Structural and emulsion stabilizing properties of pectin rich extracts obtained from different botanical sources

Neckebroeck, B.[a*], Verkempinck, S.H.E.[a], Van Audenhove, J.[a], Bernaerts, T.[a], de Wilde d'Estmael, H.[a], Hendrickx, M.E.[a], Van Loey, A.M.[a]**

[a] Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LFoRCe), Department of Microbial and Molecular Systems (M2S), KU Leuven, Kasteelpark Arenberg 22, PB 2457, 3001, Leuven, Belgium

* author to whom correspondence should be addressed during submission process:

bram.neckebroeck@kuleuven.be

+32 16 32 96 52

** author to whom correspondence should be addressed post-publication:

ann.vanloey@kuleuven.be

+32 16 32 15 67

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Abbreviations

AIR: Alcohol Insoluble Residue
AP: Apple pectin
CP: Carrot pectin
E_FE_S: Emulsifying and emulsion stabilizing
OP: Onion pectin
pSO: Purified sunflower oil
SPOS: Single Particle Optical Sizing
TP: Tomato pectin

Abstract

The presented research studied the emulsifying and emulsion stabilizing capacity of pectin samples isolated from different plant origin: apple, carrot, onion and tomato. The acid extracted pectin samples showed distinct structural properties. Specifically, apple pectin showed a high degree of methylesterification (78.41 ± 0.83%), carrot pectin had the lowest concentration of other co-eluted cell wall polymers, onion pectin displayed a bimodal molar mass distribution suggesting two polymer fractions with different molar mass and tomato pectin was characterized by a high protein content (16.48 ± 0.05%). The evaluation of the emulsifying and emulsion stabilizing potential of the pectin samples included investigating their ability to lower the interfacial tension next to a storage stability study of pectin stabilized o/w emulsions. Creaming behavior as well as the evolution of the oil droplet size were thoroughly examined during storage using multiple analytical techniques. Overall, smaller oil droplet sizes were obtained at pH 2.5 compared to pH 6.0 indicating better emulsifying capacity at lower pH. The lowest emulsion stability was observed in emulsions formulated with tomato pectin in which weak flocculation and relatively fast creaming affected emulsion stability. Onion pectin clearly showed the most promising emulsifying and emulsion stabilizing potential. At both pH conditions, emulsions stabilized by the onion pectin sample displayed highly stable oil droplet sizes during the whole storage period. The presence of the two polymer fractions in this sample can play an important role in the observed stability. In future work, it could be evaluated if both fractions contribute to emulsion stability in a synergistic way. In conclusion, this work showed that pectin samples extracted from different plant origin display diverse structural properties resulting in varying emulsifying and emulsion stabilizing potential. Polymer molar mass potentially plays a major role in the structure-function relation.

Keywords: Pectin, Acid extraction, Molecular structure, Emulsifier, Emulsion stabilizer, Storage stability

1 Introduction

Fresh fruits and vegetables as well as side streams of the fruit and vegetable processing industry rich in cell wall material contain pectin. Pectin is defined as a group of polysaccharides rich in galacturonic acid (GalA) that are present in the cell wall of higher plants and most abundantly in the middle lamella. The function of pectin in the plant cell wall is primarily linked to cell wall integrity and cell adhesion but it can play a role in signal transduction as well (Caffall & Mohnen, 2009; Voragen, Coenen, Verhoef, & Schols, 2009). Besides its biological importance and structure engineering potential in plant tissues, extracted pectin is of great interest to food researchers and manufacturers. Today, pectin is used both in home kitchens and on an industrial scale to manufacture jams and jellies and furthermore to industrially produce confectionary products and bakery fillings because of pectin's gelling and texture modifying properties (Voragen et al., 2009; Willats, Knox, & Mikkelsen, 2006).

Pectin is considered one of the most complex natural macromolecules as it consists out of multiple structurally different pectin domains that contain all together up to 17 different monosaccharides (Voragen et al., 2009). Moreover, different structural properties can be obtained using different extraction methods and furthermore, pectin extracts originating from different botanical sources can vary in structure as well. Additionally, it was found that pectin structure even changes during plant development processes such as fruit ripening (Alba & Kontogiorgos, 2017; Caffall & Mohnen, 2009; Chen et al., 2015; Müller-Maatsch et al., 2016; Willats et al., 2006). As pectin functionality largely depends on the structural composition and organization of the polymer chains, it is clear that the structural complexity of pectin creates challenges to obtain the desired functionality (Alba & Kontogiorgos, 2017; Voragen et al., 2009; Willats et al., 2006). Further understanding of this structure-function relationship, is what triggers researchers studying pectin and pectin functionality.

Besides the use of pectin as gelling or structure modifying agent, the potential of pectin as emulsifying and emulsion stabilizing (E_FE_S) agent was explored in the last three decades, as recently reviewed by Alba and Kontogiorgos (2017) and Ngouémazong, Christiaens, Shpigelman, Van Loey and Hendrickx (2015). Most research on this E_FE_S functionality however, focusses on the familiar and widely used citrus pectin and on sugar beet pectin which shows good E_FE_S potential (Alba & Kontogiorgos, 2017; Funami et al., 2007; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Verkempinck et al., 2018).

