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Rheological and chemical properties of pectin enriched fractions from different sources extracted with citric acid

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ABSTRACT

Yield, properties and functionality of pectins depend on the source material and method of extraction. The objective of this work was to compare pectinolytic enzyme activity in fresh pulp as well as the physicochemical and rheological properties of polysaccharides extracted with citric acid from six new potential sources: fruit materials – peach, blackcurrant, raspberry, strawberry, plum and a vegetable Source: carrot. The uronic acid content of polysaccharides extracted in citric acid depended on pectinolytic enzymes activity in fresh plant tissues and ranged between 16.5 and 37.1%; which are slightly lower values than those of commercial pectins isolated from citrus and apple. The values of examined rheological parameters (viscosity, thixotropic effect, flow behaviour) demonstrated quality and possibility of pectin enriched fraction application as a food texture modifier. Pectin enriched fractions extracted from seasonal fruit and carrot with citric acid showed considerable potential as thickeners and gelling agents.

1. Introduction

Pectins are one of the most intensively studied groups of cell wall polysaccharides due to their properties and complex structure. They are composed of a large number of different monosaccharides linked by many different chemical bonds (Lira-Ortiz et al., 2014). A more complete knowledge of their properties may be useful to the food processing and pharmaceutical industries (Pagan & Ibarz, 1999). Pectic substances mainly consist of D-galacturonic acid units (GalA) which are the main components of three pectic polysaccharides: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Caffal & Mohnen, 2009). The structure and composition of pectin polysaccharides forming plant cell walls depend on the source and conditions of extraction as well as their location and other environmental factors (Pérez, Carvajal, & Doco, 2003).

HG, which makes up the "smooth region" of pectin chains, is composed of GalA residues with 1,4-linkages and an α-configuration between the units. Residues of GalA in HG are methylesterified at the C-6 carboxyl group and in some cases they are also acetylated at O-2 or O-3 (Pérez Espitia, Du, Avena-Bustillos, Ferreira Soares, & McHugh, 2014). The degree of methyl-esterification (DM) and the degree of acetylation are features that affect pectin properties and they normally depend on the pectin source. Highly methyl-esterified pectin (DM > 50%), may gel in acidic conditions, when sugar concentrations are high, while low methoxyl pectin chains can form a gel structure by interaction with divalent calcium cations (Caffall & Mohnen, 2009; Lira-Ortiz et al., 2014). The "hairy regions" of pectin consist of RG-I and in some pectins, RG-II can also be found. Generally, RG I is composed of a [→4)-α-D-GalA-(1 → 2)-α-L-*R*ha-(1 →] repeating disaccharide sequence with neutral sugar side chains such as galactan, arabinan and arabinogalactan attached to the rhamnose units. Rhamnogalacturonan II consists of a HG backbone and side chains of 28 monosaccharides such as rhamnose and unusual sugars like Kdo (3-deoxy-D-manno-oct-2-ulosonic acid), Dha (3-deoxy-D-lyxo-2-heptulosaric acid), aceric acid and apiose, attached to the GalA residues (Mazeau & Pérez, 1998; Pérez et al., 2003; Pose, Kirby, Mercado, Morris, & Quesada, 2012; Ralet, Lerouge, & Quéméner, 2012).

Pectin structure and functionality is affected by the action of pectinolytic enzymes (Jayani, Saxena, & Gupta, 2005) responsible for the hydrolysis of the pectic substances during the fruit ripening process. Several enzymes are known to initiate the degradation of pectins in different locations of the pectin chain. Two of the best known fruit pectinases are pectin methylesterase (PME) and polygalacturonase (PG) (Wei et al., 2010). PME hydrolyzes the methyl-ester group at the C-6 carboxyl group producing low-methoxyl pectin chains. PME activity results in the release of methanol as well as preparing the HG chain for the action of

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PG (Arancibia & Motsenbocker, 2006). PG is responsible for the hydrolytic cleavage of α-(1,4) glycosidic linkages of the polygalacturonan acid chain (Torres, Sayago, Ordonez, & Isla, 2011).

An extraction of pectins from plants is usually carried out using acid solvents, i.e. hydrochloric acid, nitric acid (Methacanon, Krongsin, & Gamonpilas, 2014), oxalic acid (Kaya, Sousa, Crépeau, Sørensen, & Ralet, 2014) or citric acid (Yuliarti, Goh, Matia-Merino, Mawson, & Brennan, 2015). Conditions of extraction play an essential role in the yield, chemical composition and rheological properties of the pectic fractions obtained, with a direct effect on the functionality of the pectins. The ability of pectin to form a gel is especially important with regard to its applications in the food and pharmaceutical industries (Lima, Paiva, Andrade, & Paixão, 2010).

Most studies focus on the analysis of the properties of pectin isolated from a single source of raw material for use as a potential one-component matrix in the food processing or pharmaceutical industry. Since apple and citrus pectin have shown many useful features as gelling and stabilizing agents, there is an increasing interest in their industrial applications. Therefore, other raw materials, for example: banana (Oliveira et al., 2016), gold kiwi fruit (Yuliarti et al., 2015), sugar beet (Chen, Fu, & Luo), passion fruit (Seixas et al., 2014) and mango peel (Kermani, Shpigelman, Pham, Van Loey, & Hendrickx, 2015) have been extensively studied from this point of view. The properties of isolated pectin depend on the solvent used for extraction. The use of citric acid in the study is justified by the ecological aspects of this organic acid, which is more environmentally friendly and more acceptable as a food additive. The aim of this work is to compare the physicochemical and rheological properties of polysaccharides isolated from six new potential sources and to relate these properties to the activity of cell wall pectinolytic enzymes like PME and PG. Fruit materials – peach (*Prunus persica*), blackcurrant (*Ribes nigrum*), raspberry (*Rubus idaeus*), strawberry (*Fragaria ananasa*) and plum (*Prunus domestica*), and a vegetable source – carrot (*Daucus carrota*) were used, as they have not been extensively studied for this purpose yet. These plant sources have been selected due to their availability, popularity and relatively low cost of purchase. Moreover, a literature review has shown that there is very little information available about the polysaccharides isolated from these plant sources. To the best of our knowledge, this is also the first time that properties of pectin isolated from natural plant sources have been related to the activity of cell wall pectinolytic enzymes like PME and PG.

