Physicochemical characterization of pectin of rose grape Red Globe variety

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Summary
In order to generate useful information for the food industry, adding value to the national production chain of a "commodity", physico-chemical characteristics of the pectins extracted from the Red Globe rose grape were evaluated using several analytical methods and compared to commercial pectin. From this analysis, it was inferred that pectin of Red Globe grape are of low methoxyl grade, taking into account the behaviour of the characteristic signals for the esterified (COO-R, 1 745 cm^{-1}) and free (COO^-, 1 640 cm^{-1}) carboxyl groups and the area ratio of the Fourier transform infrared spectra. These results were in agreement with the analytical determinations of equivalent weight (1 441.32 ± 15.92 $mg \cdot meq^{-1}$), free acidity (0.69 ± 0.01 $meq \cdot g^{-1}$), degree of methoxylation (1.7 ± 0.1 %), degree of esterification (44.7 ± 1.3 %) and anhydrous galacturonic acid percentage (34.6 ± 0.6 %). These grape pectin characteristics are suitable for obtaining jellies or jams with low sugar content, adding value to the primary production chain.

Keywords
Red Globe*;* rose grape, pectins properties, degree of esterification, Fourier transform infrared spectrocopy

The Red Globe variety grape is the largest table berry grape. This variety has its origins at the University of Davis (Davis, California, USA) where it was created by interbreeding of the Emperador, Hunisa and Nocera varieties. Currently, the consumption of Red Globe grape has increased in America, Europe and other regions of the world, the variety being highly appreciated. Its racemes stand out for their large size, having an average weight of approximately 800 g, with long and thin peduncles. In general, its berries have a weight of 6.27–6.57 g per fruit [1] and can reach higher values of 9.25–10.75 g per fruit [2]; they are thick-skinned, pinkish-red in colour, with seeds, spherical in shape and of high calibre, approximately between 24 mm and 32 mm [1].

Grapes are characterized by being rich in water and sugars, vitamins, minerals and polyphenolic compounds with powerful antioxidant action, which makes them attractive to consumers. The grape is a very perishable fruit, like almost all foods with high water content. Therefore, it is important to preserve it through technologies that extend its useful life and add value to primary production. One of the technological alternatives in the food industry is obtaining preserves, such as jams, sweets and jellies, where fruits are used in combination with sweeteners of various types. In the formulation of this type of food, it is essential to know the properties of the fruit to define the appropriate conditions that allow obtaining a high-quality product, given that the characteris-

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tics of pectins and other components of the fruit influence the gelling capacity.

Pectins are a complex group of structural heteropolysaccharides that mainly contain units of galacturonic acid. They are present in the cell walls of vegetables, associated with other carbohydrates, such as hemicellulose. There are mainly two classes of pectic substances: pectinic acids (which contain a small portion of methyl esters of their galacturonic acids) and pectic acids (which contain only esterification-free galacturonic acid molecules). Pectins are constituted by a backbone of galacturonic acid molecules linked by glycosidic bonds [3]. Many of the galacturonic acid residues are esterified to form methyl esters. The degree of esterification (*DE*), which represents the percentage of carboxyl groups esterified with methanol, can range from 0 % to 100 %. Pectins in which more than half of the carboxyl groups (*DE* > 50 %) are in the form of methyl ester (-$COOCH_3$) are classified as high-esterification pectins. On the other hand, when pectins have less than half of the carboxyl groups esterified (*DE* < 50 %), they are called low-esterification pectins, their carboxyl groups being mostly in the form of methyl ester ($COOCH_3$) [3].

The methoxylation grade of pectins is very important. According to the methoxyl content, pectins with methoxyl content greater than 7 % are called high-methoxyl pectins; otherwise, they are known as low-methoxyl pectins [4].

Pectic substances have a close relationship with the calcium levels present in the medium [5], since, depending on the available COO^- groups, ionic bonds are formed between the pectin molecules and bivalent ions, mainly Ca^{2+}. The performance and application of pectins in the food industry depends both on intrinsic factors of pectins (their molecular weight and degree of esterification) and on extrinsic factors of the medium (pH, dissolved salts and presence of sugars) [3].

The grapes have diverse characteristics due mainly to the variety, and, to a lesser extent, to the growth conditions of the vineyard. At the moment of maturity, the grapes begin to show changes in the texture, due to softening caused by modifications that occur in the pectic substances of the cell walls.

