Physicochemical properties of modified citrus pectins extracted from orange pomace

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Abstract Modified pectin is a polysaccharide rich in galacturonic acid altered by pH adjustment and thermal treatment used especially as an anti-cancer agent. The aim of this work was to study the physical and chemical properties of modified pectins extracted from orange pomace with citric and nitric acids. The galacturonic acid content, degree of esterification, Fourier Transform Infrared Spectroscopy profile, molecular weight, intrinsic viscosity, rheological properties and antioxidant activity of the pectins were evaluated. The modification process caused the de-esterification of pectins and a decrease of molecular weight due to removal of neutral sugars, maintaining the linear chain of galacturonic acid. Such changes also caused a significant increase in the *in vitro* antioxidant activity ($p \leq 0.05$) and influenced the rheological properties of pectin, reducing its viscosity. This work showed that the modification of pectin from orange pomace with citric

and nitric acids altered its structural and physical characteristics as well as its biological activity toward a free-radical.

Keywords Pectin · Modified pectin · Degree of esterification · Rheological properties · Antioxidant activity · Fourier transform infrared spectroscopy

Introduction

Brazil is responsible for about 30 % of the production of fresh orange and 60 % of the worldwide production of orange juice and, in 2010, Brazil produced 19,112,300 tons of oranges. Orange pomace is a byproduct from the orange juice industry and accounts for up to 50 % (w/w) of the fruit. The pomace is obtained after extraction of juice after two pressings which restrict the moisture content to around 65 to 75 %. The pomace is then subjected to drying to be pelletized and marketed. The pomace can be used in the manufacture of animal feed, the production of biscuits, flavorings or extraction of pectins, thus increasing its commercial value in the market and decreasing the industrial wastes.

Pectins are complex heteropolysaccharides on the cell wall of plants that provide consistence and mechanical resistance to vegetal tissues (Canteri-Schemin et al. 2005). Pectic polysaccharides are mainly composed of polymers rich in galacturonic acid, frequently with significant amounts of rhamnose, arabinose, galactose and around thirteen other different monosaccharides. Three major chains are recognized: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Fissore et al. 2009). The main chain of pectin may or may not be esterified with methyl-ester groups in the carboxylic acid units. Pectins are commonly classified according to their degree of esterification (DE) as high (HM) or low (LM) methoxyl pectin (Luzio Ga 2013), respectively, with a DE >50 % and <50 %. HM may produce a

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gel under acidic conditions with high sugar concentrations (Evageliou et al. 2000); whereas LM forms gels by the interaction of divalent cations, especially Ca^{2+} , between free carboxyl groups (Cardoso et al., 2003).

Pectin is mainly used as a gelling, thickening and stabilizing agent in different types of foods and beverages (Videcoq et al. 2011; Fissore et al., 2012). Additionally, it has many uses in the pharmaceutical industry, with great potential in the treatment against many diseases, such as obesity, diabetes, vesicle calculus, in addition to other health benefits associated with dietetic fibers (Liu et al. 2010). Modified pectins have also been used in cancer treatment, especially as an anti-cancer agent (Videcoq et al. 2011; Maxwell et al. 2012).

Commercial pectins are generally produced by hot acid extraction from orange and apple pomaces due to their high pectin polysaccharide contents (Guo et al. 2012). Pectin with galacturonic acid contents higher than 65 % are classified as having high purity (Liang et al. 2012). The chemical structure of pectins varies according to the source, environmental factors, conditions of extraction and modification techniques, which affect pectin yield and molecular characteristics, such as the degree of esterification, galacturonic acid content, molar mass and rheological behavior. Particularly, this complexity and variability of structure makes their characterization a difficult and important task.

Modified pectin is a polysaccharide altered by pH adjustment and thermal treatment, which breaks its chain into smaller fragments (Platt, 2009). The mechanisms involved are not well understood, although evidence suggests that pectin fragments with a small molar mass, but rich in galactose, bind themselves to the protein linked to galectine-3 (GAL3). This binding may block GAL3 interactions with other proteins and peptides, inhibiting their capacity to promote cell adhesion and migration hence preventing tumor growth (Glinsky and Raz 2009; Maxwell et al. 2012).