2. Material and methods

Six potential pectin sources, i.e. peach (*Prunus persica*), blackcurrant (*Ribes nigrum*), raspberry (*Rubus idaeus*), strawberry (*Fragaria ananasa*), plum (*Prunus domestica subsp. Italica*) and carrot (*Daucus carrota subsp. Karotka*) were used in this study. These materials were purchased from the local market. 1000 g of each material was homogenized in a laboratory homogenizer. About 10 g of the homogenized tissue was used for the biochemical analyses of the pulp, while the rest of the sample was used for pectin extraction.

2.1. Chemical analyses of the pulp

The dry matter content was determined by weighing the samples, followed by drying them in an oven at 70 °C for 24 h and afterwards at 105 °C for 3 h. Then the samples were weighed again and the percentage of dry matter was determined. The soluble solid content in Brix degrees was determined using a digital refractometer (Atago, Japan).

Enzyme extraction from cell walls and determination of enzyme activity were carried out according to the method proposed by Wei et al. (2010), with slight modifications. In order to extract cell wall enzymes, frozen and powdered pulp was stirred into 6 mL of cold 12% polyethylene glycol M_n 400 (Sigma-Aldrich, St. Luis, MO) containing 0.2% sodium bisulphate (Sigma-Aldrich, St. Luis, MO). Then the homogenate was centrifuged for 10 min at 6,000 × *g* and the pellets were washed with 4 °C aqueous 0.2% sodium bisulphate (Sigma-Aldrich, St. Louis, MO). The pellets were collected and used for the extraction of PG and PME. The following solution was used to extract the enzymes: 6 mL of cold extraction buffer [1 M sodium acetate (Sigma-Aldrich, St. Louis, MO) (pH 5.2), 1 M NaCl, 2% (v/v)-mercaptoethanol (Sigma-Aldrich, St. Louis, MO), and 5% (w/v) polyvinylpyrrolidone (PVP, Mn 40,000) (Sigma-Aldrich, St. Louis, MO)] at 4 °C for 1 h. The homogenate was centrifuged for 10 min at 6,000 × *g*, and the supernatant was used to determine enzyme activity.

PG activity was determined from the reaction of the enzyme extract (0.2 mL) with 0.8 mL of 0.5% polygalacturonic acid (Sigma-Aldrich, St. Louis, MO) in 50 mM sodium acetate (Sigma-Aldrich, St. Louis, MO) buffer (pH 5.2). Then the mixture was incubated at 37 °C for 2 h. After incubation, 2 mL of borate buffer (≥90%, enzymatic, Sigma-Aldrich, St. Louis, MO) (0.1 M, pH 9.0) and 0.3 mL of cyanoacetamide (Sigma-Aldrich, St. Louis, MO) were added to the reaction mixture. This was boiled for 10 min and then cooled in an ice bath. Absorbance was measured at 320 nm. GalA (~95%, enzymatic, Sigma-Aldrich, St. Louis, MO) was used as a standard and the controls for the boiled extract were run in the reaction buffer. One unit of activity was defined as a μg of uronic acid per g of fresh weight of pulp (FW)*min^{-1}.

The determination of PME activity was carried out by reacting 1 mL of crude extract with 4 mL of 1% (w/v) citrus pectin (pectin from citrus peel, galacturonic acid ≥74% (based on dried material), Sigma-Aldrich, St. Louis, MO). The mixture was titrated with 0.01 M NaOH (Stanlab, Lublin, Poland) to maintain the pH at 7.4 while the mixture was incubated at 37 °C for 1 h. One unit of activity was calculated as μmol NaOH/g of fresh weight of pulp (FW)*10 min.

2.2. Pectin enriched fractions extraction

After preliminary drying in a laboratory dryer at 40 °C for 24 h (Memmert, Schwabach, Germany), about 1000 g of flesh sample was stirred with citric acid (monohydrate, Chempur, Piekary Slaskie, Poland) solution (pH 2.5) in the ratio of 1:50 for 30 min at 90 °C. After extraction the solution was filtered in order to separate insoluble polysaccharides like cellulose and most of hemicelluloses in the sediment from polysaccharides soluble in a water solution of citric acid. After filtration, the sample was concentrated using an evaporator and adjusted to pH 4.5 with aqueous ammonia (Stanlab, Lublin, Poalnd). Pectin enriched fractions were precipitated with isopropyl alcohol and stirred with 70% ethanol using a magnetic stirrer. Stirring with ethanol was repeated three times. Fruit pectin enriched fraction samples were dried in a laboratory dryer (convective oven; Memmert, Schwabach, Germany) and micronized in a mixer mill (MM400, Düsseldorf, Germany).

2.3. Uronic acid and calcium content in pectins

The samples were washed with alcohol repeatedly until all sugars were completely removed. Free reducing sugars content was determined according to method of Luff-Schoorl (Van Voorst, 1948). The test was negative, which proves the effectiveness of alcohol in removing soluble sugars. The amount of uronic acid in the pectin enriched fraction was determined using a Continuous Flow Analyzer (CFA), SanPlus (Skalar, The Netherlands), according to the colorimetric method used by Blumenkrantz and Asboe-Hansen (1973). The samples were decomposed using di-sodium tetraborate (Sigma-Aldrich, St. Louis, MO)/96% H_2SO_4 (Stanlab, Lublin, Poland) solution. The products of decomposition were transformed

into furfural derivatives, which were reacted with 3-phenylphenol (Sigma-Aldrich, St. Louis, MO) to form a coloured dye which was analyzed at 530 nm. Calcium content was determined by complexation of calcium with cresolphtalein complexone (Sigma-Aldrich, St. Louis, MO) in an alkaline medium. The absorbance of the complex was measured at 580 nm (Zdunek, Kozioł, Pieczywek, & Cybulska, 2014).

1.1.Rheological properties of pectin enriched fractions.

We used pectin enriched fraction solutions at concentrations of 2% and 5%. A concentration of 2% was chosen due food industry practice (Eshtiaghi & Kuldiloke, 2013; Strom, Schuster, & Goh, 2013). A concentration of 5% was chosen as well to relate results with our previous studies on carrot and apple material (Mierczyńska, Cybulska, Pieczywek, & Zdunek, 2015; Mierczyńska, Cybulska, Sołowiej, & Zdunek, 2015).

