


Extraction and physicochemical characterization of pectin from tomato processing waste

Matías M. Alancay¹  · Manuel O. Lobo² · Claudia M. Quinzio¹ · Laura B. Iturriaga¹

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Abstract The pectins extracted from tomato processing waste (TW) by the acid method using hydrochloric acid (ATP) and by water (WTP) were analyzed for their chemical composition, galacturonic acid (GalA) content, degree of methylation (DM), solubility (%S), intrinsic viscosity ($[\eta]$), molecular weight-viscosity average (Mw_v), flow and gelling properties as well as gel texture and color, and were compared against commercial high-methoxyl pectin from citrus (HMC). The maximum yields were: 280 g kg⁻¹ dry TW, obtained at 85 °C, 60 min, pH 2.0 for ATP, and 87 g kg⁻¹ dry TW at 95 °C, 180 min for WTP. The GalA content and DM of pectin extracted from TW were higher than that of HMC. The $[\eta]$ and Mw_v of ATP were in the same order as that of HMC and higher than that of WTP. All the pectin solutions presented a pseudoplastic flow behavior. The consistency coefficient (K) of ATP was significantly the highest at all pH values studied. The initial (IST) and critical structure temperature (CST) values of WTP (92–85 °C) were higher than in ATP (74–52 °C). The elastic modulus (G') values of the ATP gels showed weaker gels than that of HMC but higher than WTP ones. The hardness of ATP gels was similar to that of HMC and higher than that of WTP. Tomato pectin gels presented reddish color compared to the HMC gel. The acid method extraction was the best method to reach the maximum yield

extraction of TW pectin and provided pectin with physicochemical properties similar to that of HMC.

Keywords Tomato waste · Pectin · Yield · Gelation · Rheology · Texture

Introduction

Pectins are polysaccharides present in the primary cell wall and middle lamella of all plants. They consist of a backbone of D-galacturonic acid (GalA) units partly methyl-esterified and integrated by various neutral sugars such as, L-rhamnose, L-arabinose, and D-galactose that are present in the backbone structure as side chains [1]. The side chain, which is denominated arabinogalactan II and composed of units of L-arabinose and D-galactose, is mainly associated with proteins, 30–80 g kg⁻¹ pectin [2].

Depending on their degree of methylation (DM) pectins are referred to as high-methoxyl if $DM \geq 50\%$ and as low-methoxyl if $DM \leq 50\%$. The high-methoxyl pectins can form gels after heating in sugar solutions at a concentration higher than 55 °Brix and pH lower than 3.5, and the low-methoxyl pectins form gels in the presence of calcium ions [3].

Pectins are industrially obtained mainly from citrus peel and apple pomace; however, some nontraditional pectin sources such as fruit and vegetables or food industry by-products have recently been investigated [4–7]. In this way, the extraction process could represent an efficient and environmentally friendly route for the production of functional compounds [7–9].

Tomato is a vegetable crop widely consumed in the world. More than a third of the production is grown for the processing industry, where the waste of tomato processing

✉ Matías M. Alancay
matiasalancay@yahoo.com; marumi1786@hotmail.com

¹ Centro de Investigación y Transferencia Santiago del Estero (UNSE-CONICET), ruta 9 km 1125, Villa Zanjón, Santiago del Estero, Argentina

² Centro de Investigación y Transferencia de Jujuy (UNJu-CONICET), Italo Palanca 10, San Salvador de Jujuy, Jujuy, Argentina

represents around 4–13 g kg⁻¹ tomato fruit consisting mainly of peels, seeds and part of pomace, and is usually discarded or utilized as animal feed or fertilizer [10–13]. In 2012 the world tomato production was around 162 million metric tons [13]. However, in recent years the tomato by-products have been considered as a low-cost raw material based on their various functional ingredients such as vitamins, carotenoids, essential oils, and pectin [14, 15].

Among the extraction agents used to obtain pectin, water, ethylenediaminetetraacetic acid (EDTA) or cyclohexanediaminetetraacetic acid (CDTA), hot dilute acid, and alkali can be mentioned. The industry prefers the use of hot dilute acid as extraction agent due to the highest yield and quality in selected extraction conditions [16–19]. In the pectin extracted by a chelating agent, an amount of the agent remains, which will affect pectin functionality, while the extraction by alkali provides pectin with low degree of esterification and reduced length [18]. Pectin extraction using water is an attractive environmentally friendly process, because it would not generate waste [20].

The main objectives of this research were to find the optimum conditions for the extraction of waste tomato pectin, and to study the effect of these processes on the yield and physicochemical properties of the obtained pectin.

Materials and methods

Materials

The tomato processing waste (TW) composed of skin, seed and pulp was provided by OTITO Inc. (Jujuy, Argentina). The TW with 80 g water kg⁻¹ TW was quickly packed and stored in high density polyethylene bags at -18 °C for analysis. The commercial high-methoxylated pectin from citrus (HMC) was provided by CP Kelco (San Diego, USA) and purchased by GELFIX Inc. (Buenos Aires, Argentina). The rest of the chemicals and reagents were of analytical grade.

Extraction of pectin

Two extraction methods were used: one using HCl solution as extracting agent (acid method) and the other, distilled water (aqueous method).

Pectin extraction by the acid method was based on the method proposed by Kulkarni and Vilayanand [5]. The TW was dispersed in distilled water at a ratio of 1:100 (w/w, solid:water) in a stainless steel container equipped with a shaft-drive agitator. Enough amount of HCl 6 M L⁻¹ was added to the blend to reach pH 2. Afterwards, it was heated with gentle agitation for 1 h at different temperatures (65, 75 and 85 °C). The resulting slurry was then cooled at 25 °C and filtered with a cloth filter. The filtrate containing the

solubilized pectin was precipitated using ethyl alcohol at a ratio of 1:1.5 (v/v). The precipitated pectin was filtered and washed three times with ethanol 50:50 (v/v; ethanol:water). After that, the sample was dried at 45 ± 1 °C and ground once dry.