Although several studies have dealt with the importance of modified pectin in cancer treatment (Nangia-Makker et al. 2002; Jun Yan and Katz 2010; Maxwell et al. 2012), there is a lack of studies that deal with its physical and chemical properties.

The knowledge of the physical and chemical properties of pectin after modification, would propose new modification techniques, find new sources of extraction to obtain pectin with similar chemical structure with modified pectin, simplifying the process of obtaining and its high cost, and facilitate their application in other branches of the food industry, for example, in the production of functional foods because of their health benefits.

Therefore, the objective of this work was to investigate the physical and chemical properties of commercial and experimental citrus pectin obtained by different extraction methods, and modification process.

Material and methods

Raw material

Orange pomace was used as the raw material for the pectin extraction, and it was obtained from the pressing of the fruits obtained from the local market of Medianeira, Paraná State, Brazil. Nitric and citric acids (Merck, Brazil) were used to extract the pectin from the citrus pomace, and samples were named “nitric experimental pectin” (NEP) and “citric experimental pectin” (CEP), respectively. Commercial citrus pectin (CCP) was kindly supplied by CPKelco® (LI04050, Limeira- SP, Brazil) to compare the results. All reagents were of analytical grade.

Obtaining of the orange pomace flour

Pomace (15 kg) from oranges was dried to obtain the flour for pectin extraction. After extracting the juice orange, the orange pomaces were cut and subjected to bleaching by immersion into boiling water for 3 min, followed by cooling in an ice bath, for inactivation of enzymes (Kulkarni and Vijayanand 2010). The sample was dried at $55 \pm 5^\circ\text{C}$ for approximately 24 h in a drying cabinet with air-circulation until constant mass, and ground in a knife mill.

Pectin extraction

Pectins were obtained by acid extraction where citric and nitric acids were used as extraction solvents (Fig. 1). Extraction with citric acid was performed according to the methodology proposed by Canteri-Schemin et al. (2005), where approximately 50 g of flour was suspended in 1 L of acidified water ($\text{pH } 2.5 \pm 0.5$), with maceration for 30 min, room temperature. The pH was adjusted to 2.5 ± 0.5 using a 1 mol L^{-1} citric acid solution, before and after maceration. After maceration, this acid suspension was carried out to extraction at boiling temperature (97°C), by vigorously stirring for 30 min and the process was interrupted by immersion in a water-ice bath. Based on the methodology of Canteri et al. (2012), 50 g of flour was hydrated with distilled water for 10 min by magnetic agitation. The suspension was then completed with a solution of nitric acid, both at 80°C to obtain a final concentration of 0.05 M acid. The extraction was performed in a condensation system at 80°C for 20 min, and the process was interrupted by immersion in a water-ice bath.

Citric and nitric suspensions were then vacuum-filtered in synthetic tissue (silk cloth) and stored at 4°C . Two volumes of commercial ethanol 96 °GL were added to the filtered liquid to form a gel of pectin. The obtained gel was collected, conditioned in small cloth bags and immersed in acetone for approximately 15 h for the partial removal of the acid. The pectins were dried in a drying cabinet with air-circulation at 40°C for approximately 5 h, until a constant weight was

achieved (moisture between 8 and 10 %). Samples were ground, homogenized and sieved in order to obtain powdered pectin.

Modification of pectins

The pectins obtained by different extraction methods and the commercial citrus pectin were chemically modified as described by Platt, (2009) and Nangia-Makker et al. (2002) with some modifications. The powdered pectin was solubilized as a 1.5 % w/v- solution in distilled water, and its pH was adjusted to 10.0 by adding NaOH (3 mol L⁻¹). The mixture was stirred mechanically for 1 h at 55±3 °C. The solution was cooled at room temperature and the pH was adjusted to 3.0 with 3 mol L⁻¹ HCl and then stored overnight. Finally, the pectin samples were precipitated with two volumes of 95 % ethanol, filtered in synthetic tissue (silk cloth), washed with acetone and dried at 50°C.