Rheological properties of 5% and 2% (mass/volume percentage) aqueous solutions of pectin enriched fractions were evaluated using an R/S Plus rheometer (Brookfield, Middleboro, MA) with a cone-plate sensor (60 mm diameter, 2° angle) with a 0.5 mm gap between the cone apex and the plate. Measurements were performed at 20 °C ± 0.5 °C using a constant shear rate (1200 1/s) for the estimation of viscosity and a variable shear rate (60–1800 1/s; 1800–60 1/s) for the estimation of flow curves. The relationship between shear stress and shear rate is useful for the determination of a thixotropic effect. In order to estimate the thixotropic effect, the surface area of the hysteresis loop between the upward flow curve (shear rate 60–1800 1/s) and the downward flow curve (1800-60 1/s) was calculated (Dolz, Hernández, Delegido, Alfaro, & Muñoz, 2007). The Power law model, also called the Ostwald de Waele's model, was applied to describe the flow curves obtained. The model is described by the following equation:

$$\sigma = K\gamma^{n} \quad (1)$$

where σ – shear stress (Pa), K – consistency index (Pas^n), γ – shear rate (s^{-1}), n – flow behaviour index.

2.4. FT-IR spectra of pectins

FT-IR spectra were recorded using a Nicolet 6700 FT-IR (Thermo Scientific, Waltham, MA, USA) spectrometer equipped with an ATR attachment. The pectin enriched fraction samples were completely dried, after they reached a constant mass they were kept in a vacuum dryer prior to measurement. The samples were placed on the ATR spectrometer attachment and pressed with the ATR head. The spectra were recorded in the 4000–650 cm^{-1} range. The measurements were repeated five times for each sample, 200 scans were performed for each measurement and then averaged with a spectral resolution of 4 cm^{-1}. After that, the spectra for all repetitions were baseline-corrected and normalized. The calculations were performed using Omnic software (Thermo Scientific) and the spectra were analyzed using OriginPro 8.5.0 SR0 software (OriginLab Corporation, Northampton, MA, USA). The degree of methylation (DM) was determined from the ratio of the intensity of bands centred at 1750 cm^{-1} for the esterified carbonyl groups and at 1630 cm^{-1} for carboxylic anions (Fellah, Anjukandi, Waterlan, & Williams, 2009).

2.5. AFM imaging of pectin molecules

Pectin molecules were observed using a BioScope Catalyst II atomic force microscope equipped with a Nanoscope V controller (Bruker, Billerica, MA, US). The samples were imaged according to the procedure previously described (Cybulska, Halaj, Cepák, Lukavský, & Capek, 2015; Cybulska, Zdunek, & Kozioł, 2015). Pectin suspensions were diluted to a concentration of 10 μg/mL in water. A 3 μL droplet of the diluted solution was placed onto freshly cleaved mica. Then the samples were vacuum dried at 30 °C for 2 h and stored in a desiccator before being used for AFM measurements. The samples were imaged in the ScanAsyst™ mode using a silicon nitride cantilever ScanAsyst-Air (Bruker) of nominal spring constant of 0.4 N m^{-1}. Scan settings were optimized prior to imaging as follows: scan size: 2 × 2 μm, scan rate: 0.5 Hz (tip velocity: 2 μm s^{-1}) and resolution 512 × 512 (3.9 nm $pixel^{-1}$). Imaging was performed in air at room temperature (20–22 °C) and at a relative humidity of 26–30%. These conditions were the same for all of the images collected. More than ten images were collected for each sample. The "height" AFM images were flattened using the following steps: third order polynomial fitting to remove the bow effect of the surface; filtering using a standardized roughness filter (ISO 11562-61L-Filter 1/5th) and smoothing by Gaussian filtering (3 × 3) to reduce noise. All of the operations were carried out using SPIP 6.0.14 software (Image Metrology, Hørsholm Denmark).

2.6. Statistical analysis

Data was analyzed using one way analysis of variance (ANOVA) followed by a post-hoc test, significant differences were determined at $p < 0.05$. Statistical software STATISTICA (Statistica Version 12, StatSoft Inc., USA) was used for this purpose.

3. Results & discussion

3.1. Rheological properties of pectin enriched fractions

Fig. 1. presents the flow curves (shear stress vs. shear rate) for the solutions of pectin enriched fractions of two concentrations (2% and 5%) and various sources. The upward and downward flow curves were collected using a variable shear rate within the range of 60–1800s^{-1}. Both, the upward and downward curves were fitted to the Power law (Ostwald de Waele's) model. Parameters of the model and of the goodness of fit are presented in Table 1. The flow curves for the 5% solutions of pectins are very well described by the model as R^2 is approximately 0.99, while for the 2% solutions R^2 was slightly lower but still higher than 0.93. Based on the model, all of the samples were classified as pseudoplastic fluids due to their having n values lower than 1 in each case, i.e. lower n coefficients means higher pseudoplasticity (Penna, Sivieri, & Oliveira, 2001). The n index was lower for the 5% than for the 2% solution, which means that pseudoplasticity increases with concentration of pectin enriched fractions in solution. For most of the materials used in this study at 5% concentration of pectin enriched fractions, the n index values were similar for the upward and downward curves. Only in the case of the blackcurrant pectin enriched fraction, the downward curve presented less pseudoplastic behaviour (n = 0.61) than the upward curve (n = 0.20). In contrast, for 2% solutions of the pectin enriched fractions from the same source the flow behaviour for upward and downward curves was identical (n = 0.34).

The consistency index K is related to the viscosity of samples; higher K values mean higher viscosity. Peach and plum pectin enriched fractions solutions were found to have the highest K value, while the blackcurrant and raspberry pectin enriched fractions had the lowest one. For 5% solutions of peach and plum pectin enriched fractions the high K values were associated with a low n index value. Moreover, for raspberry and black currant 5% solutions of pectin enriched fractions, a low K index value appeared simultaneously with strong pseudoplastic behaviour. Furthermore, for 2% solutions of pectins from each material this relationship was reversed, i.e. a high K index value was combined with a higher n index value. This means that 2% pectin enriched fractions solutions became less pseudoplastic with higher viscosity.

Table 1
The Power law model parameters describing rheological properties of pectin enriched fractions.

