Characterization of Pectins Extracted from Different Varieties of Pink/Red and White Grapefruits [*Citrus Paradisi* (Macf.)] by Thermal Treatment and Thermosonication

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Abstract: The physical and chemical properties of pectin extracts obtained from different white and pink/red varieties of grapefruit [*Citrus paradisi* (Macf.)], using both conventional heating (CHE) and thermosonication (TS), were investigated. The content of galacturonic acid (GalA), degree of esterification (%DM), color and antioxidant capacity were analyzed. Fourier-Transform Infrared Spectroscopy (FTIR) associated with multivariate analysis enabled a structural comparison among the pectin extracts, and differential scanning calorimetry (DSC) completed a full landscape of the investigated extracts. Pectin extracts obtained by CHE showed mostly higher GalA than those obtained by TS. All the extracts had a high antioxidant capacity, as determined by 2,2 diphenyl 1-picrylhydrazyl (DPPH*) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS*+) assays, and a high correlation with the GalA content. The main differences observed in the FTIR spectra occurred in the 1200 to 900 cm^{-1} region (differences in GalA). The glass transition temperatures (Tgs) of all extracts were above 85 °C, making them interesting as stabilizing agents for the food industry.

Keywords: antioxidant capacity, grapefruit, infrared spectroscopy, pectin extracts, vitreous transition temperature

Practical Application: A wide database for the characterization of pectin extracts from grapefruits was obtained. The relationship between the extraction method and the source of pectins, with the physicochemical and antioxidant properties provided great support for their application in the food industry.

Introduction

The production of grapefruits [*Citrus paradisi* (Macf.)] is widely extended all over the world and like all agricultural production, citrus fruits present a large variability in chemical composition and physical properties, depending on the variety, rootstock, soil, fertilization, irrigation, age, maturity, position in the tree, among others (Berk, 2016). The citrus peel is an agro-waste arising from the production of juices, currently disposed as a solid waste or as animal feed (Grassino et al., 2016). However, citrus peels represent a rich source of bioactive compounds, mainly composed of pectins (Kaya, Sousa, Crépeau, Sorensen, & Ralet, 2014), that is, complex colloidal polysaccharides of widespread occurrence in the cell walls of plants, particularly in the middle lamella (layer between adjacent cells).

Pectins are highly valuable functional ingredients because they take part of the so-called soluble dietary fiber (Grassino et al., 2016), also having antioxidant properties (Bayar et al., 2017). In addition, they have several industrial applications, as thickening, stabilizing, or gelling agents in the elaboration of jams, jellies, or beverages, which are directly related with their chemical structure and physical properties, which in turn, are highly dependent on the *Citrus* origin.

Taking into account the high nutritional and technological value of pectins and the large variety of citrus fruits, obtaining pectins from citrus peels represents a cost-effective strategy to add value to these agro-wastes. Pectins can be commercially extracted from citrus peels by using hot water (60 to 100 °C) for 0.5 to 6 hr, at pH 1.5 to 3. The combination of heating extraction with ultrasound (thermosonication, TS) is an innovative technology that improves the extraction efficiency. However, the cost and bulkiness of equipment, noise generated by cavitation and structural alterations should be carefully considered when large scale applications are pursued (Wang et al., 2015).

Although pectins have been discovered more than 210 years ago, their physical-chemical and structural properties are still a subject of investigation due to the great diversity of this polymer family and the close relationship between structure and function. Pectins consist in linear chains of partially methyl-esterified (1→4) linked α-d-galacturonic acid residues, also containing neutral sugars such as glucose or galactose. Depending on the percentage of methoxyl groups, pectins can be classified into pectins of high or low degree of methoxylation (%DM), the first group having more than 50% esterification. The %DM is directly related with the functional and technological properties of pectins. In this regard, pectins of high %DM are able to form gels under acidic conditions whereas those of low %DM can better interact with divalent cations through the free carboxyl (Venzon et al., 2015).

Considering the importance of physical and structural properties on the functionality of pectins, reliable and quick

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characterization methods become necessary. Fourier-transform infrared spectroscopy (FTIR) is a consistent technique to ascertain structural and physical properties of carbohydrates. As no exogenous chemical reagents are needed, samples require almost no preparation and analytical testing do not generate hazardous waste, FTIR is definitely a useful tool to determine the composition of complex polysaccharides in a quick and environmentally friendly way (Santos, Araujo-Andrade, Tymczyszyn, & Gomez-Zavaglia, 2014). The use of FTIR in tandem with multivariate analysis has enabled an expeditious determination of the oligo and polysaccharide composition of different products, including commercial sugars, cellulose, starch, hemicelluloses, carrageenans, and hyaluronates (Fellah, Anjukandi, Waterland, & Williams, 2009; Kačurakóvá & Wilson, 2001).

Considering the particular structure of pectins, rich in polar groups, FTIR associated with multivariate analysis has also been used for their characterization in olive and orange pulps (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998), tissues of broccoli, mango, and apple (Kyomugasho, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015), carrot puree, broccoli (Kyomugasho et al., 2015), tomato (Chylińska, Szymańska-Chargot, & Zdunek, 2016), and citrus (Wang et al., 2016).

The ability of sugars to form glassy matrices of high viscosity and low molecular mobility explains their numerous functional and technological applications. The stabilization of such glassy matrices occurs below the glass transition temperatures (Tg) (Tymczyszyn et al., 2012). Therefore, carbohydrates with high Tg are generally used to stabilize food matrices (Sosa et al., 2016).

For this reason, the goal of this work was to investigate pectin extracts from different white and red/pink varieties of grapefruits, obtained by using both CHE and TS. The extracts were analyzed for structural aspects, namely content of galacturonic acid (GalA), %DM, color, and antioxidant capacity. A combined approach of FTIR, multivariate analysis and differential scanning calorimetry (DSC) enabled a full characterization of grapefruits pectins, thus providing a background for potential industrial applications.