The viscosity increasing capacity of pectin is often suggested as an important stability mechanism of o/w emulsions formulated with pectin samples (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002; Ngouémazong et al., 2015; Williams et al., 2005). However, it has been evidenced before that pectin can adsorb at the oil-water interface as well. Subsequently, both steric and electrostatic interactions can reduce oil droplet coalescence contributing to long term emulsion stability. Many structural characteristics of pectin polymers were suggested to influence its E_FE_S capacity. Some structural characteristics increase the amphiphilic character of the pectin chains such as a higher degree of methylesterification (DM) and/or a higher degree of acetylation (Leroux et al., 2003; Schmidt et al., 2015; Schmidt, Schütz, & Schuchmann, 2017; Verkempinck et al., 2018), and the presence of ferulic acid in sugar beet pectin (Chen et al., 2016). Also pectin related proteinaceous moieties are often suggested to improve the E_FE_S capacity as proteins can improve the adsorption of the overall pectin structure at the oil-water interface (Akhtar et al., 2002; Chen et al., 2016; Funami et al., 2007, 2011; Leroux et al., 2003; Williams et al., 2005). Lastly, many studies mentioned the influence of the pectin molar mass (MM) but the results so far were rather inconsistent. The pectin MM may influence adsorption kinetics (Leroux et al., 2003; Schmidt et al., 2015), accessibility of surface active groups (Akhtar et al., 2002; Schmidt et al., 2015) and viscosity changes in the aqueous continuous phase (Alba &

Kontogiorgos, 2017). Moreover, Zhao et al. (2020) indicated that lower molar mass pectin polymers obtained after hydrolysis can display superior interfacial activity compared to the original non-hydrolyzed sample while stabilizing 5% o/w emulsions to a lower extent.

Scientific studies investigating structural and/or functional properties of extracted pectin often use various botanical sources. Regarding the E_FE_S potential of pectin, the majority of studies focused on citrus pectin and sugar beet pectin. Although often structurally characterized, pectin extracted from other plant materials is only to a limited extent meticulously studied for its E_FE_S potential. Therefore, the presented research aimed to investigate the E_FE_S potential of four pectin rich samples extracted from four different raw plant materials: apple, carrot, onion and tomato. Literature on pectin research indicates differences in several structural properties between pectin samples obtained from these different sources. Sato et al. (2011) described apple pectin exhibiting high DM values higher than 70%. Tomato pectin has been previously characterized as a highly linear pectin with high molar mass values compared to carrot pectin which displayed more extensive branching and lower molar masses (Houben, Jolie, Fraeye, Van Loey, & Hendrickx, 2011). The degree of branching for apple pectin samples was slightly lower compared to carrot pectin (Houben et al., 2011; Sato et al., 2011). An acid extracted polysaccharide fraction from onion was characterized by a high protein content and a high galactose content (Golovchenko, Khramova, Ovodova, Shashkov, & Ovodov, 2012). Structural differences among the four pectin rich extracts were hypothesized to affect the emulsifying and emulsion stabilizing potential. Therefore, the storage stability of o/w emulsions formulated by the extracted pectin samples was studied at two pH values using a multimethod approach. Evaluating both creaming behavior and oil droplet size evolution during storage provides in-depth insight into the physical stability of these pectin based o/w emulsions. The results of this multimethod

148 research strategy will allow to relate the structure of pectin samples of different plant origin
149 to their $E_F E_S$ capacity.

150 2 Materials and Methods

151 2.1 Materials

152 Batches of apples (*Malus domestica* var. Jonagold), tomatoes (*Solanum lycopersicum* var.
153 San Marzano) and chopped onions (*Allium cepa* L.) were purchased from a local
154 supermarket. Blanched and frozen carrot (*Daucus carota* L. subsp. *sativus*) dices were kindly
155 donated by D'Arta Nv (Ardooie, Belgium) and stored at -40 °C until use. Commercial
156 sunflower oil was purchased at a local supermarket. Ultrapure water (organic free, 18.2 MΩ
157 cm resistance) was supplied by a Simplicity™ 150 water purification system (Millipore,
158 Billerica, USA). Unless mentioned differently, all chemicals used were of analytical grade.

159 2.2 Pectin extraction

160 2.2.1 Isolation of Alcohol Insoluble Residue

161 Prior to the actual AIR production, some starting materials required an extra preparatory step.
162 In case of fresh apples, the core and peel were removed and the apple flesh was chopped. The
163 fresh tomatoes were sliced and instantly blanched (95 °C, 8 min) after purchase (Rodrigo et
164 al., 2006). Hereafter, the blanched tomato slices were mixed in a kitchen blender (2 times for
165 3 s) and subsequently sieved (mesh size 1 mm) to remove seeds and peel fragments. This
166 sieved tomato tissue was used as starting material for AIR production. The chopped onion
167 and carrot dices were used without further preparation.

168 The AIR was produced for all matrices based on the procedure of McFeeters and Armstrong
169 (1984). When starting from chopped apple flesh, onion pieces and sieved tomato tissue, 60 g
170 of wet tissue was suspended in 192 ml 95% ethanol using a high speed mixer (3 times for 6 s)
171 (Buchi mixer B-400, Flawil, Switzerland). After filtration over a filter paper (Machery-Nagel

MN 615 Ø 90 mm) the residue was resuspended in 96 ml 95% ethanol, mixed and filtered again. Finally, the residue after this step was resuspended in 96 ml technical acetone and filtered to obtain the AIR.

Due to the high carotenoid content of carrots, two extra isolation steps were executed in the AIR isolation procedure starting from carrot. Firstly, the carrot dices were grounded using a knife mill (Grindomix GM 200, Retch, Germany), refrozen using liquid nitrogen and lyophilized (Alpha 2–4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). Subsequently, 15 g of this lyophilized carrot material was wetted with 22.5 ml demineralized water, suspended in 192 ml 95% ethanol using a high speed mixer and filtered. Consequently, the residue was resuspended in 96 ml 95% ethanol, mixed and filtered. This step was repeated and the obtained residue was resuspended in 96 ml technical acetone and filtered. This step was repeated a second time as well to obtain the AIR (Neckebroeck et al., 2020).

The obtained AIR fractions of all four matrices were dried overnight at 40 °C, grounded and stored in a desiccator until use.