Concentration	Sample	Upward curve			Downward curve		
		K	n	R^2	K	n	R^2
2%	Peach	$1.49^{a} \pm 0.04$	$0.58^{a} \pm 0.01$	0.99	$1.63^{a} \pm 0.09$	$0.57^{a} \pm 0.01$	0.99
	Blackcurrant	$1.00^{b} \pm 0.00$	$0.34^{b} \pm 0.03$	0.93	$1.00^{b} \pm 0.00$	$0.34^{b} \pm 0.04$	0.93
	Raspberry	$1.00^{b} \pm 0.00$	$0.38^{c} \pm 0.01$	0.95	$1.00^{b} \pm 0.00$	$0.38^{b} \pm 0.01$	0.95
	Plum	$1.97^{c} \pm 0.15$	$0.56^{ad} \pm 0.01$	0.99	$2.22^{c} \pm 0.11$	$0.55^{a} \pm 0.00$	0.99
	Strawberry	$1.00^{b} \pm 0.00$	$0.52^{d} \pm 0.00$	0.97	$1.00^{b} \pm 0.00$	$0.52^{c} \pm 0.01$	0.98
	Carrot	$1.00^{b} \pm 0.00$	$0.53^{d} \pm 0.00$	0.99	$1.07^{b} \pm 0.13$	$0.53^{ac} \pm 0.01$	0.99
5%	Peach	$75.99^{a} \pm 7.67$	$0.27^{a} \pm 0.01$	0.99	$78.56^{a} \pm 7.02$	$0.25^{a} \pm 0.01$	0.99
	Blackcurrant	$2.09^{b} \pm 0.24$	$0.20^{b} \pm 0.03$	0.99	$2.20^{b} \pm 0.75$	$0.61^{b} \pm 0.04$	0.99
	Raspberry	$2.37^{b} \pm 0.17$	$0.59^{c} \pm 0.01$	0.99	$2.67^{b} \pm 0.47$	$0.58^{bc} \pm 0.03$	0.99
	Plum	$74.80^{a} \pm 6.37$	$0.29^{a} \pm 0.01$	0.99	$78.50^{a} \pm 5.38$	$0.28^{a} \pm 0.01$	0.99
	Strawberry	$8.07^{b} \pm 0.07$	$0.54^{e} \pm 0.01$	0.99	$8.49^{b} \pm 0.33$	$0.53^{c} \pm 0.01$	0.99
	Carrot	$10.26^{b} \pm 0.42$	$0.45^{d} \pm 0.01$	0.99	$9.96^{b} \pm 0.11$	$0.46^{d} \pm 0.01$	0.99

K – consistency index (Pas^{n}); n – flow behaviour index; R^2 – determination coefficient.

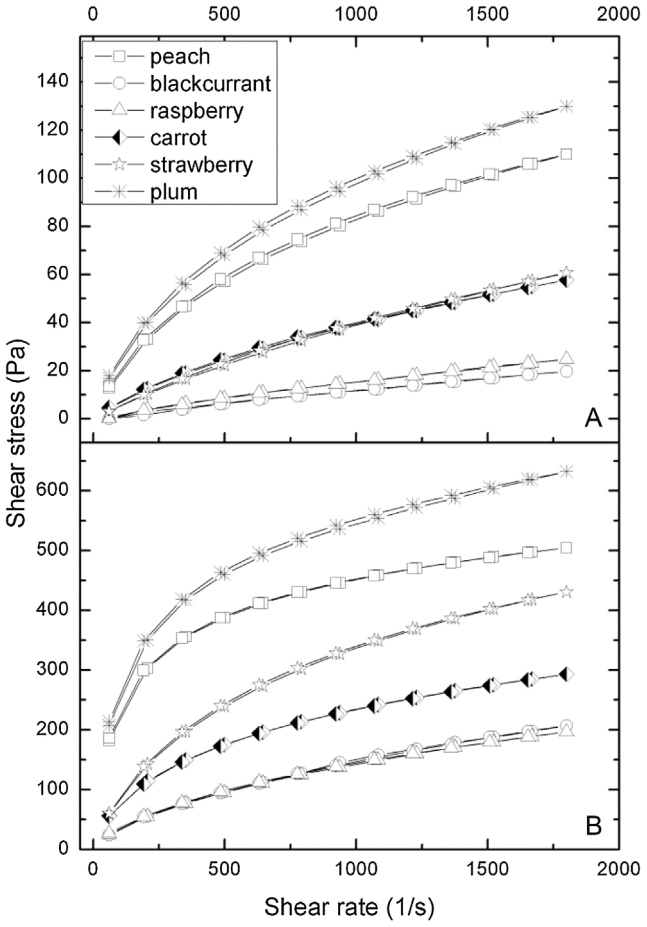

Fig. 1. Shear stress vs. shear rate relationship (the upward and downward flow curves) for 2% (A) and 5% (B) solutions of pectin enriched fractions from different sources.

Mean values with standard deviations (n = 3 replicates) are presented. Different letter indices correspond to statistical significance at $p < 0.05$ The same lower case letter in the data reported in a column means non significant differences ($p < 0.05$).

The viscosities of analyzed solutions that are presented in Table 2 vary depending on the source of pectin enriched fractions. Both solutions (2% and 5%) of pectin enriched fractions extracted from plum and peach had the highest viscosity, whereas blackcurrant and raspberry pectin enriched fractions solutions had the lowest viscosity. When polysaccharide is used as a food thickener and gelling agent, it should improve the texture of fluid and semi-fluid food products and ingredients. Compared to the viscosity of pure water at 20 °C, 0.001 Pas (not presented), the studied solutions of pectin enriched fractions, (2% and 5%) had at least a tenfold higher viscosity than water, for instance the 2% solution of blackcurrant pectin enriched fraction had a viscosity of 0.011 Pas. However, it should be noticed that applied method of pectin extraction involved treatment at 90 °C for 30 min what could influence on the functionality of the extracted pectin enriched fractions.

The gelling properties of pectin enriched fractions were indicated according to their ability to form a gel-structure of low-methoxyl pectin with calcium ions. In this case the viscosities of the samples (2% and 5% solutions) were determined with the addition of 6 mM $CaCl_2$ (Table 2). An increase in the viscosity of all of the pectin enriched fractions indicates the gelling properties of analyzed polysaccharides.

The studied pectin enriched fractions also displayed thixotropic properties (Table 3). For the plum and peach pectin enriched fractions solutions the thixotropic effect was highest, whereas, for the solutions of blackcurrant and raspberry pectin enriched fractions it was lowest. The thixotropic effect can be estimated from the relationship between shear stress and shear rate as the surface area between upward and downward flow curves. The hysteresis loop describes a change of fluid or semi-fluid from gel to sol during the application of force and return to the previous gel state after the force is withdrawn (Lee, Moturi, & Lee, 2009; Ottone, Peirotti, & Deiber, 2009). The consequences of thixotropy are of considerable interest to the food industry when designing new food processing techniques and products. For instance, knowledge of the magnitude of the thixotropic effect is very useful in predicting the flow behaviour of fluid and semi-fluid food ingredients during processing. This is a very important property because it allows for the determination of the pressure required to initiate the flow of substances through pipes, and also the minimum pressure required to resume the flow during technological process following a rest period (Barnes, 1997).