The pectin content reported by various authors for the *Vitis vinifera* grape varieties varies in the range from 0.9 $g \cdot kg^{-1}$ to 6.5 $g \cdot kg^{-1}$, expressed as calcium pectate [6]. However, the functionality and quality of the pectins present in the fruit depend on the variety, processing and environmental factors [7]. The Concord grape variety (native to North America) is one of the richest in pectins, which form 4 % on fresh weight basis [8]. However, this does not exceed the pectins content of citrus (15–20 % on fresh weight basis), where they are mainly located in the peel, or the pectins of the pomaceous (apples), where they are distributed in the skin and the seeds [9]. For this reason, Concord stands out for its ability to produce jellies [8]. In wine grapes, pectins are high-grade methoxyl [10] and the pectin content of the pulp is double that of the skin [11].

Pectin gels consist of a three-dimensional network that immobilizes the aqueous component. Solvent (water), pH, and solutes (usually sugars) influence the intermolecular forces that contribute to the gel structure. The gelling mechanism of high-methoxyl pectins is produced by non-covalent binding of adjacent pectin chains, leading to an interconnected three-dimensional network. These bonds occur in the binding zones, which are stabilized by hydrogen bonds and hydrophobic interactions between the methyl ester groups of the pectin chains. Sugars (or other co-solutes) reduce the activity of water, promoting hydrophobic interactions.

Low-methoxyl pectins can also form gels but by a different mechanism than that described above. These pectins do not require high sugar levels or low pH to start gelling, instead, prefer gelling in the presence of divalent cations such as calcium. These divalent cations can form associations between charged species sequences on adjacent chains. That is, they can also form acid-sugar gels. However, the pH requirements for the formation of these gels are such that they make them inapplicable to most foods. Low-methoxyl pectins with *DE* near the upper limit of the range require the presence of some sugar for gelation, which is a further sign of their intermediate properties. These pectins, which have the ability to form gels with low sugar contents, allow the production of dietary jams or jellies.

Free carboxyl groups, available on the galacturonic acid residues of low-methoxyl pectin, can form calcium bridges with adjacent pectin polymers. This leads to a stronger and firmer gel, and reduces the syneresis effect. Low methoxyl pectins can be generated from high-methoxyl pectins by various treatments, such as acid-, alkali- or enzyme-catalysed de-esterification. The pectins of low-methoxyl grade require much less activation energy for the formation of gels than that needed by the high-methoxyl grade pectins. This indicates that the shortest sections of the polymeric vertebral column are precisely those that are involved in the process of gelation of low-methoxy pectins [12].

High-methoxyl pectins form gels with sugar contents greater than 550 g·kg^{-1}. When *DE* of the pectin falls bellow 50 %, the amount of sugar present in the mixture becomes less significant in the gelation process because the availability of COO^- groups begins to be high. In consequence, ionic bonds appear between pectic molecules supported by bivalent cations, mainly Ca^{2+}. If the chain is long enough, the gel can form in the total absence of sugar and acid, provided the calcium content is less than 1 g·kg^{-1}. For high-methoxy pectins, any sugar, alcohol or polyol facilitates gelation. A low pH value allows that the carboxylates be present in a non-ionized state, thereby reducing electrostatic repulsion between adjacent pectin chains. High-methoxyl pectins can gelate at higher pH values than low-methoxyl pectins [12].

Based on the presented knowledge and taking into account that no studies have yet been reported on the gelation properties of rose grape pectin of Red Globe variety, we found it necessary to evaluate the quality and physico-chemical characteristics of the pectins of this grape variety. The results of the present research could generate useful information for its application in the food industry, adding value to the national production chain of a multi-origin commodity that requires competitiveness to access the main world markets.

MATERIALS AND METHODS

Raw material

Grapes of the Red Globe variety produced and harvested in the Mendoza Province (Argentina) were used. Samples were purchased in the distributor market Central Tandil (Tandil, Buenos Aires Province, Argentina).

The fruits were kept in a chamber at a temperature between –1 °C and 0 °C, relative humidity from 90 % to 95 %, with air circulation speed lower than 1 m·s^{-1} for approximately 1 month in cardboard boxes containing perforated plastic bags with bunches [13]. Before carrying out the tests, the grapes were removed from the refrigerated chamber to allow them to reach equilibrium at the ambient temperature of 25 °C. For comparative purposes in the spectrocopy analysis, a commercial low-grade methoxyl pectin Genu (CP Kelco, Atlanta, Georgia, USA) donated by the company Cicloquímica (Buenos Aires, Argentina) was used.

