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Chemical modification of citrus pectin: Structural, physical and rheologial implications

Aline Francielle Fracasso[a,*], Camila Augusto Perussello[b], Danielle Carpiné[a,*], Carmen Lúcia de Oliveira Petkowicz[c], Charles Windson Isidoro Haminiuk[d]

[a] Programa de Pós-Graduação em Engenharia de Alimentos, Departamento de Engenharia Química, Universidade Federal do Paraná, CEP 81531-980, Curitiba, PR, Brasil
[b] Department of Food Chemistry and Technology, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland
[c] Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, CP 19046, CEP 81531-990, Curitiba, PR, Brasil
[d] Programa de Pós-Graduação em Ciência e Tecnologia Ambiental, Departamento Acadêmico de Química e Biologia, Universidade Tecnológica Federal do Paraná, CEP 81280-340, Curitiba, PR, Brasil

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ABSTRACT

The present study aimed to investigate the physical, structural and rheological modifications caused by the chemical modification process of citrus pectin. Therefore, three commercial citrus pectins with different degree of esterification were chemically modified by sequential alkali and acidic hydrolytic process to produce modified citrus pectins (MCP) with special properties. The molar mass (M_w), degree of esterification (DE), monosaccharide composition, ^{13}C NMR spectra, homogeneity, morphology (SEM) and rheological behavior of both native and modified citrus pectins (MCP) were investigated. The chemical modification reduced the acid uronic content (up to 28.3%) and molar mass (up to 29.98%), however, showed little influence on the degree of esterification of native pectins. Modified citrus pectins presented higher amounts of neutral monosaccharides, mainly galactose, arabinose and rhamnose, typical of the Ramnogalacturonana-I (RG-I) region. Rheological tests indicated that the native and modified citrus pectins presented pseudoplastic behavior, however, the MCP samples were less viscous, compared to the native ones. Modified samples presented better dissolution in water and less strong gels, with good stability during oscillatory shearing at 25 °C. This study aims to better understand the implications that chemical modifications may impose on the structure of citrus pectins.

1. Introduction

Pectins are a family of structurally complex polysaccharides found on the primary cell walls and intracellular regions of plants where they have several roles [1]. They are extensively employed in the food industry as gelification agents [2–4]. Pectins consist of main fractions, or rather, the linear fraction, also known as homogalacturonan fraction (HG), and the branched fractions, also known as rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) [4–7]. The HG fraction is the smooth region of the pectin chains and consists of homopolymers of D-galacturonic acid bonded by α-(1 → 4) glycosidic links in which many acid groups are methyl-esterified. Depending on the plant, they may also be partially O-acetylated in C-3 or C-2 [1]. RG I consists of a main chain of alternating units of D-galacturonic acid and rhamnose respectively connected by α-(1 → 4) and α-(1 → 2) links to which neutral side chains of arabinan, galactan and arabinogalactan are bonded [8–10]. Other sugars that may be linked to lateral chains are D-xylose, D-glucose, D-mannose, L-fucose and D-glucuronic acids [9,11]. On the other hand, RG II is a complex pectic polysaccharide of low molar mass, formed by twelve different monosaccharides, including certain rare sugars such as apiose, acetic acid, 2-*O*-methyl-fucose, 2-*O*-methyl-xylose, 2-keto-deoxy-D-manno-octulosonic and 2-keto-3-deoxy-D-lyxo-heptulosaric acid (Dha) [12].

Commercial pectins are classified by the methoxylation degree: pectins with a high degree of methoxylation (HM) with ≥50% of methyl-esterified carboxylic groups form gels with a cosolute at pH lower than 3.5, and pectins with a low degree of methoxylation (LM) with <50% of methyl-esterified carboxylic groups form gels with calcium ions [2,13]. The methyl-esterification standard has

* Corresponding authors.
E-mail addresses: aline_fracasso@hotmail.com (A.F. Fracasso), daniellecarpine@gmail.com (D. Carpiné).

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relevant commercial implications due to their impact on the pectins functional properties [14,15].

Up to the moment, apple pomace and citrus peel are the major source of commercial pectins. The commercial citrus pectin has been used to obtain the modified citrus pectin (MCP). MCP is a dietetic supplement produced from citrus pectin via pH and temperature changes. This causes the breakage of pectin chains into fragments of low molecular weight and low esterification, which are more readily absorbed into the blood stream [16–19]. Further, it has also a great bonding affinity with galectin from the surface of cancerigenous cells which inhibit the aggregation of cancer cells and metastases [18]. Although several authors have already discussed the relevance of modified pectins [17,18,20,21] studies on their characteristics and properties are rare.

In the literature, they are few reported studies approaching structural, physical and rheological properties of chemical modification citrus pectins using advanced analytical techniques. Knowledge on the properties of MCPs broadens their applicability, especially in food and pharmaceutical industries. In this context, the objectives of this were to perform chemical modification of citrus pectin by alkali treatment and evaluate the chemical and physical properties.

2. Materials and methods

2.1. Raw material

The raw material used in this study comprised three commercial citrus pectins: two samples presenting high degree of methoxylation (greater than 50%, HM), namely S1-N and S3-N and one sample with low degree of methoxylation (less than 50%, LM), namely S2-N. These pectins were used as substrate for the proposed chemical modifications.

2.2. Purification of native commercial pectins

Samples of commercial pectins were dissolved in deionized water, precipitated in two volumes of ethanol and kept under refrigeration for 16 h. The precipitate was centrifuged, washed in ethanol and dried in a vacuum oven (EDGCON 5P) at 25 ± 1 °C, obtaining the samples of native pectins.