Finally, the extracted pectin was subjected to a purification step. An extracted pectin solution was prepared by adding distilled water to obtain a 10 g kg⁻¹ solution, heating at 60 °C for 60 min under gentle agitation. Once cooled at 25 °C, the solution was centrifuged at 2700g for 10 min. The soluble fraction was precipitated again with ethanol at the same ratio, filtered, dried and ground, as was described before.

In the aqueous method, the TW was blended with distilled water at a ratio of 1:100 (w/w, solid:water) in a stainless steel container, heated with gentle agitation at different temperatures: 75, 85 and 95 °C, and times: 30, 60 and 180 min. The slurries obtained followed the same precipitation and purification steps as was described in the previous method and is shown in Fig. 1.

The yield of both methods was calculated by the following equation:

$$\text{Yield} = \frac{m_1}{m_2} \quad (1)$$

where m_1 (g) and m_2 (kg) are the mass of pectin and dry TW, respectively.

Physicochemical properties

The chemical composition of pectin was determined by measuring the moisture, ash, protein and lipid contents according to AOAC [21]. The nitrogen contents were multiplied by 6.25 to obtain the protein of the samples.

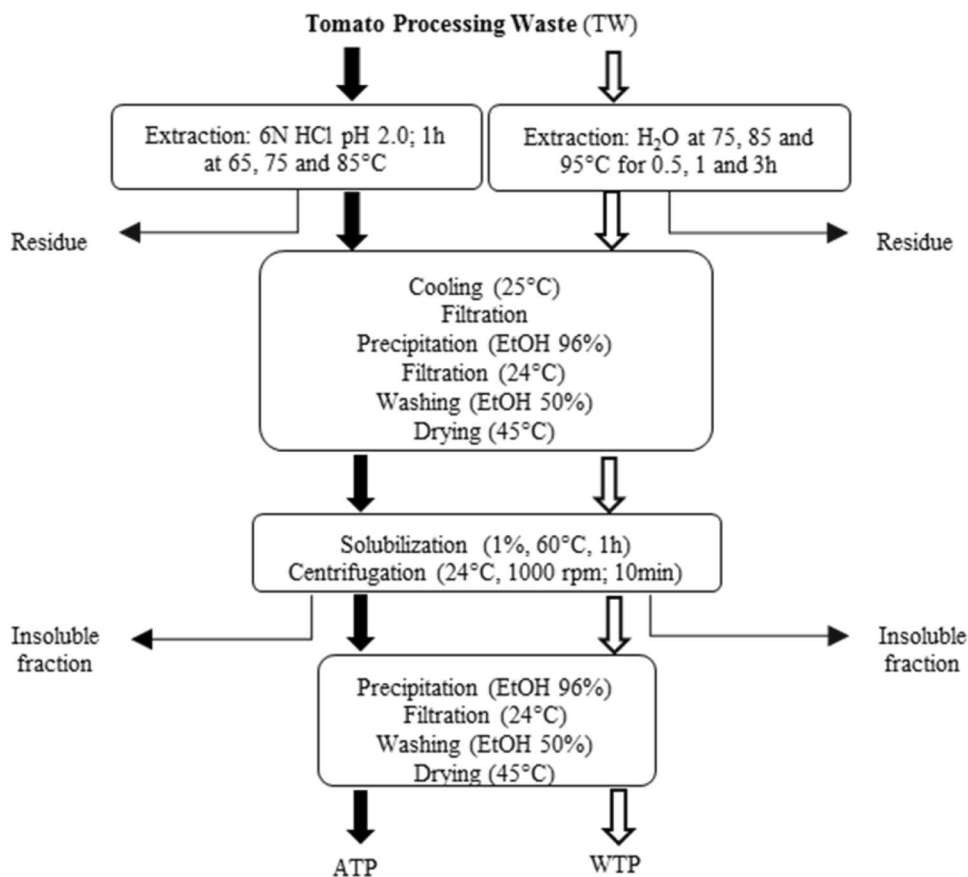
The galacturonic acid content (GalA) was determined using the spectrophotometric method proposed by Ibarz et al. [22]. The samples were hydrolyzed in a solution of 12.5 M L⁻¹ sodium tetraborate in concentrated sulfuric acid, heated at 80 °C for 6 min and then cooled to room temperature. Finally, 20 μL μL⁻¹ m-hydroxydiphenyl 1.5 g kg⁻¹ solution was added to form the colored complex

$$\text{GalA} = \frac{C \times V \times d}{1000 \times m} \quad (2)$$

where C (mg mL⁻¹), V (mL), d and m (kg) are the concentration, dilution volume, dilution factor and weight of pectin (ash-free and dried basis), respectively.

The degree of methylation (DM) was determined spectrophotometrically (412 nm) using the method suggested by Klavons and Bennett [23]. A stock solution of pectin was prepared at a concentration of 1000 μg mL⁻¹ of distilled water. It was diluted with 0.5 M L⁻¹ NaOH to achieve a 600 μg mL⁻¹ pectin solution, and hydrolyzed for

Fig. 1 Extraction process of ATP and WTP from TW using acid and water as extracting agents, respectively



30 min at room temperature. Finally, the solution was neutralized at pH 7.5 with 1 M L⁻¹ HCl, and diluted with a 0.25 M L⁻¹ phosphate buffer pH 7.5 to a final concentration of 300 µg mL⁻¹ of pectin. The methanol content in the samples was determined as follows: first, 1 mL of the final solution at 300 µg mL⁻¹ was mixed with 1 mL of alcohol oxidase (1 U mL⁻¹) in hemolysis tubes that were incubated for 15 min at 25°C. Then, 2 mL of 2,4-pentanedione 0.02 M L⁻¹ dissolved in ammonium acetate 2 M L⁻¹ and acetic acid 0.05 M L⁻¹ was added to the test tube and mixed by vortex. The tubes were capped, placed in a water bath at 58–60°C for 15 min, and cooled to room temperature. The DM of pectin was calculated as the ratio of the molar amount of methyl groups (such as methanol) to the molar amount of GalA per gram of sample, and expressed in percentage.