The presence of a thixotropic effect may also indicate complex structure of the materials. The presence and size of the hysteresis loop can be taken as a proof of complicated polysaccharide structure and the interactions between the components of the biopolymer. There is some evidence that the thixotropic effect of the pectin fraction extracted with diluted alkali is connected with its chemical structure (Mierczyńska, Cybulska, Pieczywek, et al., 2015; Tabilo-Munizaga & Barbosa-Cánovas, 2005). The pectin fraction extracted with diluted alkali is composed of polysaccharides connected to the cell wall with covalent bonds, which exhibit a

Table 2
Viscosity of 2% and 5% solutions of pectin enriched fractions.

	Viscosity (Pas)			
	2%	2% with 6 mM $CaCl_2$	5%	5% with 6 mM $CaCl_2$
Peach	$0.073^{a} \pm 0.001$	$0.079^{a} \pm 0.004$	$0.391^{a} \pm 0.021$	$0.591^{a} \pm 0.087$
Black currant	$0.011^{b} \pm 0.002$	$0.013^{b} \pm 0.0002$	$0.151^{b} \pm 0.007$	$0.160^{b} \pm 0.004$
Raspberry	$0.015^{c} \pm 0.001$	$0.017^{c} \pm 0.002$	$0.131^{c} \pm 0.003$	$0.179^{c} \pm 0.001$
Plum	$0.086^{f} \pm 0.001$	$0.095^{d} \pm 0.001$	$0.480^{f} \pm 0.021$	$0.641^{d} \pm 0.082$
Strawberry	$0.036^{e} \pm 0.001$	$0.049^{e} \pm 0.009$	$0.305^{e} \pm 0.008$	$0.487^{e} \pm 0.015$
Carrot	$0.038^{d} \pm 0.001$	$0.045^{f} \pm 0.001$	$0.227^{d} \pm 0.005$	$0.313^{f} \pm 0.017$

Mean values with standard deviations are presented. Different letter indices correspond to statistical significance at $p < 0.05$.

Table 3
Thixotropic effect of 2% and 5% solutions of pectin enriched fractions.

	Thixotropic effect (kPa s^{-1})	
	2%	5%
Peach	$8.398^{a} \pm 0.240$	$45.968^{a} \pm 5.654$
Blackcurrant	$1.701^{b} \pm 0.388$	$29.190^{b} \pm 2.640$
Raspberry	$2.394^{b} \pm 0.066$	$17.494^{c} \pm 0.477$
Plum	$10.078^{e} \pm 0.373$	$53.924^{a} \pm 1.563$
Strawberry	$4.795^{d} \pm 0.419$	$38.840^{a} \pm 4.651$
Carrot	$5.946^{c} \pm 0.204$	$35.419^{b} \pm 1.822$

Mean values with standard deviations (n = 30: replicates for viscosity; n = 3: replicates for thixotropic effect) are presented. Different letter indices correspond to statistical significance at $p < 0.05$. The same lower case letter in the data reported in a column means non-significant differences ($p < 0.05$).

strong ability to reconstruct the initial gel structure. Additionally, an atomic force microscope study showed that molecules composed of this fraction of pectin were organized into a regular network and formed 50–70 nm spaces (Cybulska et al., 2015).

3.2. Physical analyses of the flesh and enzyme activity

Wet physical analyses were performed on the flesh from all of the materials used in this study to explain the rheological properties of pectin solutions. The general characteristics of the plant material revealed differences between physicochemical and biochemical properties of fresh pulps. Basic biochemical characterization involved the determination of dry matter content and soluble solid content measured in Brix degrees (Table 4). The water content of the analyzed materials was variable. The highest water content was determined for strawberry fruit (also the lowest value of dry matter content – 7.04 g/100 g). Moreover, the highest content of dry matter in fruit tissue was determined for the blackcurrant fruit (20.95 g/100 g). For peach, raspberry, carrot and plum the content of dry matter was comparable and varied from 12.21 to 15.56 g/100 g.

Soluble solid content expressed in Brix degrees displayed a similar trend as the dry matter content in analyzed samples. The lowest content of soluble solid was determined for strawberry (5.6°Brix). Also, blackcurrant was characterized by the highest soluble solid content (15.3°Brix).

PG and PME activities measured during pectin extraction are presented in Table 4. Units of enzyme activity are expressed in comparison to fresh weight (FW). Designation of fresh weight (FW) refers to the pulp from the examined plant sources, which was used for enzyme extraction. During the fruit maturation process PME is the first enzyme that acts on pectin, preparing the polygalacturonic acid chain for the degradation of glycosidic bonds by PG (Kohli, Kalia, & Gupta, 2015). Activities of PME for peach, blackcurrant, raspberry and strawberry had similar levels (8.49–11.09 μmol NaOH/g FW*10 min). The highest PME activity was measured for carrot (14.74 μmol NaOH/g FW*10 min), while the lowest was measured for plum (2.98 μmol NaOH/g FW*10 min). PME, an enzyme that plays an important role in the first step of the pectin modification process, causes hydrolysis of the methyl-ester group at the C6 carboxyl (Jayani et al., 2005; Wei et al., 2010). PG activities for different kinds of fruit were more variable than PME activities. PG activity of peach (13.9 μg GalA/g FW*1 min) appears to be very low in comparison to blackcurrant (the highest PG activity – 110 μg GalA/g FW*1 min). PG activities of other fruit samples were similar and ranged from 33.9 μg GalA/g FW*1 min for strawberry to 87.2 μg GalA/g FW*1 min for plum. Following the action of PME, PG cleaves glycosidic linkages between galacturonic acid residues in homogalacturonan. Depending on the active site of the enzyme, polygalacturonases are classified into two types: *endo*-polygalacturonase, which is able to hydrolyze the internal α-1,4 glycosidic bond and to release oligalacturonic acid residues, and *exo*-polygalacturonase, which acts on the non-reducing end of polygalacturonic acid chains by causing the release of mono- and digalacturonic acid residues (Pan et al., 2015). The high amount of uronic acid in blackcurrant, as shown in Table 5, might be related to high PG activity in fresh blackcurrant tissue, which could lead to the release of monogalacturonic acid. PG is widely used in the food industry in the production of fruit and vegetable juices as a clarification agent, as well as an ingredient that reduces the viscosity of juice (Pan et al., 2015). Lower viscosity of pectin solutions in the case of blackcurrant and raspberry pectin might be a result of high PG activity in these materials (compare Tables 1 and 4).

