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Structurally modified pectin for targeted lipid antioxidant capacity in linseed/sunflower oil-in-water emulsions

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Effect of pectin structure on emulsion oxidative stability

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Abstract

The present work explored the lipid antioxidant capacity of citrus pectin addition to 5% (w/v) linseed/sunflower oil emulsions stabilized with 0.5% (w/v) Tween 80, as affected by pectin molecular characteristics. The peroxide formation in the emulsions, containing tailored pectin structures, was studied during two weeks of storage at 35 °C. Low demethylesterified pectin ($\leq 33\%$) exhibited a higher antioxidant capacity than high demethylesterified pectin ($\geq 58\%$), probably due to its higher chelating capacity of pro-oxidative metal ions (Fe^{2+}), whereas the distribution pattern of methylesters along the pectin chain only slightly affected the antioxidant capacity. Nevertheless, pectin addition to the emulsions caused emulsion destabilization probably due to depletion and/or bridging effect, independent of the pectin structural characteristics. These results evidence the potential of structurally modified citrus pectin as a natural antioxidant in emulsions. However, optimal conditions for emulsion stability should be carefully selected.

Keywords

Citrus pectin; emulsion stability; antioxidant capacity; degree of methylesterification; linseed/sunflower oil; lipid oxidation

1. Introduction

Many lipid based food products are oil-in-water (O/W) emulsions, in which oil droplets are dispersed into an aqueous phase (McClements & Decker, 2000). Oils rich in poly-unsaturated fatty acids (PUFAs), especially ω -3 PUFAs, are interesting to be incorporated in O/W emulsions as they are related to positive health benefits such as decreased risk of cardiovascular diseases, cancer or inflammatory illnesses (Riediger, Othman, Suh, & Moghadasian, 2009). However, PUFAs are susceptible to lipid oxidation, which negatively affects the quality and shelf-life of the food product (McClements et al., 2000). Moreover, lipid oxidation products are known to be cytotoxic and genotoxic at high doses and therefore

harmful for consumer health (Kanner, 2007). Auto-oxidation of lipids comprises a reaction between unsaturated fatty acids and oxygen-active species with the formation of peroxides and proceeds through three steps: initiation, propagation and termination (Frankel, 1980). This oxidation reaction is typically accelerated in O/W emulsions by transition metal ions, located in the aqueous phase, because of their ability to decompose lipid peroxides, which are located at the oil droplet surface, into peroxy and alkoxy radicals (McClements et al., 2000; Mei, Decker, & McClements, 1998). These reactive species can further react with unsaturated lipids with the formation of lipid radicals and promote the lipid oxidation propagation (McClements et al., 2000). The presence of transition metal ions in O/W emulsions can be largely attributed to water contamination, food fortification, packaging materials or addition of food ingredients (Hu, McClements, & Decker, 2004; Zou & Akoh, 2015). Fe^{2+} is believed to be the most important lipid oxidation pro-oxidant due to its high solubility and reactivity (Mei et al., 1998). Consequently, chelation of these pro-oxidative metal ions either to remove them from the vicinity of oil-water interphase, either to decrease metal reactivity is a common strategy to retard lipid oxidation (Chen, McClements, & Decker, 2010; Hu et al., 2004; McClements et al., 2000). Currently, ethylene diamine tetraacetic acid (EDTA) is widely used as a commercial antioxidant as it has shown to effectively reduce lipid oxidation in several O/W emulsions due to its high chelating capacity (Haahr & Jacobsen, 2008; Hu et al., 2004; Mei et al., 1998). However, the use of such synthetic additives is associated with a negative perception of the consumer because of its reported cytotoxic and genotoxic effects (Lanigan & Yamarik, 2002). Particular interest consists on replacing synthetic antioxidants by natural additives to chelate Fe^{2+} in lipid-based food products, thus contributing towards the design of *clean label* products, which are more appealing to consumers (McClements et al., 2000; Shahidi, 2000).