Materials and Methods

Material

Different varieties of pink/red and citric combinations of white grapefruits [*Citrus paradisi* (Macf.)], ratio (Brix/acid) = 5.5, possessing uniform skin coloration, free of cuts and having similar weight and size, were provided by the Estacion Experimental INTA Bella Vista (Corrientes, Argentina, 28° 30′ 52.43′′ N, 59° 1′ 47.94′′ S). Citric combinations of "Parana" on different rootstocks (used at stem) were investigated: "Citrumelo Swingle," "Duncan," "Tangelo Orlando" and "Lima Rangpur." Red/pink varieties included "Star Ruby" (pink), "Foster" (red), "Red Blush" (pink), and "Red Shambar" (pink).

Methods

Extraction of pectins. A total of 10 fruits for each variety were washed with tap water and sanitized (HClO, 200 mg/L/5 min). The peel of grapefruits including flavedo (the external layer of the peel) and albedo (the middle layer) was first separated and immersed in a water bath at 90 °C for 5 min to inactivate enzymes, and then cut in 1 cm^2-slices. Subsequently, it was dried in an oven under vacuum at 50 °C overnight. Commercial pectins from citrus peel (Sigma-Aldrich) with galacturonic acid ≥74% (dried basis) were used as controls.

To extract pectins from the peels, deionized water adjusted with 2 N H_2SO_4 to reach pH 1.5 was used as the extraction solvent. Extracting pectins using CHE was carried out according to Geerkens et al. (2015) with slight modifications. Briefly, twenty grams of peel particles were mixed with 400 mL extraction solvent under continuous stirring (Precytec modelo AE-29, Argentina) and heated at 90 °C for 150 min without sonication.

TS was applied in a 6 L-ultrasonic tank (Cleanson model KT 1106, Argentina. 200 W, 40 KHz) with thermostatic control. Twenty grams of peels were mixed with 400 mL extraction solvent and immersed into the ultrasonic bath at 60 °C for 30 min, under periodic agitation (10 s on, 50 s off). The temperature was measured with a digital thermometer located in the center of the extraction vessel.

The extracted mixtures were filtered and pressed using a nylon cloth. Alcohol insoluble residues were precipitated with ethanol (96%) for 3 hr at 20 °C. The clots were filtered, pressed, washed with ethanol, and pressed again. The alcohol insoluble residues were dried at 45 °C for 14 hr, milled, and sieved. Thereafter, the alcohol insoluble residues were freeze-dried on a Heto FD4 equipment (Heto Lab Equipment, Denmark) (condenser: −45 °C; chamber pressure: 0.04 mbar, 48 hr). The freeze-dried samples were kept in desiccators containing silica gel until analysis.

FTIR spectra. Approximately 5 mg of freeze-dried samples were placed on the sample holder of an ATR-FTIR Thermo Nicolet iS10 spectrometer (Thermo Scientific, MA, U.S.A.). Spectra were registered in the 4000 to 500 cm^{-1} range by co-adding 100 scans with 4 cm^{-1} spectral resolution, using OMNIC software (version 8.3, Thermo Scientific). An average of 9 to 10 spectra were recorded for each sample.

The obtained spectra were preprocessed using mean centering correction (MSC) (The Unscrambler® software, version 10.2, CAMO, Norway). Principal component analyses (PCA) were performed on the FTIR spectra corresponding to the different extracts obtained either by CHE or by TS, as described in the previous section.

In addition, the bands at approximately 1740 cm^{-1} (arising from the $\nu C=O$ from ester group) and at approximately 1630 to 1600 cm^{-1} (due to the νCOO^- from the carboxylate group) were used to determine the %DM (Kyomugasho et al., 2015), according to Eq. (1):

$$\%DM = \frac{A_{1740}}{A_{1740} + A_{1630}} \times 100 \quad (1)$$

where A_{1740} is the area of the band at 1740 cm^{-1}, and A_{1630}, that of the band at 1630 cm^{-1}.

Determination of GalA. The GalA content was determined using the carbazol method, according to Filisetti-Cozzi and Carpita (1991). The absorbance of the pinkish complex obtained was read at 525 nm in a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The content of GalA was expressed as g D-galacturonic acid equivalents kg^{-1} pectin extract.

Color. The CIELab coordinates (L*, a*, b*) of the AIR samples were read in a Tristimulus Colorimeter Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan), with a white tile as the reference. The L* value represented the lightness, ranging from 0 (black) to +100 (white); a* value ranges from −100 (green) to +100 (red) and b* value ranges from −100

(blue) to +100 (yellow). The hue angle (H^*_{ab}) and chroma (C^*) were calculated according to Eqs (2) and (3):

$$H^*_{ab} = \tan^{-1}\left(b^*/a^*\right) \quad (2)$$

$$C^* = \sqrt[2]{(a^{*2} + b^{*2})} \quad (3)$$

The UV-absorbance of the pectins was determined spectrophotometrically, by registering the spectrum of samples dissolved in deionized water (1% w/v) between 300 and 420 nm (Shimadzu UV-1800 Spectrophotometer, Kyoto, Japan).

Antioxidant capacity. Total antioxidant capacity was determined using the DPPH• (2,2-Diphenyl-1-picrylhydrazyl) and ABTS•+ [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] assays, according to Magalhães et al. (2012). The antioxidant capacity was expressed as EC50% (effective concentration), the lower the value the higher the antioxidant activity. The EC50% values were calculated by non-linear regression of plots where the abscissa represented the concentration of the tested samples, and the ordinate was the average percentage of antioxidant activity.

Determination of Tg. Twenty % w/v solutions were prepared with the freeze-dried pectin extracts previously obtained. The solutions were then freeze-dried following the same protocol.

Glass transitions of the freeze-dried samples were determined on samples, immediately after freeze-drying, by DSC (onset values, heating rate: 10 °C/min) in a Q100 calorimeter (TA Instruments, New Castle, DE, U.S.A.), calibrated with indium, lead and zinc. Hermetically sealed 40 μL medium pressure pans, containing 3 to 5 mg of samples, were used (an empty pan served as reference). The runs were carried out within –20 and 180 °C.

Reproducibility of results

All experiments were performed on duplicate samples using 3 independent extractions. Significant differences were evaluated by Analysis of Variance (ANOVA) and Duncan test ($P < 0.05$) using the Info-Stat Statistical Software (Cordoba-Argentina, 2009). The Pearson correlation coefficient (R) was used ($P < 0.01$) to explain the relationship between antioxidant capacity and the GalA content and Hue angle value.

