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Physicochemical properties of pectin from *Malus domestica* 'Fălticeni' apple pomace as affected by non-conventional extraction techniques

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Abstract

Six non-conventional techniques (microwave-assisted extraction – MAE, ultrasound-assisted extraction – UAE, enzyme-assisted extraction – with cellulase, EAE1, and Celluclast 1.5L, EAE2, ultrasound-assisted extraction – heating treatment – UAEH, and enzyme-assisted extraction – ultrasound treatment – EAU) and conventional citric acid extraction – CE were applied to extract pectin from *Malus domestica* 'Fălticeni' apple pomace, and were compared in terms of extraction yield and physicochemical properties of pectin. MAE led to the highest extraction yield and ; the lowest pectin recovery was found for EAE2. Pectin samples obtained by MAE showed color parameters comparable to commercial apple (AP) and citrus (CP) pectin, and had high galacturonic acid content, increased equivalent weight and high degree of esterification. High galacturonic acid content and degree of esterification were also found in UAE pectin samples. On the opposite side, EAE1, EAE2 and EAU pectin had high equivalent

weight, but lower degree of esterification that classified EAE1 and EAU pectin as low-methoxylated pectin. UAEH and MAE pectin showed thermal properties that were similar toto that of commercial AP and CP . The rheological characterization of pectin samples highlighted the high viscosities of UAE and MAE pectin solutions, which were positively correlated with their galacturonic acid content.

Keywords: apple pomace; pectin; extraction; *Malus domestica* 'Fălticeni'; comparison

1. Introduction

Pectins are macromolecular polysaccharides widely distributed in the middle lamella and primary cell walls and act as hydrating agent and serve to cement the cellulose network (Dranca & Oroian, 2018; Mualikrishna & Tharanathan, 1994). Structurally, pectin is formed of three main regions: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). HG region is composed of α-(1,4)-linked *D*-galacturonic acid (GalA) units (Cameron, Kim, Galant, Luzio, & Tzen, 2015) that may be methyl-esterified at the C-6 carboxyl or acetylated at the O-2 and/or O-3 (Caffall & Mohnen, 2009); according to the degree of methoxylation (DM), pectins are classified as high-methoxylated (DM>50%) and low-methoxylated (DM<50%) (Einhorn-Stoll, 2018). The backbone of the branched RG-I region is composed of repeating disaccharide units of [→4)-α-*D*-Gal*p*A-(1→2)-α-*L*-Rha*p*-(1→] and side chains of neutral sugar, including galactan, arabinan, and arabinogalactan, linked at C-4 of the *L*-rhamnosyl residues (J.-S. Yang, Mu, & Ma, 2019). The third main region, RG-II, consists of a polygalacturonic acid backbone containing unusual sugars such as the rarely observed apiose, 2-

O-methylxylose and 2-*O*-methylfucose, 3-deoxy-*D*-manno-2-octulosonic acid, 3-deoxy-*D*-lyxo-2-heptulosaric acid and aceric acid (Cui et al., 2019; Pérez, Rodríguez-Carvajal, & Doco, 2003; Darvill, McNeil, & Albersheim, 1978;). The proportions of HG, RG-I and RG-II in pectin structure vary with the plant source, however, it was considered that in general HG is the most abundant pectic polysaccharide (~65% of pectin), RG-I represents around 20–35% and the remaining proportion is comprised by RG-II (Mohnen, 2008).

Pectin structure is a determinant factor on its physicochemical properties and applications. We can consider for example the degree of esterification of pectin, which is important to the functional properties of the polysaccharide in the plant cell wall and dictates the gel-forming properties of aqueous solutions of pectins with acid and sugar and thus its applications as gelling agent, stabilizer, emulsifier and thickener in the food industry (Güzel & Akpınar, 2019; Marić et al., 2018). The actual structure of pectin depends on the plant source and the method of extraction (Morris, Gromer, Kirby, Bongaerts, & Patrick Gunning, 2011; Ridley, O'Neill, & Mohnen, 2001). Industrial scale pectin extraction is mainly focused on two sources: citrus peel and apple pomace. It was considered that apple pectin produces a more viscous gel and is suitable for bakery fillings, while the lighter colored citrus pectin can be used to obtain confectionery jellies (May, 1990). While citrus peel and apple pomace remain the main sources for commercial pectins, various other by-products and wastes have been considered with the purpose of studying pectic polysaccharides with unique and diverse functional properties and possibly introducing new viable sources for pectin extraction on a growing global market. As reported in the last few years, some of the plant sources for pectin extraction were eggplant peel (Kazemi, Khodaiyan, & Hosseini, 2019a), artichoke by-products (Sabater, Corzo, Olano, & Montilla, 2018), mango peel (Nagel et al., 2017), watermelon rinds (Romdhane et al., 2017),

banana peel (Oliveira et al., 2016), papaya peel (Maran & Prakash, 2015), sunflower head (Kang, Hua, Yang, Chen, & Yang, 2015), jackfruit peel (R. Begum, Aziz, Uddin, & Yusof, 2014), pumpkin biomass (Yoo et al., 2012) and cacao pod husks (Vriesmann, Teófilo, & Petkowicz, 2011).

The second factor that determines the structure of pectin is the method of extraction. An important subject of pectin research is to study the application of one extraction technique, as well as conducting a comparative study between two or more techniques used to isolate pectic polysaccharides from a plant source, together with an investigation on the changes of its physicochemical, thermal and rheological characteristics. The methods of pectin extraction reported in the literature include conventional acid extraction with a mineral or organic acid (mostly citric acid) (Colodel & De Oliveira Petkowicz, 2018; Patova et al., 2019), microwave-assisted extraction (Košťálová, Aguedo, & Hromádková, 2016; Maran & Prakash, 2015), ultrasound-assisted extraction (Hosseini, Khodaiyan, Kazemi, & Najari, 2019; Moorthy, Maran, Surya, Naganyashree, & Shivamathi, 2015), enzymatic extraction (Sabater et al., 2018; Wikiera, Mika, & Grabacka, 2015), subcritical water extraction (Liew, Teoh, Tan, Yusoff, & Ngoh, 2018; Muñoz-Almagro, Valadez-Carmona, Mendiola, Ibáñez, & Villamiel, 2019), and also combined techniques such as microwave heating extraction (Rodsamran & Sothornvit, 2019), ultrasound-microwave assisted extraction (Liew, Ngoh, Yusoff, & Teoh, 2016) and enzymatic-ultrasonic extraction (Yang, Wang, Hu, Xiao, & Wu, 2018).

Some previously published studies have reported a comparison between techniques used for pectin extraction (Rodsamran & Sothornvit, 2019; Bagherian et al., 2011). However, the present work represents a more complex holistic approach to this subject since it aims at comparing different extraction techniques, namely conventional citric acid extraction and non-conventional

methods (microwave-assisted extraction, ultrasound-assisted extraction, enzymatic extraction with cellulase and Celluclast 1.5L, respectively, combined ultrasound heating extraction and combined enzymatic (cellulase)-ultrasonic extraction) to extract pectin from *Malus domestica* 'Fălticeni' apple pomace. The physicochemical properties of the pectin obtained by each technique were also compared to those of commercial apple and citrus pectin samples.

2. Materials and methods

2.1. Materials

Apple pomace used for pectin extraction was obtained by processing *Malus domestica* 'Fălticeni' apples into juice in a small-scale plant, in the Fălticeni area of Suceava (47°27'10.5"N, 26°17'38.8"E), Romania. After juice extraction, apple pomace was dried at 60 °C in an oven with air circulation until constant weight. The dried pomace was powdered and passed through an analytical sieve shaker Retsch AS 200 (Retsch GmbH, Germany). The pomace with particle sizes of 125-200 µm was used to extract pectin.

Commercial apple and citrus pectin were purchased from Merck KGaA (Germany). All chemicals and reagents, including citric acid, ethyl alcohol, *D*-galacturonic acid, m-hydroxydiphenyl, sodium hydroxide and hydrochloric acid were of analytical grade and were purchased from Merck KGaA (Germany).

2.2. Pectin extraction

A. Conventional citric acid extraction (CE). The extraction mixture was prepared by mixing 10 g of apple pomace powder with 100 mL of distilled water in which citric acid was added to reach a pH value of 1.9. This mixture was kept in a water bath at the temperature of 90 °C for 148 min.

B. Microwave-assisted extraction (MAE). 10 g of apple pomace powder were mixed with 100 mL of water-citric acid solution with a pH of 2.2. The extraction was performed in an experimental microwave oven (MO17DW, Gorenje, Slovenia) at a power of 560 W for 120 s.

C. Ultrasound-assisted extraction (UAE). The extraction mixture, prepared by mixing 10 g of apple pomace powder with 100 mL of water-citric acid solution with a pH of 1.8, was sonicated for 30 min at 100% amplitude (20 kHz, maximum power of 70 W) using an ultrasonic device (Sonopuls HD 2070, Bandelin, Germany) with a flat tip probe (KE 76, Bandelin, Germany) that was submerged 15 mm deep into the mixture.

D. Enzyme-assisted extraction with two different enzyme preparations (EAE1 and EAE2). For the extraction process involving the use of cellulase (EAE1), 6.7 g of apple pomace were mixed with 100 mL of water brought to a pH of 4.5 with citric acid. A dose of 7.5 mg cellulase/g apple pomace was added to the mixture and the extraction was conducted at 47 °C for 20 h with constant shaking (200 rpm).