The intrinsic viscosity ($[\eta]$) of the samples was determined by capillary viscometry using a Micro-Ubbelohde viscometer DIN 55 350 (capillary no.: M I; internal diameter: 0.40 mm) at 25 ± 0.1°C using pectin solutions at 0.2, 0.4, 0.6, 0.8 and 1.0 g L⁻¹ containing 0.1 M L⁻¹ NaCl at pH 7.0 and prepared by stirring for 12 h. The $[\eta]$ was estimated by extrapolating the Huggins and Kraemer curves to zero concentration [4]. The viscosimetric

average molecular weight ($M_{w,v}$) was calculated using the Mark–Houwink equation (Eq. 4), where K and α are constants, 9.55 × 10⁻⁵ g L⁻¹ and 0.73, respectively [24].

$$[\eta] = K [M_{w,v}]^\alpha \tag{3}$$

The solubility (%S) of pectin in water was determined at pH 4.5 and 6.5 obtained with 0.1 M L⁻¹ phosphate buffer, following the gravimetric method of Li et al. [25]. Solutions at 5 g pectin kg⁻¹ were prepared by stirring a proper amount of pectin in buffers for 12 h at room temperature and filtered under vacuum using filter paper (Whatman 93) previously dried at 100°C for 1 h. Afterwards, the filter paper with insoluble solids was dried in the same conditions. The solubility was determined by Eq. (4),

$$\%S = 100 \times \left(1 - \frac{(m_3 - m_1)}{m_2} \right) \tag{4}$$

where m_1 , m_2 , m_3 are the weight of the dried filter paper, the weight of pectin, and the weight of the filter paper with the insoluble fraction, respectively.

Rheological properties of pectin solutions and gels

The rheological properties were determined using a rheometer AR 1000 (TA instruments Ltd, New Castle, UK) and the results obtained were analyzed with Rheology Advantage Data software.

The thickening properties were studied by preparing high concentration systems of pectin at 30 g kg^{-1} and analyzing the effects of pH on viscosity. To study the effect of pH, solutions were prepared by stirring pectin in 0.1 M L^{-1} phosphate buffer, to obtain pH 4.5 and 6.5, for 12 h at 25°C . Measurements were performed using a stainless steel cone-plate geometry, 40 mm diameter, 53 microns of truncation and 2° cone angle, at 25°C . The samples were subjected to the following shear program: (a) increasing the shear rate from 0 to 500 s^{-1} in 2 min (up curve), (b) holding the peak at 500 s^{-1} for 2 min, and (c) decreasing the shear rate from 500 to 0 s^{-1} in 2 min (down curve).

The gels of ATP, WTP and HMC, made for the dynamic measurements, were prepared by dispersing pectin in distilled water to obtain a concentration of 0.5 or $1.0 \text{ g pectin kg}^{-1}$ gel at room temperature. A solution of pectin-sugar, ratio 1:4 (w:w), was prepared by dissolving the mixture in distilled water. The remaining of the sugar, necessary to achieve 65°Brix , was added in three fractions at 60°C . Once these solids were dispersed, the system was heated at 95°C for 5 min. An adequate amount of citric acid solution was added to obtain pH 3. Finally, the volumes were corrected with water to achieve the appropriate concentration.

Gelation kinetic and frequency sweep measurements were carried out using a stainless steel plate–plate geometry of 40 mm and a peltier plate with controlled temperature. In order to examine the structure development rate (SDR) defined as dG'/dt , a temperature sweep at a constant rate was applied using small amplitude oscillatory shear. An aliquot of 2 mL of samples prepared to make pectin gel were placed on the peltier plate and preheated at 102°C . The dispersion was cooled down to 20°C at 1°C min^{-1} by applying a frequency of 1 Hz within the linear viscoelasticity range at a constant stress of 0.1 Pa. A solvent trap device was used to prevent dehydration of the sample during measurement. The elastic modulus (G') and storage modulus (G'') were collected from the runs. The values of dG'/dt were determined using Origin 8.5.0 SR software (OriginLab Corporation, Northampton, USA) and plotted against temperature ($^\circ\text{T}$). Two characteristic temperatures were determined from the first derivative of G' : the initial structuring temperature (IST) and critical structuring temperature (CST). The parameter IST is the temperature at which the dG'/dt value is different from zero for the first time, while the CST is the extrapolated temperature of the first strong increase of dG'/dt as is shown in Fig. 2 [26].

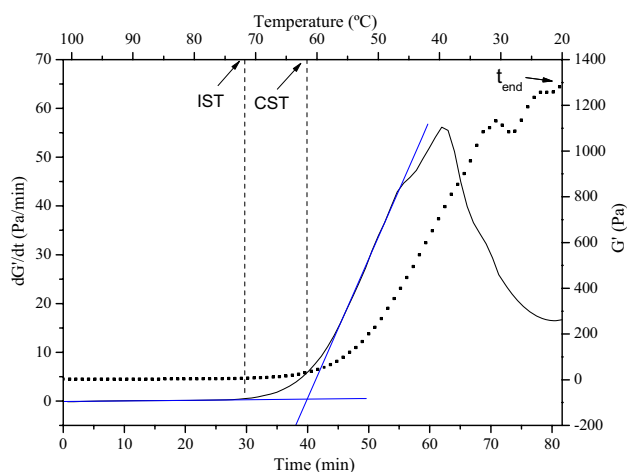


Fig. 2 Structure development rate (dG'/dt) and G' (Pa) as a function of time and temperature of ATP $10 \text{ g pectin kg}^{-1}$ gel, respectively. The solid curve = dG'/dt ; dotted vertical lines are IST and CST and the start of structuring phases

The average SDR (SDR_a) was calculated from differences of the storage moduli during cooling time for the total gel-ling process, Eq. (5).

$$\text{SDR}_a = (G'_{\text{end}} - G'_{\text{IST}}) / (t'_{\text{end}} - t'_{\text{IST}}) \quad (5)$$

where G'_{IST} and t'_{IST} are parameters at IST, and G'_{end} and t'_{end} are the final values at 20°C [26].