Polysaccharides from natural sources such as xanthan, alginate and pectin have been reported to exhibit antioxidant capacities which can be attributed to their ability to chelate metal ions (Chen et al., 2010; Salvia-Trujillo, Decker, & McClements, 2016; Shimada, Okada, Matsuo, & Yoshioka, 1996). The chelating capacity of pectin is largely related with its degree of methylesterification (DM), which is a measure of the number of methylesterified galacturonic acid (GalA) units in homogalacturonan (HG), the predominant and linear pectin domain consisting of α -1,4 linked GalA units. In addition to HG, pectin comprises more branched domains, defined as rhamnogalacturonan I and II (Voragen, Coenen, Verhoef, & Schols, 2009). Non-methylesterified GalA units of HG can be negatively charged at a pH above the pK_a of pectin (3.38 - 4.10) thereby exhibiting the ability to interact with metal ions (Kohn, 1987; Kyomugasho, Willemsen, Christiaens, Van Loey, & Hendrickx, 2015b). Such ionic interactions are assumed to be influenced not only by the number (DM) but also by the distribution pattern of the non-methylesterified GalA units, which is quantified as the (absolute) degree of blockiness (DB or DB_{abs}). The DB and DB_{abs} are defined as the ratio of the number of non-methylesterified GalA units present in blocks versus the amount of non-methylesterified GalA units and versus the total amount of GalA units, respectively (Daas, Meyer-Hansen, Schols, De Ruiter, & Voragen, 1999; Guillotin, Bakx, Boulenguer, Mazoyer, Schols & Voragen, 2005). In the context of gel formation, several studies have shown that a higher number of successive non-methylesterified GalA residues (high DB) promotes greater pectin- Ca^{2+} interactions (Fraeye, Doungha, Duvetter, Moldenaers, Van Loey & Hendrickx, 2009; Ralet, Dronnet, Buchholt, & Thibault, 2001; Willats, Knox, & Mikkelsen, 2006). Consequently, targeted modification of pectin DM and DB can contribute to direct its chelating capacity and therefore lipid antioxidant activity. Structural modification of pectin DM can be achieved enzymatically or chemically. Enzymatic demethylesterification occurs by pectin methylesterase (PME), of which PMEs with an alkaline pI (mostly plant PME) are

known to hydrolyse methylesters at C-6 of GalA units blockwise, while demethylesterification with microbial PME, with an acidic pI, results in more random distributed demethylesterified GalA units (Cameron, Luzio, Goodner, & Williams, 2008; Limberg, Körner, Buchholt, Christensen, Roepstorff, Mikkelsen, 2000). Chemical demethylesterification is achieved by use of an alkali treatment, leading to random distributed non-methylesterified GalA units and thus a lower DB compared to demethylesterified pectin by plant PME with the same DM (Limberg et al., 2000).

Pectin has been used in food industry as a functional ingredient such as a thickening, stabilizing or gelling agent, with the latter the most extensive application pectin (May, 1990). The stabilizing capacity of pectin, and polysaccharides in general, can be attributed to their ability to increase the viscosity of the aqueous phase in O/W emulsions (Dickinson, 2003). However, the addition of sufficiently high concentrations of polysaccharides (*> critical flocculation concentration*) to the aqueous phase of emulsions stabilized with previously adsorbed surfactants might cause physical instability by either depletion or bridging flocculation (Dickinson, 2003). In addition to the stabilizing effect of polysaccharides, the increased viscosity of the continuous phase in O/W emulsions is hypothesized to also influence the oxidative stability by restricting the movement of metal ions or oxygen through the aqueous phase towards the oil droplet (Shimada et al., 1996).

To date, only few studies explored the antioxidant capacity of pectin (Chen et al., 2010; Huang, Lu, Wang, & Wu, 2011; Qiu, Zhao, Decker, & McClements, 2015; Shimada et al., 1996; Xu, Liu, Luo, Liu, & Julian, 2017). However, these studies did not investigate the role of pectin structural properties on the lipid antioxidant capacity by systematically modifying the pectin structural properties, in terms of DM and DB_{abs}. In this sense, process-induced changes of pectin structural properties are hypothesized to alter its cation binding capacity and subsequently its antioxidant capacity. The addition of pectin to food emulsions as an

antioxidant can contribute to *clean label* food products and to dual functionalities, as pectin can exhibit several functionalities. However, fundamental insight into the influence of pectin addition on emulsion stability is necessary for desired food formulation. Therefore, this study aims at investigating the role of targeted citrus pectin modification, in particular the degree of methylesterification and blockiness, on the oxidative and physicochemical stability of linseed/sunflower emulsions. This goal is achieved by targeted production of pectin structures enzymatically (carrot PME) or chemically and subsequently addition of these structures in the aqueous phase of linseed/sunflower oil emulsions.

2. Material and methods

2.1. Materials

High methylesterified citrus pectin (DM 95%) was obtained from Sigma-Aldrich Belgium and used for the production of pectin samples with distinct degrees and patterns of methylesterification. Carrots (*Daucus carota* cv. Nerac) and kiwis (*Actinidia deliciosa* cv. Hayward) were purchased from a local shop and further used as a source for pectin methylesterase (PME) and PME inhibitor (PMEI) extraction, respectively. A blend of linseed/sunflower oil (30/70%) was kindly donated by Vandemoortele (Izegem, Belgium) and was used as it contained a high amount of poly-unsaturated fatty acids in order to study the lipid oxidation. FeSO_4 , FeCl_3 , BaCl_2 , Tween 80, iso-octane, methanol and ammonium thiocyanate were obtained from Sigma-Aldrich Belgium. 2-propanol was purchased from VWR Chemicals (Leuven, Belgium). Ultrapure water (organic free, 18.2 M Ω cm resistance) was supplied by a SimplicityTM water purification system (Millipore, Billerica, USA) and used for emulsion and sample preparation as well as for analytical experiments. All chemicals used were of analytical grade.