Pectin extraction with the multicatalytic enzyme preparation Celluclast 1.5L (EAE2) was carried out, as follows: 10 g of apple pomace powder were mixed with 100 mL of water (pH=4.5), an enzyme dose of 42.5 µL/g apple pomace was added to the water-pomace mixture and the extraction was carried out for 18 h 14 min at 48 °C under constant shaking.

After extraction, the samples were heated at 121 °C for 5 min to inactivate the enzyme and then cooled to room temperature.

E. Ultrasound-assisted extraction – heating treatment (UAEH). For this combined technique, ultrasound-assisted extraction was first conducted under the conditions presented in section C, and after that the sample was exposed to a heating treatment at 86 °C for 2h and 27 min.

F. Enzyme-assisted extraction – ultrasound treatment (EAU). In the case of this combined technique, enzyme-assisted extraction with cellulase was made as presented in section D, and was followed by a sonication at 62% amplitude for 21 min.

The precipitation and purification steps were identical for all extraction methods that were applied to isolate pectin from the plant material. After each extraction, pectin was separated from the remaining solid material by centrifugation at 4000 rpm for 40 min, the supernatant was collected, filtered and transferred in a laboratory glass bottle were it was precipitated by adding cold concentrated ethyl alcohol, and it was kept at 4-6 °C for 12 h to complete the precipitation. The precipitated pectin was separated by centrifugation (4000 rpm, 40 min) and was washed 3 times by concentrated ethyl alcohol and finally dried in an oven with air circulation at 50 °C to a constant weight. Pectin was finally powdered with a food processor to obtain particles <200 µm.

The extraction yield was calculated using the equation:

$$Pectin\ yield\ (\%) = \frac{m_p}{m} \times 100 \quad (1)$$

Where: m_p – weight of dried pectin (g), m – weight of dried apple pomace powder (g).

2.3. Characterization of pectin samples

2.3.1. Color

The color of the pectin samples extracted by the methods described previously, and the color of the commercial apple and citrus pectin samples were analyzed in triplicate at 25 °C with a CR-400 chromameter (Konica Minolta, Japan) after calibration with the standard white plate. CIE L*, a*, b* coordinates, hue (h^*_{ab}) and chroma (C^*_{ab}) (CIE, 1986) were obtained from the reflection spectra of the samples with illuminant D65 and 2° observer.

2.3.2. Galacturonic acid content

The galacturonic acid content (GalA) of pectin was determined in triplicate by the m-hydroxydiphenyl spectrophotometric method developed by Filisetti-Cozzi and Carpita (Melton & Smith, 2001). As described in a previous study (Miceli-Garcia, 2014), pectin samples were prepared by dissolving pectin powder (20 mg) in distilled water at 50 °C and then diluting to a constant volume of 100 mL. 400 µL of pectin solution were mixed with 4 M sulfamic acid and hydrolyzed with a solution of sulfuric acid containing 75 mM of sodium tetraborate for 20 min in a water bath, then cooled down for 10 min in an ice bath. To each sample a solution of m-hydroxydiphenyl in 0.5% sodium hydroxide was added and the content was vortexed. The absorbance was read at 525 nm using a UV-Vis-NIR spectrophotometer (Shimadzu Corporation, Japan).

2.3.3. Equivalent weight

The equivalent weight (Eq.W) of pectin samples was measured in triplicate as follows: 0.5 g of pectin powder was completely dissolved in 100 mL of distilled water under continuous stirring (300 rpm) for 1 h. 1 g of sodium chloride was added, followed by 5 drops of phenol red indicator and the solution was titrated against 0.1 N NaOH until the color changed to pink and persisted for at least 30 s (Ranggana, 1986). Eq.W was calculated with the equation:

$$Equivalent\ weight\ (Eq.W) = \frac{1{,}000 \times Weight\ of\ sample\ (g)}{Volume\ of\ alkali\ (mL) \times Normality\ of\ alkali} \quad (2)$$

2.3.4. Methoxyl content

The neutralized solution containing 0.5 g pectin, resulted from the determination of Eq.W, was mixed with 25 mL of 0.25 M NaOH in a stoppered flask, shaken thoroughly, and allowed to

stand for 30 min at room temperature. An equal volume (25 mL) of 0.25 M HCl was added and titrated against 0.1 N NaOH as before (Ranggana, 1986). Methoxyl content (MC) was calculated using the equation:

$$Methoxyl\ content\ (\%) = \frac{Volume\ of\ alkali\ (mL) \times Normality\ of\ alkali \times 3.1}{Weight\ of\ sample\ (g)} \quad (3)$$

2.3.5. Degree of esterification

The degree of esterification (DE) of pectin samples was estimated in triplicate by means of Fourier transform infrared spectroscopy (FT-IR) analysis using a Spectrum Two infrared spectrophotometer (Perkin Elmer, USA). The spectra were recorded in transmission mode within the wavenumber range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1}. SpectraGryph – spectroscopy software (Version 1.2.11) was used to display the spectra.

Since the DE is defined as the number of esterified carboxylic groups over the number of total carboxylic groups multiplied by 100, it is inferred that the ratio of the area of the band at 1730 cm^{-1}, which corresponds to the number of esterified carboxylic groups, to the sum of the areas of the bands between 1730 and 1600 cm^{-1} that corresponds to the number of total carboxylic groups, should be proportional to the DE (Wai, AlKarkhi, & Easa, 2010; Manrique & Lajolo, 2002):

$$DE\ (\%) = \frac{A_{1730}}{A_{1730} + A_{1600}} \quad (4)$$

2.3.6. Thermal properties

Thermal analysis was carried out in triplicate with the differential scanning calorimetry (DSC) technique. A small quantity (5 mg) of each pectin sample, previously dried in an oven with air

circulation, was weighted and then hermetically sealed in aluminum pan and placed in the instrument (DSC 8500, Perkin Elmer, USA) alongside an empty pan used as reference. The DSC measurements were performed over a temperature range of 0-300 °C, at a constant heating rate of 10 °C/min using nitrogen as purge gas at a flow rate of 20 mL/min.

2.3.7. Microstructure

The microstructure of the pectin samples was examined by scanning electron microscopy (SEM; SU-70, Hitachi, Tokyo, Japan). Dried pectin powder was fixed to the sample table with conductive double-sided adhesive carbon tape and analyzed using an accelerating voltage of 5 kV with a magnification of 300×.

2.3.8. Rheological properties

Pectin samples at 3% (w/w) were completely dissolved in deionized water adjusted to pH=4 by continuous stirring at 40 °C for 12 h. The samples were cooled to room temperature (25 °C) and stored under refrigeration for 12 h prior to being analyzed.

The dynamic viscosity of pectin samples was analysed with a Mars 40 rheometer (Thermo Haake, Germany) using a cone (Ø 35 mm, 2º) – plate system. In order to allow the recovering of the structure and to achieve the desired temperature, each pectin sample was left to rest for 10 min prior to the measurement which was performed at 20 °C in triplicate. The shear rate (γ, s^{-1}) was ranged between 0 – 100 s^{-1} while measuring the shear stress (τ, Pa) and dynamic viscosity (η, Pa·s).

To obtain the loss modulus *G''* (Pa) and elastic modulus *G'* (Pa) stress sweeps were performed at 1 Hz to determine the viscoelastic region. The stress was chosen within the linear viscoelastic region and the frequency ranged from 0.1 to 10 Hz.

Creep and recovery analysis was performed at a constant stress of 1 Pa, which was applied and maintained for 180 s, then released to allow sample recovery for another 180 s. Creep parameters were determined by computing a constant stress *(σ)* over time *(t)* and were expressed using the creep compliance *(J)* function in terms of shear deformation (γ), as shown in the equation:

$$J\ (t) = \frac{\gamma(t)}{\sigma} \tag{5}$$

2.3.9. Statistical analysis

Results were submitted to analysis of variance (ANOVA) using Statgraphics Centurion XVI software (Manugistics Corp., Rockville, Md.). Fisher′s least significant difference (LSD) procedure was used at the 95% confidence level.

The Spearman correlation and principal component analysis (PCA) were calculated using Unscrambler X version 10.1 (Camo, Norway).

3. Results and discussion

3.1. Extraction yield

When comparing different extraction methods applied to obtain pectin from a plant material it is important to consider the maximum extraction yield achieved with each method because this is likely to have a major influence on its industrial feasibility. The maximum yield obtained by means of each technique, for the conditions of pectin extraction detailed in section 2.2, is presented in Table 1. As it can be observed, the lowest pectin recovery from apple pomace

(6.76%) resulted when the multicatalytic enzyme preparation Celluclast 1.5L was used for the EAE (EAE2), while the highest yield (23.32%) was achieved when MAE was applied. Conventional citric acid extraction (CE) also produced a high pectin yield (23.26%). Between CE and MAE the solid-to-liquid ratio (SLR) was the same (10 g in 100 mL, 1:10), however, the extraction time was significantly shorter for MAE (120 s) than CE (~150 min). A similar result was obtained by Bagherian et al. (2011), who reported for pectin extracted from grapefruit a higher pectin yield resulted for microwave extraction (27.81%) by comparison to the conventional method (19.16%). The higher extraction yield of MAE might be attributed to the fact that microwave radiation is known to loosen the cell wall matrix and cause the severing of the parenchymal cells (Kratchanova, Pavlova, & Panchev, 2004) leading to increased interaction between the plant material and the extracting solvent.