A non-isothermal kinetic model was used to describe the structure development process during cooling of pectin gels. It was based on the combination of the classical rate equation, the Arrhenius equation, and the time–temperature relationship, Eq. (6):

$$\ln\left(\frac{1}{G^n} \frac{dG'}{dt}\right) = \ln k_0 - \left(\frac{E_a}{RT}\right) \quad (6)$$

where G' is the storage modulus, taken as a measure of the junction zones in the network, n is the reaction rate order, t is time, k_0 is the Arrhenius frequency factor, E_a is the activation energy of the process, R is the universal gas constant, and T is the temperature in $^\circ\text{K}$ [27].

Once the gels were formed in situ, and since the temperature sweep was carried out preserving the structure, a frequency sweep was performed. The samples were subjected to a frequency range of 0.1–100 Hz at 20°C within the linear viscoelastic range ($\tau=0.1 \text{ Pa}$). Rheological parameters, elastic modulus (G') and viscous modulus (G'') were collected.

Textural analysis

The textural parameters of the pectin gels were determined using a texture analyzer TA.XT Plus (Stable Micro System Ltd., Godalming, UK) coupled with a 20 mm diameter

measuring cylinder. The hot pectin gel was transferred to 8 mm diameter containers and cooled at 25 °C for 12 h. Samples of 15 mm height were subjected to two cycles of compression under the following operating parameters: pretest speed = 2 mm s⁻¹, test speed = 1 mm s⁻¹, posttest speed = 2 mm s⁻¹, strain 30%, and trigger force of 5 g. Textural parameters, such as firmness, cohesiveness and adhesiveness, were collected.

Color measurement

The colorimetric parameters of the pectin gels prepared were determined using a colorimeter (Minolta, Model CR300, Tokyo, Japan) at 10° observer angle with D65 illuminant. The results were expressed as the Hunter Lab scale parameters L* (Lightness: 0 = black, 100 = white), a* [greenness (-), redness (+)] and b* [blueness (-), yellowness (+)].

Statistics

The number of repetitions of each measurement is indicated under the graphs and tables. The data were analyzed by one-way analysis of variance (ANOVA) using a statistical software program Infostat (version 2015, Córdoba University, Argentina). Tukey's test was used to compare mean values when the variance was found to be significant. Significant values $p < 0.05$ were considered.

Results and discussion

Pectin extraction from TW by the acid method

The yield obtained by the acid method increased with the temperature, as shown in Table 1. However, the Tukey's test indicated that only the highest yield at 85 °C presented significant differences from those obtained at lower temperatures. A lower extraction yield from apple pomace (150–200 g pectin kg⁻¹ dry material) and a higher one from citrus peel (300–350 g pectin kg⁻¹ dry material) in acid conditions were informed by Rao and Lopes da Silva [28]. Grassino et al. [15] reported an extraction yield of 160–190 g pectin kg⁻¹ dry material using oxalic acid/ammonium oxalate to extract pectin from tomato waste.

Pectin extraction from TW by the aqueous method

The extraction yield of TW is listed in Table 1. Yields were high when both the time and temperature increased. In fact, the highest yield corresponded to the process carried out at 95 °C and 180 min. The temperature in the experiments carried out for 30 min did not have any effect on the yield

Table 1 Effect of temperature (°C) and time (min) on extraction yield (g pectin kg⁻¹ dry TW) of ATP and WTP

	Time			
	ATP	WTP		
Temperature	60	30	60	180
65	241.9 ^B ± 5.5	–	–	–
75	250.4 ^B ± 11.9	31.0 ^d ± 12.2	41.0 ^{cd} ± 3.4	48.0 ^{cd} ± 3.2
85	280.9 ^A ± 12.8	36.0 ^d ± 1.3	32.0 ^d ± 9.3	73.0 ^{ab} ± 8.5
95	–	32.0 ^d ± 15.4	56.0 ^{bc} ± 4.5	87.0 ^a ± 2.6

Capital letters in the columns show significant differences for ATP; lower case letters show significant differences for WTP. Mean ± standard deviation (n = 3)

and gave the lowest yield. Chou and Kokini [29] reported yields of 29 and 70 g pectin kg⁻¹ dry material at 65 and 104 °C, respectively, for pectin extracted from tomato pastes and using water as extracting agent. Using the same method, the yields of extraction from mango and lime dry peel were 46–76 and 67 g kg⁻¹ dry material, respectively [30].

Then, the extraction conditions were optimized at pH 2, 60 min and at 85 °C for the acid method, and 95 °C and 180 min for the aqueous method. Even though no acid was added in the extraction with water (aqueous method), it could be considered acid since the resultant pH was 4.5, but less harsh than in the acid method.

By analyzing these results, it is clear that the increase of temperature increased the yield in both extraction methods, and it was higher using acid as extracting agent. The difference between the yield of pectin by acid hydrolysis and water has been reported earlier. Since the pectin extracted by water is the biopolymer weakly attached or linked by hydrogen bonds to the cell wall, lower yields have been found. On the other hand, the pectin extracted by acid conditions is the polymer strongly attached to the cell wall through covalent bonds, which consequently increases the yield [6, 31, 32].

Chemical composition of pectin

The chemical composition of ATP, WTP and HMC samples is listed in Table 2. The moisture content of ATP and WTP were higher than in HMC, WTP being the sample with the highest value. The protein content decreased in the following order: ATP > WTP > HMC. The ash contents of ATP and WTP were considerably higher than in HMC. The high protein and ash contents in ATP and WTP are in agreement with the values reported by Guil-Guerrero and Rebollosa-Fuentes [33] for pectin from different varieties of tomato (*Lycopersicon esculentum*), i.e., between 131 and

Table 2 Chemical composition (g kg⁻¹ pectin), DM (%) and GalA (g kg⁻¹ pectin) of ATP, WTP and HMC

	ATP	WTP	HMC
Moisture	129.5 ^a ± 3.5	143.0 ^d ± 0.7	72.5 ^b ± 1.7
Protein ¹	81.1 ^d ± 0.5	42.7 ^c ± 0.5	31.5 ^b ± 0.3
Ash	65.4 ^a ± 1.7	100.4 ^c ± 1.6	16.6 ^b ± 0.2
Lipid	16.0 ^a ± 2.3	130.8 ^b ± 4.4	18.6 ^a ± 0.4
Purity ⁴	70.9	57.5	86.1
DM ³	76.3 ^c ± 1.8	87.8 ^d ± 1.9	60.2 ^b ± 4.2
GalA ²	800.5 ^b ± 27.3	813.0 ^b ± 26.5	762.8 ^b ± 21.7