Results and Discussion

GalA content and %DM

The structure of pectins strongly determines their functionality and technological properties (Wang et al., 2016), and the GalA content is a key factor, affecting their gellifying, stabilizing and thickening properties. In this work, the GalA content of citrus peel obtained by CHE varied from 724.3 to 894.0 g/kg (Table 1). These values are above 650 g/kg GalA, which is the limit required by the Joint FAO/WHO Expert Committee on Food Additives, for a pectin to be considered as food grade (JECFA, 2007), and is slightly higher than the GalA contents reported for commercial pectins from citrus peel (712.9 g/kg) (Kaya et al., 2014; Wang et al., 2016). Among the white grapefruits, "Parana on Lima Rangpur" and "Parana on Duncan," where those with the highest percentages of GalA, and among the pink/red varieties, "Foster" and "Red Shambar" (Table 1).

GalA contents of the extracts obtained by TS were mostly lower than those obtained by conventional heating. The exceptions were "Parana on Tangelo Orlando" and "Star Ruby" grapefruits, for which the GalA content was not significantly affected by the extraction method ($P > 0.05$) (Table 1). It must be mentioned that within the TS extracts, only 3 of them ("Parana on Tangelo Orlando," "Parana on Duncan," and "Star Ruby") had GalA contents above 650 g/kg, and thus, can be considered as food grade (JECFA, 2007). Other authors also reported that obtaining of pectins from citrus peel by CHE leads to higher GalA contents than TS extraction (Wang et al., 2015). The results obtained in this work, demonstrated that the GalA contents were strongly determined not only by the extraction method but also by the variety of grapefruit used as a source of pectins.

The parameter %DM, used to classify pectins, can be determined using FTIR spectroscopy. Figure S1 shows the FTIR spectral differences between pectins of high and low %DM in the 1800 to 1550 cm^{-1} region ('Parana on Citrumelo Swingle" pectin extracts obtained by CHE and by TS were selected as representative examples of high and low %DM). The increase of the relative intensity of the νCOO^- band, at approximately 1630 to 1600 cm^{-1}, indicates the lower %DM.

The %DM is the main parameter affecting not only gelling but also influencing the surface tension and the formation of emulsions. In this work, all the pectin extracts can be classified as high methoxy (%DM ≥ 50%), with exception of those obtained by TS from white "Parana on Citrumelo Swingle" and pink/red "Red Shambar" varieties, which also had a low GalA content (Table 1). Some authors suggest that the slight changes in the %DM of pectins extracted by TS may be related to the mechanical effects of cavitation, producing stronger shear force, which could break the C–O bond of carboxyl groups (Zhang et al., 2013).

The white grapefruits "Parana on Lima Rangpur" and "Parana on Duncan" and the pink-red varieties "Star Ruby" and "Foster" did not have statistically significant differences in the %DM, ascribable to the extraction method (Table 1). On the contrary, "Parana on Citrumelo Swingle" grapefruit, "Red Shambar" and "Red Blush" varieties had lower %DM when TS was applied as extraction method. In turn, "Parana on Tangelo Orlando" extracts obtained by TS had significantly higher %DM than those extracted by conventional heat (56.84 ± 5.35 compared with 49.64 ± 1.29) (Table 1). In light of these results, it can be concluded that the extraction method led to structural changes in the obtained extracts, probably because of the hydrolysis of the esterified pectins.

FTIR spectra and multivariate analysis

The major FTIR spectral differences among the pectin extracts were observed in the so-called "fingerprint region" of sugars (1200 to 900 cm^{-1}) (Santos, Araujo-Andrade, Tymczyszyn, & Gómez-Zavaglia, 2014), a complex region rich in bands attributed to the C–O–C glycosidic linkage, the δCOH and the νC–C vibrational modes. Even when it is difficult to assign the vibrational modes corresponding to each individual band, the bands in this region collectively provide a complex pattern that univocally characterizes each carbohydrate and can be used to identify it in a pure sample. According to the loading plots in PC1 and PC2, the main differences among pectin extracts were observed in that region (Figure S2). Hence, a PCA was carried out on 153 different FTIR spectra in that region (Figure 1). PC1 and PC2 explained 61% and 17% of the variance, respectively (Figure 1) and no clearly defined groups were observed. This

Table 1–Galacturonic acid content (g d-GalA/kg) and percentage of methoxylation (%DM) of white and pink/red grapefruit pectin extracts obtained by conventional heat extraction (CHE) and thermosonication (TS).[1]

	CHE		TS	
	g/kg GalA	%DM	g/kg GalA	%DM
White grapefruits[2]				
"Lima Rangpur"	821.3 ± 27.6^{a}	51.10 ± 4.81^{A}	555.0 ± 32.2^{b}	50.80 ± 4.61^{A}
"Tangelo Orlando"	729.0 ± 26.0^{a}	49.64 ± 1.29^{A}	709.0 ± 23.9^{a}	56.84 ± 5.35^{B}
"Citrumelo Swingle"	724.3 ± 12.6^{a}	50.66 ± 7.07^{A}	539.7 ± 20.5^{b}	39.83 ± 1.07^{B}
"Duncan"	780.0 ± 13.9^{a}	50.98 ± 2.15^{A}	702.7 ± 34.0^{b}	51.05 ± 2.69^{A}
Pink/red grapefruits				
"Red Shambar"	894.0 ± 43.5^{a}	54.89 ± 5.30^{A}	550.0 ± 16.5^{b}	44.00 ± 1.62^{B}
"Star Ruby"	749.3 ± 26.1^{a}	50.03 ± 5.14^{A}	727.0 ± 16.1^{a}	50.48 ± 2.90^{A}
"Red Blush"	744.0 ± 7.2^{a}	59.95 ± 3.33^{A}	455.3 ± 8.1^{b}	51.98 ± 2.81^{B}
"Foster"	849.3 ± 13.3^{a}	54.61 ± 3.88^{A}	608.7 ± 5.5^{b}	56.69 ± 1.43^{A}

[1]Values represent means ± standard deviation of 3 replicates. Values with different lowercase superscript letters in the same rows indicate significant differences ($P < 0.05$) between treatments for GalA. Superscript capital letters in the same rows indicate significant differences between treatments for %DM.
[2]Parana variety of different rootstocks.