2.2.2 Nitric acid pectin extraction

After AIR production, an acid extraction was performed to obtain extracts rich in pectin. Starting from the AIR obtained from apple, onion and tomato, acid extractions were performed using nitric acid following the procedure of Willemsen et al. (2017). Briefly, 60 g of AIR was pre-homogenized in 4 L of demineralized water at 80 °C for 30 min after which the pH was adjusted to 1.6 using 7 M HNO_3. Pectin extraction proceeded for 60 min at these conditions under mild stirring. After extraction, the suspension was immediately cooled in an ice bath and centrifuged for 10 min at 4000 g and room temperature. The pectin rich supernatant was separated from the pellet and the pH was instantly brought to pH 6.1.

In case of carrot AIR, a slightly different procedure was followed (Neckebroeck et al., 2020). Briefly, 2.5 g of AIR was homogenized in 200 ml of demineralized water at 80 °C for 30 min after which the pH was adjusted to 1.6 using 7 M HNO_3. Only 30 minutes of pectin extraction was performed after which the pectin rich extract was separated from the residual suspended solids by vacuum filtration after cooling. Finally, the extract rich in pectin was brought to pH 6.1.

All pectin rich extracts were extensively dialyzed against demineralized water for 48 h (Spectra/Por® Dialysis Membrane, 3.5 kDa MWCO), lyophilized (Alpha 2–4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) and stored in a desiccator until use. Due to high polyphenol content and dark reddish color of the apple pectin extract, this extract was vacuum filtered before dialysis. The lyophilized pectin samples extracted from apple, carrot, onion and tomato tissue will be further referred to as AP, CP, OP and TP respectively.

2.3 Pectin structure and structure related properties

2.3.1 Galacturonic acid content

Samples were hydrolyzed using sulfuric acid according to the method described by Ahmed and Labavitch (1978) before measuring the GalA content by the spectrophotometric analysis of Blumenkrantz and Asboe-Hansen (1973). The acid hydrolysis was performed in duplicate for each pectin sample, followed by a triplicate analysis of the GalA content of each hydrolysate (25 °C, wavelength 520 nm, GENESYS^{TM} 30 Visible Spectrophotometer, Thermo Scientific, Massachusetts, USA).

2.3.2 Monosaccharide content

High performance anion exchange chromatography (HPAEC) coupled to pulsed amperometric detection (PAD) was applied to quantify the content of the neutral sugars

fucose, rhamnose, arabinose, galactose, glucose, xylose and mannose in the pectin samples. First, the pectin samples were hydrolyzed in 4 M trifluoroacetic acid at 110 °C for 1.5 h and immediately hereafter cooled in an ice bath. Subsequently, the remaining TFA was neutralized by consecutively drying the samples under N_2 at 45 °C, washing the samples with 1 M ammonium hydroxide and finally drying the samples again under N_2 at 45 °C (De Roeck, Sila, Duvetter, Van Loey, & Hendrickx, 2008; Stolle-Smits, Beekhuizen, Recourt, Voragen, & Van Dijk, 1997). Hydrolyzed samples were redissolved in ultrapure water and filtered (Chromafil® A-45/25, 0.45 µm pore size, Macherey-Nagel, Düren, Germany) before analysis. The hydrolysis was carried out in duplicate.

The HPAEC-PAD analysis was performed exactly as described in Neckebroeck et al. (2020) using a Dionex ICS-6000 system equipped with a Dionex ICS-6000 Single Pump, a CarboPac™ PA20 column (150 x 3 mm) protected by a CarboPac™ PA20 guard column (30 x 3 mm) and an ED50 electrochemical detector (Dionex, Sunnyvale, CA, USA).

2.3.3 Degree of methylesterification

Fourier transform infra-red spectroscopy (FT-IR; IRAffinity-1, Shimadzu, Kyoto, Japan) was used to determine the DM of the pectin samples following the procedure described by Kyomugasho, Christiaens, Shpigelman, Van Loey and Hendrickx (2015). The calibration curve constructed by Kyomugasho et al. (2015) was used to calculate the DM after analysis. The FT-IR analysis was performed in quadruplicate.

2.3.4 Protein content

The total nitrogen content of each pectin sample was determined in duplicate by use of an elemental analyzer (EA 1108 CHNS–O elemental analyzer, CE- Instruments/Thermo Scientific, Waltham, MA, USA) and the Dumas method (Santiago et al., 2018a).

Subsequently, a conversion factor of 6.25 was applied to calculate the protein content of each pectin sample (Mariotti, Tomé, & Mirand, 2008).

2.3.5 Molar mass distribution

By means of high performance size exclusion chromatography coupled to a multiangle laser light scattering detector (HPSEC-MALLS) the molar mass distribution of each pectin sample was determined in duplicate as described by Shpigelman, Kyomugasho, Christiaens, Van Loey and Hendrickx (2014). Overnight, lyophilized pectin samples were dissolved in ultrapure water at a concentration of 2 mg/ml. The dissolved samples were filtered (Chromafil A-45/25, 0.45 mm pore size, Macherey-Nagel Gmbh, Duren, Germany) before injection. During HPSEC, 100 µl of each sample was eluted over a series of three columns kept at 35 °C: Ultrahydrogel 250, 1000 and 2000 with exclusion limits of 8×10^4, 4×10^6 and 1×10^7 g/mol, respectively (Waters, Milford, MA). Pectin polymers were eluted using a 0.1 M acetic acid buffer (pH 4.4) containing 0.1 M $NaNO_3$ at a flow rate of 0.5 ml/min. After elution, the polymers passed through three detectors: a MALLS detector (PN3621, Postnova analytics, Germany), a refractive index (RI) detector (Shodex RI-101, Showa Denko K.K., Kawazaki, Japan) and a diode array detector (DAD) (G1316A, Agilent technologies, Diegem, Belgium). The concentration of the eluted polymers was calculated based on the RI signal and a dn/dc value of 0.146 ml/g. The molar mass (MM) was calculated from the MALLS signal applying the Debye fitting method (up to second order) by the NovaMals software (version 1.2.0.0, Postnova analytics, Germany).