2.3. Modification of pectins

Citrus pectin was initially dissolved in a 1.5% solution in distilled water and pH raised to 10.0 with NaOH (3 N) and incubated for 1 h at 50–60 °C. The sample was then cooled up to room temperature and its pH adjusted to 3.0 with HCl (3 N). After being stored overnight, the samples were precipitated the next day with ethanol 95% and incubated at 20 °C during 2 h. The material was filtrated, washed with acetone and dried in a vacuum buffer at 25 °C [20]. The modified pectins were denominated S1-MCP, S2-MCP, S3-MCP, referring to the native samples S1-N, S2-N and S3-N, respectively.

2.4. Chemical characterization of native and modified citrus pectins

2.4.1. Determination of uronic acid content and monosaccharide composition

Dosage of uronic acid was performed by the colorimetric method, using described by Blumenkrantz and Asboe-Hansen [22], with galacturonic acid as standard, in triplicate. The monosaccharide composition was performed following the methodology of Vriesman and Petkowicz [23]. The native and MCP pectin samples were hydrolyzed with trifluoroacetic acid 2 M (6 h, 100 °C) and, after the drying the chloroform phase containing alditol acetates, the samples were re-dissolved in acetone for liquid-gas chromatograph.

2.4.2. Determination of molar mass (M_w)

The molar mass was determined by dissolving the samples in a solution of sodium nitrite ($NaNO_2$) 0.1 mol L^{-1} containing sodium azide (NaN_3) 200 mg L^{-1}, at, and filtered in a 0.22 μm porous cellulose acetate membrane (Millipore). Samples were injected in a high-performance size-exclusion chromatograph (HPSEC-Waters), equipped with a differential Refractive Index (RI) detector (Waters 2410) and multiangle laser-light scattering (MALLS) detector (Wyatt Technology, Dawn DSP) with 18 series locked channels. The calculations of dn/dc molar mass were performed using the software ASTRA 4.70.07.

2.5. Structural analysis

2.5.1. Nuclear magnetic resonance spectroscopy (RMN)

The ^{13}C RMN spectra of commercial and modified pectin samples were obtained using a BRUKER AVANCE DRX-400 spectrometer incorporating Fourier transform, at 70 °C. Displacements (δ) were given in ppm relative to resonance of CH_3 of acetone used as internal standard (^{13}C, δ 30.2).

2.5.2. FTIR analysis and degree of esterification (DE)

Fourier transform infrared spectroscopy (FTIR) analyses were carried out using a Vertex 70 (Brucker) spectrophotometer, at the absorbance mode in the frequency range of 4000–400 cm^{-1}, according to Vriesmann and Petkowicz [23]. The spectral values of the samples were means obtained by 32 scans at a resolution of 4 cm^{-1}.

FTIR spectra were obtained in triplicates and the areas corresponding to the esterified carboxylic groups, COO—R (1749 cm^{-1}) and to free carboxylic groups, COO— (1630 cm^{-1}) were used to determine the degree of esterification, according to Eq. (1). Since DE is defined as the (number of esterified carboxylic groups/number of total carboxylic groups) × 100, it is inferred that the ratio of the bands (Eq. (1)) should be proportional to the degree of esterification [24]. In order to quantify the% of DE of native and modified pectins, a calibration curve was constructed based on pectin standards of known% of DE.

$$DE(\%) = \frac{A_{1749}}{(A_{1749} + A_{1630})} \times 100 \quad (1)$$

2.5.3. Scanning electron microscopy (SEM)

Commercial and modified samples were analyzed by scanning electron microscopy (SEM) – JEOL 6010LA. Samples were vacuum-metalized with gold-palladium and the microscopic images of the mixtures elucidated the network characteristics, following Almrhag et al. [25]. SEM produced highly amplified (up to 300.000×) and high resolution images.

2.6. Rheological analysis

Polymer solutions of commercial and modified pectins were prepared in water at a 5%. Samples were solubilized in deionized water for 16 h at room temperature. Polymer solutions were kept at rest for 1 h and then used to the rheological analyses using a Haake Mars Rheometer coupled to a thermostatic bath Haake K 15, a water thermocirculator DC5 and a thermal controller TC 81 (Peltier) using a cone-plate (C60 2Ti) and plate-plate (PP 20 Ti; PP 35 Ti) sensors. Inertia was determined before perform the rheological analyses to subtract the values of centrifuge and centripetal forces produced during the experiments. The analyses were carried out at 20 ± 1 °C, in triplicate.

Table 1
Monosaccharide composition of native and modified pectins.

Monosaccharide (%)	Samples					
	S1-N	S1-MCP	S2-N	S2-MCP	S3-N	S3-MCP
Rha[a]	4.5	2.1	1.7	4.6	3.6	1.4
Xyl[a]	2.3	0.8	2.3	2.9	–	–
Man[a]	1.6	3.5	2.8	2.6	–	1.7
Gal[a]	7.0	18.0	12.0	15.1	8.5	10.0
Glc[a]	11.1	11.2	5.4	10.9	2.9	7.0
Fuc[a]	1.2	–	–	1.1	–	4.3
Ara[a]	9.0	19.1	3.3	3.7	3.6	12.0
Uronic acids[b]	63.3	45.4	71.8	59.2	80.1	63.2

S1-N (DE of 64.1%), S2-N (DE 47.7%) and S3-N (DE 58.3%): native citrus pectin samples.
S1-MCP, S2-MCP and S3-MPC: modified citrus pectin samples.
[a] Neutral sugars determined by gas chromatography, where: Rha = rhamnose; Fuc = fucose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose.
[b] Uronic acids determined according to Blumenkrantz and Asboe-Hansen [23].