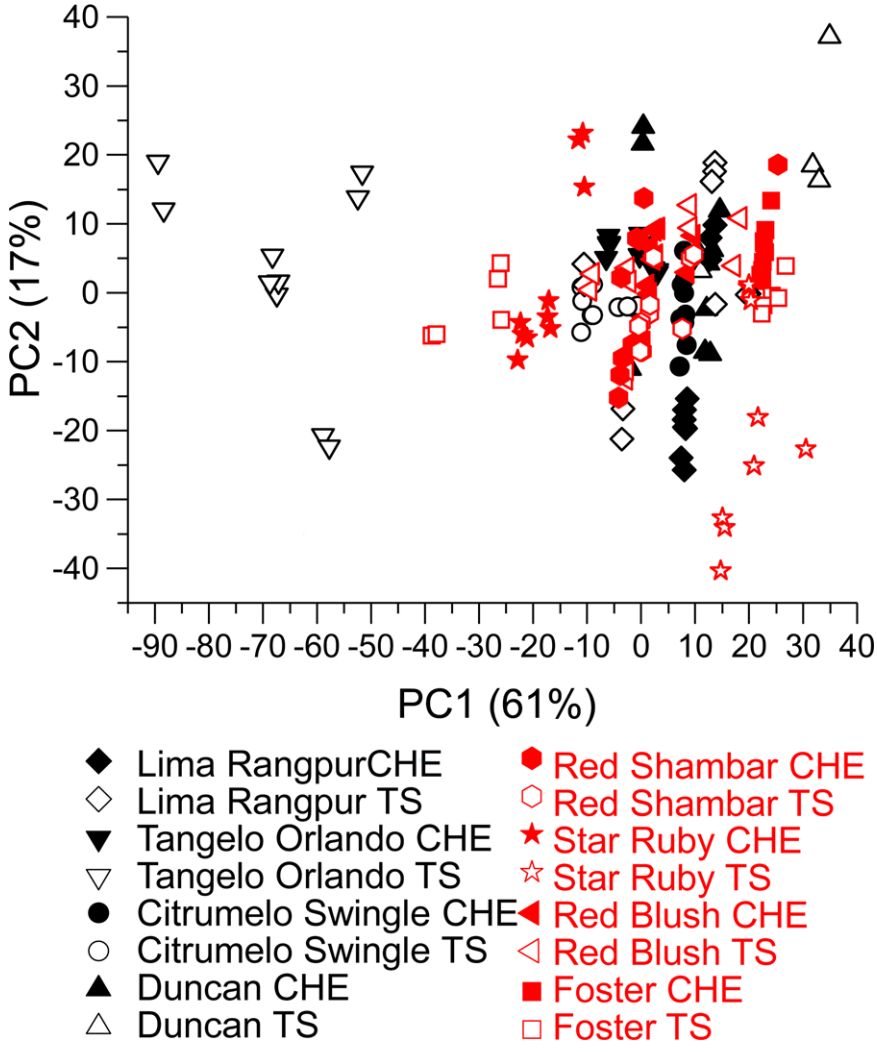

Figure 1–PCA of the FTIR spectra of pectin extracts in the 1200 to 900 cm^{-1} region. Full symbols: CHE; empty symbols: TS. Black symbols: white varieties: rhombus: "Parana on Lima Rangpur"; down triangles: "Parana on Tangelo Orlando"; circles: "Parana on Citrumelo Swingle"; up triangles: "Parana on Duncan." Red symbols: pink/red varieties: diamonds: "Red Shambar"; stars: "Star Ruby"; left triangles: "Red Blush"; squares: "Foster." The loading plots in PC1 and PC2 are shown in Figure S2.

indicates that most of the samples (regardless the origin and the type of extraction) showed only slight differences. In spite of that, it is important to notice that pectin extracts obtained by TS from 'Parana on Tangelo Orlando' cultivar were those showing the greatest differences along PC1. In turn, extracts obtained by TS from "Star Ruby" cultivar were those showing the greatest differences with regard to the extracts obtained from the former cultivar (Figure 1).

In spite of the complexity of the 1200 to 900 cm^{-1} region of carbohydrates, Kačuráková, Capeka, Sasinková, Wellner, and