Yield

The yield of pectin extraction was calculated as a function of the pectin mass obtained from the raw material (dry basis) used, according to Eq. 1:

$$\% \text{Yield} = \frac{M_{\text{pectin}}}{M_{\text{raw material}}} \times 100 \quad (1)$$

where, M_{pectin} is the pectin mass obtained and $M_{\text{raw material}}$ is the raw material utilized for extraction.

Galacturonic acid content

The galacturonic acid content of the pectins was determined using a spectrophotometer (PerkinElmer, MA, USA) at

520 nm by the alkaline m-hydroxydiphenyl method, according to a classical methodology outlined by Blumenkrantz and Asboe-Hansen (1973) using monohydrated D-galacturonic acid (Sigma, USA) as a standard.

Determination of degree of esterification

The degree of esterification was estimated by the methodology proposed by Bocek et al. (2001). Samples of dried pectin (0.05 g) were dissolved in 50 mL of distilled water for 12–15 h in a drying cabinet at 50 °C in closed flasks. The solution was titrated with 0.05 mol L⁻¹ NaOH until a pH of 8.5±0.2 was reached using a digital pH meter (Hanna, pH 21 pHmeter, Brazil). The used volume was named V_1 . The saponification process was carried out by adding 10 mL of 0.5 mol L⁻¹ NaOH for 30 min at 30°C in a drying cabinet. The solution was then neutralized by the addition of the same volume of 0.5 mol L⁻¹ HCl. The excess of HCl was titrated with 0.05 mol L⁻¹ NaOH, and the result was expressed as the final volume (V_2). The degree of esterification was calculated by Eq. 2:

$$\text{DE}(\%) = \left(\frac{V_2}{V_1 + V_2} \right) \times 100 \quad (2)$$

Determination of molar mass

The average molar mass of unmodified and modified pectin samples was estimated using the Mark Houwink-Sakurada equation (Eq. 3) (Arslan 1995).

$$\eta = K \times M^a \quad (3)$$

Fig. 1 Flowcharts of acid extraction: ^anitric acid ^bcitric acids

where, K ($L g^{-1}$) and a are constants; M ($g mol^{-1}$) is the molar mass and η ($L g^{-1}$) is the intrinsic viscosity defined according to Eq. 4:

$$[\eta] = \lim_{c \rightarrow 0} \left(\frac{\eta_r - 1}{C} \right) \quad (4)$$

where η_r is the relative viscosity (solution for solvent) and C ($g L^{-1}$) is the pectin concentration. Both constants K and a depend on the temperature and characteristics of the solvent and solute. In the case of the pectin solution in 0.1 M NaCl at pH 7.0, we may assume the value of K as $4.36 \times 10^{-5} L g^{-1}$ and of a as 0.78 (Garnier, Axelos and Thibault 1993). The kinematic viscosities of pectin solutions at different concentrations (among 0.3 and 2.0 $g L^{-1}$) were measured by a capillary viscometer Cannon Fenske ($n^\circ 100$) at 25°C¹¹. The intrinsic viscosity of pectins was calculated by fitting the experimental data to Huggins ($\eta_{red} = \eta + K_H \cdot \eta^2 \cdot C$) and Kraemer ($\frac{\ln(\eta_{red})}{C} = \eta + (K_H - \frac{1}{2}) \cdot \eta^2 \cdot C$) mathematical models (Table 1).

Rheological analysis

Non-oscillatory rheological analysis of the pectin solutions was performed in a Rheometer Brookfield (DV-III+), with spindle SC4-18, (Brookfield Engineering Laboratories, MA, USA), connected to a thermostatic bath for temperature control. Shear stress (τ) and rate ($\dot{\gamma}$) values were obtained by Rheocalc V 3.1-1 software (Brookfield Engineering Laboratories, MA, USA).