Stress sweep at 1 Hz was initially performed to verify linear viscoelastic region and the selection of stress that would be employed in the oscillatory frequency sweep and temperature ramps to preserve the gelís structure. Frequency sweep was carried out in a pre-selected stress, with an increase of oscillatory frequency over time, ranging between 0.01 and 10 Hz. The flow curves were obtained at 25 °C with the shear rate ranging to 0.001 from 300 s^{-1}. The flow curves of the pectin solutions were fitted by the Power Law model (Eq. (2)):

$$\tau = K\gamma^n, \quad (2)$$

where: τ is the shear stress (Pa), γ is shear rate (s^{-1}) K is the coefficient of consistency (Pa s^n) n is the flow behavior index (dimensionless).

2.7. Statistical analysis

Data were assessed by analysis of variance ANOVA and Tukey's test ($p \leq 0.05$). Results were given in means ± standard deviation.

3. Results and discussion

3.1. Chemical characterization of native and modified pectins

Table 1 presents the results of the monosaccharide composition of native (S1-N, S2-N and S3-N) and modified citric pectins (S1-MCP, S2-MCP and S3-MCP). Uronic acid was the main component of pectins, with higher values for native ones (63.3, 71.8 and 80.1%) than for modified pectins (45.4, 59.2 and 63.2%). A decrease of uronic acid content can be attributed to the chemical β-elimination, one of the mechanisms of nonenzymatic degradation of pectins, which occur mainly in alkali conditions. This reaction cleaves and de-esterifies the homogalacturonan (HG) backbone, generating oligomers of polygalacturonic acid and rhamonogalacturonan I (RG-I) fractions [17]. This trans-elimination reaction that proceeds on uronic acids can results in the removal of activated hydrogen atom at C-5 and the glycosidic residue at C-4 of galacturonic acid residues, leading to un-stable, intermediary compounds, as unsaturated compounds [26,27]. These secondary reactions products can be indicated by absorbance changes at 235 nm [28–30]. The methodology applied in this study was performed by the colorimetric method at 520 nm and a decrease in the uronic acids content, can suggest that it have been partly degraded in β-eliminative products, as unsaturated compounds that absorbs in another wavelength.

Samples of modified pectins had a higher galactose concentration (18.0% for S1-MCP; 15.1% for S2-MCP; 10% for S3-MCP) when compared to native ones (7.0; 12.0%, 8.5; respectively). Overall, galactose and glucose was the major neutral sugar pectin in all samples, followed by arabinose and rhamnose, indicating that neutral sugars are predominantly arabinogalactans. In the branched regions of pectins, xylogalacturonan structure could also be present. The same was also observed by Schols et al. [11]. In fact, xylose was present in S1 and S2 samples (native and modified). Albersheim et al. [31] reported that pectins in the edible parts of fruits and vegetables may only constitute the HG and RG-1 regions, with Rha-GalA sequence. The statement seems to be true for the analyzed samples since there is the possibility that they also contain xylogalacturonan regions.

Fucose, a rather rare sugar in pectins, was also identified, besides the sugars which constitute the pectin structure. According to Kravitchenko et al. [32] the sugars arabinose, galactose, xylose and glucose are bonded to carbon 4 of rhamnose, even though the first three were linked to the units of galacturonic and fucose acids. The latter is a rare sugar but detected in some pectin compounds without the link site being determined. The presence of glucose suggests that starch may exist in the samples at low proportions, which was confirmed by the lugol test. Brigand et al. [33] also found starch in the pectins of apple pomace. Considering that the degree of esterification is greater than 50%, these data suggest that the structure of native and modified pectins may consist mainly of a homogalacturonana (HG) and the highly esterified ramnogalacturonana type I (RG-I) with side chains of galactans or arabinogalactans.

The sample S2 presented a different behavior from the other samples with respect to the sugars rhamnose, xylose and arabinose. In this case, the chemical modification caused an increase in the proportion of rhamnose and xylose sugars, while the arabinose content was practically unchanged. This difference in the behavior of the monosaccharide composition of sample S2 can be attributed to the pectin extraction process. Levigne et al. (2012) concluded that the extraction conditions (pH, time and temperature of extraction) have important effects on the characteristics of the extracted pectin (neutral and acidic sugars, degrees of esterification, physicochemical properties and intrinsic viscosity). The authors verified that pectin samples extracted at pH 3.0 presented lower amounts of rhamnose than at other conditions (pH 1 and 2) [34]. Furthermore, the type of acid extractant influenced the physicochemical parameters of pectins [35]. In our study, the commercial pectin S2-N presented the lowest content of rhamnose (1.7%) compared to the other native samples (4.5% and 3.6%, S1-N and S3-N, respectively), which may suggest that the conditions of extraction were probably different. Therefore, the frequency that rhamnose interrupt the HG sequence in the S2-N pectin macromolecule was lower than the other samples (S1-N and S3-N). Thus, extraction method used in S2-N native pectin may have caused structural modifications, which directly influenced the monosaccharide composition obtained after chemical modification. In all these cases the monosaccharide content increased after the chemical modification, while the galacturonic acid content was reduced.