2.3.6 ζ-potential

Capillary electrophoresis (Zetasizer NanoZS, Malvern Instruments, Worchester, UK) was used to determine the ζ-potential of the pectin samples as described in Neckebroeck et al. (2020). Overnight solubilized pectin solutions were measured at a concentration of 0.1 mg/ml and at nine different pH values ranging between 1.5 and 7.0. Analyses were executed in

capillary cells and all samples were inserted twice into the equipment with each capillary cell being measured in duplicate with a minimum of 12 runs per measurement. Reported values are averages of these four analytical values.

2.4 Emulsifying and emulsion stabilizing potential

The E_FE_S potential of the acid extracted pectin samples was thoroughly evaluated by a multimethod approach. The interfacial tension as function of contact time between the oil phase and a pectin solution was determined by pendant drop tensiometry. Next to this, a storage stability study was performed with emulsions containing the pectin samples as sole E_FE_S agent. At predetermined time moments during refrigerated storage, emulsion stability was evaluated. Firstly, an accelerated and direct stability analysis was performed on the day of emulsion preparation (day 0). Further, multiple parameters linked to emulsion stability were analyzed on day 0; 1; 4; 8 and 14 of storage. More specifically, the particle size distribution and the volume-weighted mean particle size (d_{43}) of each emulsion were determined by laser diffraction. Furthermore, the individual oil droplet size was studied by Single Particle Optical Sizing (SPOS) and the microstructure of each emulsion was visualized by light microscopy. Finally, macroscopic pictures were taken each analysis day to evaluate the visibility of a cream layer in the emulsions stored at refrigerated temperature.

2.4.1 Dynamic interfacial tension

Firstly, commercial sunflower oil was purified with the inorganic adsorbent Florisil® exactly as described by Neckebroeck et al. (2020) to prevent surface active compounds inherently present in the oil (e.g. polyphenols) influencing emulsion stability. Hereafter, a pendant drop tensiometer (CAM 200, KSV Instruments, Finland) was employed to determine the change in interfacial tension of a droplet of purified sunflower oil (pSO) formed in a 0.2% w/v (i.e. 2 mg/ml) pectin solution at the end of a J-shaped needle. Analysis was performed at room

292 temperature and in duplicate for pH 2.5 and 6.0 exactly as described before by Neckebroeck
293 et al. (2020).

294 **2.4.2 Flow behavior**

295 A stress-controlled rheometer (MCR 302, Anton Paar, Graz, Austria) was used to study the
296 flow behavior of 1% w/v pectin solutions based on a method described by Bernaerts et al.
297 (2018) with slight modifications. Samples were prepared by solubilization overnight followed
298 by pH adjustment to pH 2.5 or pH 6.0 and finally the volume was adjusted to obtain a final
299 concentration of 1% w/v. The rheometer was equipped with a double wall Couette geometry
300 (DG26.7, internal radius 12.3 mm, external radius 13.3 mm and measuring height 40 mm).
301 First, the samples were presheared for 30 s at a shear rate of 10 s-1 followed by 300 s of rest.
302 Next, steady-shear measurements were performed by increasing the shear rate logarithmically
303 from 1 to 100 s-1. Each shear rate was applied until steady state was reached, with a maximal
304 measuring time of 40 s per shear rate. A minimum torque of 0.1 µN m was set to ensure
305 reliability of the data and each sample was measured in duplicate.

306 **2.4.3 Emulsion preparation**

307 All 5% w/v oil-in-water (o/w) emulsions were prepared using pSO as the dispersed phase and
308 a 1% w/v pectin solution at pH 2.5 or pH 6.0 as the continuous phase. Both liquids were
309 mixed (10 min; 9500 rpm) to form a course emulsion using a high speed mixer (Ultra-Turrax
310 T25, IKA, Staufen, Germany). Coarse emulsions were further subjected to high pressure
311 homogenization at 100 MPa (STANSTED SPCH-10, Homogenising Systems, UK) for one
312 cycle. The resulting emulsions were transferred in test tubes (resulting in two test tubes for
313 each analysis day) which were stored at 4 °C for 14 days. Four pectin samples (i.e. AP, CP,
314 OP and TP) evaluated at two pH conditions resulted in a total of eight distinct emulsions
315 studied which are denominated based on the pectin sample used to stabilize the emulsion and
316 the pH of this emulsion, e.g. AP pH 2.5.

2.4.4 Physical storage stability of emulsions

2.4.4.1 Accelerated physical stability

An accelerated and direct stability analysis was performed with an analytical centrifuge (LUMiFuge, LUM GmbH, Berlin, Germany) as described previously in Neckebroeck et al. (2020). The Space- and Time-resolved Extinction Profiles (STEP®) Technology® supports the calculation of an instability index as described by Detloff, Sobisch and Lerche (2014) in full detail. Briefly, the instability index at time t is calculated based on the difference between the transmission profile at time t and the transmission profile at the start of analysis. In other words, the more this difference in laser transmission increases over time due to oil droplet migration, the higher the instability index will become. During centrifugation (1308 g at 20 °C) a total of 800 transmission profiles of the total height of each emulsion were recorded, one profile every minute. The stability analysis was performed in duplicate.

2.4.4.2 Volume-weighted particle size

The particle size distribution and d_{43} of each emulsion were determined on each analysis day by means of laser diffraction (Beckman Coulter Inc, LS 13 320, Miami, Florida, USA). A detailed and complete description of the analysis can be found in Neckebroeck et al. (2020) The presented results are averages of four measurements as two different test tubes of each emulsion were analyzed in duplicate. It was preferred to report d_{43} values as the average particle size as it is sensitive to the presence of larger oil droplets, making it a good parameter to evaluate emulsions stability.

2.4.4.3 Individual oil droplet size

Combining light extinction and light scattering, the SPOS technique (AccuSizer 780 APS, Particle Sizing Systems, Santa Barbara, California, USA) allows the measurement of individual oil droplet size while laser diffraction estimates a distribution based on the scattering of multiple oil droplets at the same time. Analysis was performed exactly as

described in Neckebroeck et al. (2020), the results of this analysis are displayed as a volume percentage of oil droplets larger than 1 µm present in the emulsion. Each analysis day, two separately stored test tubes for every emulsion were analyzed.

