IR spectra of PE exhibited similarities on the absorption patterns to those of commercial pectin standards; therefore, it is confirmed that the polysaccharide extracted from Mexican lime bagasse and pomace is pectin. FT-IR spectra at wavelength between 950 and 1200 cm^{-1} are considered as the portion of the 'fingerprint' region for carbohydrates (Kalapathy and Proctor, 2001; Cerna *et al*., 2003).

Analysis of the FT-IR spectra revealed that the broader band of absorption between 3600 and 2500 cm^{-1} was due to O-H stretching whereas strong absorbance observed at 1730-1760 and 1600-1630 cm^{-1} were attributed to the ester carbonyl (CaO) groups and carboxyl ion stretching band (COO^{-}), respectively (Kamnev *et al*., 1998). In order to determine whether Mexican lime bagasse and pomace pectin is a low or high methoxy pectin, the FT-IR spectra of PE were compared against to commercial citrus pectin with 70% degree of esterification. The intensity of the absorbance of the ester carbonyl groups (1730-1760 cm^{-1}) increased as the degree of esterification was higher; in contrast, the intensity of the free carboxyl stretching band decreased (Manrique and Lajolo, 2002). The qualitative comparison of absorbencies at the two characteristic peaks of pectin from Mexican lime bagasse and pomace suggest that is a high-methoxyl pectin. Quantitative determination degree of esterification of Mexican lime bagasse and pomace PE is also reported (**Table 4**).

A relation between the integration of areas under the curve at two different wavelengths can be used to confirm if pectin found in the extracts is high-methoxyl pectin (Dorman *et al*., 2004), as described in the following formula:

$$DE = A_{1730} / \left(A_{1730} + A_{1600}\right) x\ 100$$

where, A_{1730} is area under the curve at 1730 cm^{-1} signal and $A_{1600\ si}$ area under the curve at 1600 cm^{-1} signal.

The qualitative comparison of the absorbance in both peaks characteristic confirms the degree of esterification detected chemically above and suggests the existence of high methoxyl pectins in PE.

Another important parameter in the description of pectin obtained from the Mexican lime bagasse and pomace, is the molecular weight of the pectin polymer and the distribution of the pectin compounds present, as determined by gel filtration chromatography (**Fig. 3**). Microwave pectic extracts (PM and BM) presented their higher peaks around fraction 26 to 28 corresponding to retention time of 104 min. Conventional PE (PC and BC) presented higher peaks around fraction 18 to 20 (72 min). This results indicates that PE obtained by conventional methods have a molecular weights close to 670 kDa and PE extracted with microwave technology, presented a depolymerization so that the most abundant component had a molecular weight of 12 kDa. This results also indicate that pomace extracts had higher pectin yields than bagasse extracts.

3.5. Antioxidant Activity

Citrus fruits are known for their rich sources of bioactive compounds, including organic acids, vitamin C, phenolic compounds and flavonoids, with potential health-promoting properties (Bocco *et al*., 1998; Kawaii *et al*., 1999; Manthey and Grohmann, 2001; Yapo *et al*., 2007; Wang *et al*., 2007; Gonzalez-Molina *et al*., 2010; Hayat *et al*., 2010b; Londono-Londono *et al*., 2010). The interaction of those compounds contributes to the overall antioxidant activity and it is difficult to measure total antioxidant activity on the basis of individual components (Yapo *et al*., 2007). Therefore, the antioxidant activity expressed in this study was in the form of total activity. Although numerous techniques are available to evaluate antioxidant activity, there is no single procedure capable of integrating the full set of mechanisms typical of an antioxidant (Frankel and Meyer, 2000; Lee *et al*., 2003). Three methods, DPPH scavenging, lipid oxidation inhibition and Fe^{2+} chelating activity, based on different principles were selected for measurement of antioxidant activity of pectic extracts. Results from antioxidant activity are shown in **Fig. 4** as percentage of inhibition or chelating activity.