Characterization of fresh grapes

Gravimetric (average fruit weight), geometric (characteristic dimensions such as equatorial and meridian diameter) and physico-chemical parameters (moisture content, soluble solids content, pH value, acidity value, calcium content) were determined for the fresh grape. These properties were considered of interest as descriptive quality parameters of the raw material. Grapes were characterized in terms of their average weight of whole fruit using an analytical balance with precision ± 0.001 g (Pioneer, Zhejiang, China) in 25 randomly selected representative units of the lot. To realize a dimensional description of the grape, two representative characteristic lengths of its geometry were defined: the equatorial diameter and the meridian diameter. These dimensions were determined with a manual vernier caliper with a precision of ± 0.02 mm (Labor, Fujian, China) on 25 representative units selected at random. The moisture content was determined by drying in a forced convection oven DH6-9123a (ICSA, Hong Kong, China) at a temperature of 70 ± 1 °C until constant weight, on a sample of 5 g, in triplicate (adaptation of the method AOAC 934.06) [14].

The soluble solids content of the fruit (expressed as degrees Brix) was measured in filtered grape juice according to AOAC 942.15 by refractometer Atago (Atago, Tokyo, Japan) with a precision of ± 0.05 °Brix, in triplicate [14]. The pH value was determined in triplicate with a pH meter Orion 720A (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with a precision of ± 0.01 pH units in a carbon dioxide-free grape juice sample [15].

The acidity value of the Red Globe grape was determined by potentiometric technique, in order to avoid possible errors or deviations associated with the colouring of the fruit juice, which could interfere with the visualization of the colour change of the acid-base indicator [16]. The determinations were realized in triplicate. The method of Gran [17] was used. This method allows to use the data obtained before the end point for subsequent determination of the end point (equilibrium) using the following Eq. 1 of the Gran graph:

$$V_b \times 10^{-\mathrm{pH}} = \frac{\gamma_{\mathrm{HA}}}{\gamma_{\mathrm{A-}}} K_a (V_e - V_b) \quad (1)$$

where V_b is the added volume (in millilitres) of NaOH solution (0.0974 mol·l^{-1}), K_a is the dissociation constant of the acid (in moles per litre), γ_{HA} is the activity coefficient of the acid, γ_{A-} is the activity coefficient of the conjugate base of the acid and V_e is the added volume (in millilitres) of NaOH solution (0.0974 mol·l^{-1}) at the equivalence point. A volume of 25 ml of the filtered grape juice was transferred to a 150 ml beaker along with a magnetic bar and the corresponding pH meter glass

electrode. Titration was started by initially adding 5 ml of NaOH solution (0.0974 mol·l^{-1}) and 1 min was waited (with the stirrer running continuously) to allow the pH value to stabilize, and then the reading was made. Next, 1 ml aliquots of NaOH solution (0.0974 mol·l^{-1}) were continued to be added by repeating the pH stabilization procedure with continuous stirring before measurement, until there was a noticeable jump in the pH value. With the obtained data, the graph of $V_b \times 10^{-pH}$ as a function of V_b was constructed. The equivalence volume (V_e) was extrapolated from this representation. From the sample volume used and the concentration of NaOH solution used, the acidity of the sample was calculated in terms of grams of tartaric acid per litre.

For the determination of Ca concentration of the fruit, the standard method of American Public Health Association – American Water Works Association – Water Pollution Control Federation [18] was used. The determinations were realized in triplicate. To this end, 20 ml of grape juice taken using double-gauging pipette were placed in a 250 ml Erlenmeyer flask and diluted to 50 ml with distilled water. Then, 2 ml (or sufficient volume to obtain a pH value of 12 or 13) of NaOH solution of adequate concentration and a spatula tip of the calconcarboxylic acid indicator were added. It was titrated using ethylene diamine tetra acetic acid (EDTA; Merck, Darmstadt, Germany) until the test solution turned from reddish to greenish blue, with no reddish tint. Calcium concentration was calculated using Eq. 2, considering that the EDTA solution was prepared (and titrated) so that 1 ml corresponds to 1 mg of $CaCO_3$. Results were expressed in milligrams of calcium per litre.

$$CA = \frac{400.8\,AB}{V_s} \tag{2}$$

where CA is de concentration of Ca in the sample (in milligrams of calcium per litre), 400.8 is the factor that arises when considering that 1 ml of the standard titration reagent EDTA 0.0100 mol·l^{-1} is equivalent to 400.8 μg Ca, A is the volume of the titration reagent for the sample (in millilitres), B is the mass of $CaCO_3$ equivalent to 1 ml of EDTA titration reagent at the end point of the indicator for calcium (in milligrams) and V_s is volume of sample (in millilitres).