2.4.4.4 Microstructure

Light microscopy was used to visualize the microstructure of all o/w emulsions produced. An Olympus BX-51 light microscope (Olympus, Optical Co. Ltd., Tokyo, Japan) equipped with an Olympus XC-50 digital camera (Olympus, Optical Co. Ltd., Tokyo, Japan) and coupled to an image-analysis software (cellSens Standard, Olympus, Optical Co. Ltd., Tokyo, Japan) was utilized. Micrographs were taken using a 40x magnification and an exposure time set at 10 ms.

2.5 Statistical analysis

Significant differences in structural characteristics of all samples were evaluated by one way ANOVA and Tukey's Studentized Range Post-hoc test with a 95% level of significance ($p < 0.05$).

3 Results and discussion

To evaluate the E_FE_S potential of the generated pectin samples of different origin, both the structure of the pectin polymers as well as their capability of stabilizing emulsions were investigated. In section 3.1, the structural characteristics and the ζ-potential are discussed and compared among the four pectin samples. Subsequently, in section 3.2, the potential of the pectin samples to lower the interfacial tension was investigated in addition to the storage stability of o/w emulsions formulated with each pectin sample acting as sole E_FE_S agent at two different pH values.

3.1 Pectin structure and structure related properties

The structural properties of the different pectin samples are represented in **Table 1**. When comparing the results of the GalA and neutral monosaccharide content among the different samples, it is apparent that all samples contain considerable high amounts of GalA and pectin related monosaccharides such as rhamnose, arabinose and galactose. However, the relatively low GalA content for OP is noteworthy, especially since both the galactose and glucose content of this sample are significantly higher compared to the other pectin samples. Most probably, the OP extract consists of other polymers next to pectin. The presence of galactans in onion tissue has been suggested before by Golovchenko et al. (2012) who studied the effect of onion pectin polysaccharides on intestinal protein adsorption. Considering the glucose content of all samples, it can be stated that CP contains the lowest amount of residual glucose-containing polymers, possibly starch and hemicellulose. The presence of starch granules in apple and tomato tissue was demonstrated before (Berüter, 2004; Luengwilai & Beckles, 2009; Obiadalla-Ali, Fernie, Kossmann, & Lloyd, 2004), consequently it is very likely that a residual starch fraction is still present in the AP and TP extracts. Ng et al. (2000) reported high glucose levels in isolated cell wall material of onion tissue which were not attributed to the presence of starch however the exact origin was not further investigated.

Regarding the DM, all pectin samples displayed a DM higher than 50% and are therefore considered high DM pectin samples. This indicates that the blanching step during tomato handling and the industrial blanching of carrot cubes were effective in at least partly inactivating endogenous pectin methylesterase, which could induce deesterification of metlyesterified GalA units. Furthermore, a significantly higher DM for AP (78.41 ± 0.83%) was observed in comparison to OP (56.41 ± 0.21%), TP (54.29 ± 0.32%), and CP (63.02 ± 2.26%). A high DM is an interesting characteristic for the $E_F E_S$ capacity of pectin polymers as good emulsifying properties were reported for citrus pectin samples with DM values

between 55% and 84% by Schmidt et al. (2017). Additionally, Verkempinck et al. (2018) reported better emulsifying potential for high DM citrus pectin in comparison to medium and low DM citrus pectin.

Concerning the protein content of the samples, a high protein content was observed for TP (16.48 ± 0.05%), while CP contained the lowest amount of protein (5.28 ± 0.37%). It has been described previously in open literature that the protein content of pectin plays a major role in its E_FE_S capacity by increasing the surface activity of the overall structure (Akhtar et al., 2002; Funami et al., 2007; Leroux et al., 2003). Based on these results it can be expected that the presence of significantly more protein in the structure of TP may lead to an improved E_FE_S potential for this sample.

Lastly, the structural characterization analyses provided data on the MM distributions, which are depicted in **Figure 1**, while the average MM of the pectin samples are represented in **Table 1.** Overall, the MM distributions of AP, CP and TP are monomodal and display similar average MM values of about 500 kDa. By contrast, the MM profile of OP displays a remarkable bimodal concentration curve, suggesting the presence of two polymer fractions with different MM and possibly different structural properties. Compared to the average MM of the other pectin samples, OP consists of a polymer fraction with significant higher MM and a fraction with significant lower MM. Alba and Kontogiorgos (2017) stated that a MM region of 100 to 200 kDa for pectic polymers is beneficial for its emulsifying properties. They elucidated that if the MM is too low, the adsorbed layer might be too thin to ensure sufficient stability. In case of pectin polymers with a relatively high MM, the hydrophobic groups might be inaccessible due to the chain conformation and subsequently lower the emulsifying capacity of such polymers. Considering this, it is possible that most polymers present in AP, CP and TP have a too high MM to exhibit good emulsifying capacity. The OP sample however, consists of both a high MM polymer fraction (1150.00 ± 42.43 kDa) and a

low MM polymer fraction (64.40 ± 5.37 kDa) that can possibly each contribute to emulsion stability by different mechanisms.

Beside the structural properties, external factors, such as the pH of the medium, can influence the E_FE_S potential of pectin polymers as well. The pH of the medium determines the charge density and subsequently the conformation of pectin polymers. The ζ-potential of all samples was determined based on the electrophoretic mobility as a function of pH (**Figure 2**). The effect of pH on the charge density of the polymers is clearly visible in the sigmoidal shape of the curves displaying a more negative ζ-potential with increasing pH. Higher charge density with increasing pH values leads to more electrostatic repulsion and can consequently enhance emulsion stability when the pectin polymers adsorb at the oil-water interface. Overall, no distinct differences were detected among the pectin samples. Based on the ζ-potential measurements, pH 2.5 and pH 6.0 were selected to further investigate the E_FE_S functionality of the pectin samples as these two pH conditions result in distinctly different charge densities on the pectin polymers.