2.2. Preparation of pectin samples with different degree and pattern of methylesterification

Pectin samples with distinct degrees and patterns of methylesterification were produced starting from high methylesterified, linear citrus pectin with a DM of $95.1 \pm 1.8\%$ (M95). This sample preparation was achieved by enzymatic or chemical demethylesterification of the mother pectin to obtain blockwise or randomly distributed methylesterified carboxylic groups, respectively as described in our previous study (Celus et al., 2017). In brief, the enzymatic demethylesterification was performed by incubation of a solution of M95 (0.8% w/v in sodium phosphate buffer, pH 7) with purified carrot PME at 30 °C for different time periods. After incubation, the pH of the pectin solutions was adjusted to 4.5 and a thermal treatment at 85 °C for 4 min was performed to inactivate PME. The chemical demethylesterification of mother pectin (M95) was achieved by titration at pH 11 using 0.1 M NaOH. The alkaline demethylesterification reaction was performed at 4 °C to exclude undesired pectin depolymerisation. After addition of predetermined amounts of NaOH, the reaction was stopped by lowering the pH of the pectin solution to 4.5.

The pH of both enzymatic and chemical demethylesterified pectin samples was subsequently adjusted to pH 6, followed by dialysis (Spectra/Por[®], MWCO = 12-14 kDa) for 48 h against demineralized water and further lyophilization. In this study, the enzymatic (from plant extracts) and chemical demethylesterified pectin samples will be denoted as P-pectins and C-pectins, respectively.

2.3. Pectin sample characterization

The modified pectin samples were characterized for their degree of methylesterification (DM), galacturonic acid (GalA) content, molar mass distributions and intrinsic cation concentration (^{24}Mg , ^{44}Ca , ^{56}Fe and ^{66}Zn) as described previously by Celus et al. (2017).

The absolute degree of blockiness (DB_{abs}) was estimated as a quantification of the distribution pattern of methylesters in the pectin samples. The DB_{abs} is defined as the ratio of non-methylesterified GalA units present in blocks to the total amount of GalA units (Guillotin et al., 2005) and is predicted based on polynomial functions, describing the DB_{abs} as a function of the DM of tailored pectin samples as determined by Ngouémazong et al. (2011). The pectin samples in the present work are prepared in exactly the same way as described by Ngouémazong et al. (2011). The estimated parameters of the polynomial functions used in this study to predict the DB_{abs} of the demethylesterified pectin samples are as follows:

$$\text{P-pectins: } DB_{abs} = 0.472 (\pm 0.03) DM^2 - 1.485 (\pm 0.04) DM + 1.009 \quad [1]$$

$$\text{C-pectin: } DB_{abs} = -1.998 (\pm 0.10) DM^3 + 4.595 (\pm 0.20) DM^2 - 3.598 (\pm 0.06) DM + 1.000 \quad [2]$$

2.4. Emulsion preparation

Stock O/W emulsion

First, a coarse O/W emulsion was prepared by blending 20% (w/v) linseed/sunflower oil (LSSO) and 2% (w/v) Tween 80 with 78% (w/v) of ultrapure water at 9500 rpm for 10 min using an Ultra-Turrax (T25, IKA, Staufen, Germany). This O/W emulsion was further stabilized by high-pressure homogenization at 100 MPa for one pass (Pressure Cell Homogenizer, Stansted Fluid Power LTD., UK) and is referred as the stock emulsion.

O/W emulsions with pectin addition

Stock pectin solutions of 1.33% (w/v) were prepared for addition to the stock emulsion by dissolving pectin samples in ultrapure water overnight to ensure complete dissolution. Subsequently, these pectin solutions were mixed with the stock emulsion by stirring for 10 min to obtain a final concentration of 5% (w/v) LSSO, 0.5% (w/v) Tween 80 and different pectin concentrations (0.1 and 1% w/v). A 0.1% pectin concentration was chosen as this was the concentration at which some polysaccharides have been reported to exhibit a significant

antioxidant capacity (Chen et al., 2010; Salvia-Trujillo et al., 2016) while concentrations higher than 1% could increase the viscosity substantially thus possibly affecting the lipid oxidation results. In the emulsions without pectin, the stock emulsion was mixed with equal amounts of ultrapure water.