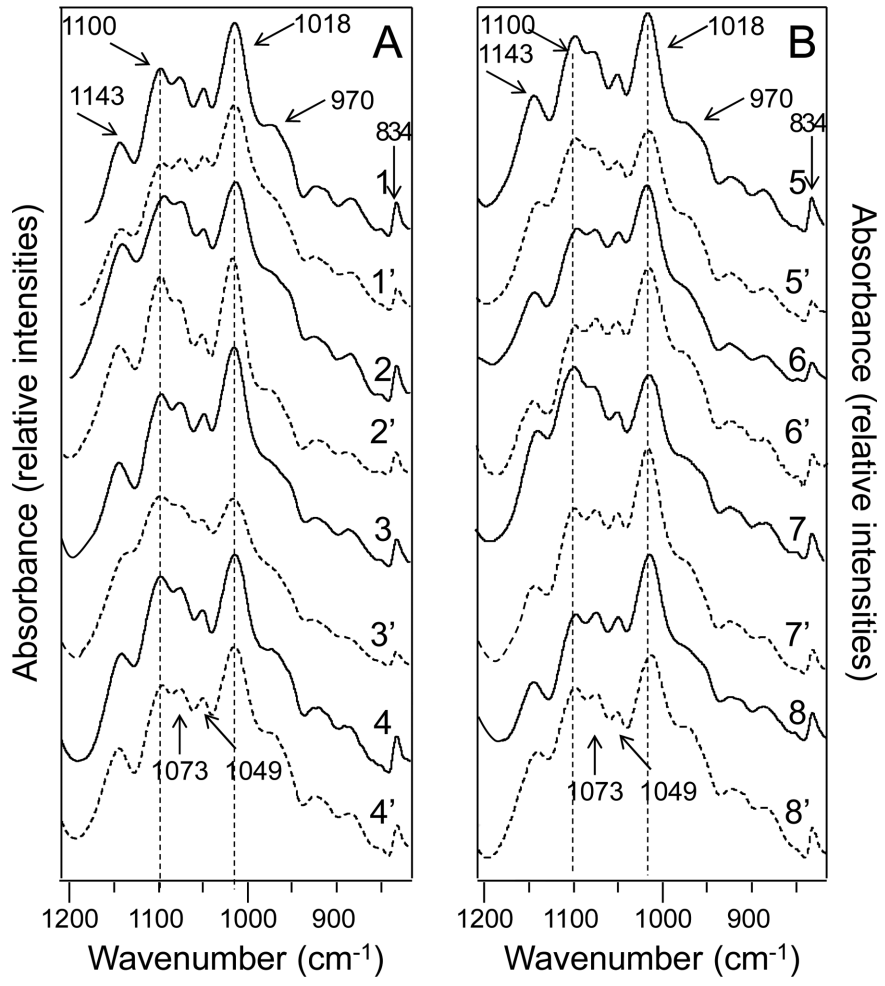

Figure 2–FTIR spectra of pectin extracts in the 1200 to 900 cm^{-1} region. Full line spectra: CHE; dash line spectra: TS. (A) white grapefruits; (B) pink/red grapefruits. 1: Parana on "Lima Rangpur"; 2: "Parana on Tangelo Orlando"; 3: "Parana on Citrumelo Swingle"; 4: "Parana on Duncan"; 5: "Red Shambar"; 6: "Star Ruby"; 7: "Red Blush"; and 8: "Foster."

Ebringerová (2000) reported that pectic compounds from different origins have different band patterns in this region. In this sense, samples rich in uronic acid (like GalA) show 2 intense bands at 1100 and 1018 cm^{-1} (Barros, Coimbra, Barros, Rutledge, & Delgadillo, 1997), and a shoulder at 1143 cm^{-1} (Coimbra et al., 1998). The intensity of these bands on the investigated grapefruits' extracts (Figure 2) was directly related with their contents of %GalA and %DM (Table 1). For example, the intensities of such bands for "Parana on Citrumelo Swingle" extracts obtained by thermosonication, which were those with the lowest content of both %GalA and %DM (Table 1), were the lowest ones (spectrum 3 in dash lines, Figure 2). On the contrary, all the extracts obtained CHE, had higher %GalA and %DM and in consequence, the above mentioned bands were more intense (full line spectra in Figure S1). The bands at 1073 and 1049 cm^{-1}, and the shoulder at 970 cm^{-1} were ascribed to neutral sugars, namely arabinose and galactose (Coimbra et al., 1998). Grapefruits have higher contents of galactose than other citrus fruits, which take part of short lateral chains (Kaya et al., 2014). The variable intensity of these bands in the investigated extracts indicates that the contents of galactose and arabinose do not seem to have any relation to either the grapefruit variety or the type of extraction. In turn, the band at 834 cm^{-1} can be ascribed to α-glycosidic linkages (Chylińska et al., 2016), thus indicating that the linkage among monomeric units is α.

As a whole, the FTIR spectra and multivariate analysis denoted structural differences in the GalA and esterification of the investigated extracts, thus complementing the colorimetric determinations shown in Table 1.

Color

The color of the investigated extracts is the result of the presence of various colored compounds and can be measured through the parameters L*, a*, and b*. These parameters can be used to evaluate color changes associated to biochemical processes, for example to monitor browning resulting from storage or from thermal treatments (Berardini, Knödler, Schieber, & Carle, 2005; Geerkens et al., 2015).

The color differences of the studied pectin extracts are shown in Table 2. Among white grapefruits, extracts obtained by CHE had mostly lower values of L* parameter and Hue* angle and greater values for a* than those extracted by TS. The b* and Chroma* values were varied in the former group. These results are typical from samples undergoing browning reactions (Berardini et al., 2005; Geerkens et al., 2015; Limbo & Piergiovanni, 2006). The exposure of the extracts to high temperatures (90 °C) for prolonged time (150 min) upon CHE could stimulate a wide number of reactions, including caramelization, Maillard reactions and chemical oxidation of phenolic compounds (Berardini et al., 2005), thus explaining the yellowish color of these samples (Figure 3A). Furthermore, in this work, the absorption of pectin extracts obtained using CHE was maximal in the UV region, at 300 and 320 nm (possibly due to colorless browning intermediates produced