3.3. Chemical properties of pectin enriched fractions

Chemical properties of pectin enriched fractions obtained using citric acid are presented in Table 5. The degree of methylation determines the conditions and the mechanism of gel formation. Low-methylated (LM) pectin chains with DM < 50% form a gel structure in the presence of calcium, via cross-linking of negatively charged uronic acid residues (that are not methyl-esterified at C-6 carboxyl) and positively charged cations (Grant, Morris, Rees, Smith, & Thom, 1973; Mierczyńska, Cybulska, Sołowiej, et al., 2015). In contrast, high-methylated (HM) pectins are able to form gels in the presence of soluble solids e.g. sucrose, at a relatively high concentration of 55–75% and at low pH <3.5. The mechanism of gel formation in this case is related to the formation of connection zones as a result of water being trapped between HG chains (Sousa, Nielsen, Armagan, Larsen, & Sørensen, 2015; Srivastava & Malviya, 2011). Analyzed pectin enriched fractions had a similar DM value, which varied closely around the value of 50% (Table 4). Therefore, citric acid aided extraction used in this study allowed pectins that have similar chemical properties to be obtained. However, raspberry, carrot, strawberry and black currant pectin enriched fractions can barely be classified as LM pectins with DM values of 45.8%, 45.2%, 46.0% and 49.2%, respectively. Despite the relatively high calcium content in raspberry, black currant and carrot polysaccharides (7.67 μg/mg, 5.40 μg/mg and 5.59 μg/mg, respectively) in comparison to other analyzed pectin enriched fractions,

Table 4
Dry matter content, soluble solid content and enzyme activity of six materials used for pectin enriched fractions extraction.

	Enzyme activity			
	Dry matter (g/100 g)	Soluble solid content (°Brix)	PME (μmol NaOH/g FW*10 min)	PG (μg GalA/g FW*1 min)
Peach	$12.21^{a} \pm 0.45$	$10.9^{a} \pm 0.1$	$11.09^{a} \pm 0.55$	$13.9^{a} \pm 1.8$
Black currant	$20.95^{b} \pm 2.54$	$15.3^{b} \pm 0.1$	$8.76^{b} \pm 0.15$	$110.0^{b} \pm 1.5$
Raspberry	$15.56^{c} \pm 1.16$	$8.8^{c} \pm 0.1$	$8.49^{b} \pm 0.40$	$64.7^{c} \pm 2.5$
Plum	$14.65^{ac} \pm 0.27$	$15.0^{e} \pm 0.1$	$2.98^{d} \pm 0.17$	$87.2^{f} \pm 3.4$
Strawberry	$7.04^{d} \pm 0.23$	$5.6^{d} \pm 0.1$	$10.42^{a} \pm 0.23$	$33.9^{e} \pm 1.5$
Carrot	$13.92^{ac} \pm 0.71$	$10.8^{a} \pm 0.1$	$14.74^{c} \pm 0.85$	$42.2^{d} \pm 2.4$

Mean values with standard deviations (n = 3: replicates) are presented. Different letter indices show statistical significance at $p < 0.05$. The same lower case letter in the data reported in a column means-non significant differences ($p < 0.05$). Dry matter content was expressed as a g of dry substance per 100 g of fresh weight. PME – pectin methylesterase; PG – polygalacturonase; FW – fresh weight of pulp.

all the aqueous solutions (2% and 5%) of LM pectins showed significantly lower viscosity than pectins with DM >50% (Table 1). The highest viscosity was observed for the peach pectin enriched fraction and plum pectin enriched fraction, which had a DM value slightly above 50%. This may suggest that, a mechanism other than calcium-induced gelling could be responsible for the cross-linking of extracted pectin enriched fractions. The DM value of the pectin chain is related to the activity of PME, which catalyzes the removal of the methyl ester group from homogalacturonan (Yoo et al., 2009). The highest activity of PME among the materials analyzed was determined for carrot pectin which had the lowest DM level, but in the case of peach, relatively high PME activity was observed at DM > 50%. The fresh pulp of plum has the lowest PME activity and its DM value was the highest among all of the materials used. This lack of an unambiguous relationship between PME activity and the degree of methylation suggests that extraction with citric acid would affect the DM value of pectin enriched fractions.

The content of uronic acid and calcium ions varied between extracted pectin enriched fractions (Table 5). The calcium content ranged from 0.166 ± 0.001% for plum to 0.767 ± 0.011% for raspberry. The amount of uronic acid varied between 37.1 ± 1.06% for blackcurrant and 16.5 ± 0.188% for carrot. The method of extraction may influence pectin properties including uronic acid content. Strong mineral acids are very often used in industry due to their low price and efficacy although these chemicals may impact negatively on the environment. Therefore organic acids are being widely investigated as an alternative for pectin extraction (Oliveira et al., 2016). Yuliarti et al. (2015) compared galacturonic acid content in gold kiwi fruit pectin using citric acid, water and enzymes for extraction and demonstrated very slight differences in the GalA content of extracted pectin. Chan & Choo (2013) demonstrated higher GalA content in pectin extracted from cocoa husk using hydrochloric acid, pH 2.5 at 95 °C (GalA – 54.69%) than in pectin extracted with citric acid under the same conditions (GalA – 31.19%). Methacanon et al. (2014) proved that pectin extracted using a nitric acid solution of pH 2 was characterized by a higher GalA content than pectin extracted with a pH 3 acid solution. The literature analyzed that report on pectin extraction using acid, especially citric acid, showed that extraction conditions are very important for pectin yield. Pectin extraction using citric acid is a more ecological and less hazardous alternative from the food technology point of view compared to strong mineral acids, however it results in different properties of extracted pectin. Specifications for quality and physicochemical parameters for commercial apple pectins require that galacturonic acid content should not be less than 65% of dry weight (Yapo & Gnakri, 2014). The uronic acid content, obtained for this paper were lower (from about 16.5% for carrot to about 37.1% for blackcurrant) than for the commercial pectin substances. This is especially true when comparing citrus peels and apple pomace, however, this study shows that the search for new sources of pectins and a more ecological method of extraction could benefit the food industry. Their potential use is supported by their rheological properties presented in Table 1.