The influence of Fe^{2+} on both lipid oxidation and emulsion stability was studied in this work. For this, a stock Fe^{2+} solution of 0.5 M was freshly prepared by dissolving FeSO_4 into ultrapure water and added to the emulsions to obtain a final concentration of 0.1 mM Fe^{2+} (based on preliminary experiments, results not shown). For the physicochemical stability study, emulsions were prepared both with and without Fe^{2+} , whereas for the oxidation studies all samples contained Fe^{2+} (0.1 mM)

For the physicochemical stability study, emulsions without or with high (M95), intermediate (P60) and low (P13) DM pectin and without or with Fe^{2+} addition were evaluated. Samples were divided into small glass tubes containing 5 mL aliquots. On the one hand, tubes were stored for creaming index measurements, so that they remained undisturbed and on the other hand and for particle size distribution, particle electrical charge, pH and microscopic measurements. Emulsions were stored in the dark at 25 °C.

For the oxidative stability study, samples were prepared as described above and additionally chemical demethylesterified (C58) pectin and low DM pectin (P33) were incorporated. Samples were stored in the dark at 35 °C for 15 days and the peroxide formation was determined.

2.5. Physicochemical characterization of emulsion stability

The physicochemical stability of all LSSO emulsions was determined by measuring the particle electrical charge, creaming index, particle size distribution and the microstructure.

2.5.1. Particle electrical charge

The electrical charge (ζ -potential) of the oil droplets in all LSSO emulsions and of the 0.1% (w/v) pectin solutions was determined by phase-analysis light scattering using an automated capillary electrophoresis equipment (Zetasizer NanoZS, Malvern Instruments, Worcestershire, UK). Before each measurement, samples were diluted 1:100 (v/v) with ultrapure water and subsequently transferred into a capillary cell equipped with two electrodes. During the measurement, a potential was applied and the electrophoretic mobility was determined based on the particle velocity through the sample. The electrophoretic mobility is converted into ζ -potential values by the instrument's software, using the equation of Henry. The ζ -potentials of the emulsions and pectin samples were determined immediately after being produced. The sample preparation and analysis were performed in duplicate.

2.5.2. Creaming index

The creaming index of all LSSO emulsions, with and without Fe^{2+} and/or pectin addition, was measured after two weeks of storage. The capped glass tubes, filled with 2 mL of the emulsion, were not shaken or opened during storage time. The total height of the emulsion (H_e) and the height of the cream layer (H_c) were measured using a caliper (Mitutoyo Belgium, Kruibeke, Belgium). The creaming index (%) is expressed as follows (Guerra-Rosas, Morales-Castro, Ochoa-Martínez, Salvia-Trujillo, & Martín-Belloso, 2016):

$$\text{Creaming index (\%)} = \left(\frac{H_c}{H_e} \right) \cdot 100 \quad [3]$$

2.5.3. Particle size distribution

The particle size distributions of all LSSO emulsions, with and without Fe^{2+} and/or pectin addition, were measured using a laser diffraction equipment (Beckman Coulter Inc., LS 13 320 Miami, FL, USA). The measurement was performed in duplicate and at day 0, 7 and 14 of storage. Emulsions were agitated prior to particle size measurements and a small

amount was poured into a stirring tank which was filled with demineralized water. The sample was pumped into the measurement cell where the laser light, with the wavelength of the main illumination source 750 nm and wavelengths of halogen light for Polarization Intensity Differential Scattering (PIDS) 450 nm, 600 nm and 900 nm, is scattered by the particles. The intensity of the scattered laser light is detected and analyzed by the Fraunhofer model. The particle sizes of the emulsions are reported as the $D[v;0.5]$ value, which defines the median volume-weighted particle size. In other words, $D[v;0.5]$ is the particle diameter at which 50% of the particles are smaller and 50% bigger.

2.5.4. Microstructure

The microstructure of representative LSSO emulsions mixed with 1% (w/v) pectin solutions and Fe^{2+} (0.1 mM) was determined at day 0, 7 and 14. Emulsions were gently agitated before placing a drop on a microscope slide. Visualization of the microstructure was performed at different magnifications with an optical microscope (Olympus BX41, Olympus Corporation, Tokyo, Japan) equipped with a digital camera (Olympus BX51, Olympus Optical Co. LTD., Tokyo, Japan).

2.6. Determination of lipid oxidation stability

The oxidative stability of LSSO emulsions was studied as a function of storage time by assessing the formation of peroxides spectrophotometrically as a measure for primary oxidation products. The procedure is based on the method described by Shantha & Decker (1994) with some modifications. A 0.3 mL aliquot of the agitated emulsion was added to 1.5 mL iso-octane/2-propanol (3:1 v/v) in glass tubes and vortexed three times for 10 s. The tubes were then centrifuged (Sigma 4-16KS centrifuge, swing-out rotor, Osterode am Harz, Germany) at 1000 g for 2 min at 20 °C and 0.2 mL of the upper organic layer was added to 2.8 mL of a methanol-2-propanol (2:1 v/v) solution. Subsequently, 15 μ L of a 3.94 M ammonium thiocyanate solution and 15 μ L of a 0.0072 M Fe^{2+} solution were added and the

mixture was vortexed. After 20 min in absence of light, the absorbance of the mixture was measured at 510 nm. The analysis was performed in triplicate and the Fe^{3+} concentration in the emulsion was determined from a standard curve of FeCl_3 solutions (0-2.7 μM). The peroxide concentration, expressed in mEq peroxide/L emulsion, was calculated by dividing the concentration of Fe^{3+} in the emulsion ($\mu\text{g/mL}$) by 55.845 g/mol, the atomic weight of Fe^{3+} , and by two to express the PV as mEq of peroxide instead of mEq of oxygen as explained by Shantha et al. (1994).