Pectins (1 $g L^{-1}$) were dissolved in 0.1 mol L^{-1} NaCl solution by mechanical stirring for 6 h at room temperature (Min et al. 2011; Liang et al. 2012). Flow curves of pectin samples were obtained at different temperatures of processing (10, 30 and 50°C). Each analysis had a duration of 4 min, with

40 points; whereas, 20 points were in the ascending curve (0–20 s^{-1}) and 20 points were in the descendent curve (20–0 s^{-1}). All flow curves of pectins at different temperatures were fitted to the Power Law model.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of unmodified and modified pectins were recorded on a Shimadzu, FTIR – 8300 spectrophotometer in the 4,000–500 cm^{-1} region using potassium bromide (KBr) pellets (Jiang et al. 2012).

Evaluation of the antioxidant activity of pectin samples

The free radical scavenging activity was assessed with the DPPH• (1,1-Diphenyl-2-picrylhydrazyl radical) method as previously described by Mensor et al. (2001). Seven different concentrations (0.025, 0.050, 0.125, 0.250, 0.500, 0.750 and 1 mg mL^{-1} in 0.1 mol L^{-1} NaCl) of the extract were used to perform the DPPH assay. A 0.3 mmol L^{-1} DPPH ethanolic solution (1 mL) was added to 2.5 mL of the sample and the mixture was vortexed at room temperature. After 30 min, the absorbance values were measured at 518 nm, and they were converted into the antioxidant activity percentage (AA%) using the following equation (Eq. 5):

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}} \right] \quad (5)$$

where, Abs_{sample} is the absorbance of the sample; Abs_{blank} is the absorbance of the ethanol (1.0 mL) mixed with the pectin solution (2.5 mL) and $Abs_{control}$ is the absorbance of the 0.3 mmol L^{-1} DPPH solution (1.0 mL) mixed with ethanol (2.5 mL). The EC_{50} (effective concentration) values were calculated by non-linear regression of plots where the abscissa

Table 1 – Intrinsic viscosity and molecular weight of pectins

		Pectin			Modified pectin		
		CCP	CEP	NEP	MCCP	MCEP	MNEP
Huggins	Intrinsic viscosity ($mL g^{-1}$)	329.82 ^{b*} ±0.01	300.82 ^c ±0.01	447.21 ^a ±0.01	242.98 ^d ±0.14	283.96 ^c ±0.002	228.51 ^d ±0.01
	Molar mass ($g mol^{-1}$)	93,937 ^b ±3,284	83,486 ^c ±3,434	138,787 ^a ±2,824	63,485 ^d ±757	77,528 ^c ±779	58,686 ^d ±2,325
	R ²	0.99	0.95	0.99	0.90	0.96	0.98
Kraemer	Intrinsic viscosity ($mL g^{-1}$)	369.07 ^a ±0.01	308.55 ^b ±0.01	337.06 ^{ab} ±0.01	268.16 ^c ±0.01	267.26 ^c ±0.007	225.97 ^d ±0.01
	Molar mass ($g mol^{-1}$)	108,499 ^a ±2,585	86,256 ^b ±5,242	96,589 ^{ab} ±3,057	72,043 ^c ±2,113	71,736 ^c ±2,450	57,855 ^d ±3,309
	R ²	0.97	0.76	0.93	0.98	0.95	0.99

* Each value is expressed as mean ± standard deviation of triplicate tests. Means within the same line with different letters are significantly different ($p \leq 0.05$), according to Tukey's Test.

CCP Commercial citrus pectin, CEP Citric experimental pectin, NEP Nitric experimental pectin, MCCP Modified commercial citrus pectin, MCEP Modified citric experimental pectin, MNEP Modified nitric experimental pectin

represented the concentration of tested samples, and the ordinate was the average percent of antioxidant activity. BHT (butylated hydroxytoluene) and alpha-tocopherol were used as antioxidant standards.

Activation energy measurement

Pectin samples (1 g L^{-1}) were dissolved in 0.1 mol L^{-1} NaCl solution. The apparent viscosity was calculated according to the methodology of Haminiuk et al. (2006). The effect of temperature on the apparent viscosity of fluids at constant shear rates may be described by the Arrhenius equation. The shear rate of 10.53 s^{-1} was chosen to calculate activation energy (E_a) of the pectin samples.