3.2. FTIR spectra

The FTIR spectra of native citrus pectin and modified citrus pectin are presented in Fig. 1. A slight difference in the structure and composition of a molecule may cause significant alterations in the intensity of absorption peaks in FTIR [36]. The spectral data were analyzed by comparing the following characteristics regions: ester carbonyl (1760–1645 cm^{-1}) and carboxylate ions stretching bands (1640–1620 cm^{-1}); C—H stretching (3000–2800 cm^{-1}) and O—H stretching (3600–2500 cm^{-1}), which reflect common groups of pectin composition.

The examination of the spectral regions revealed the existence of two bands occurring at 1760–1745 cm^{-1}, and at 1640–1620 cm^{-1}, which were assigned to the ester carbonyl groups

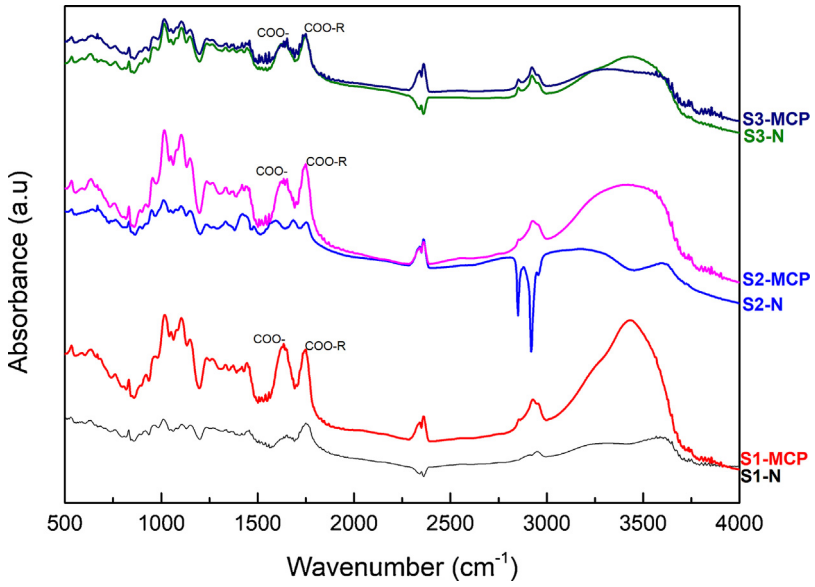

Fig. 1. FTIR spectra of natives (S1-N, S2-N and S3-N) and modified citrus pectins (S1-MCP, S2-MCP and S3-MCP).

Table 2
Degree of esterification of native and modified pectins.

Samples	Native citrus pectin (%)	MCP (%)
S1	64.1 ± 6.2^{a}	53.0 ± 2.7^{b}
S2	47.7 ± 6.8^{a}	55.7 ± 5.8^{a}
S3	58.3 ± 9.0^{a}	57.1 ± 3.4^{a}

Different letters on the same line indicate statistically significant differences among the samples, according to Tukey's test ($p \leq 0.05$).

(COO—R) and the carboxylate ion stretching band (COO—), respectively. The correlation between the intensity of carbonyl and carboxyl groups established the basis for the quantitative analysis of degree of esterification (DE) of pectins by FTIR. The intensity of the absorbance of the ester carbonyl groups (1760–1645 cm^{-1}) increases along with the DE, in contrast with the decrease in the intensity of bands related to carboxyl ion stretching groups (1640–1620 cm^{-1}). In the same way, the intensity of the absorbance of free carboxyl groups increases with lower DE [24]. It was observed that the FTIR spectra of modified S1-MCP (native DE: 64%) and S2-MCP (native DE: 47.7%) citrus pectin samples, revealed more intense bands at 1760–1730 cm^{-1} (esterified carboxylic groups) and at 1630–1600 cm^{-1} (free carboxylic groups) compared to the native ones (S1-N and S2-N).

Table 2 shows mean values of the degree of esterification of native and modified pectins. Commercial native citrus pectins S1-N and S3-N were classified as pectins of high degree of methoxylation (HM), i.e, pectins containing ≥50% of methyl-esterified carboxylic groups, with DE values of 64.1% and 58.5%, respectively. By the other hand, commercial native citrus pectin S2-N presented DE <50% (47.7%), being considered a pectin of low degree of methoxylation (LM).

After the chemical modification, a statistically significant reduction of 17.3% on DE ($p \leq 0.05$) was observed in sample S1-N. This result agrees with those found in the FTIR spectrum, where the free carboxylate groups (COO—) band was slightly higher than the esterified carboxylic band. This behavior corroborates with the study of Wai et al., where the free carboxylate groups increased in modified durian rind pectin [20]. However, in the S2-N sample it was not observed a significant reduction in DE after the chemical modification, probability because the esterified carboxylic groups predominated over the free carboxylate groups. Thus, for the S1 sample the differences in% DE indicate the occurrence of successful modifications.

Bands around 3000 and 2800 cm^{-1} were observed in the spectra (C—H absorption), which is an indicative of the presence of —CH, $—CH_2$ and $—CH_3$ methyl esters of galacturonic acid [36,37]. Bands around 3600 and 2500 cm^{-1} refers to O—H stretching absorption and in the case of pectins, they can be attributed to the intra and intermolecular hydrogen bonding of the galacturonic acid backbone [36,38]. The O—H stretching vibrations can indicate several features of a compound, including free hydroxyl groups stretching bonds that occur in samples in vapor phase and bonded O—H bands of carboxylic acid [39]. Thus, an increase in the O—H stretching band is related to the increase of hydroxyl groups in pectin samples after chemical modification, behavior clearly seen in samples S1 and S2.