Physico-chemical characterization of grape pectin

For the extraction of pectin from the Red Globe grape, an adaptation of methods proposed for other fruits was used, such as passion fruit [4] and lemon [19]. The grape was cut into small pieces and subjected to ultrasound treatment in bath-type equipment TB24TACA 40 kHz (Testlab, Bernal Oeste, Argentina) for 20 min at 45 °C. Subsequently, acid hydrolysis was carried out. For this purpose, the sample was treated with water acidified with 37% hydrochloric acid until reaching a pH value equal to 2, in a sample-to-acid solution ratio of 1:3 for 30 min at 80 °C with continuous stirring by means of a magnetic stirrer 78HW-1 (Arcano, Beijing, China). After that time, the mixture was filtered through a small strainer. The liquid filtrate was precipitated with 96% ethanol using a 1:1 volume ratio. The precipitated gel was filtered through a quantitative filter paper Quanty JP41 (JProlab, Sao José dos Pinhais, Brazil) and dried in a forced draft oven.

To determine the equivalent weight (EW) of Red Globe grape pectin, 5 ml of ethanol, 1 g of sodium chloride, 100 ml of distilled water and two drops of phenol red as indicator were added to a 0.5 g sample of pectin. The mixture was titrated with 0.1 mol·l^{-1} NaOH solution until the colour change was observed. The neutralized solution was stored for the subsequent determination of the methoxyl percentage of pectin. EW and free acidity (FA) of pectin were calculated using Eq. 3 and Eq. 4 [20]:

$$EW = \frac{100\,W_t}{N_{\mathrm{NaOH}} V_{\mathrm{NaOH}}} \tag{3}$$

where EW is expressed in milligrams per milliequivalent; W_t is the acid component (in grams of pectin); N_{NaOH} is the concentration of the NaOH solution (in milliequivalents per millilitre) and V_{NaOH} is the volume of the NaOH solution used in the titration (in millililtres).

$$FA = \frac{M_{eqA\ \mathrm{NaOH}}}{W_t} \tag{4}$$

where FA is expressed in milliequivalents per gram; M_{eqANaOH} are the milliequivalents of the NaOH solution used in the titration and W_t is the acid component (in grams of pectin) [21].

The methoxyl groups content of the pectin of the Red Globe grape was determined by means of an adaptation of the technique proposed by Owens [20]. To the neutral solution from the determination of the equivalent weight, 25 ml of NaOH solution (0.25 mol·l^{-1}) were added, stirred and kept standing for 30 min. Next, 25 ml of HCl solution (0.25 mol·l^{-1}) were added and finally it was titrated with NaOH solution (0.01 mol·l^{-1}) until the colour change (pH 7.5). The percentage of methoxyl was calculated using Eq. 5:

$$MeO = \frac{3.1\,N_{\mathrm{NaOH}} V_{\mathrm{NaOH}}}{W_t} \tag{5}$$

where *MeO* is the percentage of methoxyl and 3.1 is the equivalent weight of the methoxyl group CH_3O- (in milligrams per milliequivalent).

The percentage degree of esterification (*DE*) of pectin was calculated by relating the milliequivalents of NaOH solution spent in the methoxyl determination titration (milliequivalents of *B*) with the sum of total milliequivalents of NaOH solution spent together between the equivalent weight determination titration (milliequivalents of *A*) and the percentage of methoxyl determination titration (milliequivalents of *B*), according to Eq. 6 [21]:

$$DE = \frac{meqA}{meqA + meqB} \times 100 \quad (6)$$

where meq*A* are the milliequivalents of NaOH solution (0.1 mol·l^{-1}) used in the titration to determine the equivalent weight (first titration) and meq*B* are the milliequivalents of NaOH solution (0.01 mol·l^{-1}) used in the titration to determine the methoxyl content (second titration).