Statistical analysis

All of the experiments were done in triplicate except for the antioxidant activity analysis, which was done in duplicate. The data was analyzed using analysis of variance (ANOVA) by OriginPro 8.0 (OriginLab Corporation, Northampton, USA), and expressed as mean value and standard deviation, compared using Tukey's test at a 5 % confidence level.

Results and discussion

The yield of extraction with citric acid was 17.75 % and a mild condition of extraction with nitric acid was 10.9 %. Canteri-Schemin et al. 2005 stated that nitric acid is an excellent extraction agent. The pectin concentration in different materials vary quantitatively according to the source of raw material, but usually is between 2.9 and 22 % in apples; 9–30 % in lemons; 17 and 25 % in mangoes and 5 and 30 % in oranges (Koubala et al. 2008; Min et al. 2011; Rha et al. 2011).

High rates of pectin extraction by hot diluted acid, HCl or HNO_3 , is suggested as the best approach for production on an industrial scale (Canteri-Schemin et al. 2005; Liang et al. 2012).

The pectins without the chemical modification showed values of galacturonic acid of 70.00 ± 3.27 % for commercial citrus pectin (CCP), 54.86 ± 1.13 % for citric extraction (CEP) and 60.63 ± 2.29 % for the nitric extraction (NEP). On the other hand, for the chemically modified pectins, values were found for galacturonic acid of 87.82 ± 1.16 %, 56.10 ± 4.10 % and 62.03 ± 0.19 % for modified commercial citrus pectin (MCCP), modified citric experimental pectin (MCEP) and modified nitric experimental pectin (MNEP), respectively.

As specified by Food Chemical Codex - FCC, the standard of purity was found for commercial citrus pectin (CCP) used in this work. Nitric (NEP) and modified nitric pectins (MNEP) may also be considered of high purity, since a statistical

difference was not found ($p \leq 0.05$) when compared to the commercial citrus pectin. The content of galacturonic acid, which is predominant in the primary structure of the pectin (Ovodov 2009) was higher for nitric acid than for citric acid extraction.

Pectin modification increases the galacturonic acid content by the removal of impurities due to the treatment with hydrochloric acid, which enhances the solubilization of the minerals in the sample. The galacturonic acid content of modified commercial citrus pectin (MCCP) was significantly higher than that of unmodified pectin. The galacturonic acid content of citric (CEP) and nitric (NEP) pectins has not changed significantly with modification.

All pectin samples presented high methoxylation (DE >50 %). Commercial citrus pectin (CCP) had the highest degree of esterification (70.00 ± 0.65 %). Citric (63.11 ± 0.25 %) and nitric (59.92 ± 3.22 %) pectins were statistically different between them and CCP ($p \leq 0.05$). De-esterification promoted by citric acid in the extraction process was slightly lower than that promoted by nitric acid due the greatest strength of this acid.

Modified commercial citrus pectin (MCCP) had the degree of esterification (65.73 ± 0.52 %). Modified Citric (61.18 ± 1.41 %) and modified nitric (58.57 ± 0.23 %) pectins were statistically different of MCCP ($p \leq 0.05$).

The modification applied in this work caused the de-esterification of pectins commercial and citric. NaOH treatment during modification caused the de-esterification of pectins and replaced a methyl with a hydroxyl group (Fajardo et al. 2012). The reactions involved in the process of modification of pectin, the methyl ester and free carboxyl groups of pectin are replaced by a hydroxyl group.

The intrinsic viscosity of pectins calculated by data fit to Huggins and Kraemer mathematical models are showed in Table 1. The mathematical model of Huggins showed a better fit to the experimental data of unmodified pectins, whereas the model of Kraemer showed higher values of determination of coefficient (R^2) for modified pectins.

According to the Huggins equation, nitric experimental pectin (NEP) had the highest intrinsic viscosity followed by that of commercial citrus pectin (CCP), and citric experimental pectin (CEP). The chemical modification decreased the intrinsic viscosity of pectins, caused by the lower degree of esterification. The values of intrinsic viscosity of pectin modified with respect to unmodified pectins were statistically different ($p \leq 0.05$), except for CEP and MCEP.