3.2 Emulsifying and emulsion stabilizing potential

3.2.1 Dynamic interfacial tension

The ability to lower the interfacial tension is an important property for emulsifying compounds as a lower interfacial tension facilitates droplet disruption during homogenization. Moreover, compounds adsorbed at the oil-water interface protect oil droplets against coalescence and in that way increase emulsion stability (McClements, 2015). The ability of pectin samples to lower the interfacial tension depends on both the pH conditions and pectin structure, which was evidenced in recent open literature (Chen et al., 2016; Funami et al., 2011; Neckebroeck et al., 2020; Verkempinck et al., 2018). The dynamic interfacial tension of a pSO droplet in all aqueous pectin solutions is represented in **Figure 3**. Firstly, when the interfacial tension was measured in ultrapure water, so in absence of any

439 E_FE_S compounds, the interfacial tension did not drop and remained constant at both pH
440 values. This indicates that nearly all emulsifying compounds inherently present in the
441 sunflower oil were removed during purification. Consequently, this result indicates that the
442 interfacial tension changes observed when evaluating pectin solutions can be attributed to the
443 adsorption of these pectin samples only. Of all pectin samples tested, AP showed the lowest
444 potential to decrease the interfacial tension at both pH values. In case of CP and OP, the
445 interfacial tension drop was similar at both pH values. Oppositely, TP presented a lower
446 interfacial tension at pH 6.0 compared to pH 2.5. Specifically for TP this might be explained
447 by a better availability of the abundantly present protein moieties at pH 6.0 compared to pH
448 2.5, as at pH 6.0 the pectin structure is opened up due to intramolecular electrostatic
449 repulsion. This increased availability can facilitate their adsorption at the interface (Akhtar et
450 al., 2002; Williams et al., 2005). Comparison of the obtained final values of CP at both pH
451 values with literature values indicate that the results obtained are comparable to citrus pectin
452 samples of varying DM (Verkempinck et al., 2018) and to whey protein isolate (Bai, Huan,
453 Gu, & McClements, 2016) while gum arabic lowered the interfacial tension to a lower extent
454 compared to CP (Bai et al., 2016). The described final values of gum arabic appear to be
455 similar to the values obtained for AP (Bai et al., 2016).

456 **3.2.2 Viscosity increasing capacity**

457 An increased viscosity of the continuous phase of an o/w emulsion can contribute to emulsion
458 stability as oil droplet movement is slowed down and consequently oil droplet collisions are
459 decreased. Ultimately, this results in slower creaming rates and possibly even decreased
460 coalescence and flocculation (Ngouémazong et al., 2015).

461 The viscosity of 1% w/v pectin solutions at pH 2.5 and pH 6.0 at a shear rate of 10 s^{-1} is
462 presented in **Figure 4** (same trends were observed at other shear rates). A clear effect of
463 solution pH was observed for pectin from all botanical sources. At pH 6.0, higher charge

densities on the polymers (see also **Figure 2**) could initiate electrostatic interactions and subsequent complex formation of pectin chains with trace amounts of divalent cations such as Ca^{2+} resulting in higher viscosities. In other words, residual cation concentrations could play an important role in the observed differences between pH conditions. The influence of pH on the viscosity was most outspoken for CP, and to a lesser extent for OP and TP, which could (partly) be related to different residual cation concentrations in these pectin samples. In contrast, the branched structure of AP combined with a high DM (see **Table 1**) reduces the possibility for this pectin structure to form strong and extensive electrostatic complexes while increasing chain entanglement. This can explain the high viscosity of AP observed at pH 2.5 compared to the other samples and the small difference between the values at both pH conditions for AP.

3.2.3 Creaming behavior

The stability of 5% w/v o/w emulsions was evaluated during 14 days of refrigerated storage by combining several analytical techniques. The results of the different analyses complement each other allowing to obtain in-depth insight into both creaming stability of the emulsions as well as stability against coalescence and flocculation (explained below). The former phenomenon being more a macroscopic process while the latter phenomena encompass microscopic changes in oil droplet size and organization. The macroscopic creaming behavior of the emulsions was evaluated by performing a direct and accelerated physical stability test using an analytical centrifuge and by taking macroscopic pictures of the emulsions stored on the shelf at 4 °C. For all emulsions, the instability index as function of analysis time is presented in **Figure 5**. A slow increase of the instability index when experiencing large centrifugal forces indicates a good stability of the emulsion against creaming. Of all emulsions analyzed, AP pH 6.0 and CP pH 2.5 displayed the slowest increase as function of analysis time, thus presenting best stability against creaming. Oppositely, TP pH 2.5 and TP

pH 6.0 creamed faster, indicating a lower stability against creaming. The creaming behavior of TP pH 6.0, and to a lower extent TP pH 2.5, was different in comparison to all other samples as the instability index increased fast in the beginning and also reached the plateau value rapidly. An explanation that can be put forward is the occurrence of pectin-protein interactions during storage due to the high protein content of TP. Most probably, a network will be formed at pH 2.5 by electrostatic interactions between the negatively charge pectin polymers and the positively charged proteins (Oduse, Campbell, Lonchamp, & Euston, 2017). This hypothesis seems plausible when observing the macroscopic picture of the TP pH 2.5 emulsion after 14 days of storage in **Figure 6**, since this emulsion is the only one with visible phase separation. In all other cases, no creaming layer could be visually detected during two weeks of refrigerated storage (**Figure 6**). At high pH however, a different mechanism is proposed as both pectin and protein are negatively charged at this pH value. It is hypothesized that depletion flocculation might cause the instability index to increase fast while the emulsion undergoes external centrifugal forces. The microstructure of this particular emulsion displayed in **Figure 10** substantiates this hypothesis (see also section 3.3.4). A similar observation was made by Santiago et al. (2018b). Overall, a clear pH effect regarding creaming could not be established based on these evaluations.