The bands detected between 1100 and 1200 cm^{-1} represent R—O—R ether bonds and C—C cyclic bonds present in the ring structure of pectins. An increase in the intensity of these peaks was observed in modified citrus pectins with similar molecular weight, which could indicate the presence of more cyclic chains and the reduction of pectin ramifications.

Based on the FTIR spectra obtained for the native and modified pectins, it be concluded that the chemical modification caused structural differences in the pectin molecules, observed mainly by the proportion of esterified carboxylic groups and free carboxylic groups. However, the reduction in the degree of esterification was only observed for the pectin with higher degree of methoxylation (S1).

3.3. Structural analysis

3.3.1. ^{13}C RMN spectroscopy

Pectin samples were analyzed by ^{13}C RMN spectroscopy after solubilization in D_2O. Fig. 2 shows the ^{13}C RMN spectra for the native and modified sample 1 (S1-N and S1-MCP, respectively). Several common signs and similar results for MCP and native pectin were observed. The signals at 170.6 ppm and 173.5 ppm respectively correspond to the methyl-esterified and free carboxylic groups, whereas that at 52.8 ppm represent the methyl-ester groups of the carboxylic groups. Comparing the spectra of native pectin (S1) and modified pectin (S1-MCP), it is possible to notice that the methyl esterified group (170.6 ppm) had its signal reduced after the chemical modification. This means that after the modifi-

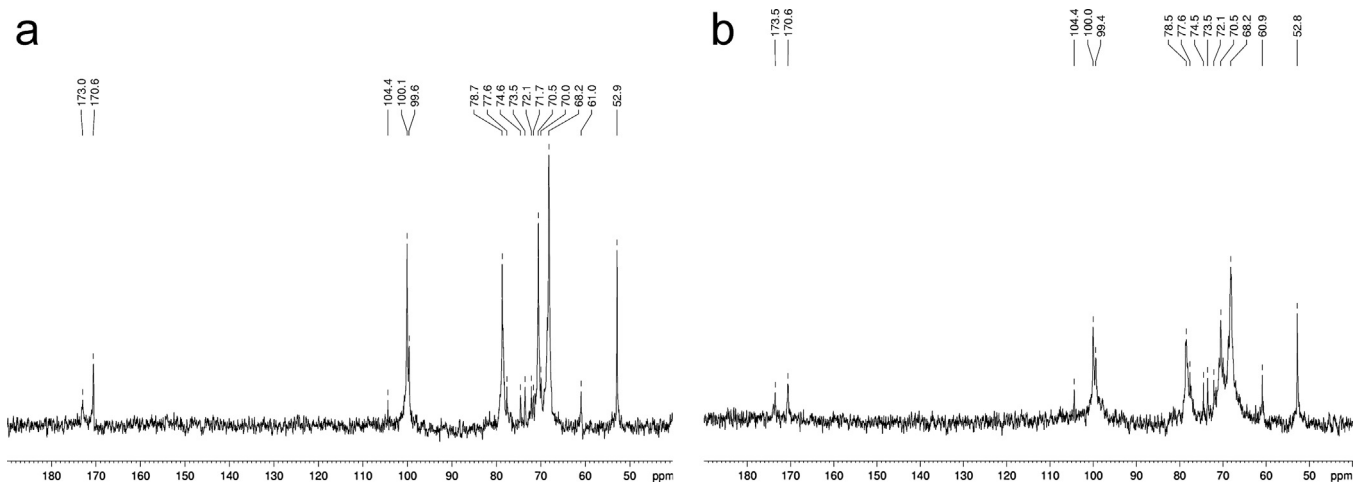

Fig. 2. Spectra of ^{13}C RMN (70 °C, 100 MH_z) for citrus pectin: (a) S1 native and (b) S1 modified. *Chemical displacements in ppm.

cation there was a reduction in the esterified groups, which may justify the reduction in the degree of esterification of the modified sample (64.1% to 53.0%, S1-N and S1-MCP, respectively). The spectrum obtained corroborates to the results obtained in the FTIR analysis.

Further, α-D-galacturonic acid, corresponding to C-1 of the esterified and non-esterified units, was evidenced by signs of 100.0 ppm and 99.4 ppm, respectively. In Fig. 2b, relative to the S1-MCP spectra, the same assignments were observed. However, there was a decrease in these signals (C-1 groups), which means there was a decrease in the acid uronic content after chemical modification.

Signals of carbons 2–5 of the galacturonic acid units are evidenced at 68.2, 70.5, 78.5, 77.5 and 74.5 ppm. The signal at 104.4 ppm was attributed to C-1 of α-L-rhamnose units. The signal of CH_3 of the rhamnose units occurred at 16.7 ppm. The spectra were similar to those of other pectins. The assignments were carried out according to literature [40,41]. These results indicate the presence homogalacturonan and rhamnogalacturonan with arabinose and galactose in samples of native pectins and especially in modified pectins, wich agrees with the results found in the monosaccharide composition.