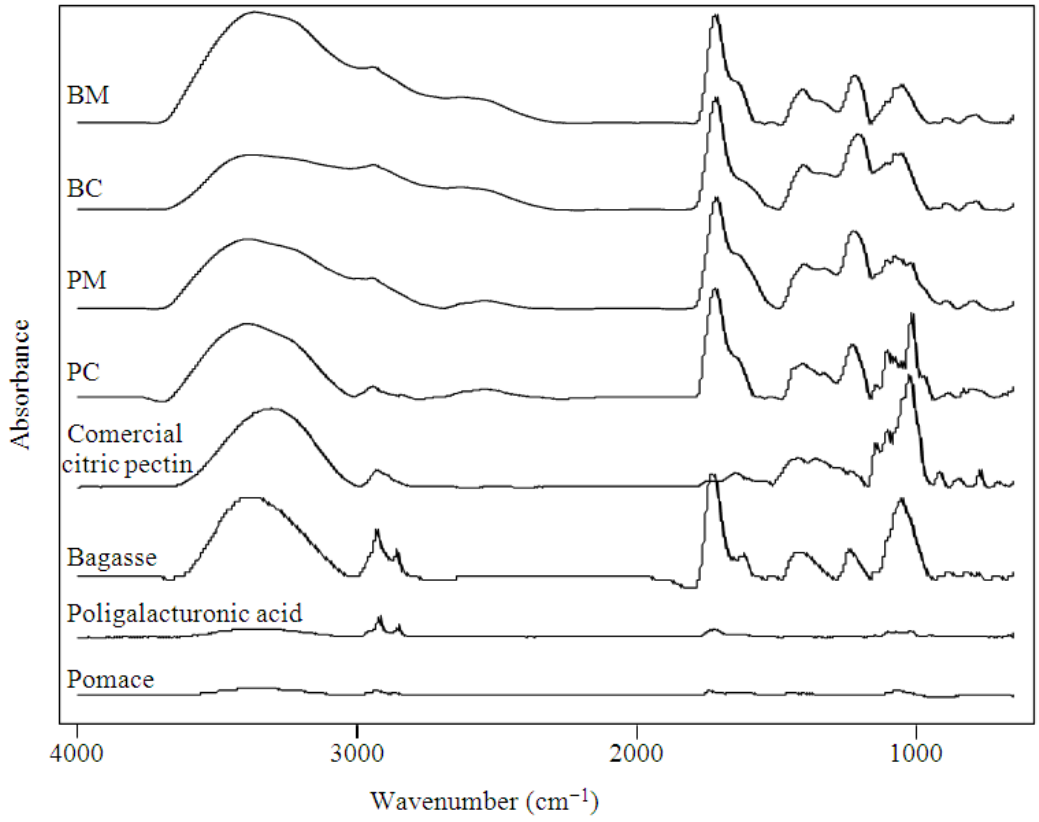

Fig. 2. FTIR/ATR spectrums for pectic extracts and raw materials from Mexican lime bagasse and pomace

Table 4. Degree of esterification estimation of PE by FT-IR measurements

PE	$A_{1730cm-1}$ (mAU)	$A_{1730\text{-}1600\ cm-1}$ (mAU)	DE* (%)	DE** (%)
PM[a]	92.33	132.70	69.58±0.95 [a]	69.11±2.38
PC[a]	62.61	90.62	69.09±0.49 [a]	72.79±2.70
BM[a]	107.24	174.51	61.45±0.38 [b]	65.46±5.35
BC[a]	161.23	230.35	69.99±1.01 [a]	68.44±3.24

* IR method esterification of degree; ** Titration method esterification of degree; A Area under a curve