The percentage of anhydrous galacturonic acid (*AGA*) was determined by Eq. 7 taking into account the free acidity and the methylated units [21]:

$$AGA = \frac{176\,[100 - (meqA + meqB)]}{W_t} \quad (7)$$

where 176 is the molecular weight of anhydrous galacturonic acid expressed in milligrams per milliequivalent; *meqA* are the milliequivalents used in the first titration with NaOH solution (0.1 mol·l^{-1}); *meqB* are the milliequivalents of NaOH solution (0.01 mol·l^{-1}) used in the second titration to determine the percentage of methoxyl and W_t is the weight of the sample (in milligrams).

Taking advantage of the Fourier transform infrared spectrocopy (FTIR) technique that provides an absorption spectrum corresponding to the vibrational levels of the functional groups present in a sample, an analysis of the pectins extracted from the Red Globe grape was carried out by mean of this methodology. The objective was to qualitatively estimate the degree of esterification of the pectin sample from the signals of the free carboxyl (COO^-) and esterified (COOR) groups, distinguishing pectins of high and low methoxyl content.

Likewise, for comparative purposes, the spectrum of commercial pectin was also obtained, selected particularly based on the results of analytical tests regarding the degree of methoxylation and esterification of the pectin of the Red Globe grape. The dry pectin samples were analysed by FTIR in the range of 4000–400 cm^{-1} using a Magma-IR 550 Spectrophotometer (Nicolet, Markham, Ontario, Canada), in 4 cm^{-1} resolution, applying the pelletization technique with KBr (pure and dry) [22].

Statistical analysis

The results were submitted to the Chi-Square test to verify their normality. Then, one-way analysis of variance (ANOVA) and Tukey's test were applied to for compare means at a confidence level of 95%.

RESULTS AND DISCUSSION

Fresh grape characterization results

Tab. 1 shows the data obtained by characterization of fresh fruit, in terms of gravimetric, dimensional and physico-chemical properties - average fruit weight, characteristic dimensions (meridian and equatorial diameters), moisture content, soluble solids content, pH, acidity and calcium

Tab. 1. Characteristics of the Red Globe grape.

Characteristic	Experimental results		Literature	
	Value	*n*	Value	Reference
Average fruit weight [g]	10.95 ± 3.08	25	9.25–10.75	[2]
Meridian diameter [mm]	27.46 ± 2.39	25	24.72–27.60	[2]
Equatorial diameter [mm]	24.90 ± 2.52	25	22.47–24.13 23.00	[2] [23]
Moisture content [%]	81.8 ± 0.9	3	81.6	[23]
Soluble solids content [°Brix]	16.37 ± 0.26	3	16.00–18.32	[23]
pH	4.12 ± 0.10	3	3.58	[23]
Acidity value [g·l^{-1}]	2.44 ± 0.60	3	1.19	[23]
Calcium content [mg·kg^{-1}]	170 ± 0.2	3	100–120	[1]

Values represent mean ± standard deviation. Acidity value is expressed as tartaric acid equivalents.

concentration, compared with data from literature [1, 2, 23]. As can be seen, the experimental values obtained were in the typical ranges reported by various bibliographic sources.

Physico-chemical properties of grape pectin

Tab. 2 shows the results of the physico-chemical characterization of the pectin extracted from the Red Globe grape. Its free acidity was found to be slightly above that of some commercial pectins (ranging from 0.28 meq·g^{-1} to 0.62 meq·g^{-1}) [4, 21]. This might have been due to the fact that free acidity increases as the extraction pH is lower because the carboxyl groups undergo chemical modifications, reduce their presence as salts or esters and increase their presence as acid groups [24].

The degree of methoxylation (*MeO*) of Red Globe grape pectin was less than 7 %, which was indicative that it is a low-methoxyl grade pectin [4]. The *MeO* value found for grapes was in the range reported for guava in different stages of maturity (1.0–2.5 %) [25], but it was less than the value reported for apple (*Malus domestica)* variety Fălticeni (which was in the range of 3.0–4.8 %, depending on the extraction method) [26], although the pectins of those fruits are also low-methoxyl ones like the grape in this study.