3.3.2. Molar mass (HPSEC-MALLS analysis)

Degradation as a result of the chemical modification of the pectin is related to its properties, such as pH, esterification grade and molecular mass [5]. The molar mass of pectins was calculated from the elution profiles obtained by HPSEC-MALLS and dn/dc values (Table 3). The chemical modification of pectins resulted in a decrease in their molar mass of 10.50%, 29.98% and 25.29%, for S1, S2, and S3, respectively. Neutral sugars were not reduced with the chemical modification, but galacturonic acid content was instead. Thus, the decrease in molecular mass suggests that the linear chain of galacturonic acid was altered (as also observed in Table 1). The molar mass of pectin samples was similar to values reported by Mesbahi et al. [42] for high esterification degree pectin extracted from beetroot (1.53×10^5 g mol^{-1}) and high esterification degree of commercial citrus pectin (1.16×10^5 g mol^{-1}), both analyzed with HPSEC-MALLS. Mean molar mass of pectins from several fruit sources lies between 10^4 and 10^5 Da. The values found in this study are consistent with these values [43].

In general, however, the homogeneity of the modified samples increased with respect to native pectins, as expected. Diaz et al. [29] report that the molar mass of citrus pectin obtained at pH 4.5 and temperature at 95 °C for 30 min decreased approximately 10%. The authors concluded that acid conditions may cause hydrolysis, whereas β-elimination occurs in alkaline conditions. In fact, β-elimination only occurs with methoxylated groups and therefore inhibition with saponification. Since the predominance of a reaction over another one is controlled by temperature and pH, higher temperatures in alkaline conditions favor β-elimination. Further, an increase in pH causes the predomination of de-methylation over β-elimination [30,44].

The modification of the citrus pectins is intended primarily to reduce its molecular weight in order to promote important benefits to human health when consumed in food and pharmaceutical formulations. These benefits include reduction in tumor growth and inhibition of metastasis [17,45,46]. It has been reported that the medicinal function of pectins is closely related to its structural characteristics. Pectins in their native structure are not digestible by humans. However, when the average molecular weight is reduced to 10–20 kDa, studies point out an increase in their solubility, which in turn may enhance their uptake and circulation in the blood [21,46]. In this study it can be verified that the molar mass obtained after the chemical modification was higher than the value referenced for its medicinal function (115.5–165.2 kDa, S3-MCP and S1-MCP, respectively). Thus, most likely the pectins of the present study have not been modified enough to be absorbed by the human organism.

Although there was no significant decrease in the DE of the S2-MCP and S3-MCP (Table 2), it is possible to verify that there was a decrease in the molar mass of all the pectins samples after the modification, indicating that structural modifications occurred in the samples. Since the neutral sugars increased with de chemical modification, independent of the native sample, the reduction in the molar mass can be attributed to the partial degradation of galacturonic acids chains.

3.3.3. Scanning electron microscopy (SEM)

Measurements by scanning electron microscopy (SEM) were carried out to characterize the surface of native and modified pectin samples, visualize their structures and morphology, and understand their modification level. Fig. 3 demonstrates that native and modified samples are highly heterogeneous with multiform particles and different sizes, having the aspects of fibers. It is actually hygroscopic material and triggers the formation of groats which are difficult to solubilize.

The observation of the particles in the samples of modified pectins shows a fibrous or at least a rough structure, with mean sizes between 5 and 100 μm (Fig. 3B and D). The surface is uneven

Table 3
Molar mass (M_w) of native and modified pectin samples.

Pectin	M_w (g mol^{-1}) and ED – native	M_w (g mol^{-1}) and ED – modified	dn/dc – native	dn/dc – modified
S1	1.846×10^5 (0.6%)	1.652×10^5 (0.6%)	0.130	0.155
S2	1.871×10^5 (0.5%)	1.310×10^5 (0.5%)	0.150	0.124
S3	1.546×10^5 (0.7%)	1.155×10^5 (0.7%)	0.113	0.124

ED: Error of determination; dn/dc: differential index of refraction.