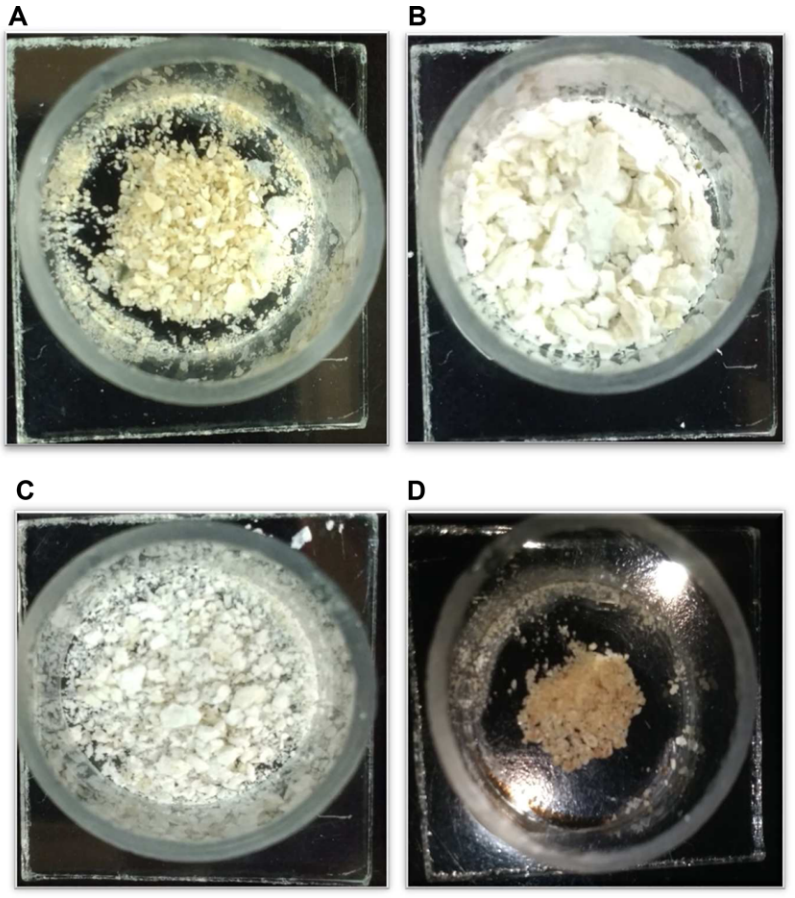

Figure 3–Representative images of pectin extracts from white ("Parana on Lima Rangpur") and pink/red ("Star Ruby") grapefruits. Pectin extracts from "Parana on Lima Rangpur" obtained by CHE (A); pectin extracts from "Parana on Lima Rangpur" obtained by TS (B); pectin extracts from "Star Ruby" obtained by CHE (C); pectin extracts from "Star Ruby" obtained by TS (D).

during early stage of Maillard reaction), and in the visible region at 420 nm (data not shown), probably because of the presence of dark-brown polymeric compounds (Yu, Zhou, & Yang, 2016). Other authors have also reported a brown color for pectins extracted from citric and mango peels using CHE (Geerkens et al., 2015; Wang et al., 2015). On the contrary, pectin extracts from the white variety obtained by TS, in which the thermal treatment (60 °C, 30 min) was much softer, did not show color changes (Figure 3B).

Among colored cultivars, the color of the peel results from the combination of various pigments present in the epicarp, and is of extremely importance for a good first impression on consumers. Some of those pigments have bioactive properties, and may undertake deterioration reactions. Within the pink/red variety, pectins extracted by CHE were colorless whereas those extracted by TS were colored (Figure 3C, D and Table 2). In fact, the extracts obtained by TS mostly had higher a*, b*, and Chroma* and lower L* parameters than those obtained by CHE (Table 2). Taking into account that the observed color patterns were related with both the grapefruit variety and the extraction procedure, an average of each color parameter was calculated for each of these four patterns (Table S1). Hence, Hue* angle values of pectins extracted by TS were within 51.61° and 71.51°, whereas those of pectins extracted by CHE were within 74.11° and 82.87° (Table 2). These results indicate coloration in the pectin extract (Figure 3D). Taking into account the widely accepted international criterion to assign the angle 0° to the +a* semiaxis (redness) and the angle of 90 °C to the +b* semiaxis (greenness), pectin extracts from red/pink varieties obtained by TS showed a greater redness (Limbo & Piergiovanni, 2006). This coloration may be attributed to the co-extraction of pigments present in plastids of the epicarp, which are in higher amounts in mutants of different *Citrus* species. Although the subsequent washing of pectins isolated with ethanol reduced the content of these pigments, they may still be partially associated to the extracted pectins (Berardini et al., 2005).

Antioxidant activity

As the extraction procedures employed in this work are physical treatments, a concomitant extraction of other compounds having free hydroxyl groups and/or phenolic compounds, which are present in grapefruit peels and have antioxidant activity may also occur (Berardini et al., 2005). In this regard, it has been reported that the pectic polysaccharides from apple, commercial and citrus peel have high antioxidant activity, as determined by the DPPH• free radical scavenging assay (Wang & Lu, 2014). In this work, most of the extracts from white and pink/red varieties obtained by CHE showed greater antioxidant capacity than those obtained by TS (DPPH• and ABTS•+ assays, Table 3). This indicates that the extraction method strongly determines the antioxidant capacity of the samples. In this regard, it was reported that acoustic cavitation produced during ultrasound treatments could lead to the production of free radicals, thus explaining the lower antioxidant capacity of the TS extracts (Piyasena, Mohareb, & McKellar, 2003).

The Pearson correlation coefficient (R) and *P* values enabled a better comparison of the antioxidant capacities of the different extracts (Tables S2 and S3). Regardless the extraction procedure, extracts from white grapefruits had higher inverse correlations

Table 2–Color parameters (L*, a*, b*, Chroma*, and Hue*) of white and pink/red grapefruit pectin extracts obtained by conventional heat (CHE) and thermosonication (TS).[1]