Different letters in the same row indicate significant differences ($p < 0.05$). Mean ± standard deviation ($n = 3$)

¹Protein content was calculated using the factor 6.25

²GalA content free of ash and moisture

³Ratio of the molar amount of methyl groups to the molar amount of GalA per gram of sample and expressed in percentage

⁴Purity was calculated as: $[100 - (\% \text{Moisture} + \% \text{Protein} + \% \text{Ash} + \% \text{Lipid})]$

Table 3 Intrinsic viscosity (dL g⁻¹), viscosimetric average molecular weight (kDa) and solubility (%) at different pH values of pectin (ash-free and dry basis) determined by Huggins and Kraemer equations

	ATP	WTP	HMC
$[\eta]$	4.2 ^b ± 0.2	1.8 ^a ± 0.03	3.9 ^b ± 0.1
Mw _v	94.6 ^c ± 1.8	30.5 ^a ± 0.1	86.7 ^b ± 0.4
S _{pH 6.5}	74.1 ^a ± 1.8	83.3 ^c ± 0.4	78.2 ^b ± 1.5
S _{pH 4.5}	89.7 ^{cd} ± 0.5	85.8 ^d ± 0.2	86.5 ^e ± 2.3

Values with different letters show significant differences between samples ($p < 0.05$). Mean ± standard deviation ($n = 3$)

262 and 187.5–195 g kg⁻¹ dry matter for protein and ash, respectively.

The lipid content showed differences among the samples and was low as is expected for vegetables; however, WTP presented the highest value. Guil-Guerrero and Reboloso-Fuentes [33], who attributed the lipid content to the presence of lycopene, reported the same result.

The main differences in the chemical composition were observed in the purity of these samples, shown in Table 2. The commercial pectin HMC presented a higher purity than ATP and WTP. The pectin extracted using water, WTP, was the sample with the lowest purity. The acid method allows one to obtain a pectin with good purity.

GalA content and DM of pectin

Table 2 shows that the GalA contents of ATP and WTP are close to each other and with similar statistical values ($p < 0.05$). These results of GalA content were in the same order as that of HMC sample. Thus, the polysaccharide obtained from tomato waste can be considered

as pectin since these values are much higher than about 650 g GalA kg⁻¹ pectin (ash-free and dry matter), set for pectin by the Food and Agriculture Organization (FAO) of the United Nations [34, 35].

The DMs of ATP and WTP were higher than 50%, significantly different and even higher than those of HMC. The difference in the DM between extracted pectins might be due to the acid strength and high temperatures, which boost the demethoxylation and promote the hydrolysis, respectively [36, 37].

Intrinsic viscosity, viscosimetric average molecular weight and solubility of pectin

Intrinsic viscosity is a parameter that reflects the hydrodynamic volume, which in turn depends on the molecular weight and the conformation of the polymer in the solution. Table 3 shows the intrinsic viscosity of pectin in 0.1 M L⁻¹ NaCl and the viscosimetric average molecular weight that was calculated with the Mark–Houwink equation using the parameters suggested by Anger and Berth [24]. The $[\eta]$ informed is the mean value of those obtained through the Huggins and Kraemer equations as was suggested by Li et al. [25].

The ATP sample presented an intrinsic viscosity similar to the values reported by Chou and Kokini [29] for tomato paste water-soluble pectin (3.4 dL g⁻¹). However, nonconventional pectin extracted by hydrochloric acid at pH 1.5, such as ambarella and lime pectin, showed lower values of 1.8 and 3.4 dL g⁻¹, respectively [38]. The pectin extracted from conventional sources such as apple and citrus exhibited values of intrinsic viscosity slightly higher than that of ATP, 4.5 and 3.8 dL g⁻¹, respectively [39]. However, the intrinsic viscosity of pectin from *Akebia trifoliata* var. *Australis* peel extracted by hydrochloric acid was higher than that extracted by citric acid [4].

The viscosimetric average molecular weight was 93,000–96,000 and 30,000 Da on an ash-free basis, for ATP and WTP, respectively. Besides, the Mw_v of ATP was close to the one corresponding to HMC. These results show that the extraction process affected the size of the tomato pectin molecule obtained, since the $[\eta]$ and Mw_v of ATP extracted under harsher pH conditions were higher than the ones corresponding to WTP.

Table 3 shows the solubility in water of the samples, carried out at pH 4.5 and 6.5 and at room temperature. High solubility was found for all samples, and the values were greater at pH 4.5 than at pH 6.5.

Flow properties of pectin solutions

Flow curves (not shown), obtained from steady shear assays of pectin at 30 g kg⁻¹ solution and pH 4.5, 6.5 and at

natural pH, indicated that all the samples presented a shear-thinning time-independent behavior, since the viscosity decreased with the increase of the shear rate and no thixotropic area was detected. Similar results were reported for HMC pectin from citrus pectin, cacao pod husks and apple pomace [40–42].

The Power Law model was applied to the down curve, and the coefficient of consistency K was represented against pH (Fig. 3). The K values of the samples were affected by the pH, and at pH 4.5 they were higher for ATP and HMC. The WTP sample did not present sensitivity to the change of pH, probably due to the high DM of this sample. On the other hand, the K value showed a direct relation with the Mw_v of pectin and was more pronounced at pH 4.5. This result indicates that the viscosity of these samples depended on the molecule size, the pH and the solubility (the solubility was higher at pH 4.5). It is evident that the extraction process affected the properties of the pectin since the K value of ATP was higher than that of WTP at all pH values studied, essentially at pH 4.5.

The flow behavior index “ n ” given by the Power Law model was, for all samples, in the range of 0.7–0.85 (data not shown) that corresponded to a pseudoplastic or shear-thinning behavior.