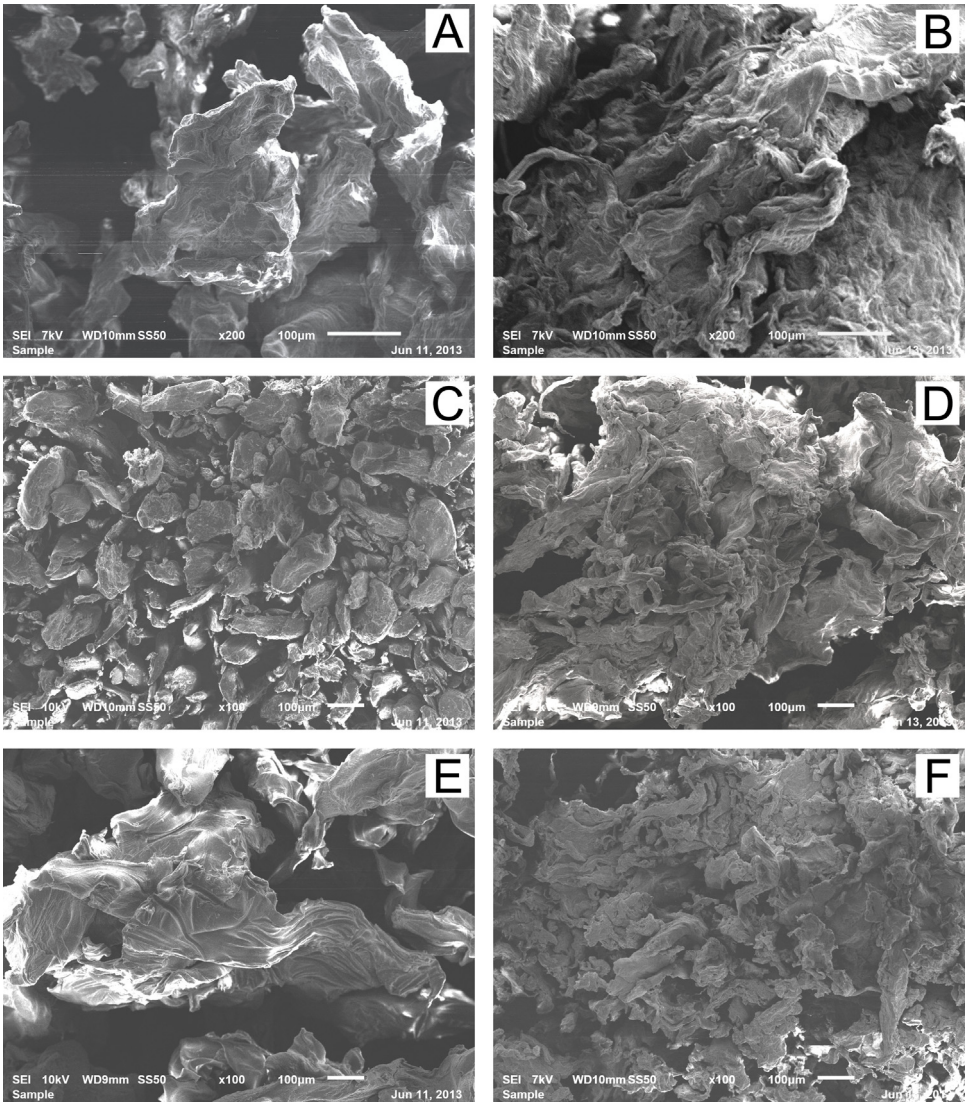

Fig. 3. SEM of citrus pectin samples: (A) S1-N; (B) S1-MCP; (C) S3-N; (D) S3-MCP; (E) S2-N (F) S2-MCP. *Magnified 200× in (A) and (B); 100× in (C) and (D); 100× in (E) and (F).

and sensitive to the touch. The moisture on the fingers wets the surface, makes it smooth, and enhances linking with other pectin particles.

3.4. Rheological properties (oscillatory and non-oscillatory tests)

Oscillatory assays are very useful to characterize macromolecular conformation and the intermolecular interactions in the solution. The rheological data of native and modified pectin samples were properly fitted by the power law model, with a high coefficient of determination (R^2), as shown in Fig. 4 and Table 4.

The flow curves revealed a pseudoplastic, non-Newtonian behavior of the samples due to the flow behavior indexes were lower than the unit. Since the flow behavior index for all pectins increased after chemical modification, the pectin solutions presented a rheological behavior closer to a Newtonian fluid. Apparent viscosity (η) of native samples was higher than that of modified ones and suggested that pectins chemical modification changed their rheological properties. The above has been confirmed by changes in DE and M_w values. Maximum and minimum viscosity values in Table 4 correspond to maximum and minimum shear rates values in the rheological test. In addition, the coefficients of consistence (K) decreased fold for all pectins. In short, the modified pectins have suffered a significant decrease in apparent viscosity and the consistency coefficient, associated with reduction of molar mass and increase the homogeneity. Similar behavior was found by Masuelli [47] studying citrus pectin. Venzon et al. [48] observed the same behavior for commercial citrus pectins modified by alka-

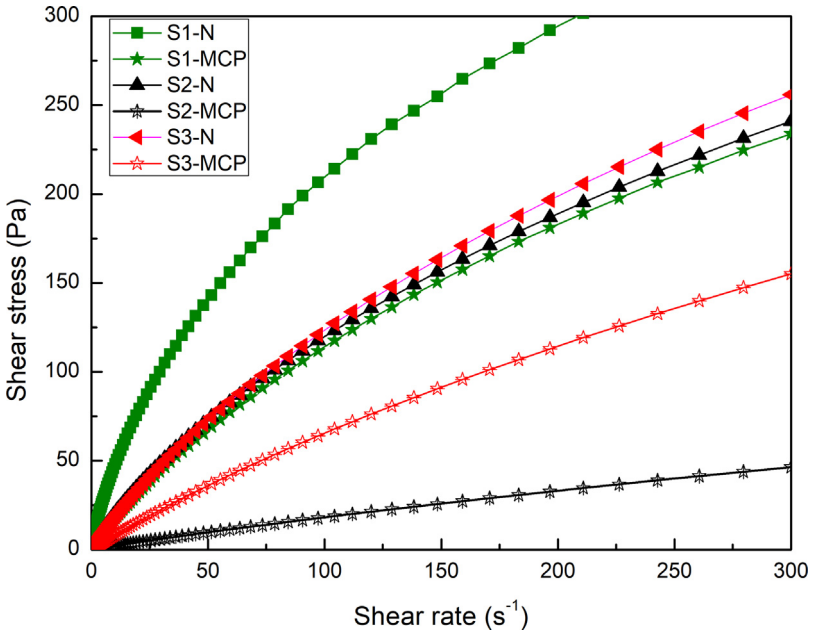

Fig. 4. Flow curves of aqueous solutions a the concentration of 5 g.100 g^{-1} of the samples of native pectins: S1-N, S2-N and S3-N; and samples of modified pectins: S1-MCP, S2-MCP and S3-MCP, adjusted by the model power law.