	L*		a*		b*		Chroma*		Hue*	
	CHE	TS	CHE	TS	CHE	TS	CHE	TS	CHE	TS
White grapefruits[2]										
"Lima Rangpur"	65.41 ± 0.03^{a}	74.61 ± 0.01^{b}	5.72 ± 0.02^{A}	1.52 ± 0.01^{B}	14.38 ± 0.19^{a}	14.37 ± 0.01^{b}	15.47 ± 0.20^{A}	14.45 ± 0.01^{B}	68.31 ± 0.28$^{\mathbf{B}}$	83.95 ± 0.05^{b}
"Tangelo Orlando"	69.12 ± 0.01^{a}	80.39 ± 0.11^{b}	3.81 ± 0.01^{A}	0.66 ± 0.18^{B}	17.26 ± 0.01^{a}	11.70 ± 0.22^{b}	17.67 ± 0.01^{A}	11.72 ± 0.20^{B}	77.54 ± 0.04^{a}	86.78 ± 0.84^{b}
"Citrumelo Swingle"	76.76 ± 0.02^{a}	66.33 ± 0.01^{b}	3.34 ± 0.02^{A}	1.79 ± 0.04^{B}	18.44 ± 0.09^{a}	18.05 ± 0.02^{b}	18.74 ± 0.10^{A}	18.14 ± 0.01^{B}	79.75 ± 0.08^{a}	84.34 ± 0.12^{b}
"Duncan"	68.87 ± 0.01^{a}	62.36 ± 0.02^{b}	5.35 ± 0.01^{A}	1.24 ± 0.01^{B}	15.41 ± 0.01^{a}	16.47 ± 0.02^{b}	18.44 ± 0.01^{A}	16.51 ± 0.01^{B}	74.11 ± 0.05^{a}	85.68 ± 0.03^{b}
Pink/red grapefruits										
"Red Shambar"	76.99 ± 0.01^{a}	55.35 ± 0.01^{b}	1.68 ± 0.01^{A}	9.44 ± 0.01^{B}	13.40 ± 0.01^{a}	21.10 ± 0.01^{b}	13.51 ± 0.01^{a}	23.11 ± 0.01^{B}	82.87 ± 0.05^{a}	65.69 ± 0.03^{b}
"Star Ruby"	80.87 ± 0.01^{a}	51.21 ± 0.05^{b}	2.56 ± 0.01^{A}	17.00 ± 0.89^{B}	16.01 ± 0.01^{a}	21.45 ± 0.03^{B}	16.21 ± 0.01^{A}	27.38 ± 0.60^{B}	80.93 ± 0.02^{a}	51.61 ± 1.40^{b}
"Red Blush"	78.63 ± 0.01^{a}	72.78 ± 0.01^{b}	2.28 ± 0.02^{A}	5.61 ± 0.04^{B}	17.08 ± 0.04^{a}	16.77 ± 0.01^{b}	17.23 ± 0.01^{A}	17.70 ± 0.01^{B}	82.39 ± 0.07^{a}	71.51 ± 0.14^{b}
"Foster"	71.27 ± 0.01^{a}	71.00 ± 0.01^{b}	5.05 ± 0.01^{A}	9.51 ± 0.02^{B}	17.74 ± 0.01^{a}	15.46 ± 0.02^{b}	18.44 ± 0.01^{A}	18.14 ± 0.01^{B}	74.11 ± 0.05$^{\mathbf{a}}$	58.39 ± 0.08^{b}

[1]Values represent means ± standard deviation of 3 replicates. Statistics was performed on rows, by comparing different treatments (TS and CHE) for similar parameters (L*, a*, b*, Chroma*, Hue*). For each case, different scripts were used (a, b; A, B; *a, b; A, B;* **a**, b). Different scripts indicate significant differences ($P < 0.05$) between treatments. [2]Parana variety of different rootstocks.

between DPPH• activity and GalA content ($R = -0.86$ $P < 0.0001$) and slightly lower compared with ABTS•+ assay and GalA ($R = -0.64$; $P < 0.0001$) (Table S2). These results indicate that the DPPH• and ABTS•+ radical scavenging capacity of pectins gradually increased with the increase of GalA content, thus explaining the greater antioxidant capacity of the CHE samples. Similar results were reported by Wang et al. (2016) for pectins from fresh grapefruit (*Citrus paradisi* Macf. cv. "Changshanhuyou").

The correlation values between antioxidant capacity and GalA content for pectin extracts obtained by TS from pink/red varieties of grapefruits were similar than those of the white grapefruits. In addition, a high direct correlation between the antioxidant capacity and the color was observed for the pink/red varieties extracted by TS. In turn, the values of DPPH• and Hue* angle showed a high correlation ($R = 0.92$; $P < 0.0001$). On the contrary, a lower correlation between the ABTS•+ and Hue angle ($R = 0.73$; $P = 0.0068$) was found, indicating that the more reddish the extracts the higher their antioxidant capacity (Table S3). The reason for this observation is that besides the hydroxyl groups and phenolic compounds, different pigments, namely β-carotenes and orange red lycopenes, are also present in pectin extracts (Zheng, Zhang, Quan, Zheng, & Xi, 2016), and could be co-extracted. These compounds give an intense red color to the peel, apart from antioxidants properties. For example, β-carotenes have an unsaturated hydrocarbon chain that could be oxidized, enhancing the nutritional value of pectins from red and pink grapefruit varieties. On the other hand, the pectin extracts obtained by CHE, were colorless and a significant correlation was not found with antioxidant capacity (Figure 3c).

Vitrification

The Tgs of most of the extracts investigated were within 85 to 95 °C, with no significant differences among extraction methods ($P > 0.05$) (Table 3). The only samples behaving differently were those obtained by TS from "Parana on Tangelo Orlando" cultivar. The Tg for such extract (105.5 ± 0.7 °C) was significantly higher than those of all the other samples. Note that this sample was also that with the greatest structural differences in the FTIR spectra (Figure 2). In addition, all the investigated pectins showed higher Tg than those reported for citric commercial pectins (reported values 48 to 60 °C) (Iijima, Nakamura, Hatakeyama, & Hatakeyama, 2000; Karaki, Aljawish, Muniglia, Humeau, & Jasniewski, 2016). Pectins from grapefruit peels contain shorter side chains than those from orange, lime and lemon (Kaya et al., 2014). High Tgs have been associated low water activities and to linear chains of high molecular weight polymers, and are strongly affected by the size and shape of carbohydrate molecules (Kaya et al., 2014). This could partially explain the higher Tgs obtained in this work.

The correlation between Tgs and %DM is controversial. While some authors reported that for a given water activity, low %DM pectins absorb more water than high %DM ones (Wallingford & Labuza, 1983), an opposite trend has also been stated (Panchev, Slavov, Nikolova, & Kovacheva, 2010). In this work, no direct correlation between the %DM and Tg was observed (Tables 1, 3), thus indicating that the obtained Tgs were the result of more complex interactions involving not only the water absorption capacity but also interactions with other compounds that may be present in the extracts.