Gelation kinetic and viscoelastic behavior of pectin gels

The gelation of high-methoxylated pectin is produced by non-covalent bonding of adjacent pectin chains, leading to an interconnected three-dimensional network. These bonds are produced in junction zones, which are stabilized by hydrogen bonds and hydrophobic interactions between the methyl-ester groups of the pectin chains. The sugar reduces water activity, promoting hydrophobic interactions. These interactions, under entropic control, dominate at high

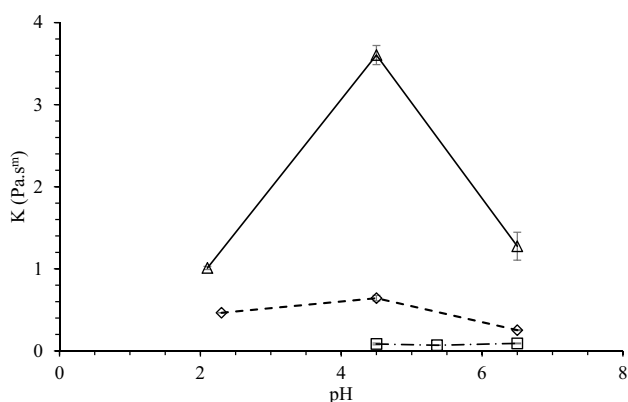


Fig. 3 a Consistency coefficients (K) of ATP (open triangle), WTP (open square) and HMC (open diamond) solutions at 30 g pectin kg^{-1} solution at different pH values were obtained from Ostwald-de Waele model

temperatures. The acid releases (H^+), which neutralizes the action of the R-COO^- groups, reducing the electrostatic repulsion between pectin chains, and therefore favoring hydrogen bonding. This mechanism, under enthalpic control, dominates at low temperature [43].

To follow and characterize the gelation process of pectin, the first derivation dG'/dt was used for the description of the gelling kinetics of pectin and the calculation of the SDR_a (Fig. 4). The IST and CST parameters obtained from dG'/dt versus time plots, corresponding to ATP, WTP and HMC gels, are listed in Table 4. The IST and CST values increased with the increase of concentration for ATP. No significant differences in the structuring temperatures at 5 and 10 g pectin kg^{-1} gel of HMC pectin were found. The IST and CST values of ATP were significantly different ($p < 0.05$) with respect to HMC at a concentration of 10 g pectin kg^{-1} gel. In fact, ATP showed a higher structuring temperature than HMC, which could be due to higher DM that contributed to the hydrophobic interactions between polygalacturonic chains. The increase of concentration increased the SDR_a of ATP sample, but did not affect the SDR_a of HMC. A special behavior was observed for WTP since this sample did not gel at 5 and 10 g pectin kg^{-1} gel. Thus, the system was studied at 20 g WTP kg^{-1} gel. Under these conditions, IST and CST were higher than in ATP. Even though the gelation of WTP sample was tested at 20 g pectin kg^{-1} gel, its SDR_a was in the same order as the SDR_a of ATP sample at 5 g pectin kg^{-1} gel and they were the lowest. Besides, by comparing the SDR_a values, it can be seen that it was higher for those samples with higher Mw_v such as ATP and HMC. The increase in pectin size would promote an increase in the number and length of interactions between pectin chains [4, 41].

Considering the complex mechanism explained earlier, it was assumed that changes in dG'/dt with time for ATP, WTP and HMC occurred in two steps at two different ranges of temperatures. Consequently, a non-isothermal kinetic model (Eq. 6) was applied at a high temperature range and low temperature range to obtain two activation energies E_{a1} and E_{a2} assuming a second order reaction, $n=2$ [27].

The values of activation energies of pectin gels are presented in Table 4. The E_{a1} values were higher than E_{a2} for all samples, which indicates that the gelation was driven by the hydrophobic interactions at the beginning of gelation. Besides, the E_{a2} values suggest that the contribution of hydrogen bonds in these samples was too low, in line with the high DM of all the samples.

The E_{a1} values did not present significant differences among all the samples, which would mean that the differences in the pectin properties such as DM, Mw_v and concentration would not be high enough to find differences in E_{a1} . The E_{a2} values did not present significant differences