2.7. Statistical analysis and data modeling

Statistical analysis of the experimental data was carried out with the statistical software JMP (JMP 13, SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) and a Tukey HSD test were carried out to analyze significant differences with a 95% confidence interval ($p < 0.05$) between the experimental data.

The peroxide formation was modeled as a function of storage time in order to quantify differences between the abilities of the different pectin samples to retard lipid oxidation. The development of the peroxide concentration can typically be described by bell-shaped curves. During an induction period, no significant increase in peroxide concentration occurs. This period is followed by a propagation step, during which a progressive increase in peroxides can be observed. As peroxides are decomposed to secondary oxidation products in the termination step, a maximum level of peroxides is observed after which the decomposition process dominates (Frankel, 1980; Manzocco, Calligaris, & Nicoli, 2010). The peroxide concentration as a function of storage time can be modeled using a simplified reaction scheme of three consecutive reactions representing the autocatalytic lipid oxidation process.

where RH represents an unsaturated lipid, R[•], a lipid free radical, ROOH, the peroxides and k₁, k₂ and k₃, the rate constants of the three reactions, respectively. Solubilized oxygen in the aqueous phase is assumed to be an unlimited factor. The concentration change of each compound in the preceding reaction scheme can be written as:

$$\frac{dC_{RH}}{dt} = -(k_1 + k_2 \cdot C_{R^{\bullet}}) \cdot C_{RH} \quad [7]$$

$$\frac{dC_{R^{\bullet}}}{dt} = (k_1 + k_2 \cdot C_{R^{\bullet}}) \cdot C_{RH} \quad [8]$$

$$\frac{dC_{ROOH}}{dt} = k_2 \cdot C_{R^{\bullet}} \cdot C_{RH} - k_3 \cdot C_{ROOH} \quad [9]$$

with C_{RH}, C_{R[•]} and C_{ROOH}, the concentrations of the unsaturated lipids, lipid free radicals and peroxides, respectively and t represents the storage time in days. At storage time 0, the initial unsaturated lipid concentration was calculated based on the fatty acid profile of the linseed/sunflower oil and equals 93 mmol/L emulsion while the concentrations of radicals and peroxides can be considered zero.

The differential equations were solved numerically while at the same time rate constants were estimated based on the peroxide profile as a function of storage time (using the SAS Proc Model procedure, SAS, version 9.4, SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

3.1. Pectin sample characterization

The tailored pectin samples were characterized for their DM, DB_{abs}, GalA content, weight-average molar mass and intrinsic cation concentration. All results are summarized in **Table 1**.

Pectin samples were demethylesterified enzymatically (P-pectins) and chemically (C-pectin).

As presented in **Table 1**, distinct DMs (%) were obtained by applying different reaction

times. In addition, distinct patterns of methylesterification (DB_{abs}) were expected to be generated by applying different demethylesterification methods. **Table 1** shows that different DB_{abs} values (%) were estimated for P60 and C58, both samples with a similar DM, with P60 showing a higher number of blocks of successive non-methylesterified GalA units. The weight average molar masses of the pectin samples varied between 48.1 ± 2.2 and 66.2 ± 0.1 kDa, but are not considered to be largely different. Therefore, it can be assumed that no depolymerisation occurred and thus, P60 and C58 were expected to only differ in DB_{abs} . In addition, the GalA content of all pectin samples was similar.

The concentration of intrinsic cations was assessed in order to ensure that no substantial contribution occurred when adding Fe^{2+} ions during the oxidation study. Results of the intrinsic Fe concentrations (sum of Fe^{2+} and Fe^{3+}) revealed that they were very low, 15 to 50 times lower, than the added amount of Fe^{2+} (**Table 1**). Ca^{2+} was present in the highest concentrations (1.0 – 2.7 mg/g pectin).

3.2. Emulsions physicochemical stability

The influence of pectin DM on the physicochemical stability of LSSO emulsions was studied by measuring the particle electrical charge, creaming index, particle size distribution and the microstructure during storage time. LSSO emulsions were prepared with and without the addition of 0.1 mM Fe^{2+} and/or pectin with different DMs at two different concentrations (0.1 and 1% w/v).