3.3.4 Oil droplet size

Characterizing and monitoring of the oil droplet size during storage is important to study emulsion stability. Coalescence and flocculation are two important destabilization mechanisms influencing droplet size but can be difficult to distinguish based on results of only one experimental analysis. Therefore, the oil droplet size of the emulsions was studied, integrating results from three analytical techniques. **Figure 7** displays the distributions of the oil droplet size for all emulsions at the beginning and the end of refrigerated storage as measured by laser diffraction. Additionally, the evolution of the volume-weighted average

514 particle size (d_{43}) during storage is depicted in **Figure 8**. An interesting complementary
515 analysis technique to laser diffraction is Single Particle Optical Sizing (SPOS) which is based
516 on the integration of both light extinction and light scattering during analysis. The main
517 advantage of this technique is that it is able to count and size every individual oil droplet as
518 they pass the detector zone one by one. The volume percentage of oil droplets larger than 1
519 µm can be calculated and is displayed in **Figure 9** for all emulsions on each analysis day.
520 Lastly, micrographs of all emulsions are presented in **Figure 10**, visualizing the oil droplet
521 size and organization.

522 Comparing for each pectin sample the results at both pH conditions, smaller droplet sizes
523 were formed at pH 2.5 compared to pH 6.0. This implies a better emulsifying capacity of all
524 studied pectin polymers at lower pH. Increased charge density might lead to insufficient or
525 incomplete oil droplet coverage due to intermolecular repulsion resulting in larger oil droplet
526 sizes after emulsification at pH 6.0. Polymers that still adsorb to the interface at these
527 conditions can contribute to emulsion stability by both steric interactions and electrostatic
528 repulsion. Another overall observation is that all oil droplet size distributions do not present
529 drastic changes during storage with the OP stabilized emulsions displaying the smallest oil
530 droplet size and best stability during storage. Possibly, emulsion stability is the result of the
531 interplay of different phenomena regarding the pectin samples. Interfacial activity and
532 increasing continuous phase viscosity are both beneficial properties for emulsion formation
533 and stability. Pectin polymers displaying both functionalities could create fine and stable
534 emulsions. Integrating all data per pectin sample, provides some specific insight and
535 interesting conclusions.

536 For AP it can be stated that after emulsion production, the emulsions were well stabilized at
537 both pH 2.5 and pH 6.0. The monosaccharide composition of AP indicated that these pectin
538 polymers contained a substantial fraction of the branched RG-I domain (**Table 1**). After

adsorption at the oil-water interface, this may have induced steric hindrance further stabilizing the AP emulsions during storage. Besides, the viscosity increasing capacity of non-adsorbed AP polymers can increase emulsion stability during storage as well (see also **Figure 4**). The oil droplet size distributions of AP pH 6.0 (**Figure 7 A**) however, suggest the presence of larger oil droplets or flocs. The microscopic images of AP pH 6.0 reveal the presence of small flocs of associated oil droplets, indicating that the oil droplet distributions of this sample comprised both individual as well as flocculated oil droplets. As during SPOS analysis flocs can be disrupted due to physical agitation and dilution, the results of the AP stabilized emulsions presented in **Figure 9** predominantly represent the individual oil droplet size which remained constant during storage. Thus, it can be concluded that AP pH 2.5 was stable during 14 days of storage, while (bridging) flocculation is the main mechanism causing destabilization in AP pH 6.0.

The results for CP clearly showed a better emulsifying capacity at pH 2.5 than at pH 6.0. SPOS analysis revealed some coalescence for both CP-based emulsions, while flocculation was not observed. Overall, CP has definitely potential as an E_FE_S agent. This sample was characterized as a high DM pectin sample with some side chains present.

OP presents the best E_FE_S potential of all pectin samples at both pH values. All analyses performed clearly indicate very stable oil droplet sizes during storage. No coalescence or flocculation could be observed for these emulsions. It can be hypothesized that the presence of two polymer fractions with different MM (*cfr.* **Table 1**), a particular property of the OP pectin sample, might positively influence its E_FE_S potential. Both fractions may actually contribute to emulsion stability in a synergistic way with the lower MM fraction adsorbing at the interface and the higher MM fraction increasing the viscosity of the continuous aqueous phase. Future research must be conducted to provide better insight in this hypothesis.

563 Finally, micrographs of TP pH 2.5 suggest the presence of (weakly) associated flocs of oil
564 droplets after 14 days of storage. For TP pH 6.0, flocculation was already visible on the day
565 of emulsion production but intensified during storage. Interestingly, the TP stabilized
566 emulsions at both pH conditions showed the largest d_{43} increase between day 8 and day 14 of
567 storage. Longer storage might have led to further fast destabilization. At pH 2.5, this was also
568 observed for the individual oil droplet size (**Figure 9**) while good oil droplet size stability
569 was observed at pH 6.0. The latter indicates that flocs are broken up during analysis.
570 Nevertheless, weakly associated flocs can certainly influence creaming behavior which was
571 found to be occurring rather fast for TP stabilized emulsions in comparison to all other
572 emulsions (**Figure 5**). This indicates that (weak) flocculation and creaming were the
573 destabilization phenomena with the largest impact on the emulsions stabilized by TP. As was
574 also suggested above, the high protein content of TP might cause network formation through
575 pectin-protein interactions explaining the flocculation and creaming behavior observed.