Table 5
Chemical characteristics of pectin enriched fractions.

	Chemical properties of extracted pectin enriched fractions		
	Ca (g/100 g)	Uronic acid (g/100 g)	DM (%)
Peach	$0.206^{a} \pm 0.002$	$26.0^{a} \pm 1.57$	$50.1^{a} \pm 0.3$
Black currant	$0.540^{b} \pm 0.009$	$37.1^{b} \pm 1.06$	$49.2^{b} \pm 0.3$
Raspberry	$0.767^{c} \pm 0.011$	$23.1^{c} \pm 0.99$	$45.8^{cd} \pm 0.3$
Plum	$0.166^{e} \pm 0.001$	$23.8^{ac} \pm 0.16$	$50.7^{a} \pm 0.5$
Strawberry	$0.266^{d} \pm 0.004$	$33.9^{e} \pm 0.48$	$46.0^{c} \pm 0.3$
Carrot	$0.559b \pm 0.008$	$16.5d \pm 0.18$	$45.2d \pm 0.4$

Mean values with standard deviations (n = 3 replicates) are presented. Different letter indices show statistical significance ($p < 0.05$). The same lower case letter in the data reported in a column means-non significant differences ($p < 0.05$). Uronic acid content (g/100 g of dried pectin-rich fraction); Ca – calcium content (g/100 g of dried pectin-rich fraction); DM – degree of methylation (the ratio of the intensity of bands centred at 1750 cm^{-1} for the esterified carbonyl groups and at 1630 cm^{-1} for carboxylic anions).

3.4. Structure of pectin enriched fractions

FT-IR spectroscopy was applied for the structural analysis of pectin enriched fractions extracted with citric acid. A set of FT-IR spectra of pectin enriched fractions in the range of 1800–650 cm^{-1} is shown in Fig. 2 Application of the same extraction method resulted in chemically uniform pectic material. For each sample, the highest peak was present at 1015 cm^{-1}–this band is characteristic for polysaccharides rich in polygalacturonic acid (Posé et al., 2015). The bands at 1730 and around 1600 cm^{-1} indicate the presence of esterified and non-esterified carboxyl groups, respectively (Cybulska et al., 2015; Fellah et al., 2009). The DM value was calculated from the ratio of absorbance intensity of esterified carboxyl groups to all carboxyl groups; the results are presented in Table 5. Shifting of bands characteristic of esterified carboxyl groups at about 1600 cm^{-1} to higher wavenumbers was clearly visible for the carrot pectin enriched fraction, this could be associated with higher DM values or numerous interconnections between pectin molecules as well as the presence of pectins complexed with calcium ions (Chatjigakis et al., 1998; Szymańska-Chargot & Zdunek, 2013). For pectin enriched fractions extracted from raspberries, carrots and plums a small band at about 1513 cm^{-1} was observed, which probably originated from aromatic skeletal vibrations of lignocellulosic residue (Xue, Wena, Xu, & Sun, 2012; Zhang et al., 2015). The bands at 1438, 1415, 1369 cm^{-1}, the most intensive for the peach pectin enriched fraction, may be ascribed to an ester group (Čopíková, Synytsya, Černá, Kaasová, & Novotná, 2001). Rasp-

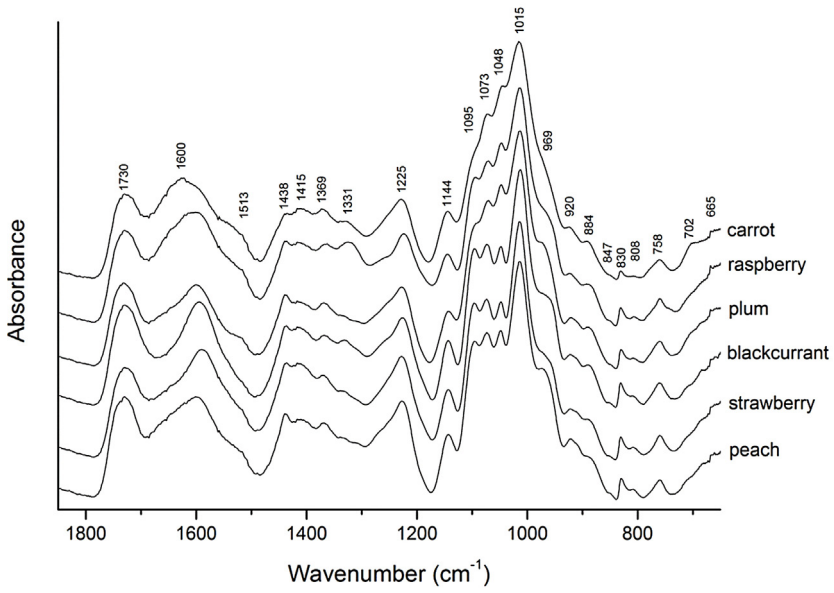

Fig. 2. FT-IR spectra of pectin enriched fractions extracted from peach, strawberry, blackcurrant, plum, raspberry and carrot using citric acid.

berry pectin enriched fraction was distinguished by a clear band at 1331 cm^{-1} which can be assigned to ring vibration (Wellner, Kacuráková, Malovíková, Wilson, & Belton, 1998). All the samples displayed a strong peak at 1225 cm^{-1} coming from CO stretching vibrations (Cybulska et al., 2015). The presence of long molecules in the samples can be deduced from the presence of a relatively strong band at ~1144 cm^{-1} which can be assigned to COC vibrations of glycosidic bonds (Kacuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). Some differences in peak intensity in the range of 1100–1015 cm^{-1} can be observed, particularly in the case of the carrot pectin enriched fraction. The bands at 1095, 1073 and 1048 cm^{-1} are distinctly separated in the case of peach, strawberry and blackcurrant, although they become overlapped in the case of plum, raspberry and carrot.

The region of 1200–800 cm^{-1} is a fingerprint region used to identify particular polysaccharides (Pose et al., 2012). In all samples, the characteristic bands for arabinose at ~1060-1040 and 975 cm^{-1} were found (Coimbra, Barros, Barros, Rutledge, & Delgadillo, 1998). Extracted pectin enriched fraction samples had almost identical FT-IR spectra; this indicates that similar pectins can be extracted from different sources when identical extraction conditions are applied.