The molar masses of the samples were 93,937; 83,486; 138,787 g mol^{-1} (Da), respectively, for CCP, CEP and NEP. These values are comparable to the molar mass of 140,68 Da for carrot and 78,60 Da of citrus pectin (Ngouémazong et al.

Fig. 2 FTIR spectra of the pectins

2012). The extraction conditions used in this work produced different types of pectins with different molar masses and conformations. The severe systems of extraction with citric acid (high temperature) are necessarily the explanation for both the low viscosity and low molecular weight (Canteri et al. 2012).

The chemical modification of pectins resulted in a decrease of their molar masses to 63,485, 77,528, 58,686 Da, respectively, this decrease has been touted to improve the intestinal absorption of nutrients (Courts 2013). Galacturonic acid content was not changed with the chemical modification; the

decrease in molecular mass can suggest that there was a partial removal of neutral sugars while the linear chain of galacturonic acid was not altered as suggested by Platt, (2009).

Pectins are highly heterogeneous with regard to their molar mass and chemical structure. The molar mass average of pectins from several fruit sources varies between 10^4 and 10^5 Da (Cui 2005), which is similar to the values in the current study.

FTIR spectra analyses identified important functional groups of unmodified and modified pectins extracted by the citric and nitric acids. These spectra were compared with the

Fig. 3 Flow curves of unmodified and modified pectins. ■ 10 °C ● 30 °C ▲ 50 °C

spectrum of commercial citrus pectin (CCP), which is shown in Fig. 2. All pectin FTIR spectra showed intense absorption at 800 and 1,200 cm^{-1} wave intervals, which is considered as the finger print region for carbohydrates and allows the identification of major chemical groups in polysaccharides as the position and intensity of the bands are specific for every polysaccharide (Nesic et al. 2011; Sivam et al. 2012). Since the FTIR spectra of pectins showed absorbance intensity standards similar to CCP, the polysaccharides extracted were confirmed as pectins.

The wide band of approximately 3,440 cm^{-1} is attributed to the distension of a $-\text{OH}$ group (Liang et al. 2012). Absorbance at approximately 2,900 cm^{-1} refers to distensions $-\text{CH}$, $-\text{CH}_2$ and $-\text{CH}_3$, methyl esters of galacturonic acid (Kowalonek and Kaczmarek, 2010; Liu et al. 2010).

Strong absorption reported at intervals of 1730–1760 and 1600–1630 is caused by distension $\text{C}=\text{O}$ of esterified carboxylic groups ($-\text{COOCH}_3$) and free carboxylic groups ($-\text{COOH}$), respectively (Nesic et al. 2011; Fajardo et al. 2012).

The FTIR spectrum of commercial citrus pectin (CCP) had a higher absorbance at 1,753 cm^{-1} than at 1,630 cm^{-1} , characteristic of the high degree of esterified pectin. The modified commercial citrus pectin (MCCP) also revealed the same behavior, unlike the other pectins with a higher absorbance at 1,630 cm^{-1} than at 1,745 cm^{-1} .

The ratio between the peak area of esterified carboxylic group and the sum of peaks of esterified and non-esterified carboxylic groups co-related linearly with the degree of methoxylation of pectin (Liang et al. 2012; Sivam et al.

2012). The ratio between these peaks shows that all pectins had high methoxylation ($\text{DE}>50\%$). CCP had the highest degree of esterification ($71.48\pm0.06\%$). Citric ($64.03\pm0.05\%$) and nitric ($62.72\pm1.06\%$) pectins were statistically different from CCP ($p\leq0.05$). The chemical modification decreased the values of the degree of esterification to 66.79 ± 0.12 , 62.03 ± 1.62 and 58.95 ± 0.08 for CCP, CEP and NEP, respectively.

Absorptions between 1,100 and 1,200 cm^{-1} in FTIR spectra correspond to the ether $\text{R}-\text{O}-\text{R}$ and cyclic $\text{C}-\text{C}$ ring links of the pectin structure (Liu et al. 2010).