Contrary to the results of previously published studies (Guandalini et al., 2018; Hosseini, Khodaiyan, Kazemi, & Najari, 2019; Hosseini, Khodaiyan, & Yarmand, 2016), UAE did not produce a pectin yield higher than that obtained for CE; this outcome that may be mostly attributed to the lower maximum working power (70 W) of the ultrasonic device used in the present study. Because sonication caused a disintegration of apple pomace, therefore affecting the separation between the solid and liquid phases, a second ultrasound treatment may be efficient in dissolving the pectin previously absorbed in the residue (Dranca & Oroian, 2018). This observation was confirmed by Wang et al. (2017), who reported an increase of pectin yield with about 25% by performing a second ultrasound extraction. These factors, alongside the absence of periodical agitation meant to keep the mixture evenly distributed (Xu et al., 2014) also explain why the combination between a heating and ultrasound treatment did not lead to a higher pectin yield. A slight increase in pectin extraction was observed when UAE was used in

combination with cellulase (EAU) as compared with the enzyme-assisted extraction (EAE1), but the change in efficiency was not as substantial as in previous studies (Yang et al., 2018).

3.2. Color

The color of pectin is an important parameter as it affects the appearance of the solution or the gel produced and therefore the appearance of the food product in which was added (Grassino et al., 2016). As can be seen in Table 2, the commercial pectin samples CP and AP had the highest lightness (L*) values, as well as higher hue, which tended to be more green in the case of CP sample and more red in the case of AP. By comparison to CP, the other pectin samples, including AP sample, were characterized by more redness, especially in EAE2 that showed the lowest hue value. CE and MAE pectin samples were similar in terms of color to AP samples (with values of lightness, chroma and hue that were not significantly different. Contrary to previous studies, microwave (Rodsamran & Sothornvit, 2019) and ultrasound-assisted extraction (Wang et al., 2015) did not produce a pectin with higher L* values as compared to CE samples, showing that in the case of the present study higher temperatures and longer extraction time did not lead to a dark color of pectin. In general, it was observed that extraction techniques that involve exposure to temperatures below 50 °C for prolonged time (EAE1, EAE2 and EAU) determined lower L* values and values of hue and chroma associated with a brown color of the extracted pectin. In a similar way, UAE and UAEH determined a darker color of the pectin sample, although the heating treatment applied for UAEH increased the lightness and reduced the redness and yellowness. Considering that *Malus domestica* 'Fălticeni' apples are red skinned, the brown color of pectin samples obtained by either enzymatic or ultrasonic treatment may be the result of

295 the presence of polyphenols and other water-soluble pigments that were trapped inside pectin
296 during extraction and precipitation (Grassino et al., 2016; Wang et al., 2016).
297
298 **3.3. Galacturonic acid content**
299 GalA is the most prevailing building block of pectin (Broxterman, Picouet, & Schols, 2017),
300 which makes its determination a very important step in the analysis of pectin's chemical
301 structure. According to the specifications on purity characteristics of the Joint FAO/WHO Expert
302 Committee on Food Additives and the European Commission, pectin should not contain less than
303 65% galacturonic acid (Müller-Maatsch et al., 2016). As Table 2 shows, this regulation regarding
304 the purity of pectin was met for all the samples analyzed in this study, in which was determined a
305 GalA content between a minimum of 69.06 g/100 g (UAEH pectin) and a maximum of 92.83
306 g/100 g (UAE pectin). The GalA content of CE pectin was similar to that of the commercial CP
307 and AP samples, while MAE and UAE pectin samples had a higher GalA content, corroborating
308 the findings of previous studies on the comparison between CE and these techniques (Yang et
309 al., 2018; Bagherian et al., 2011). The use of the multicatalytic enzyme preparation Celluclast
310 1.5L determined an increased GalA content of the extracted pectin (EAE2) by comparison to the
311 enzyme-assisted extraction with cellulase (EAE1). When enzymatic extraction with cellulase
312 was followed by ultrasound treatment (EAU), the GalA content was lower, indicating an
313 opposite effect to the increase in this chemical parameter obtained by Yang et al. (2018) when
314 using the combined enzymatic-ultrasound extraction instead of the enzymatic/ultrasound
315 treatment. This shows that the increase of extraction yield by the combined EAU was not
316 accompanied by an increase of pectin purity. On the other side, UAEH extraction led to a lower
317 GalA content as the pectin yield also decreased (Table 1).

3.4. Equivalent weight

Commercial pectin samples were very different in terms of their Eq.W as shown in Table 2.Eq.W of commercial CP sample was 1190, a value higher than that obtained for commercial AP pectin (515). The values presented in Table 2 suggest that the choice of extraction technique had a great influence on the Eq.W of the extracted pectin, as it was previously concluded by Kumar & Chauhan (2010). It can be observed that Eq.W values for the extracted pectin samples varied between a minimum of 704 (UAE pectin) and maximum of 2778(EAE1 pectin). Microwave extraction (MAE) led to a higher Eq.W than that obtained for the pectin sample extracted by the conventional method (CE). Thiswas in accordance with the study by Rodsamran & Sothornvit (2019). Both techniques involving the application of ultrasound treatment, namely UAE and UAEH, resulted in pectin samples with lower Eq.W, which may be caused by some breaking in the linear pectin molecule leading to a weaker network formation (Abid et al., 2013; Seshadri, Weiss, Hulbert, & Mount, 2003). The extraction techniques that required the use of enzymes led to the highest Eq.W values, meaning that a polymerization of pectin into a longer chain occurred, and this in turn decreased the free acid (non-esterified galacturonic acid) content. With the exception of EAE1, EAE2 and EAU, all pectin samples extracted from *Malus domestica* 'Fălticeni' apple pomace had Eq.W similar to that obtained by Kumar & Chauhan (2010) for pectin extracted from pomace of *Malus pumila* and *Spondias dulcis* apple varieties.

3.5. Methoxyl content

As pectins are classified as high- and low-methoxyl and their ability to form gels in certain conditions varies accordingly, the methoxyl content (MC) is another parameter that describes the

functionality of the extracted pectin (O'Shea et al., 2015). As shown in Table 2, AP and CP samples had a MC of about 4%, being AP samples¡ the pectin with the highest MC (4.83%) among all samples analyzed in this study. The CE method resulted in the lowest MC which might have been due to the extended heating at high temperatures that are involved in the extraction process. Shorter extraction techniques such as MAE and UAE led to higher MC in the extracted pectin. MC of MAE pectin and AP sample were not significantly different. Furthermore, MC of MAE pectin was higher than that of CE pectin, with an opposite trend than that reported for MC of lime peel pectin (Rodsamran & Sothornvit, 2019). Despite their high equivalent weight, enzymatic treatment did not lead to a high MC in the EAE1, EAE2 and EAU pectin, while the combined UAEH led to a decrease in this parameter similar to that observed for GalA content. All MC values reported in this study were comparable to others reported for the MC of pectin extracted from apple pomace from other varieties (Kumar & Chauhan, 2010; Virk & Sogi, 2004). Since MC was below 7% for all samples, the pectin extracted from *Malus domestica* 'Fălticeni' apple pomace was of low ester characteristic (Yapo & Koffi, 2013) and was considered as being "desirable" in terms of quality. In general, pectis with low MC form a thermo-irreversible gel, which means that it will stay gelled even when heated to temperatures that would normally melt it (Fakayode & Abobi, 2018).

3.6. Degree of esterification and pectin structure

Another parameter with significant influence on pectin quality and applications that presented variations in function of the extracted technique used to obtain pectin was the degree of esterification (DE). As shown in Table 2, the use of citric acid for the conventional method (CE) of pectin extraction determined a DE + similar to that of commercial AP , and significantly

higher than the DE of commercial CP. Numerous authors reported that microwave extraction (Rodsamran & Sothornvit, 2019; Bagherian et al., 2011; Fishman, Chau, Hoagland, & Hotchkiss, 2006) produces higher DE of the extracted pectin by comparison to a conventional extraction, however, that was not the case of our study probably because of the lower microwave power and shorter extraction time (560 W, 120 s). Likewise, ultrasound treatment (UAE and UAEH) application resulted in a pectin with lower DE than that found by using the conventional method, which is in accordance with previous studies (Guandalini et al., 2018; Wang et al., 2015; Bagherian et al., 2011). Of all extraction techniques, the ones based on the use of enzymes and enzymatic preparations (EAE1, EAE2 and EAU) led to the most significant differences in the DE of pectin. As seen in Table 2, EAE2 and EAU samples can be classified as low methoxyl pectins because the DE was below 50% (Giacomazza, Bulone, San Biagio, Marino, & Lapasin, 2018). In the case of the enzymatic extraction with Celluclast 1.5L (EAE2), the enzyme dose showed a major influence on the methylation and acetylation degree of the extracted apple pectin (Wikiera, Mika, Starzyńska-Janiszewska, & Stodolak, 2015). With the exception of enzymatic extraction techniques, the DE of pectin samples were higher than the values reported for pectin extracted from other apple varieties (Kumar & Chauhan, 2010).