Mild extraction of pectin enriched fractions from different sources described in this study, produced a large number of long molecules of about 100 nm in length, which are depicted in AFM images in Fig. 3. For example, pectin enriched fractions from raspberry, plum and strawberry, had similar topographic features, i.e. strong networking, rather than aggregation was observed. Similar organization of the molecules was previously observed for sodium carbonate extracted pectins from apple and carrot (Cybulska et al., 2015; Zdunek et al., 2014). This suggests that extraction with citric acid at 97 °C results in the formation of a similar network of pectins to the structures formed by pectins extracted with sodium carbonate and that, unlike the pectins extracted using well-established methods, they can self-organize on freshly-cleaved mica. Although this AFM study did not allow for the grading of the networking capability of these different pectins, the ability to cross-link may provide an explanation for the mechanisms of the viscosity of aqueous solutions, as shown by rheological measurements. Moreover, as discussed in a previous study on the rheological properties of the sodium carbonate extracted pectins from carrot (Mierczyńska, Cybulska, Pieczywek, et al., 2015) it was found that aqueous solutions of citric acid-extracted pectin enriched fractions also showed strong thixotropic properties that were not observed for the water and calcium chelator soluble pectins. The similar structure revealed by AFM images and the same thixotropic properties as for sodium carbonate extracted pectins suggest that these methods of extraction produce similar types of pectins.

Analysis of viscosity and chemical properties of the extracted pectin enriched fractions showed that DM values lower than 50% and high calcium content did not ensure the production of viscous solutions. The highest viscosity observed for peach and plum pectin enriched fractions and similarity of AFM images (Fig. 3) with the images of pectins extracted with sodium carbonate (Cybulska et al., 2015; Zdunek et al., 2014) suggest that the self-organized network of pectic polymers was probably responsible for this structuring effect. Moreover, pectins with DM > 50% had the highest viscosity, but this cannot be a result of the gelling mechanism characteristic for HM pectins due to a lack of sugars necessary for gelling. Soluble sugars were removed during pectin extraction. Therefore, further studies are necessary to interpret the mechanism of gelling of pectins with DM > 50% without the presence of sugars.

4. Conclusions

This study demonstrates that polysaccharides suitable as a food thickener can be obtained from commonly available fruit sources like peach, blackcurrant, raspberry, strawberry, plum and vegetable like carrot using citric acid extraction. Pectinolytic enzymes acting in fresh plant tissues have an influence on the properties of extracted pectin enriched fractions. Despite the fact, that isolated polysaccharides were found to have a lower uronic acid content than commercial apple and citrus pectins, pectin enriched fractions extracted using citric acid demonstrated rheological properties which are useful in gelling additives. Pectin enriched fractions extracted from different plant sources demonstrated the thixotropic effect, pseudoplastic flow behaviour and high viscosity which allow for the thickening and gelling of food fluids. The chemical properties as well as the rheological behaviour of analyzed polysaccharides from different sources differed from each other in the value of various parameters. This diversity presents great opportunities for new applications of pectins in the food industry. The desired properties of pectins, such as acting as gelling or stabi-

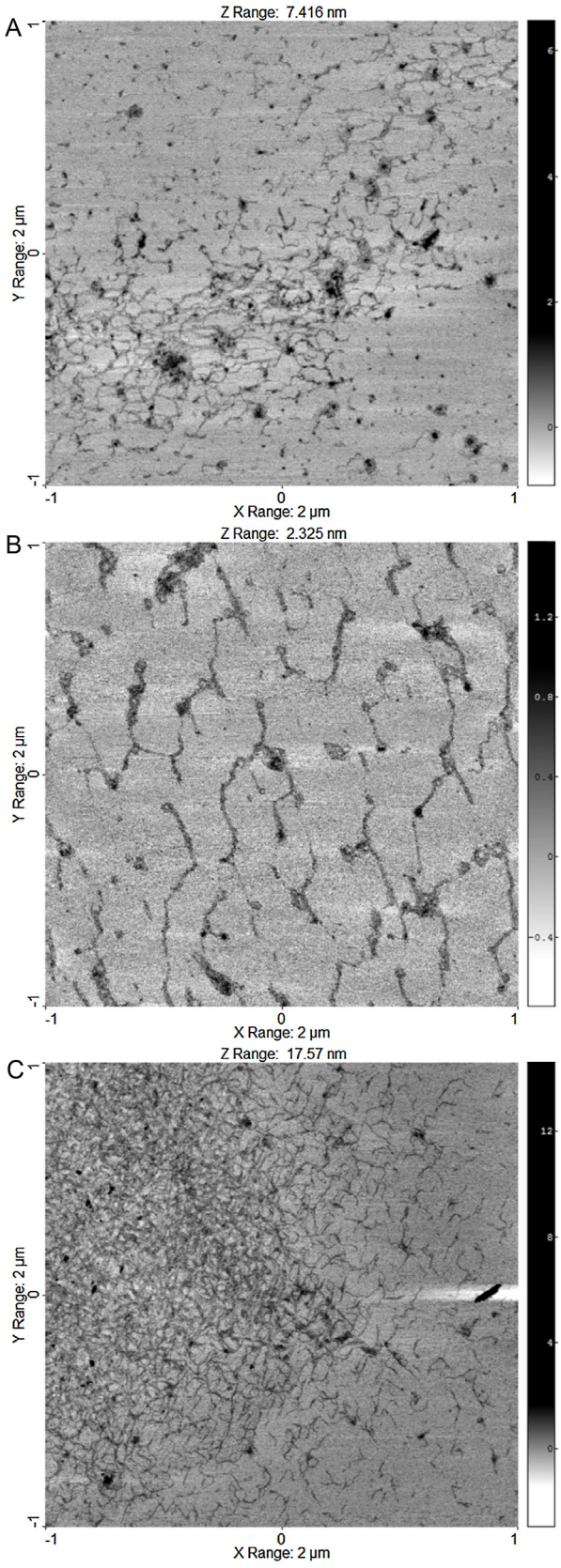

Fig. 3. AFM height images of pectin enriched fractions extracted from A) raspberry, B) plum, and C) strawberry, as examples.

lizing agents, as well as being used as functional food ingredients may be achieved by the appropriate selection of the plant source. Moreover, the use of citric acid for pectin enriched fractions extraction is an ecological alternative to the mineral acids commonly used in industry.

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