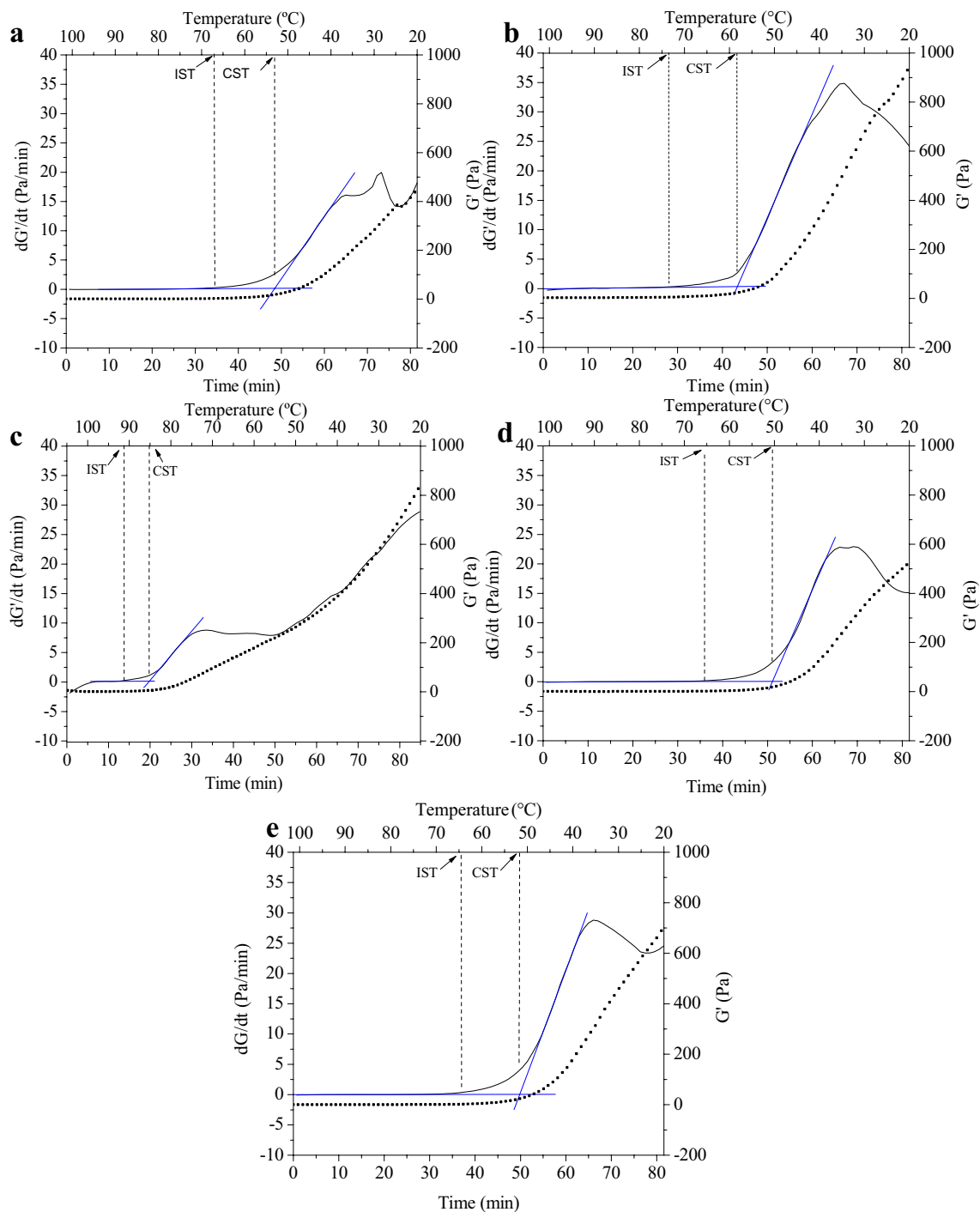


Fig. 4 Solid curve is the structure development rate (dG'/dt) as a function of time, the dotted curve is G' (Pa) as a function of temperature; dotted vertical lines give IST and CST for: **a** ATP gel at 5 g, **b**

ATP gel at 10 g, **c** WTP gel at 20 g, **d** HMC gel 5 g and **e** HMC gel at 10 g pectin kg^{-1} gel

between the ATP and HMC samples. On the other hand, the Ea_2 value of WTP did not present significant differences with respect to ATP but it was significantly different with respect to HMC. This could be explained

considering that WTP has a GM similar to that of ATP and higher than that of HMC.

In summary, these results indicated that all these samples presented the same mechanism of gelation: a first step

Table 4 IST and CST (°C), SDR_a (Pa min⁻¹) and Ea (kJ mol⁻¹) of ATP, HMC and WTP gel (g pectin kg⁻¹ gel)

	ATP		WTP	HMC	
	5	10	20	5	10
IST	69.6 ^{ab} ± 2.1	73.7 ^b ± 2.1	91.9 ^c ± 0.8	67.3 ^{ab} ± 0.3	63.4 ^a ± 3.5
CST	52.0 ^a ± 1.0	60.3 ^b ± 2.4	85.3 ^c ± 0.3	53.4 ^a ± 4.5	50.3 ^a ± 2.5
SDR _a	7.1 ^a ± 0.9	21.9 ^b ± 2.8	8.5 ^a ± 4.4	15.6 ^{ab} ± 1.5	15.1 ^{ab} ± 0.5
Ea ₁	133.2 ^a ± 15.4	168.3 ^a ± 10.2	196.7 ^a ± 34.5	183.6 ^a ± 15.6	163.9 ^a ± 4.9
Temperature range	70–30	70–40	90–65	60–30	70–30
Ea ₂	58.1 ^{ab} ± 3.9	60.1 ^{ab} ± 6.6	37.7 ^{ab} ± 8.8	74.5 ^b ± 7.3	62.7 ^{ab} ± 0.9
Temperature range	30–20	30–20	55–20	25–20	25–20

The different letters in the row show significant differences between the samples ($p < 0.05$). Mean ± standard deviation (n = 2)

at high temperatures, driven by the hydrophobic interactions, and a second step at lower temperatures, driven by the hydrogen bonds, typical of high-methoxylated pectins. The main difference was in the IST and CST, which were the highest for WTP, namely, the sample with the highest concentration and DM as well. Kinetic measurements indicated that the IST, CST and SDR_a of ATP depended on the concentration and were higher than those of HMC at the concentration 10 g pectin kg⁻¹ gel. WTP showed the lowest SDR_a at the concentration assayed. The HMC sample did not show differences in the kinetic parameters at the two concentrations studied.

Frequency sweeps of gels

The viscoelastic behavior of gels has been used to classify them as “strong” (true) or “weak” gels [27, 44]. In the former the molecular rearrangement within the network is much reduced at the time scales analyzed, G' is independent of frequency and ten times higher than G'' . On the contrary, in weak gels there is a higher dependence of G' on frequency, suggesting the existence of relaxation processes occurring at short time scales. The difference between G' and G'' is lower, which indicates that a lower percentage of the energy stored is recovered [27].

To evaluate the viscoelastic behavior of the pectin-sugar acid gels, frequency sweeps were done. The corresponding mechanical spectrum of ATP gel at 10 g pectin kg⁻¹ gel and at 20 °C is shown in Fig. 5. The rest of the samples presented the same profile (rheograms not shown). However, in order to analyze differences in the behavior of the samples, G' and $\tan \delta$ values at a frequency of 1 Hz were taken from the rheograms and are listed in Table 5. As can be observed, the storage modulus increased with the increase of the concentration for ATP and HMC samples. Additionally, they did not show significant differences between each other at the concentration of 10 g pectin kg⁻¹ gel. The WTP gel presented an intermediate G' value between those values for pectin gels with a concentration of 5 g pectin kg⁻¹