Fourier transform infrared spectroscopy, which is a fast and convenient method for the investigation of functional groups of polysaccharides (Zouambia, Youcef Ettoumi, Krea, & Moulai-Mostefa, 2017), was used in this study as a mean to identify differences in pectin structure. The FT-IR spectra presented in Fig. 1 showed that all pectin samples obtained by different extraction methods had a similar transmission pattern to those of commercial CP and AP samples. Pectin samples had characteristic chemical shifts at 3330, 2930 and 1145 cm^{-1} (Fig. 1a), which were attributed to inter- and intramolecular hydrogen stretching of O–H, C–H, CH_2

and CH_3, and C–O–C of glycoside compounds (Hosseini et al., 2019; Wang et al., 2015). The absorption bands around 1730 cm^{-1} and 1630-1610 cm^{-1} were common for all pectin samples and corresponded to stretching vibration of ester carbonyl (C=O) and carboxylate ion stretching (free carboxyl groups), respectively (Alba, Laws, & Kontogiorgos, 2015). Theincreasing trend in the intensities and the band area of esterified carboxyl groups indicated an increased DE (Rodsamran & Sothornvit, 2019; Begum, Yusof, Aziz, & Uddin, 2017), as observed for AP, CE, MAE, UAE and UAEH samples.

Another important region for the structure analysis of pectin samples by FT-IR was identified between 1200 and 950 cm^{-1}. In this region, the high absorbencies were collectively referred to as the 'finger print' region of carbohydrates because the position and intensity of the bands are unique to a compound, allowing the identification of the major chemical groups (Urias-Orona et al., 2010; Černá et al., 2003). The absorption band at 1225 cm^{-1} was from the cyclic C–C bond in the ring structure of pectin, while the characteristic bands between 1120 and 990 cm^{-1} were considered the range for the spectral identification of GalA in pectic polysaccharides (Acikgoz, 2011). For all pectin samples, the major peak at 1015 cm^{-1} was referred to the presence of pyranose in pectin molecule (Wang et al., 2015).

3.7. Rheological properties

3.7.1. Flow behavior of pectin solutions

Fig. 2 shows the flow curves of the pectin solutions, of which two were commercial pectin and seven were pectin extracted from *Malus domestica* 'Fălticeni' apple pomace using different methods. There was observed a non-Newtonian fluid behavior with a decrease of the dynamic viscosity with the increase in the shear stress applied ($n < 1$, as shown in Table 3). This shear-

thinning behavior was attributed to the weakness of the pectin intermolecular forces during the increase of the shear rate (Rodsamran & Sothornvit, 2019; Lewandowska, Dąbrowska, & Kaczmarek, 2012). The dynamic viscosity observed in this study at a shear rate of 1 s^{-1} was higher than the dynamic viscosity measured in the same conditions for solutions prepared with apple pectin (0.39 Pa·s), citrus pectin (1.35 Pa·s) and gabrioba pectin (approx. 0.2 Pa·s) (Barbieri et al., 2019). Vriesmann & Petkowicz (2013) measured the dynamic viscosity for 5% solutions of pectin from cacao pod husks and obtained a value lower than 0.2 Pa·s.

According to the Spearman correlation there was a positive correlation between dynamic viscosity and GalA content (r = 0.644*). However, if the pectin obtained from combined methods (UAEH and EAU) was not considered in the statistical análisis, the correlation observed between the dynamic viscosity and galacturonic acid was more significant (r = 0.937**). Hua, Wang, Yang, Kang, & Yang (2015) argued that the source of pectin and the extraction procedure influences the viscosity of the solutions obtained because a high methoxyl content means a small number of molecules and a greater distance between molecules, resulting in low viscosity of pectin solution; this observation was not confirmed by this study because there was no correlation between the MC and dynamic viscosity.

3.7.2. Viscoelastic properties of pectin solutions

Fig. 3 presents the viscoelastic properties (elastic modulus and loss modulus) of the pectin solutions in the linear region. The elastic modulus (*G*') is the in-phase component of stress with an oscillating strain, and the loss modulus (*G*'') is the out-of-phase (viscous) component of stress that is a measure of the energy lost through viscous flow (Padmanabhan, Kim, Pak, & Sim, 2003). It was observed that, as expected, loss modulus (Fig. 3b) followed the same trend that was

433 reported for dynamic viscosity (Fig. 2). As expected, the pectin solutions had a higher loss
434 modulus than elastic modulus in the frequency domain applied, behavior that was similar to that
435 of solutions of gabiroba pectin (Barbieri et al., 2019) and lime peel pectin (Rodsamran &
436 Sothornvit, 2019). According to the Spearman correlation there was a positive correlation
437 between GalA content and elastic modulus (r = 0.594*) and loss modulus (r = 0.595*), however,
438 if the pectin samples obtained from combined extraction techniques (UAEH and EAU) were
439 excluded the correlation observed between these two parameters was more significant (r =
440 0.883** and r = 0.884**, respectively). The extraction methods had a significant effect on the
441 rheological characteristics of pectin and of all the analyzed samples the ones extracted by
442 ultrasound (UAE) and microwave (MAE) treatment were considered suitable for use in various
443 food products as high-quality thickener or stabilizer.
444 The results of the creep and recovery analysis of pectin solutions are presented in Fig. 4. The
445 creep phase ranged from 0 to 180 s and the recovery phase from 180 s to 360 s. The creep and
446 recovery analysis parameters are shown in Table 3. As the data shows, the equilibrium
447 compliance J_e was higher in the case of EAE1 pectin, while the smallest value was determined
448 for MAE pectin. In the same way, the total recoverable deformation J_r, which is a measurement
449 of the material elasticity i.e. the mechanical energy stored in the sample during the creep phase
450 (Franck, 2005), was higher in the case of EAE1 pectin, while very low in the case of MAE
451 pectin. The shear stress was the highest in the case of EAU pectin and the lowest in the case of
452 UAE pectin and the same evolution was also observed for $d(\log(\dot{\gamma}))/d(\log(t))$. The GalA content
453 was negatively correlated with J_e (r = -0.628*), J_r (r = -0.728*) and $d(\log(\dot{\gamma}))/d(\log(t))$ (r = -
454 0.697*) and positively correlated with η (r = 0.594*), respectively. The creep and recovery

parameters had no correlation with the Eq.W, DE and MC. The viscosity measured with creep and recovery and the dynamic viscosity were positively correlated (r = 0.917**).

3.8. Thermal properties

The influence of the extraction method on the thermal behavior of pectin extracted from *Malus domestica* 'Fălticeni' apple pomace was examined by DSC between 0 °C and 300 °C. This analysis also served as a mean to compare the thermal properties of the extracted pectin samples to those of commercial pectin. As shown in the thermograms presented in Fig. 5, for all pectin samples no endothermic peaks (melting temperature) were observed, while exothermic peaks (degradation temperature) were recorded at temperatures between 230 and 255 °C. Previous studies (Priyangini, Walde, & Chidambaram, 2018; Einhorn-Stoll & Kunzek, 2009; Wang et al., 2016) argued that endothermic peaks result from water evaporation, hydrogen bonding among GalA units, and also a conformational change of the galacturonan ring i.e. the transformation from the more stable $^{4}C_{1}$ chair conformation to the $^{1}C_{4}$ reverse-chair conformation. The lack of an endothermic peak in the case of our study suggests that no water was present in the pectin samples.

Commercial AP and CP samples had exothermic peaks at 255 °C and 243 °C, respectively, and these values were similar to others found in the scientific literature for the same source materials (Wang, Chen, & Lü, 2014; Wang & Lü, 2014). For pectin samples extracted by different methods, the exothermic peaks appeared, as follows: CE – 251 °C, MAE – 248 °C, UAE – 240 °C, EAE1 – 236 °C, EAE2 – 230 °C, UAEH – 249 °C and EAU – 234 °C. As it can be deduced, the pectin samples extracted using enzymes suffered degradation in the heat processing at temperatures below that determined for AP. On the other side, pectin obtained by CE, UAEH

and MAE showed higher thermal stability than the commercial pectin, which indicates that these samples might be preferred during thermal processing. UAE pectin, which showed thermal stability comparable to that reported for CP, was the only sample with a sharper exothermic peak, which shows that this pectin has narrower degradation range, a more concentrated molecular weight distribution and ordered molecular structure (Jiang, Du, Zhang, & Li, 2018).

3.9. Microstructural analysis by SEM

SEM analysis was carried out to observe the effect of the extraction method on the morphological characteristics of pectin and to compare the physical structure of these samples with that of commercial pectin. As shown in Fig. 6, commercial AP and CP samples have less even surfaces and partly look like being built from layers, a feature that was previously described for commercial high-methoxylated pectin (Einhorn-Stoll, 2018). AP sample differed from CP through its more pronounced fragmentation and the tendency to curl. Pectin from CE had a homogenous and porous surface and was smoother by comparison to the surface of MAE pectin which appeared very rough and slightly ruptured. For MAE the morphology seemed to be influenced by the quick temperature increase and high internal pressure associated with this extraction method (Kazemi, Khodaiyan, & Hosseini, 2019b; Liew et al., 2016). UAE pectin structure was similar to MAE, but more fragmented and closely packed; similar morphological characteristics were described for potato pectin extracted by combined ultrasound-microwave assisted acid extraction (Yang et al., 2019). The combination between ultrasound and heating seemed to determine a similar fragmented, but smoother surface of UAEH pectin that also had larger size particle distribution. EAE1 and EAE2 pectin samples were both characterized by homogenous particle size distribution and fragmented structure, while EAU differed from the

other samples in terms of size because it had the smoothest structure with a tendency to curl easily. A similar structure was obtained for enzymatic demethoxylated LMP (low-methoxylated pectin) (Einhorn-Stoll, 2018), which is expected since we consider that EU pectin had DE<50%.