As shown in Table 3, the investigated extracts had antioxidant capacity, thus indicating the co-existence of hydrophilic pectins with antioxidants, which are generally hydrophobic phenolic compounds. A recent study in which pectins were grafted with

Table 3–Antioxidant activity expressed as EC50% and vitreous transition temperatures (Tg) of pectin extracts obtained by conventional heat extraction (CHE) and thermosonication (TS).[1]

	DPPH• (g/mg)		ABTS•+ (g/mg)		Tg (°C)	
	CHE	TS	CHE	TS	CHE	TS
White grapefruits[2]						
"Lima Rangpur"	0.150 ± 0.07^{a}	0.320 ± 0.01^{b}	0.028 ± 0.01^{A}	0.050 ± 0.01^{B}	92.5 ± 2.3^{a}	94.2 ± 0.4^{a}
"Tangelo Orlando"	0.140 ± 0.01^{a}	0.152 ± 0.01^{B}	0.044 ± 0.01^{A}	0.078 ± 0.01^{B}	86.5 ± 2.5^{a}	105.5 ± 0.7^{b}
"Citrumelo Swingle"	0.104 ± 0.01^{a}	0.259 ± 0.01^{b}	0.048 ± 0.02^{A}	0.107 ± 0.01^{B}	89.7 ± 2.2^{a}	92.3 ± 0.6^{a}
"Duncan"	0.140 ± 0.01^{a}	0.202 ± 0.02^{b}	0.046 ± 0.01^{A}	0.055 ± 0.02^{B}	89.8 ± 0.3^{a}	91.4 ± 1.7^{a}
Pink/red grapefruits						
"Red Shambar"	0.289 ± 0.01^{a}	0.443 ± 0.01^{b}	0.140 ± 0.01^{A}	0.285 ± 0.01^{B}	85.5 ± 2.1^{a}	91.3 ± 0.9^{a}
"Star Ruby"	0.156 ± 0.04^{a}	0.112 ± 0.02^{b}	0.028 ± 0.01^{A}	0.139 ± 0.08^{B}	91.0 ± 2.9^{a}	94.4 ± 0.7^{a}
"Red Blush"	0.279 ± 0.02^{a}	0.489 ± 0.01^{b}	0.119 ± 0.01^{A}	0.239 ± 0.01^{B}	92.2 ± 0.4^{a}	92.7 ± 0.6^{a}
"Foster"	0.139 ± 0.01^{a}	0.105 ± 0.02^{b}	0.059 ± 0.05^{A}	0.097 ± 0.01^{B}	93.7 ± 2.6^{a}	92.4 ± 0.6^{a}

[1]Values represent means ± standard deviation of 3 replicates; the values of antioxidant capacity were expressed as g of extracts/mg of radical scavenging. Statistics was performed on rows, by comparing different treatments (TS and CHE) for similar parameters (DPPH•, ABTS•+, Tg). For each case, different scripts were used (a,b; A,B; *a,b*). Different scripts indicate significant differences ($P < 0.05$) between treatments. [2] Parana variety of different rootstocks.

phenolic compounds (Karaki et al., 2016), reported that such hydrophobic compounds led to a less hygroscopic polysaccharide at low water activities. This could be the reason for the higher Tg observed in the grapefruit extracts in comparison with pure pectins (Iijima et al., 2000).

The Tg is the range of temperatures at which amorphous materials pass from the glassy to the rubbery state (Santos et al., 2014). It is a key parameter for determining the optimal conditions for both food processing and storage of dehydrated products, as well as to understand the textural properties of food systems. For an adequate preservation, biological materials should be stored below Tg (amorphous state) (Miao et al., 2008). Above Tg (rubbery state) the molecular mobility is greatly increased and many amorphous compounds crystallize. Therefore, high Tgs difficult crystallization and other deteriorative reactions (that is, stickiness, caking, agglomeration), thus, they are desirable for the stabilization of carbohydrate containing products during storage. The values of Tg obtained in this work were particularly high (all of them above 85 °C, see Table 3), which is a temperature comparable with that of sucrose (Tg 77 °C), well-known as stabilizing compound (Hinrichs, Sanders, de Smedt, Demeester, & Frijlink, 2005). In particular, the Tg value for "Parana on Tangelo Orlando" extracts obtained by TS (Table 3) was comparable to that of trehalose, one of the most efficient stabilizing compounds (that is, trehalose, Tg 108 °C) (Wolkers, Oliver, Tablin, & Crowe, 2004).

To summarize the value of results presented in this section, it can be stated that the high values of Tg obtained for all the pectin extracts could be the result of the concomitant presence of pectins and antioxidants in both CHE and TS extracts. This results in technological and functional advantages, as they can be useful ingredients for the formulation of novel dehydrated functional foods.

Conclusions

The polysaccharide structure of pectins is on the basis of most of their technological applications. This work investigated the influence of both the extraction method and cultivar origin on the physical-chemical and thermophysical properties of 16 different pectin extracts obtained from grapefruit peels. Their high GalA content and %DM in most of the extracts makes them suitable candidates to be used as food ingredients. The FTIR spectra revealed that main differences observed occurred in the 1200 to 900 cm^{-1} region, and were ascribed to differences in the contents of GalA. Besides that, the color of the pectic extracts from the red/pink varieties could be an added value, supporting their use as color enhancers of some jellies, jams and coatings.

The high Tg of the all the extracts, together with the high antioxidant capacity, indicate that antioxidants were extracted concomitantly with pectins. As a whole, it could be concluded that certain cultivars (that is, "Parana on Tangelo Orlando") and certain extraction methods (that is, TS) lead to the obtaining of pectins with better functional and technological properties.

In summary, the results obtained in this study provided for the first time, a wide database for the characterization of pectin extracts from grapefruits. Furthermore, the approach employed brought a new vision of the relationship between the extraction method and the source of pectins with the physicochemical, antioxidant and thermophysical properties, thus supporting their application in food processing.

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Author Contributions

Enzo La Cava performed the experiments and also took part in writing. Esteban Gerbino contributed to experimental part of researches. Sonia Sgroppo and Andrea Gomez-Zavaglia planned the experiment and took part in writing.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Average values for color parameters.

Table S2. Correlation coefficients of GalA, DPPH•, ABTS•+, and Hue angle from white grapefruits extracted by conventional heating and thermosonication.

Table S3. Correlation coefficients of GalA, DPPH•, ABTS•+, and Hue angle from red/pink grapefruits extracted by thermosonication.

Figure S1. FTIR spectra from 'Parana on Citrumelo Swingle" pectin extracts obtained by CHE (full line spectrum), and by TS (dash line spectrum) in the 1800 to 1550 cm^{-1} region.

Figure S2. 1D loading plots in PC1 and PC2 performed on the FTIR spectra of pectin extracts.