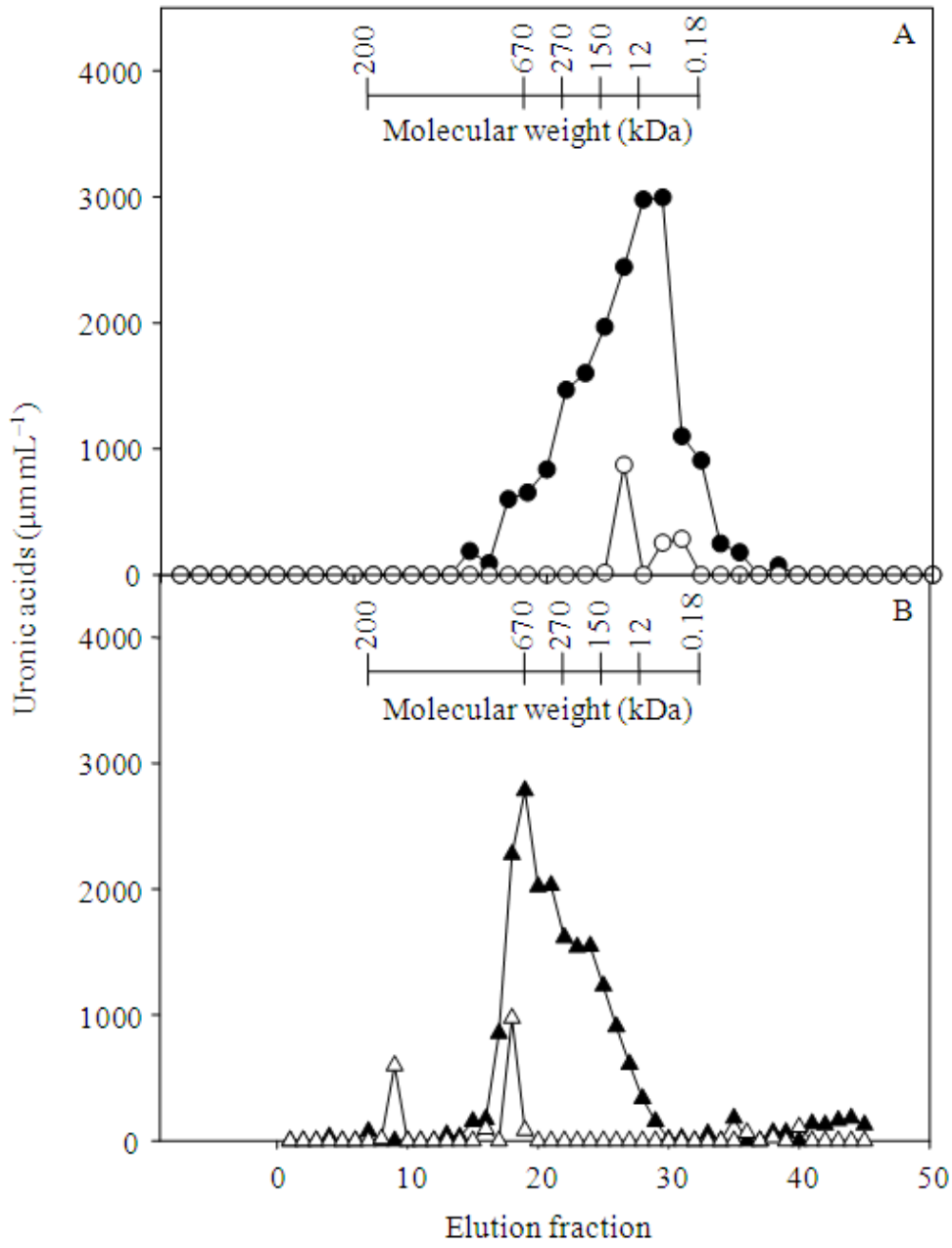

Fig. 3. Gel filtration profiles of pectic extracts from Mexican lime pomace and bagasse. Black circles, PM; Open circles, BM; Black triangles, PC; Open triangles, BC

Gallic acid standard showed excellent activity on all three antioxidant assays (>95%). Free radical scavenging is one of the most studied and reported mechanism of inhibition of lipid oxidation; The DPPH (1, 1,-diphenyl-2-picryl hydrazine) free radical scavenging assay is a rapid method and one of the commonly reported to characterize antioxidant activity of plant material (Lee *et al*., 2003). In this study, pectic extracts showed weak antioxidant activities in the DPPH assay (<10%) at 30 min reaction. Flavanones, such as naringin, due to the lack of conjugation provided by the 2,3-double bond with the 4-oxo group, are weak antioxidants (Zhou *et al*., 2006). This is consistent with the results of this study were it was found that naringin standard presented less than 40% of DPPH scavenging activity.