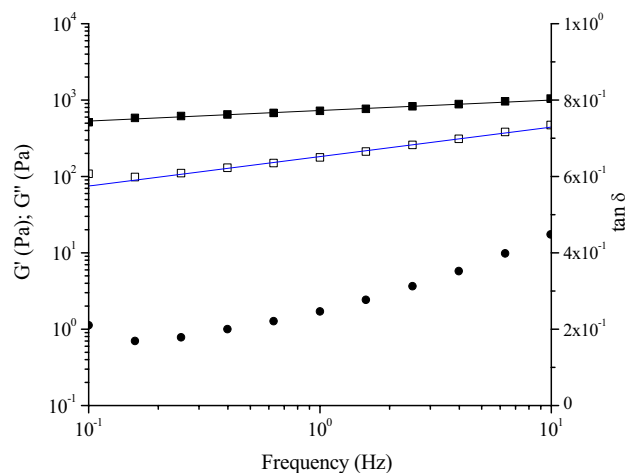


Fig. 5 Mechanical spectra of ATP at 10 g pectin kg⁻¹ gel at 20 °C. The parameters G' , G'' and $\tan \delta$ are represented by the following symbols: (filled square), (open square) and (filled circle), respectively. Linear fit of G' (black line) and G'' (blue line). (Color figure online)

gel. The $\tan \delta$ values, that is, the ratio $G'' G'^{-1}$, were between 0.1 and 0.2, which means that G' was ten and five times higher than G'' respectively. In this sense, ATP and WTP samples formed weaker gels than HMC.

These results demonstrated that the extraction process affected the viscoelastic behavior of TW pectin, where ATP gel exhibited a much higher elasticity than WTP.

Texture analysis of gels

The texture parameters of pectin gels are shown in Table 5. The hardness (also called firmness), adhesiveness and cohesiveness of ATP gels increased with the concentration. HMC did not change the hardness with the increase of concentration, but affected its adhesiveness. The WTP gel prepared at 20 g pectin kg⁻¹ gel showed hardness, adhesiveness and cohesiveness between the values of ATP at 0.5 and 10 g pectin kg⁻¹ gel. The reason for these differences

Table 5 G' values (Pa), $\tan\delta$, hardness (g), adhesiveness (g s), cohesiveness and color parameters of ATP, WTP and HMC gel (g pectin kg^{-1} gel)¹

	ATP		WTP	HMC	
	5	10	20	5	10
G'	291.9 ^a ±21.7	720.9 ^{cd} ±46.7	456.4 ^{ab} ±61.2	547.1 ^{bc} ±35.4	744.1 ^d ±47.4
$\tan\delta$	0.2	0.2	0.2	0.1	0.1
H	20.9 ^a ±0.9	92.8 ^c ±4.8	53.9 ^b ±1.4	108.8 ^d ±7.3	116.9 ^d ±0.5
A	7.5 ^c ±0.1	9.1 ^a ±0.7	9.6 ^a ±0.3	3.9 ^d ±0.2	5.9 ^c ±0.4
C	0.3 ^b ±0.02	0.6 ^a ±0.1	0.5 ^a ±0.0	0.5 ^a ±0.0	0.5 ^a ±0.0
L^*	71.4	67.6	51.6	97.8	98.7
a^*	0.96	1.5	5.3	-1.0	-1.7
b^*	3.2	4.7	2.8	2.6	2.7

The different letters in the rows show significant differences between the same samples ($p < 0.05$). Mean \pm standard deviation ($n = 3$)

¹Dynamic parameters: storage modulus (G') and $\tan \delta$ of pectin gel at 1 Hz frequency. Hardness (H), adhesiveness (A) and cohesiveness (C) and color parameters: L^* , a^* and b^*

would be related to the gel-forming characteristics of the pectins that are greatly influenced by the molecular weight [4]. High molecular weight not only strengthens the interactions between pectin chains but also enlarges the junction areas to make pectin gels stronger [45]. The hardness of all these gels was lower than that informed by Garrido et al. [43], who reported a hardness of 520 g for apple jelly prepared with citrus pectin at 10 g pectin kg^{-1} product. However, the adhesiveness and cohesiveness were in the same order. On the other hand, Javanmard et al. [46] informed that the hardness of fruit jam prepared with high-methoxyl pectin with a concentration higher than 5 g pectin kg^{-1} jam did not increase significantly ($p < 0.05$).

In addition, some authors have reported the formation of smooth regions (without neutral sugar branching) in the pectin chain with the increase of its DM. So the formation and hardness of its three-dimensional structure would be favored by a greater interaction of the pectin chains [36]. For high-methoxyl pectin, the higher the DM and the more blockwise the distribution of methoxyl-esters, the faster the gelation process [46].

Color of gels

Table 5 lists the color parameters of pectin gels. The highest experimental values of L^* were those of HMC in all studied concentrations. Gels of tomato waste pectin exhibited a lower lightness. Regarding a^* values, ATP and WTP showed positive scores, which means the color shifted towards reddish, while HMC presented negative scores, which means the opposite, that is, the absence of red color. The values of b^* were positive for all samples, i.e., the gels were yellowish. These results indicated that the gels exhibited differences in the color parameters since the pectin sources were different. Tomato pectin gels were red–brown due to the presence of lycopene [15].

Conclusions

In this study the extraction of pectin from the waste of tomato processing was carried out. Two methods were used. One of them, the acid method, was optimized by extraction with hydrochloric acid solution at pH 2, 85 °C, and 60 min. The other one, the aqueous method, used distilled water at 95 °C for 180 min. The yield and the purity of the extract obtained by the acid method were significantly higher than that of the sample obtained by the aqueous one. The pectin obtained by the former, namely ATP, and the one obtained by the extraction with water, WTP, were classified as high-methoxylated pectin due to their degree of methoxylation and their galacturonic acid content.

These results show that the process affected the properties of the pectin extracted. ATP presented $[\eta]$, Mw_v , consistency coefficient, elastic modulus and hardness higher than WTP.

The gelation kinetic parameters of WTP such as IST and CST were higher than those of ATP and HMC, in line with its highest DM value. However, its SDR_a was lower than that of the rest of the samples and could be related to its small size molecule. It was found that the mechanism of gelation of tomato pectins corresponded to those of high-methoxylated pectin. Two activation energies, Ea_1 and Ea_2 , were found, which explains a two-step mechanism, one at a high temperature range, driven by hydrophobic interactions, and a second one, driven by hydrogen bonds, at a lower temperature range.

Results suggest that the acid method gave a sample, ATP, with similar properties to those of the commercial pectin used as control and better than the properties found in WTP, which makes this pectin suitable to be used in food formulations, upgrading the waste of tomato processing.

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