3.10. Principal component analysis

Principal component analysis (PCA) of the experimental data served as a mean to emphasize the relationship between pectin samples and the most significant physicochemical properties. As shown in Fig. 7, the first and second principal component explained the greater part of the variability, with a cumulative variance contribution of 99% (PC-1: 96%, PC-2: 4%). A significant influence on the distinction between pectin samples was displayed by Eq.W, GalA content, DE, h^*_{ab} and L* value. Commercial CP, together with EAE1, EAE2, EAU and MAE pectin were correlated with Eq.W; the fact that the two pectin samples extracted by enzymatic treatment and the one extracted by combined enzymatic-ultrasound treatment appear more to the right and close to each other was due to their high Eq.W. Commercial AP, CE and UAEH pectin samples were correlated to the GalA content, DE, L* value and hue (h^*_{ab}). The placement of UAE pectin in the upper left corner was due to its higher G' and G'' values. Parameters such as J_r, MC, and chroma (C^*_{ab}) showed little variation between pectin samples and therefore had no significant contribution to the correlation between the extraction technique and the characteristics of the extracted pectin.

4. Conclusions

The pomace resulted from processing *Malus domestica* 'Fălticeni' apples was found to be a valuable source of pectin with high GalA content and DE. A compressive comparison between

different methods of extraction applied to obtain pectin from this plant material was made with the purpose of observing the changes in terms of yield and physicochemical properties. The use of MAE led to a high extraction yield, obtaining pectin samples that were very similar in terms of color to commercial pectin samples and that were characterized by high GalA content, Eq.W and DE, with a MC very close to that of commercial apple pectin. Ultrasound treatment allowed for the obtention of pectin samples (UAE) with high GalA content and DE and high apparent viscosity. Considering their global physicochemical properties, MAE and UAE pectin samples can be used in various food productions as high-quality thickeners or stabilizers. Enzymatic extraction yielded pectin samples that were darker in color, with high Eq.W but decreased DE and lower thermal stability as compared to the commercial pectin. The combined UAEH and EAU techniques did not lead to significant improvements in the overall physicochemical characteristics of the extracted pectin.

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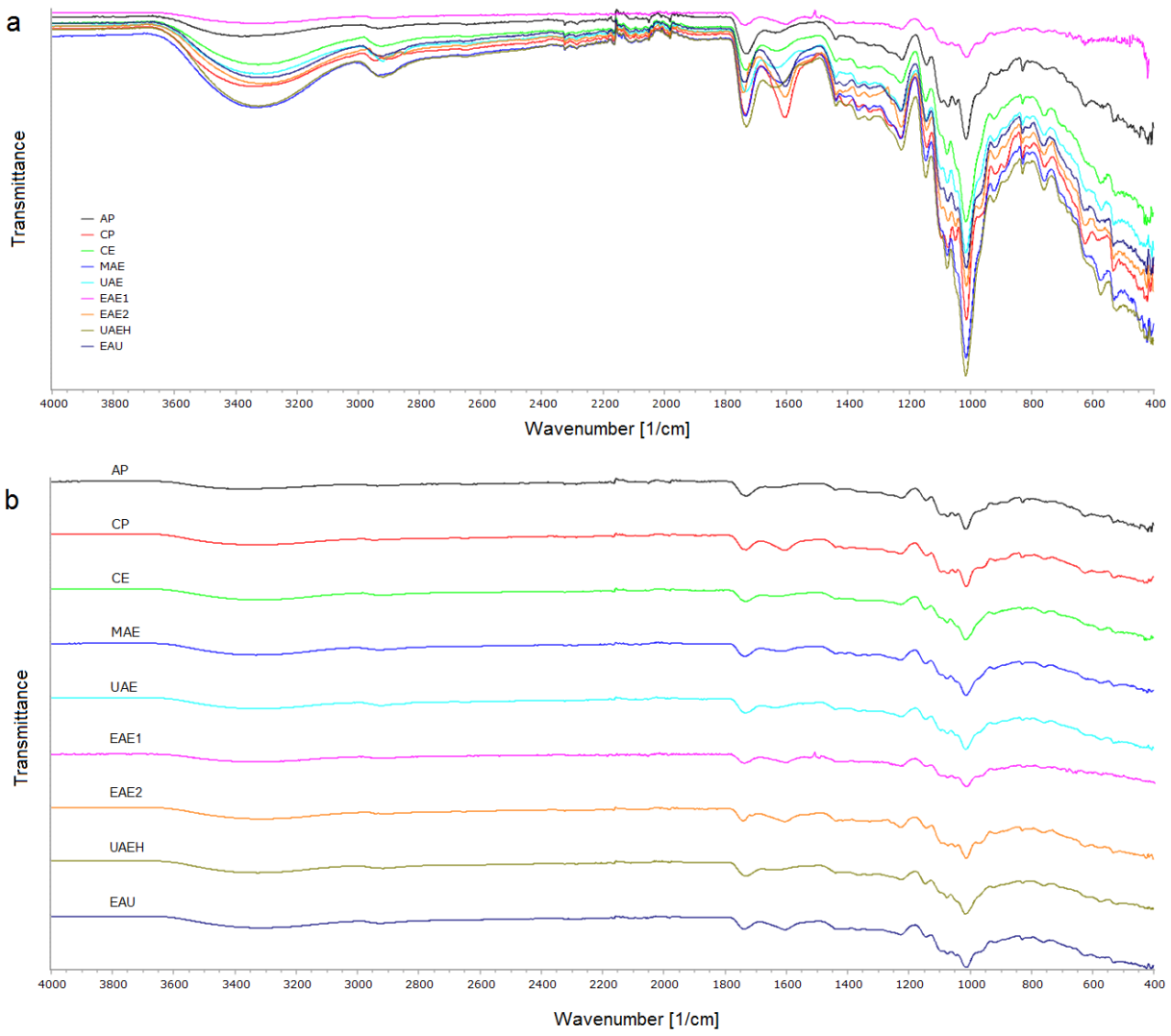


Fig. 1. FT-IR spectra of commercial pectins and pectin samples extracted from *Malus domestica* 'Fălticeni' apple pomace: (a) spectra of all samples, (b) stacked spectra for a better vision of specific wavenumbers.