3.2.1. Particle electrical charge measurements

ζ -potential measurements were assessed to determine the electrical charge of the oil droplets at the oil/water interface, which indicates the species that are adsorbed at the interface and therefore could help to understand the possible destabilization phenomena. The electrical charges (ζ -potential) of the 0.1 and 1% (w/v) pectin solutions as such were also measured and

presented in **Fig. 1 (A, B, C)**. Pectin with decreasing DM showed a more negative ζ -potential in comparison with high DM pectin due to their higher number of negatively chargeable non-methylesterified GalA units. For instance, the ζ -potential of M95 pectin solution at 0.1 % (w/v) was -16.3 ± 1.9 mV whereas the one of P13 was -41.5 ± 6.9 mV at the same concentration. In addition, pectin solutions at a higher concentration revealed a more negative ζ -potential, related to a higher number negatively chargeable particles.

The electrical charges of the LSSO emulsions are also shown in **Fig. 1 (D, E, F)** and reveal that all emulsions were characterized by anionic charged oil droplets, despite the use of a non-ionic surfactant (Tween 80). Although Tween 80 is not expected to provide a negative charge to the oil droplets, these results can be explained by the presence of impurities, such as free fatty acids, from the surfactant or from the oil (McClements, 2016). Indeed, the pH values of the emulsions were above the pK_a value of fatty acids (4.8-5) (Spector, 1975), leading to ionization of their carboxylic groups, which could contribute to the negative charge of the oil droplets. Of secondary importance in explaining the negative charges of the oil droplets is the orientation of OH^- ions of the aqueous phase in the vicinity of the oil droplets (McClements, 2016). As negative ζ -potentials are observed in the emulsions, it can be stated for this study that Tween 80 might behave as an anionic surfactant. Therefore, the addition of pectin to the LSSO emulsion led to possible electrostatic repulsion between the negatively charged oil droplet surfaces and anionic pectin molecules. Therefore, since Tween 80 is known to be a strong amphiphilic emulsifier that provides steric stabilization at oil/water interfaces, pectin could be assumed to be in the aqueous phase and not to adsorb at the oil droplet.

The addition of pectin to the LSSO emulsions led to less negative ζ -potential values, which was only significant ($p < 0.05$) in the 1% (w/v) pectin containing emulsions (**Fig. 1 D, E, F**). For instance, the ζ -potential of the LSSO emulsion without Fe^{2+} changed from -39.2 ± 7.7 mV to -22.6 ± 1.1 mV, -26.4 ± 0.3 mV and -33.1 ± 1.1 mV with addition of 1% (w/v) M95, P60

and P13, respectively. The ζ -potential is determined by measurement of the Brownian motion of particles through a path when a voltage is applied, which means that the higher the absolute electrical charge of particles, the higher their electrophoretic mobility. Therefore, the less negative ζ -potential values observed when pectin is added to the emulsions, can be attributed to two possible hypotheses. Firstly, if anionic polymers, such as pectin, are located in the aqueous phase, the difference in potential between the oil droplet surface and aqueous phase will be reduced, resulting in a less negative net charge. This implies a slower movement of particles and thus a less negative ζ -potential value. Secondly, the electrophoretic mobility of the particles might be influenced by the particle size, being that smaller particles have a higher mobility than larger particles. In this regard, the addition of pectin to the emulsions led to an increase in volume-weighted mean particle sizes (d_{43}) (**Table 2**, discussion see 3.2.2.), increasing from $0.91 \pm 0.05 \mu\text{m}$ to $1.78 \pm 0.06 \mu\text{m}$, which might have resulted in a slower movement of the particles, explaining the less negative ζ -potential observed in the emulsions with pectin in comparison with the non-pectin containing emulsions. Both hypotheses also explain the observation that higher pectin concentration (1% w/v) led to less negative ζ -potential values compared to lower concentration (0.1% w/v).

The ζ -potential was also slightly less negative with increasing pectin DM, as for example $-33.8 \pm 0.8 \text{ mV}$ for 0.1% (w/v) M95, $-36.4 \pm 0.8 \text{ mV}$ for 0.1% (w/v) P60 and $-38.1 \pm 0.4 \text{ mV}$ for 0.1% (w/v) P13. This might be explained by interference of the pectin negative charges with the measurement of the oil droplet charges. Low DM pectin contains more negative chargeable carboxylic groups, resulting in a more negative ζ -potential. In accordance with our results, Surh et al. (2006) reported that pectin is expected to be present in the aqueous phase when oil droplets exhibit a negative ζ -potential. However, they did not observe an increase in ζ -potential if 0.25 wt% pectin was added to sodium caseinate stabilized O-/W emulsions, which might be explained by the lower pectin concentration compared to the present study. In

addition, they did not observe differences between low and high DM pectin. As a result, the influence of pectin on the ζ -potential of O/W emulsions might be mainly concentration dependent, since in this study only significant differences in ζ -potentials were observed in LSSO emulsions containing 1% (w/v) pectin.