576 ## 4 Conclusion

577 Structural differences in terms of DM, monosaccharide composition, protein content and/or
578 MM were observed for pectin rich extracts from apple, carrot, onion and tomato. All pectin
579 samples were able to lower the interfacial tension of an oil droplet. During storage, the pectin
580 samples generally displayed a better emulsifying capacity at pH 2.5 than at pH 6.0. A higher
581 charge density induces interpolymer repulsion possibly leading to incomplete droplet
582 coverage. For AP, possibly bridging flocculation occurred due to the presence of long side
583 chains in the AP structure, while for TP pectin-protein interactions might explain the
584 observed flocculation and creaming behavior. In case of CP no flocculation was observed,
585 but coalescence occurred to a low extent. OP displayed the most promising $E_F E_S$ potential of
586 all pectin samples tested. The oil droplet size remained constant for 14 days in all analyses
587 performed, implying neither coalescence nor flocculation did occur in these emulsions. The

proposed hypothesis suggests that the two polymer fractions with different MM present in the OP sample may contribute to emulsion stability in a synergistic way. Yet, this hypothesis needs to be verified by a dedicated experimental set-up in follow-up studies.

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Declaration of interests

The authors of the present work declare no conflict of interests.

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Tables

Table 1. Structural properties of all pectin samples generated, expressed as average values ± standard deviation. (GalA: galacturonic acid; DM: degree of methylesterification; MM: molar mass)

	AP	CP	OP	TP
GalA content (mg/g)	601.88 ± 14.76 [a]	572.86 ± 20.85 [b]	359.26 ± 21.96 [d]	523.83 ± 8.65 [c]
Neutral monosaccharides (mg/g)				
Rhamnose	43.17 ± 2.29 [a]	32.39 ± 1.63 [b]	12.23 ± 0.54 [c]	13.00 ± 1.54 [c]
Arabinose	158.03 ± 2.79 [a]	53.45 ± 4.05 [b]	12.79 ± 0.66 [c]	13.40 ± 1.01 [c]
Galactose	57.71 ± 3.69 [b]	66.95 ± 5.94 [b]	291.11 ± 8.57 [a]	47.97 ± 5.03 [c]
Fucose	0.17 ± 0.06 [b]	2.28 ± 1.19 [a]	ND	ND
Glucose	17.27 ± 0.62 [b]	8.86 ± 1.59 [c]	52.23 ± 1.71 [a]	15.77 ± 1.44 [b]
Xylose	21.22 ± 0.95 [a]	2.34 ± 0.07 [c]	6.37 ± 0.96 [b]	5.92 ± 0.86 [b]
Mannose	1.33 ± 0.03 [c]	4.36 ± 0.84 [b]	ND	5.40 ± 0.27 [a]
DM (%)	78.41 ± 0.83 [a]	63.02 ± 2.26 [b]	56.41 ± 0.21 [c]	54.29 ± 0.32 [c]
Protein content (% w/w)	6.53 ± 0.02 [b]	5.28 ± 0.37 [c]	6.28 ± 0.08 [b]	16.48 ± 0.05 [a]
MM peak 1 (kDa)	482.50 ± 6.36 [bc]	429.50 ± 2.12 [c]	1150.00 ± 42.43 [a]	523.50 ± 14.85 [b]
MM peak 2 (kDa)	N/A	N/A	64.40 ± 5.37 [d]	N/A

ND: not detected
N/A: not applicable
Different superscript letters indicate significant differences ($p < 0.05$) between pectin samples for one specific structural property.

Figure Captions

Figure 1. Molar mass distributions displaying the molar mass (dashed lines) and concentration (full lines) profiles for AP (green), CP (orange), OP (blue) and TP (red). For better interpretation of this graph, the reader is referred to the colored version (available online).

Figure 2. ζ-potential as function of pH for 1% w/v solutions of (○) AP, (■) CP, (□) OP and (●) TP.

Figure 3. Dynamic interfacial tension of a droplet of purified sunflower oil in 0.2% w/v solutions of AP (green), CP (orange), OP (blue) and TP (red). Grey lines represent the dynamic interfacial tension of a droplet of purified sunflower oil in ultrapure water. Full lines represent measurements at pH 2.5, while dashed lines represent measurements at pH 6.0. For better interpretation of this graph, the reader is referred to the colored version (available online).

Figure 4. Viscosity of 1% w/v solutions of AP, CP, OP and TP at a shear rate of 10 s^{-1}. Full bars represent measurements at pH 2.5 while shaded bars represent measurements at pH 6.0.

Figure 5. Instability index of 5% o/w emulsions stabilized by 1% w/v AP (green), CP (orange), OP (blue) and TP (red). Full lines represent measurements at pH 2.5, while dashed lines represent measurements at pH 6.0. For better interpretation of this graph, the reader is referred to the colored version (available online).

Figure 6. Macroscopic pictures of 5% o/w emulsions stored at 4 °C stabilized by 1% w/v of different pectin samples. For each set of pictures from left to right: day of emulsion production (day 0), day 1, day 4, day 8 and day 14 of refrigerated storage at 4°C.

Figure 7. Oil droplet size distributions for 5% o/w emulsions stabilized by 1% w/v (A) AP, (B) CP, (C) OP and (D) TP for the day of emulsion preparation (full lines) and day 14 of refrigerated storage (dashed lines). Black lines represent emulsions at pH 2.5, while grey lines represent emulsions at pH 6.0.

Figure 8. Evolution of the average oil droplet size expressed as d_{43} during refrigerated storage of 5% o/w emulsions stabilized by 1% w/v (A) AP, (B) CP, (C) OP and (D) TP. Black lines represent emulsions at pH 2.5, while grey lines represent emulsions at pH 6.0.

Figure 9. Volume fraction of oil droplets larger than 1 µm present in 5% o/w emulsions stabilized by 1% w/v AP, CP, OP and TP at (A) pH 2.5 and (B) pH 6.0 measured by the SPOS technique. Increasing color intensity of bars indicates longer storage time: ■ day of emulsion production (day 0), ■ day 1, ■ day 4, ■ day 8 and ■ day 14.

Figure 10. Light microscopic images of 5% o/w emulsions stabilized by 1% w/v AP, CP, OP and TP at pH 2.5 and 6.0. Scale bars represent a length of 100 µm.