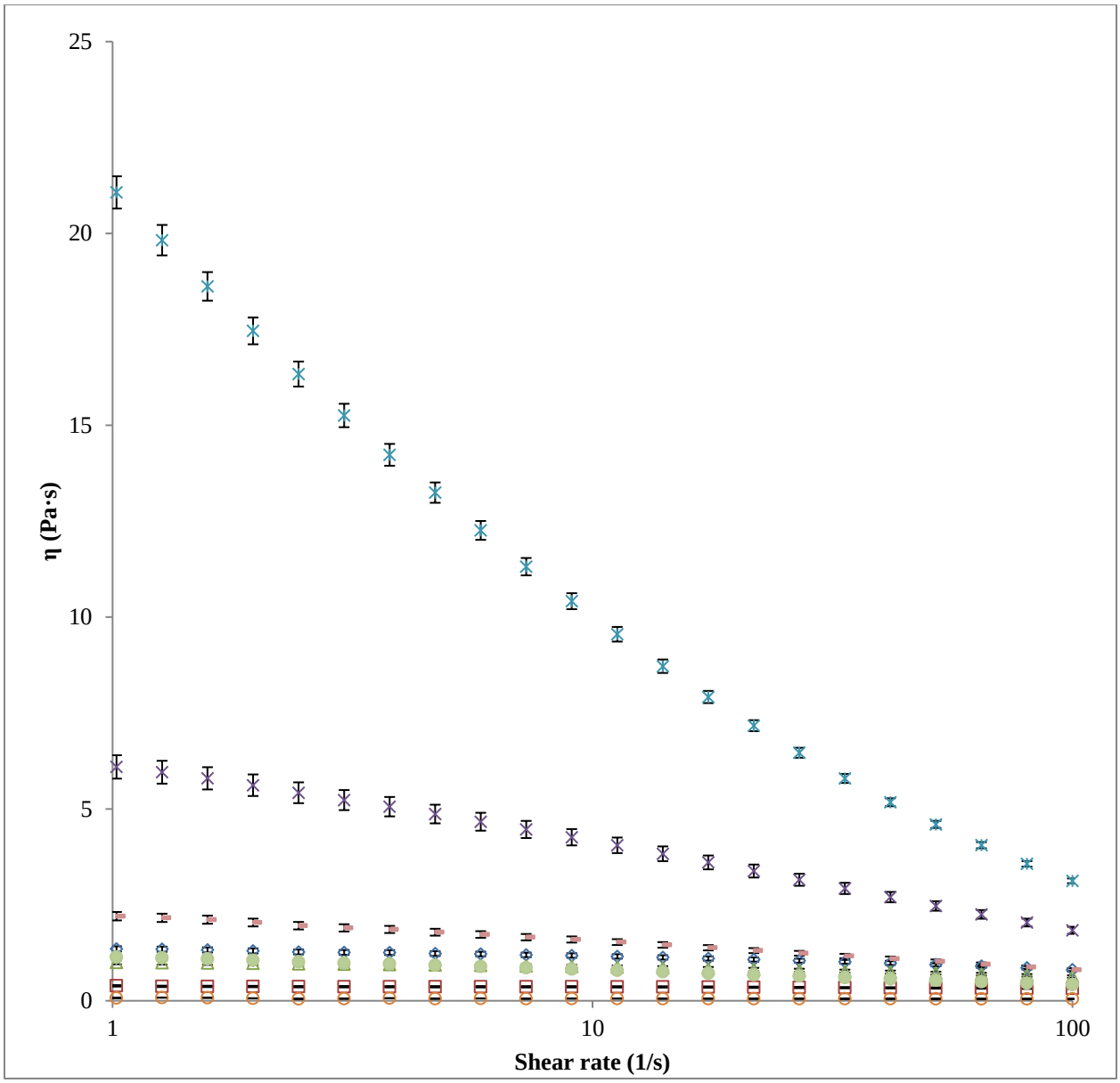


Fig. 2. Flow curves of pectin solutions: CP (◇), AP (□), CE (△), MAE (×), UAE (✳), EAE1 (○), EAE2 (+), UAEH (▪) and EAU (●).

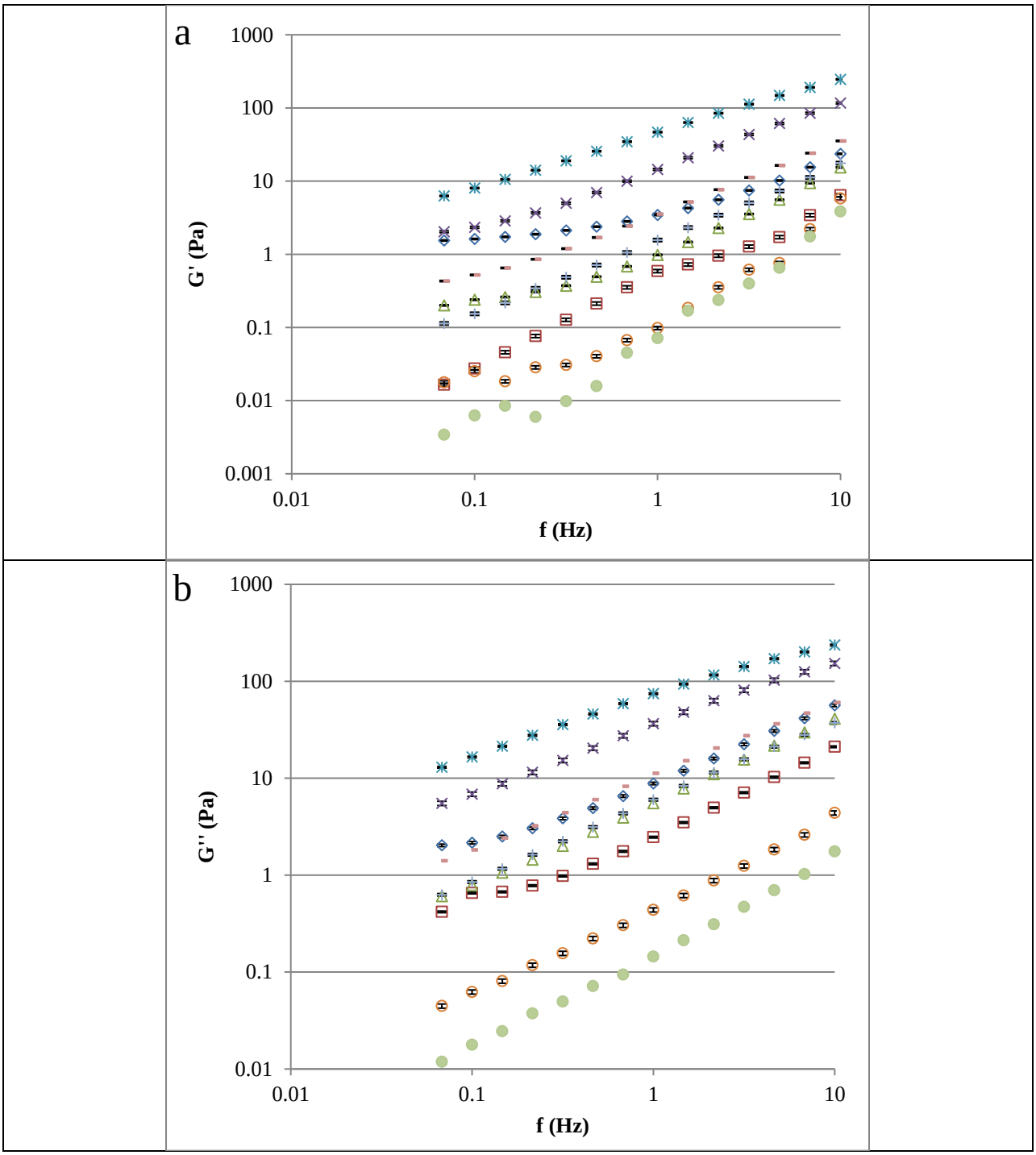


Fig. 3. Elastic modulus (a) and loss modulus (b) for different pectin solutions: CP (◇), AP (□), CE (△), MAE (×), UAE (✳), EAE1 (○), EAE2 (+), UAEH (-) and EAU (●).

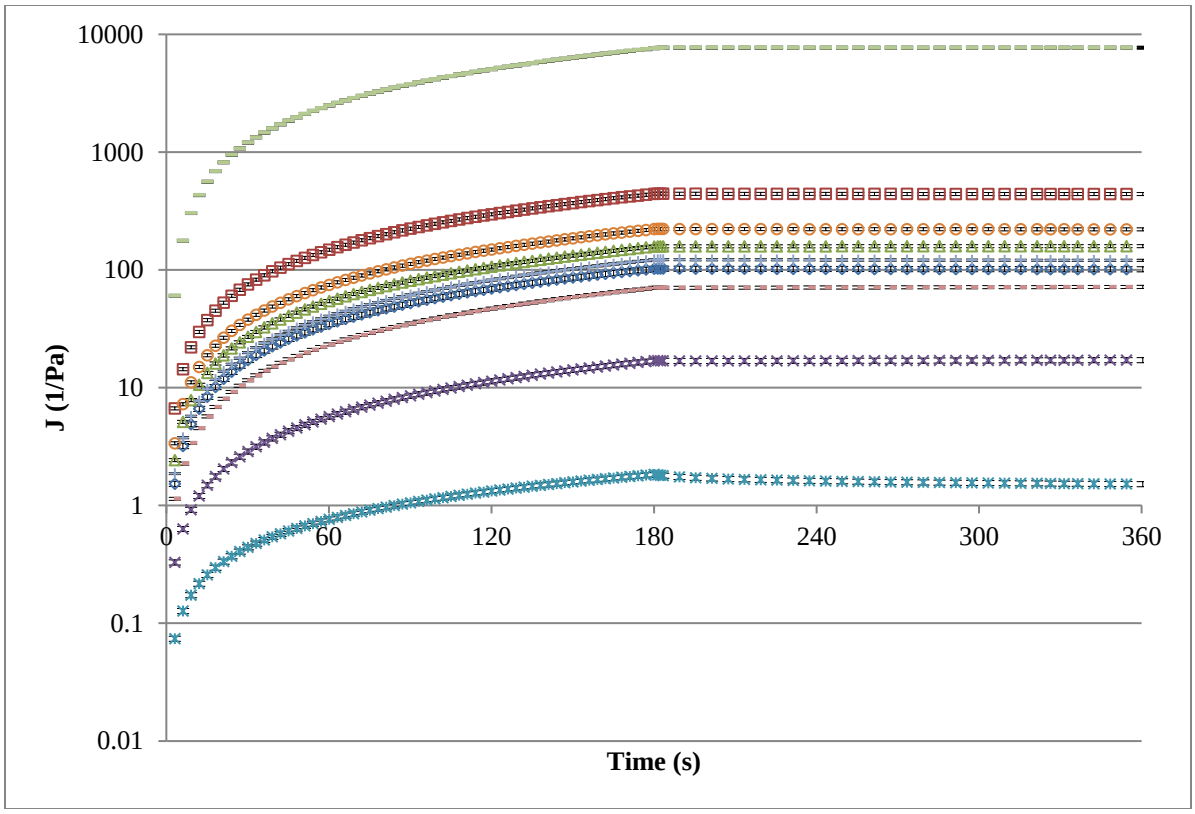
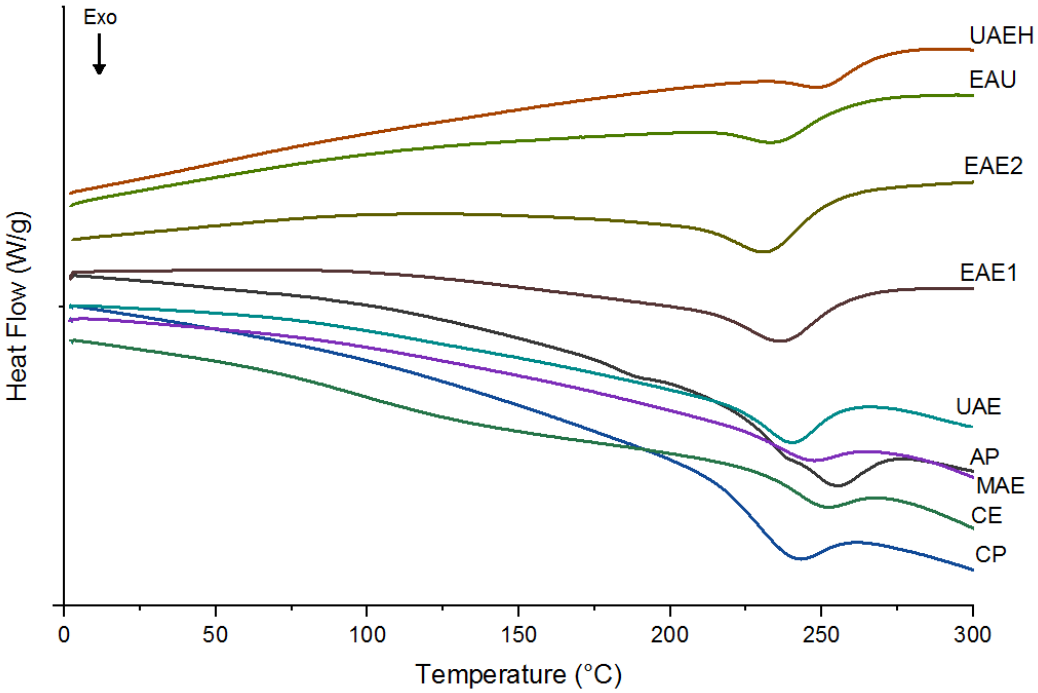