In the same sense, the degree of esterification (*DE*) was less than 50 %, confirming that it is a low methoxyl pectin [5], the same as those found in cocoa (*Theobroma cacao*) husk [27] and fig (*Ficus carica*) skin [28] among other plants. This type of low-methoxyl pectins, such as that extracted from Red Globe grapes in this study, is usually used to formulate low-sugar or low-energy jams, jellies and dairy desserts [29]. In the food industry it is important to know these parameters, since the requirements for formation of gels are different in terms of pH, presence of calcium ions and sugar content, depending on whether pectins of high or low methoxyl grade are used.

The percentage of anhydrous galacturonic acid (*AGA*) is indicative of the degree of purity of a pectic substance, given that pectin is a polysaccharide whose structure is made up not only of D-galacturonic acid but can also contain other sugars (arabinose, glucose, rhamnose) in a percentage of 10 % (or more) of the chain [21]. The pectin extracted from Red Globe grapes presented an *AGA* fraction of 34.6 %. This result was in the order of that reported for pectins extracted from lemon peel (34 %) [30] and above that reported for pectins from cocoa peel (12.5 %) [21] and guava at different stages of maturity (7.4–16.1 %) [25]. However, the international requirements established for industrial pectins indicate that they must contain a minimum of 65 % *AGA* to be considered as a high quality additive [31]. Therefore, the Red Globe grape pectin obtained through the methodology used in this work would not meet those requirements. The lower purity of the Red Globe pectin compared to commercial pectins was possibly associated with its structure containing other monosaccharides in addition to galacturonic acid, as well as the presence of other minor components not solubilized in the extraction process (antioxidants, secondary metabolites, pigments), requiring in the latter case an additional purification process [21].

Regarding the equivalent weight (*EW*) of the Red Globe grape pectin, it was in the range reported for pectins of apple variety Fălticeni (641–2778 %) [26], but it was lower than that reported by other authors for commercial pectins (range 1500–3600 mg·meq^{-1}) [4, 21]. This parameter is related to both *MeO* and *DE*. Low equivalent weight values of pectins indicate that smaller amounts of the product are needed to produce one mole H^+, which corresponds to low values of *MeO* and/or *DE* [27, 32]. In turn, equivalent weight is indicative of the firmness of a gel: the higher the equivalent weight, the greater the strength of the gel; this is due to the number of galacturonic acid residues in the molecule [33].

The determination of these physico-chemical parameters is directly related to the quality of the pectin, since they indicate its gelling and thickening properties. Based on the results observed for the pectin extracted from the Red Globe rose grape under the conditions of this study, insufficient gel firmness could be expected in the production of jams. However, it has been shown that the pectin extraction treatment affects its physico-chemical parameters, so the extraction method could be optimized to achieve the best performance of the Red Globe pectin [28].

Tab. 2. Physico-chemical characterization of the pectin extracted from the Red Globe grape.

Parameter	Pectin
Equivalent weight [mg·meq^{-1}]	1441.32 ± 15.92
Free acidity [meq·g^{-1}]	0.69 ± 0.01
Degree of methoxylation [%]	1.7 ± 0.1
Degree of esterification [%]	44.7 ± 1.3
Anhydrous galacturonic acid [%]	34.6 ± 0.6

Values represent mean ± standard deviation (n = 3).

Fourier transform infrared spectrocopy analysis

As a result of the FTIR analysis of the pectin extracted from the Red Globe grape and com-

mercial low-methoxyl pectin Genu used comparatively as a standard, the spectra were obtained (Fig. 1). The signals corresponding to the characteristic functional groups of pectins could be observed, in particular that in the range between 3400 cm^{-1} and 2500 cm^{-1}, which is the stretching of O-H groups due to the intermolecular and intramolecular hydrogen bond of the galacturonic acid polymer. The absorbance between 2900 cm^{-1} and 3000 cm^{-1} corresponds to the C-H group, being induced by the C-H stretching of the vibrations of the CH_2 and CH_3 groups. Stronger bands are observed between 1745 cm^{-1} and 1760 cm^{-1}, being assignable to the carbonyl ester group (C=O); and to the carboxylate groups (COO^-), which have two bands - one with an asymmetrical stretch at 1550–1650 cm^{-1} and the other with a weaker symmetrical stretch close to 1400 cm^{-1}. Other minor bands occur in both Red Globe grape pectin and commercial pectin: C-H flex signals, occurring at 1380 cm^{-1}, and C-O stretch occurring between 1000 cm^{-1} and 1300 cm^{-1} [34]. The signals observed in the 3300–3500 cm^{-1} region of commercial low-methoxyl grade pectin are assigned to the characteristic vibrations of the amino group, confirming the information provided by the manufacturer in the technical data sheet that partial amidation of the pectin was carried out [35].