Table 4
Rheological data of native and modified pectin fitted by the power law model.

Type of pectin	K (Pa s^n)	n	R^2	η_{max} (Pa s)	η_{min} (Pa s)
S1-N	14.033 ± 1.488	0.574 ± 0.021	0.998	397.02 ± 67.96	1.240 ± 0.201
S1-MCP	3.893 ± 0.009	0.729 ± 0.009	0.999	32.558 ± 1.91	0.830 ± 0.061
S2-N	4.625 ± 0.010	0.699 ± 0.003	0.998	48.748 ± 1.18	0.831 ± 0.012
S2-MCP	0.316 ± 0.006	0.881 ± 0.004	0.999	0.801 ± 0.03	0.160 ± 0.002
S3	4.005 ± 0.004	0.735 ± 0.004	0.995	31.855 ± 1.05	0.844 ± 0.023
S3-MCP	1.399 ± 0.051	0.830 ± 0.005	0.988	5.310 ± 0.40	0.530 ± 0.008

n = 3, R^2–coefficient of determination; K – coefficient of consistence; n – flow behavior index; η – apparent viscosity.
S1-N (DE of 64.1%), S2-N (DE 47.7%) and S3-N (DE 58.3%): native citrus pectin samples.
S1-MCP, S2-MCP and S3-MPC: modified citrus pectin samples.

line treatment. They concluded that lower viscosity values may be a positive factor, since this means a lower energy expenditure during processing.

Results of dynamic oscillatory analyses of native and modified pectin samples presented a storage module (G') superior to the loss module (G") throughout the frequency range studied, with strong gels and a predominantly elastic behavior. When the elastic module (G') is superior to the viscous module (G") and both are independent of frequency, the material has the characteristics of solids or strong gels. A weak gel has higher values for the viscous module (G") when compared with the elastic module (G') at low frequencies; an inversion occurs at high frequencies with G' greater than G" [49].

Native pectins have higher G' values than those obtained for modified pectins, as well as a greater difference between G' and G". The above characteristics reveal that the solutions of native pectins form a stronger gel than that from modified ones, under the conditions tested, probably due to de-polymerization and de-esterification of the polysaccharides caused by chemical modification and as a consequence of the fragmentation of the polygalacturonic chain compromising the gelification of the pectins [50].

Solutions of native pectin proved to be stronger when compared to the modified ones, which is associated to the amounts of galacturonic acid found during chemical analyses. Galacturonic acid is directly related to the formation of the pectin network and the difference may be attributed to values of degrees of esterification (DE) and to the molecular mass of the pectins. Several factors affect the pectins rheological characteristics and the strength of the gel. Chain length and the chemical nature of the connecting zones by pectin molecules have a strong influence on these characteristics. The gel strength of pectins is positively related to molar mass. Pectins with low molar mass do not form gels under any condition and pectins with high molar mass promotes the formation of strong gels [51,52].

However, it should be emphasized that all native and modified pectin solutions formed gels, albeit with different characteristics. Samples of modified pectins had the lowest gelification capacity. Schmelter et al. [53] observed that pectins with short lateral chains had better gelification capacities than those with longer ones. The size of the lateral chains should be sufficient to provide entanglement in the polymer network and stronger than those of linear polymers with similar molecular mass, since the main chain of branched polymers is shorter than that of linear polymers of the same mass. If too long or too short, neutral lateral chains of native and modified pectins may affect gelification. In the former, a steric hindrance occurs in the interaction between different molecules; in the latter, pectin is more compact than the respective linear polymer. Further, DE and the distribution of GalA methyl-esterified units in the pectin chain (randomized or in blocks) affect its aggregation capacity and the formation of intermolecular networks [54]. Neidhart et al. [55] reported that the distinct distribution of the ester groups among the pectin molecules strongly influence its

capacity in gel formation, with differences in molecules with similar uronic acid and methyl group levels [51].

The modification significantly influenced the rheological behavior of pectins indicating change of molecular structure.

Therefore, even in view of the similarities between samples in certain aspects, it may suggest that the differences in flow behavior observed refer to the molecular properties of samples, such as chemical composition of neutral sugars, galacturonic acid content and degree of esterification of the pectins. In short, modified pectins presented lower apparent viscosity, pseudoplastic behavior, and formed less strong gels when compared to the native samples.

4. Conclusion

Our study indicated that the proposed modification in citrus pectin influenced their structure (monosaccharide composition, molar mass, rheological properties) at some extent. The pectins modified by alkaline treatment presented lower uronic acid contents, and higher proportion of neutral sugars than native ones. Native pectins after chemical modification showed higher amounts of neutral monossaccharides, mainly galactose, arabinose and rhamanose, typical of Ramnogalacturonana-I (RG-I) fraction. The molecular mass was influenced by the modification proposed, and as expect, it was reduced after the treatment. However, this reduction was not sufficient to affirm these pectins could be improved sufficiently to be absorbed by the human body. Rheological behavior of pectin samples was significantly influenced by modification process, presenting lower viscosity, suggesting that occurred changes in the molecular structures of native pectins samples. The solutions of modified citrus pectin showed good stability at room temperature, better dissolution in water and pseudoplastic behavior. This paper contributes to better understand the behavior of the pectins submitted to alkaline treatment, and how this chemical modification can influence the structural properties of such a complex polysaccharide.

Conflict of interest

All the authors hereby declare that they do not have any conflict of interest about this manuscript.

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