Fig. 4. Creep and recovery test of pectin solutions: CP (◇), AP (□), CE (△), MAE (×), UAE (✳), EAE1 (○), EAE2 (+), UAEH (▪) and EAU (●).



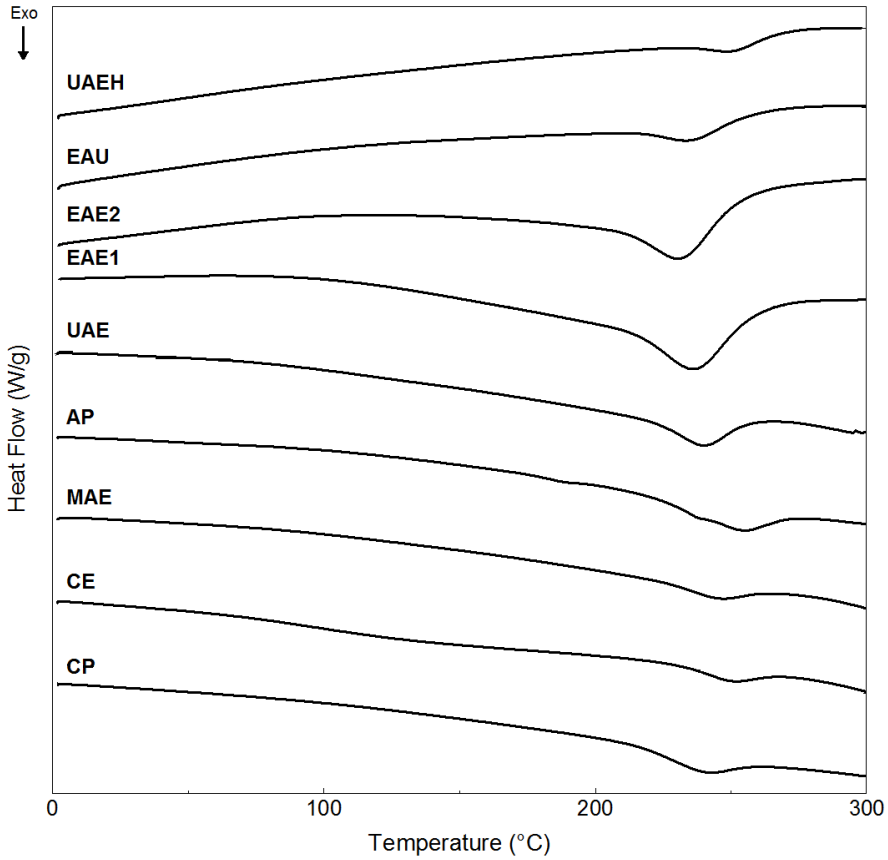


Fig. 5 DSC thermograms of commercial apple and citrus pectin and pectin samples extracted from *Malus domestica* 'Fălticeni' apple pomace.

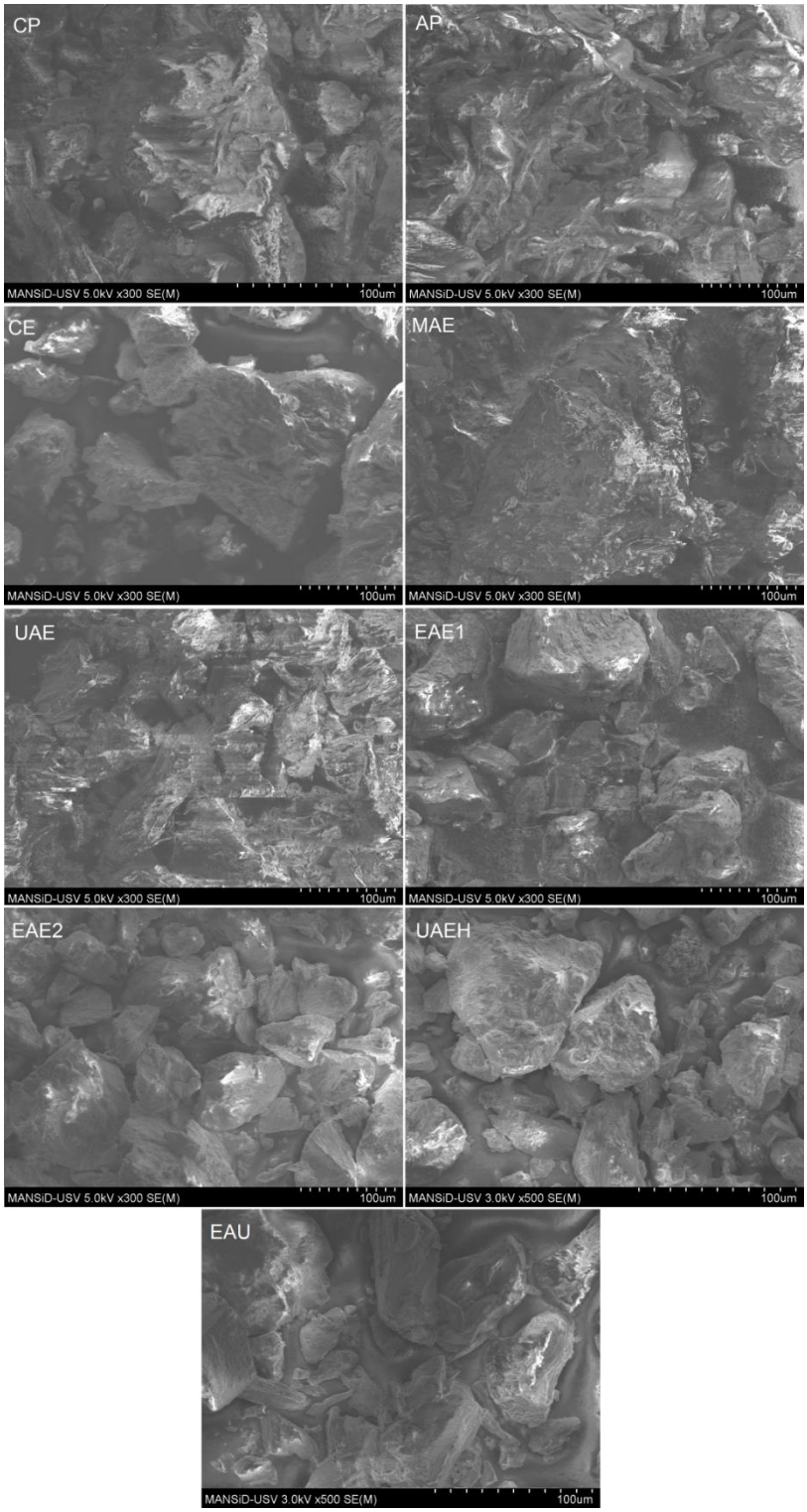


Fig. 6. SEM images of commercial pectins and pectin samples extracted from *Malus domestica* 'Fălticeni' apple pomace; 5 kV, 300× magnification