The addition of Fe^{2+} (0.1 mM) to the LSSO emulsions did not significantly influence the electrical charge of the oil droplets, except for the emulsion without pectin (**Fig. 1**). In the latter, Fe^{2+} might absorb onto the negatively charged oil droplets and lead to less negative ζ -potential values. Ogawa, Decker & McClements (2003) also observed less negative ζ -potential values when adding Ca^{2+} ions to lecithin stabilized O/W emulsions and attributed this to adsorption of Ca^{2+} ions on the negatively charged oil droplet surfaces. In the LSSO emulsions containing pectin, Fe^{2+} is probably bound to pectin, which is located in the aqueous phase, thus not being able to increase the electrical charge of the oil droplets.

3.2.2. Emulsion particle size, creaming index and microstructure

The presence of hydrocolloids in emulsions may compromise emulsion stability (Dickinson, 2003). Therefore, the emulsions particle size distribution and creaming index (%) were measured to detect possible destabilization phenomena occurring during 2 weeks of storage at 25 °C. The volume-weighted (d_{43}) and surface-weighted (d_{32}) mean particle sizes are presented in **Table 2** for each LSSO emulsion as a function of storage time (day 0, 7 and 14), together with the creaming index (%), measured at day 14. Both the d_{43} and d_{32} values of the samples showed the same trend. However, the discussion will mainly focus on the volume-weighted mean particle size, as it is more sensitive to particle size growth. Representative micrographs of LSSO emulsions with pectin (1% w/v) and Fe^{2+} addition and its changes during storage time are shown in **Fig. 2**. No clear differences were observed between the micrographs of the emulsions with and without Fe^{2+} .

The LSSO emulsion presented an initial mean particle size of 0.91 μm which remained constant during 14 days of storage, without evidences of creaming (**Table 2**). Moreover, after the addition of Fe^{2+} (0.1 mM) to the LSSO emulsion at day 0, their particle size remained unchanged. During storage time, LSSO emulsion with Fe^{2+} exhibited a significant larger particle size compared to the emulsion without Fe^{2+} . Nonetheless, no creaming could be detected after 14 days of storage in both the LSSO emulsion and the Fe^{2+} -containing LSSO emulsion. This evidenced that, despite the addition of a positively charged entity to the system (Fe^{2+}), the strong steric stabilization provided to the oil droplets by the surfactant (Tween 80) was not compromised at this Fe^{2+} concentration (0.1 mM), which was also confirmed by the microscopic images (**Fig. 2 A, B, C**). Nevertheless, preliminary studies (data not shown) revealed that the addition of an increased Fe^{2+} concentration to LSSO emulsions, up to 2 mM, lead to strong emulsion instability, which was already noticeable at day 0. This implies that Fe^{2+} at high concentrations may absorb at the oil droplet surface, resulting in a decrease of the electrostatic repulsion between the oil droplets and inducing emulsion instability. These results might be important regarding the formulation of emulsion-based food products with a high Fe^{2+} content.

The addition of pectin solutions to the LSSO emulsion led to an immediate significant ($p < 0.05$) increase in their particle sizes, varying from 1.08 ± 0.04 to 1.92 ± 0.05 μm (**Table 2**), regardless the molecular characteristics of the pectin samples. Nevertheless, this increase was in general more pronounced at higher pectin concentrations (1% w/v) in comparison with lower pectin concentrations (0.1% w/v). The increase in particle size was probably caused by droplet flocculation, which was clearly visible in the microscopy images of LSSO emulsions immediately after being mixed with pectin solutions (day 0) (**Fig. 2 D, G, J**). Pectin is considered to act as a stabilizing agent in emulsions due to the viscosity increase of the aqueous phase, resulting in the hindrance of oil droplets movement (Dickinson, 2003).

However, when emulsions are stabilized with strong amphiphilic emulsifiers, such as Tween 80, biopolymers, such as pectin, tend to be located in the bulk aqueous phase. Moreover, due to electrostatic repulsion between the negatively charged oil droplets and the anionic pectin, pectin is assumed to be in the aqueous phase. In this situation, biopolymer molecules are excluded from the vicinity of the oil droplets, thus leading to a pectin concentration depleted zone. The pectin concentration difference between the surroundings of the oil droplets and the bulk aqueous phase creates an osmotic driving force caused by the movement of water to the bulk aqueous phase, resulting in the attraction of oil droplets together. In addition to the depletion flocculation, pectin is reported to cause bridging flocculation which also may contribute to the abovementioned droplet flocculation (Dickinson, 2003). Similar results were obtained by Surh et al. (2006), who observed that the presence of pectin increased the volume-weighted mean diameter of the particle sizes substantially in emulsions exhibiting a negative ζ -potential.