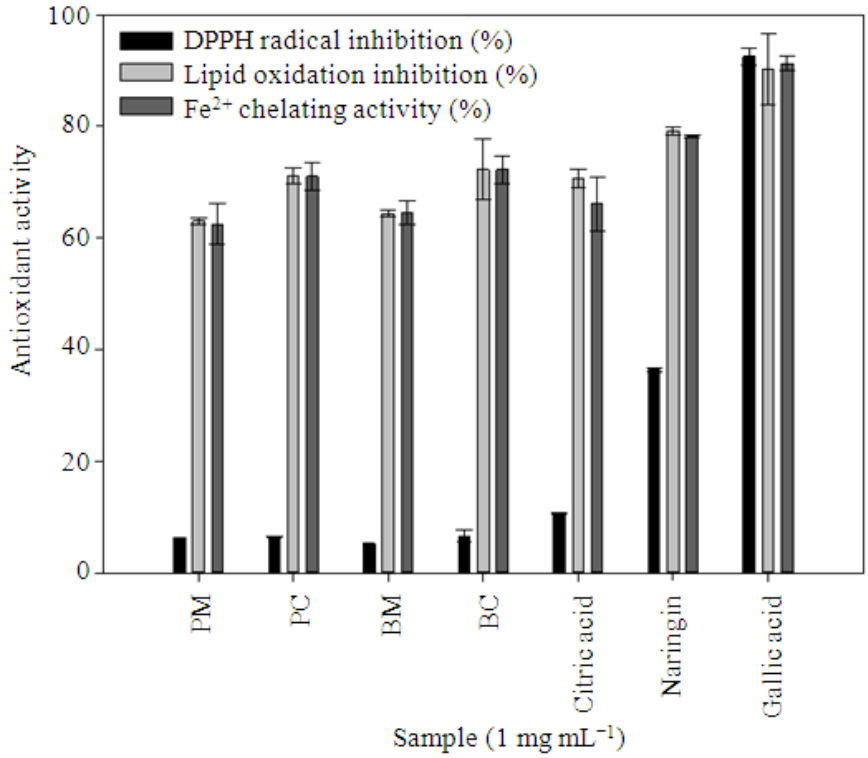

Fig. 4. Antioxidant activity comparisons of the four pectic extracts from Mexican lime pomace and bagasse. Citric acid, naringin and gallic acid are used as standards

All four pectic extracts presented similar antioxidant activities in lipid oxidation inhibition and Fe^{2+} chelating activity. Conventional pectic extracts (PC and BC) had better antioxidant inhibition than microwave pectic extracts (PM and BM), however, lipid oxidation and Fe^{2+} chelating activity did not have statistically significant differences among them; all the extracts had similar antioxidant activity as the naringin standard. The results of antioxidant activity by the three methods used, suggests that antioxidant components present in each of the four pectic extracts, could possess different mechanism of action to inhibit lipid oxidation.

The assay of inhibition of lipid oxidation simulates the oxidation of lipids in foods (Huang *et al*., 2005). It can be observed that the pectic extracts obtained by conventional extraction are capable of inhibiting linoleic acid oxidation with an effectiveness of 71.22±1.01 and 72.28±1.27% of inhibition (PC and BC, respectively). For the microwave PE lipid oxidation inhibition was lower (63.07±0.98% for PM and 64.37±1.29% for BM). Also, conventional pectic extracts bound ≥ 70% of Fe^{2+} and Microwave pectic extracts only ≥60%. Many studies on chelation of iron ions by isolated phenolic acids, flavonoids and anthocyanins, have established that suitably oriented functional groups in the structure of the ligand are essential for the formation of the metal ionphenolic compound complexes (Brown and Kelly, 2007). Phenolic compounds with a single OH group on the aromatic ring do not bind iron ions, as in the catechol group (as quercitin and hesperidin) or the galloyl group (trihydroxyphenyl) as in methyl gallate and pyrogallol (Zhou *et al*., 2006). Moreover, it is well known that pectin substances (soluble, insoluble fibers and modified pectin) bind through hydroxyl groups of uronic acids. Modified citrus pectin, alone or alginate combinations was used to decrease body heavy metal burden in patients Also, modified pectin has been used to decrease lead in children's blood serum levels. In this sense, Fe^{2+} chelating activity could be due a synergic effect between the phenolic compounds and the uronic acids presence of PE.