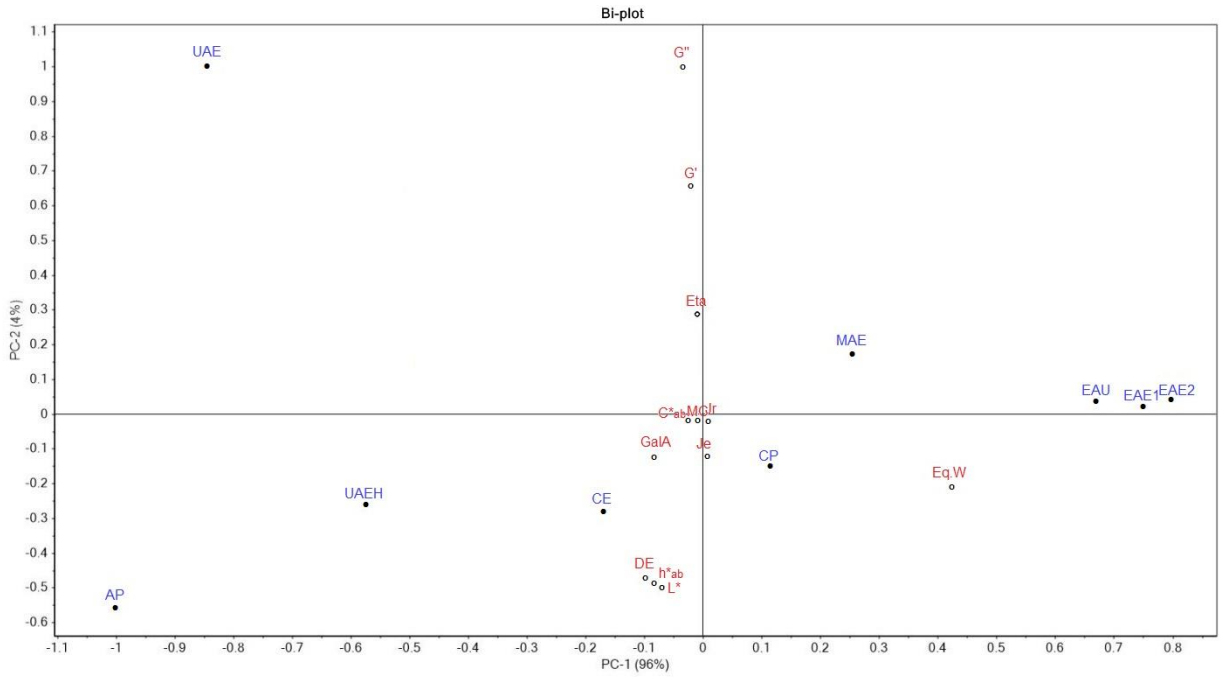


Fig. 7. PCA biplot showing the correlation between scores (pectin samples, closed symbol ●) and loadings (physicochemical properties, open symbol ○)

Table 1. Maximum yield obtained in each one of the pectin extraction techniques. Mean values and standard deviation, in brackets.

Extraction technique	Abbreviation	Maximum yield (%)
Conventional citric acid extraction	CE	23.262 (0.013)[a]
Microwave-assisted extraction	MAE	23.32 (0.08)[a]
Ultrasound-assisted extraction	UAE	9.183 (0.018)[b]
Enzyme-assisted extraction with cellulase	EAE1	7.174 (0.013)[d]
Enzyme-assisted extraction with Celluclast 1.5L	EAE2	6.76 (0.03)[e]
Ultrasound-assisted extraction – heating treatment	UAEH	6.86 (0.06)[e]
Enzyme-assisted extraction – ultrasound treatment	EAU	7.95 (0.04)[c]

[a-e] Different letters in the same column indicate significant differences among samples ($p < 0.001$)

Table 2. Color triestimulus coordinates (L*, h^*_{ab}, C^*_{ab}) galacturonic acid content (GalA), equivalent weight (Eq.W), methoxyl content (MC), and degree of esterification (DE) of pectin samples. Mean values and standard deviation, in brackets.

Sample	L*	h^*_{ab}	C^*_{ab}	GalA (g/100 g)	Eq.W	MC (%)	DE (%)
CP	80.93 (0.06)^{a}	96.55 (0.07)^{a}	17.29 (0.09)^{e}	85.5 (0.5)^{b}	1190 (20)^{c}	4.03 (0.06)^{bc}	50.5 (0.4)^{d}
AP	80.453 (0.005)^{ab}	89.95 (0.03)^{b}	23.35 (0.04)^{d}	81.4 (0.2)^{bc}	515 (5)^{e}	4.8 (0.2)^{a}	88.5 (1.5)^{a}
CE	78.18 (0.06)^{b}	89.19 (0.07)^{b}	21.62 (0.05)^{d}	86.5 (0.2)^{b}	961 (9)^{c}	3.04 (0.16)^{d}	84.4 (1.9)^{a}
MAE	77.19 (0.04)^{b}	89.26 (0.02)^{b}	24.17 (0.06)^{cd}	90.6 (0.5)^{a}	1612 (6)^{b}	4.77 (0.12)^{a}	73.8 (0.9)^{b}
UAE	65.11 (0.04)^{e}	80.47 (0.09)^{d}	29.71 (0.02)^{a}	92.83 (0.09)^{a}	704 (5)^{d}	4.22 (0.06)^{b}	77 (2)^{b}
EAE1	66.896 (0.011)^{de}	82.440 (0.07)^{c}	27.08 (0.05)^{bc}	78.8 (0.6)^{c}	2778 (70)^{a}	3.84 (0.12)^{c}	53.5 (0.8)^{c}
EAE2	60.99 (0.07)^{f}	78.13 (0.07)^{e}	26.2 (0.3)^{c}	85.2 (0.4)^{b}	2632 (44)^{a}	4.15 (0.12)^{b}	44.6 (0.5)^{f}
UAEH	68.106 (0.110)^{d}	82.346 (0.110)^{c}	26.58 (0.07)^{c}	69.1 (0.5)^{d}	641 (15)^{d}	3.162 (0.107)^{d}	80.7 (0.3)^{b}
EAU	64.05 (0.04)^{e}	80.183 (0.102)^{de}	28.103 (0.015)^{b}	75.5 (0.2)^{c}	2500 (33)^{a}	3.906 (0.104)^{c}	47.6 (0.4)^{e}
F-value	56433.37***	19829.88***	4124.00***	1021.16***	2586.42***	68.21***	669.79***

CP – commercial citrus pectin, AP – commercial apple pectin

$^{a-f}$ Different letters in the same column indicate significant differences among samples ($p < 0.001$)

Table 3. Power Law model parameters and creep and recovery parameters for pectin solutions. Mean values and standard deviation, in brackets.

Sample	n	k (Pa·s)	η (1 s^{-1}) (Pa·s)	J_e (1/Pa)	J_r(1/Pa)	$\dot{\gamma}$ (1/s)	η (mPa·s)	d(log($\dot{\gamma}$))/d(log(t)) (1/s)
CP	0.831 (0.009)^{c}	1798 (142)^{d}	1.35 (0.06)^{d}	4.4 (0.4)^{d}	1.37 (0.17)^{c}	0.543 (0.010)^{d}	1842 (36)^{c}	0.952 (0.005)^{d}
AP	0.930 (0.001)^{b}	447 (2)^{f}	0.389 (0.001)^{f}	12 (3.)^{c}	2.882 (0.108)^{c}	2.39 (0.05)^{c}	419 (9)^{c}	0.972 (0.007)^{bc}
CE	0.837 (0.005)^{c}	1359 (61)^{e}	0.989 (0.016)^{e}	1.56 (0.04)^{d}	0.517 (0.012)^{c}	0.869 (0.002)^{de}	1152 (29)^{c}	0.989 (0.006)^{a}
MAE	0.655 (0.001)^{f}	9375 (202)^{b}	6.07 (0.03)^{b}	0.224 (0.012)^{d}	0.191 (0.012)^{c}	0.503 (0.002)^{e}	10770 (268)^{b}	0.986 (0.002)^{ab}
UAE	0.536 (0.002)^{g}	28170 (480)^{a}	21.18 (0.16)^{a}	0.35 (0.03)^{d}	0.32 (0.05)^{c}	0.008 (0.001)^{e}	53100 (644)^{a}	0.744 (0.020)^{e}
EAE1	0.773 (0.003)^{d}	1481 (43)^{de}	0.073 (0.001)^{g}	26 (2)^{b}	32 (2)^{b}	14.8 (0.4)^{b}	67.6 (1.6)^{c}	0.989 (0.002)^{ab}
EAE2	0.744 (0.029)^{e}	91 (2)^{fg}	1.05 (0.05)^{e}	4.6 (0.7)^{d}	1.68 (0.09)^{c}	0.652 (0.012)^{de}	1533 (28)^{c}	0.960 (0.006)^{cd}
UAEH	0.716 (0.001)^{e}	3054 (10)^{c}	2.18 (0.05)^{c}	0.488 (0.016)^{d}	1.140 (0.004)^{c}	0.388 (0.003)^{de}	2574 (10)^{c}	0.992 (0.002)^{a}
EAU	0.963 (0.002)^{a}	23 (0.1)^{g}	1.08 (0.09)^{e}	36.7 (0.5)^{a}	46 (6)^{a}	42.5 (0.8)^{a}	23.5 (0.6)^{c}	1.005 (0.001)^{a}
F-value	304**	4837***	18332***	38.4***	28.3***	12345***	298***	70.5***

$^{a-g}$ Different letters in the same column indicate significant differences among samples ($p < 0.001$)