For identification of the degree of esterification of pectins, the characteristic bands at 1640 cm^{-1} and 1745 cm^{-1}, which indicate the presence of free and esterified carboxyl groups, respectively, are of particular importance. As defined by Manrique and Lajolo [34] and Pappas et al. [36], it is possible to infer the degree of esterification on the basis of the relation of areas of the characteristic bands, that is the ratio between the area of the band at 1745 cm^{-1} and the sum of the areas of the bands at 1745 cm^{-1} and 1640 cm^{-1}.

Tab. 3 shows the areas corresponding to the characteristic bands. The areas (representing the absorbance intensity) were determined by integration of the areas under characteristic peaks of functional groups.

The area ratios for commercial pectin and for the grape sample were 0.25 and 0.37, respectively. Red Globe grape pectin gave a value close to that of the commercial pectin, which is known to be of low-methoxyl grade. Therefore, it can be inferred that the pectin extracted in this study is also of low-methoxyl grade.

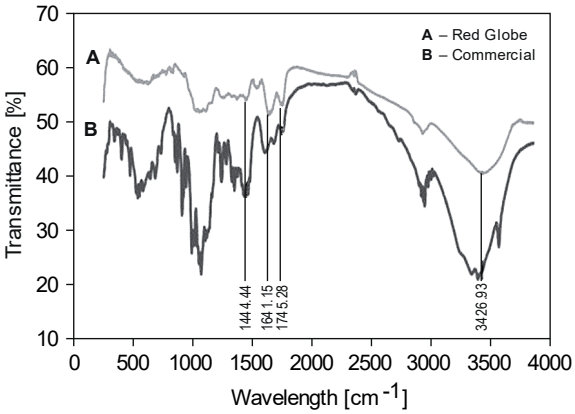

Fig. 1. FTIR spectra of pectin extracted from Red Globe grapes and commercial low-methoxyl grade pectin.

In addition, different authors [33, 36] have obtained calibration curves with standard pectins of known methoxylic grade based on the relationship of band areas at 1745 cm^{-1} and 1640 cm^{-1} where, regardless of the way of preparing the pectin standards, it was observed that *DE* of 50 % corresponded to an area ratio of approximately 0.4. Considering that the behaviour of the characteristic signals for the esterified (COO-R, 1745 cm^{-1}) and free (COO^-, 1640 cm^{-1}) groups in Red Globe grape pectin is similar to those of the low-methoxyl grade commercial pectin and also that the area ratios are similar, it can be inferred that Red Globe grape pectin is also of low-methoxyl grade. These results yield the same conclusions regarding the characteristics of the pectin extracted from the Red Globe grape reported from the analytical determinations (Tab. 2).

CONCLUSIONS

The Red Globe rose grape is an oval fruit with an average diameter of approximately 26 mm, and an average weight of approximately 11 g. Its

Tab. 3. Characteristic signals in Red Globe pectin and commercial pectin FTIR spectra.

Band [cm^{-1}]	Group type	Area	
		Pectin of Red Globe grape	Commercial pectin
1745	Esterified (COO-R)	2.788	2.702
1640	Free (COO^-)	4.669	8.175

moisture content is approximately 82 % wet basis, soluble solids content is approximately 16 °Brix, pH value of 4, acidity value of 2.44 $g \cdot l^{-1}$ and calcium content of 170 $mg \cdot kg^{-1}$. Regarding the pectins of this grape, it can be inferred that they are of low-methoxyl grade, taking into account the relation of the areas of the characteristic FTIR spectral bands for the esterified (COO-R, 1745 cm^{-1}) and free (COO^-, 1640 cm^{-1}) groups, as well as the analytically determined equivalent weight (1441.32 $mg \cdot meq^{-1}$), free acidity (0.69 $meq \cdot g^{-1}$), degree of methoxylation (1.7 %), degree of esterification (44.7 %) and anhydrous galacturonic acid percentage (34.6 %). These characteristics of the Red Globe grape pectin suggest that it is suitable for obtaining products such as jellies or jams with low sugar content, adding value to the primary production chain.

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