Bands occur at 1,012 and 1,106 cm^{-1} indicating vibration of $\text{C}-\text{C}$ and vibration $\text{C}-\text{O}-\text{C}$ of backbone, respectively (Liang et al. 2012). Modified citrus commercial pectin had an increase in peak 1,106 cm^{-1} which is consistent with an increase in the galacturonic acid unit, while for while for other modified pectins, this peak was not altered.

In the Food Science and Technology field, aqueous solutions of polymers are a source of important materials. All flow curves of pectins at different temperatures are presented in Fig. 3. The mathematical fit showed higher values of R^2 , whereas, the parameters of the rheological model are presented in Table 2.

All samples showed pseudoplastic behavior due to the fact that the values of the flow behavior index (η) were lower than 1 for all temperatures, as reported by Sengkhamparn et al. (2010) and Bélafi-Bakó et al. (2012).

The consistence coefficients values were statistically different ($p\leq0.05$) for all pectins with an increase in temperature,

Table 2 Rheological parameters of pectins

Samples	Temp. ($^{\circ}\text{C}$)	Consistency coefficient K (Pas^n)	Flow behavior index n (ad)	R^2
CCP	10	$10.50^{\text{a}*}\pm0.50$	$0.91^{\text{a}}\pm0.02$	0.99
	30	$3.75^{\text{b}}\pm0.40$	$0.92^{\text{a}}\pm0.02$	0.99
	50	$1.53^{\text{c}}\pm0.02$	$0.97^{\text{a}}\pm0.01$	0.99
CEP	10	$24.33^{\text{a}}\pm5.75$	$0.68^{\text{a}}\pm0.06$	0.99
	30	$3.45^{\text{b}}\pm0.08$	$0.78^{\text{a}}\pm0.001$	0.99
	50	$1.73^{\text{b}}\pm0.01$	$0.77^{\text{a}}\pm0.003$	0.99
NEP	10	$11.93^{\text{a}}\pm0.83$	$0.83^{\text{b}}\pm0.02$	0.99
	30	$3.66^{\text{b}}\pm0.14$	$0.89^{\text{a}}\pm0.003$	0.99
	50	$1.85^{\text{b}}\pm0.001$	$0.90^{\text{a}}\pm0.006$	0.99
MCCP	10	$1.35^{\text{a}}\pm0.04$	$0.89^{\text{ab}}\pm0.005$	0.99
	30	$0.36^{\text{b}}\pm0.005$	$0.86^{\text{b}}\pm0.01$	0.97
	50	$0.17^{\text{c}}\pm0.007$	$0.92^{\text{a}}\pm0.01$	0.93
MCEP	10	$0.94^{\text{a}}\pm0.11$	$0.81^{\text{a}}\pm0.03$	0.99
	30	$0.69^{\text{ab}}\pm0.12$	$0.64^{\text{a}}\pm0.07$	0.95
	50	$0.38^{\text{b}}\pm0.07$	$0.65^{\text{a}}\pm0.07$	0.87
MNEP	10	$1.47^{\text{a}}\pm0.36$	$0.71^{\text{a}}\pm0.06$	0.99
	30	$0.58^{\text{b}}\pm0.06$	$0.68^{\text{a}}\pm0.04$	0.92
	50	$0.28^{\text{b}}\pm0.05$	$0.73^{\text{a}}\pm0.06$	0.93

* Each value is expressed as mean $\pm$ standard deviation of triplicate tests ($n=3$)

The mean values of consistency coefficient and flow behavior index of pectins, related to temperature variation, with different letters are significantly different ($p\leq0.05$) according to Tukey's Test

Table 3 Antioxidant activity of pectins

Unmodified	AA (%) [*]	EC ₅₀ mg mL ⁻¹	Modified	AA (%)	EC ₅₀ mg mL ⁻¹
CCP	11.30 ^b ±0.29	0.70 ^a ±0.02	MCCP	14.51 ^{ab} ±0.94	0.68 ^a ±0.03
CEP	13.44 ^{ab} ±0.72	0.77 ^c ±0.01	MCEP	14.92 ^a ±1.08	0.73 ^c ±0.02
NEP	13.14 ^{ab} ±0.29	0.73 ^b ±0.01	MNEP	15.17 ^a ±1.29	0.70 ^{ab} ±0.02