3.6. Film Forming Capacity Assay

Films obtained by using PE from microwave-assisted method (BM and PM) in the casting solution, had undesirable characteristics; with 0.5 and 1.0 % glycerol were very brittle films and with 1.5 % glycerol were very elastic and difficult to handle. On the other hand, films made from conventional method were easy to peeled off and handle (**Fig. 5**), presented good appearance, homogeneity and integrity. Microwave assisted PE, which have a lower molecular weight than the one obtained by conventional methods, was not able to form a matrix solution, appropriate for a latter film formation.

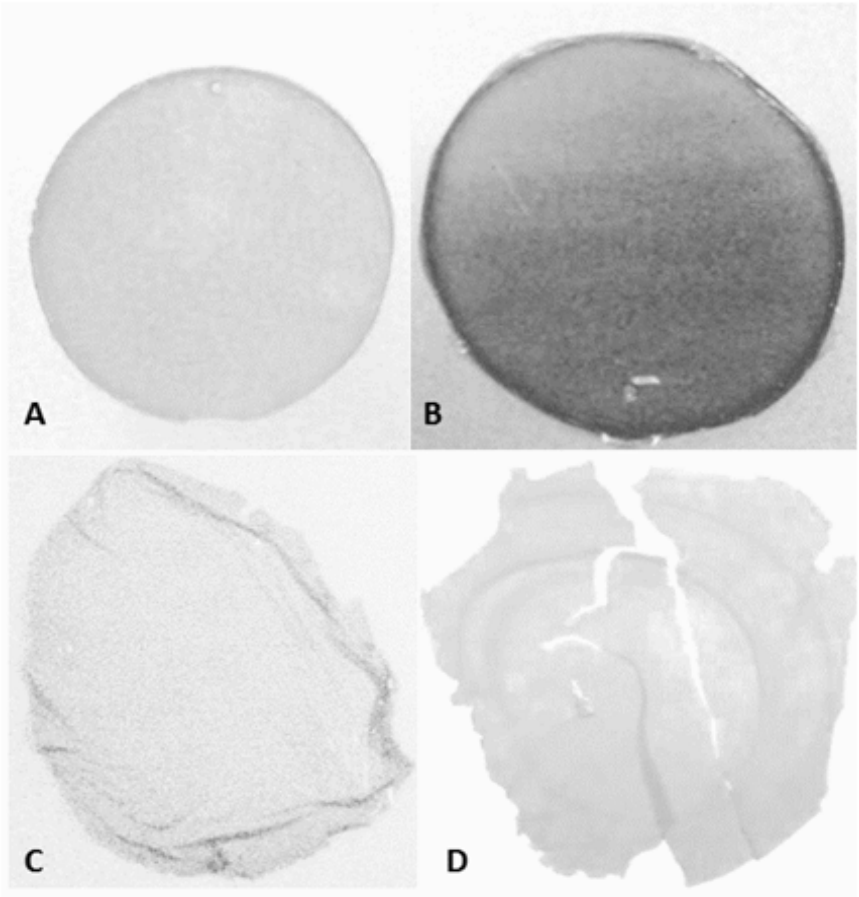

Fig. 5. Pectic extracts Film appearance. (A) PC 1.0 % glycerol, (B) BC 1.0 % glycerol, (C) PM 1.0 % glycerol and (D) BM 1.0 % glycerol

4. CONCLUSION

Conventional and microwave methods were used to obtain pectic extracts from two raw materials: pomace and bagasse from Mexican lime. The yield of pectin from pomace was greater than that from bagasse. The extraction method showed to have an impact on the molecular weight and polymerization of pectin compound. The polyphenols of the four pectic extracts from Mexican lime bagasse and pomace were also examined; pomace extracts showed the highest pectin yield and the lower polyphenolic content including naringin and hesperidin. Mexican lime Bagasse pectic extracts here presented, can have potential as raw material to form edible films and coatings.

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