Each value is expressed as mean ± standard deviation of duplicate tests ($n=2$)

Means with different letters are significantly different ($p \leq 0.05$) according to Tukey's Test

^{*}The values of the antioxidant activity are represented in Table 3 at a concentration of 0.05 mg mL⁻¹. EC₅₀ values of BHT and α -Tocopherol (standards) were 0.10 and 0.12 mg mL⁻¹, respectively

according to the one-factor analysis of variance (ANOVA). The consistence coefficient values (K) decreased when the temperature increased for all pectins, with almost no changes in the flow behavior index. A similar behavior for citrus pectin was found by Masuelli (2011).

The chemical modification significantly affected the rheological behavior of pectins. Figure 3 shows that the flow curves of unmodified and modified pectins belong to distinct groups. When compared to the group of pectins without modification, the group of modified pectins had a fast shear-stress fall with an increase in the shear-rate values. After modification, decreases in the values of consistence coefficient (K) and flow behavior index were observed. This fact revealed changes in molecular structures and the non-Newtonian behavior of the sample (Steffe 1992). In the modified pectins, the consistence coefficient did not show a statistically difference at 10 and 30°C ($p > 0.05$) showing some independence with respect to the extraction method and solvents employed.

A decrease in apparent viscosity of the samples with an increase in shear rate and temperatures was observed (data not shown). The same behavior was reported by Agoda-Tandjawa et al., (2012) and Sengkhamparn et al. (2010). A distinction between unmodified and modified pectin groups was again observed in which the apparent viscosity was lower for modified pectins. A lower viscosity in the modified pectin is a positive factor meaning less energy expenditure during processing which does not require increased viscosity.

The viscosity of the samples decreased for all pectins when the temperature was increased. The decrease in viscosity can be attributed to an increase in intermolecular distances, because of the thermal expansion caused by the increase in temperature (Constenla et al. 1989).

Table 4 shows the activation energy calculated for all pectins, whereas the Arrhenius model properly described the relation of apparent viscosity and the inverse of absolute temperature at 10.53 s⁻¹. The activation energy values of the pectin samples were statistically similar ($p > 0.05$), except to the citrus pectin (modified and unmodified). The modification did not alter the Ea of pectins.

The antioxidant capacity of pectin samples was evaluated by the antioxidant methodology of the DPPH^{*}. The data were presented at a concentration of 0.05 mg/mL and EC₅₀ (mg/mL) values. According to the data of Table 3, the antioxidant activity (AA) of all samples increased with an increase in the polymer concentration. The chemical modification caused a slight increase in the antioxidant capacity of the pectins, which was also reported by Rha et al. (2011). This fact corroborates the fact that the antioxidant activity of pectin follows the same behavior of donating electron of phenolic compounds (Serrano-Cruz et al. 2013, Haminiuk et al. 2012). Indeed, the modification causes the de-esterification of the methyl-ester groups of the samples with an increase in the number of hydroxyls and consequent increase of antioxidant activity.

Table 4 Activation energy (Ea) values of unmodified and modified pectins

Pectins	Unmodified			Modified		
	CCP	CEP	NEP	MCCP	MCEP	MNEP
Ea (KJ mol ⁻¹)	36.49 ^{b*} ±1.07	50.37 ^a ±4.73	35.49 ^b ±1.32	38.75 ^b ±1.28	16.76 ^c ±1.12	31.10 ^b ±1.60
R ²	0.99	0.90	0.97	0.97	0.91	0.95

^{*} Each value is expressed as the mean ± standard deviation of triplicate tests ($n=3$)

Means with different letters are significantly different ($p \leq 0.05$) according to Tukey's Test

Conclusion

The results obtained in this study brought a new vision of the modification of pectin regarding to physicochemical and antioxidant properties. Comparing the modified and unmodified pectins of orange pomace, it can be seen that the modification process promoted the de-esterification of pectins causing the decrease in molecular weight due to removal of neutral sugars, maintaining its linear chain of galacturonic acid. Such changes caused a slight, however significant, increase in in vitro antioxidant activity and influence the rheological properties of pectin, reducing its viscosity.

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