During storage time, the mean particle sizes of the LSOO emulsions containing 0.1% (w/v) pectin (with or without Fe^{2+}) did not significantly change. However, at higher pectin concentrations (1% w/v), the particle size became gradually larger during the two weeks storage time (**Table 2**). Namely, the oil droplet size of the emulsions with 1% (w/v) M95, P60 and P13 increased from 1.76 ± 0.10 to 3.81 ± 0.23 μm , from 1.78 ± 0.06 to 15.20 ± 2.33 μm and from 1.14 ± 0.08 to 5.84 ± 0.59 μm , respectively. Despite that the oil droplet size remained constant during storage after the addition of 0.1% (w/v) pectin solution, a noticeable emulsion creaming was observed at both pectin concentrations as reflected by the creaming index values (**Table 2**). This evidences that the flocs formed immediately after mixing the LSOO emulsion with pectin solutions, led to eventually gravitational separation and ultimately to the creaming of the oil droplets. Moreover, at a high pectin concentration (1% w/v), the flocculation and creaming led to the coalescence of oil droplets, thus explaining the

increase in particle size during storage time. In fact, the coalescence phenomena, with visible larger oil droplets, was also observed in the microscopy images (**Fig. 2 F, I, L**). During the measurement of the particle size by light scattering technique, samples were diluted in a stirring tank filled with distilled water, thus causing the decrease in pectin concentration and disrupting the flocs formed. However, when the flocculation led to the coalescence, this is reflected by an increase in the oil droplet size (Salvia-Trujillo et al., 2016). In addition, the higher instability of LSSO emulsions containing 1% (w/v) pectin was confirmed by the reported creaming indices, which were generally higher than those from the 0.1% (w/v) pectin containing emulsions (**Table 2**). These results are in agreement with the findings of Guerra-Rosas et al. (2016), who observed a substantial increase in average particle size as a function of storage time (up to 56 days) in oregano and thyme nanoemulsions containing 1% (w/v) high methylesterified pectin.

In general, the DM of the pectin samples had only little influence on the emulsion destabilization observed. For all pectin types, depletion flocculation and coalescence was observed in LSSO emulsions containing 0.1 and 1% (w/v) pectin, respectively. According to Surh et al. (2006), this may be explained by similar driving forces for depletion flocculation for each pectin sample due to their similar observed molar masses (**Table 1**). Emulsions with 1% (w/v) P60 exhibited higher particle sizes during storage time (**Table 2**). However, no clear differences were observed by visualization of the microstructure compared to the other DMs (**Fig. 2**). At lower DMs, slightly lower the creaming indices were observed, which can be related to more compact creaming layers (**Table 2**).

In bulk oils and emulsions, lipid oxidation occurs at very low rate, therefore Fe^{2+} was added to the emulsions to accelerate the lipid oxidation process, thus allowing the observation of the antioxidant capacity of citrus pectin. In preliminary experiments (data not shown), high destabilization of LSSO emulsions (without pectin) was observed after Fe^{2+} addition, at

concentrations of 0.1 mM Fe^{2+} and higher. Therefore, the influence of Fe^{2+} on both lipid oxidation and emulsion stability was studied in this work. The incorporation of Fe^{2+} (0.1 mM) to the LSSO emulsions containing pectin had no additional significant influence on droplet flocculation based on the measured particle sizes at day 0 (**Table 2**). However, a large increase in particle size was observed for samples containing 1% (w/v) M95 or P60 as the storage time increased, which could indicate that Fe^{2+} adsorbed onto the negatively charged oil droplet surface and decreased the electrostatic repulsions between the oil droplets, leading to larger particle sizes. In general, thinner cream layers were observed in the emulsions with Fe^{2+} in comparison with those not containing Fe^{2+} . This might be due to the screening effect that Fe^{2+} can induce to the negatively charged pectin molecules, leading to pectin molecules with less intramolecular repulsion within the anionic groups and therefore more compact layers of pectin between oil droplets may be formed.

3.3. Emulsions oxidative stability

The influence of pectin DM and DB_{abs} on the Fe^{2+} catalyzed lipid oxidation was studied in LSSO emulsions by measuring the peroxide concentration during storage, as a measure of the primary lipid oxidation products. LSSO emulsions were prepared with the addition of 0.1 mM Fe^{2+} as a pro-oxidant and pectin with distinct DMs and DB_{abs} at two different concentrations (0.1 and 1% w/v). Experimental results of the peroxide measurements and predicted curves from **Equations 4, 5 and 6** are shown in **Fig. 3 (A-E)**. For all systems studied, lipid oxidation could be described by three consecutive reactions consisting firstly of a lag phase where the formation of free radicals occurs (initiation), secondly the formation of lipid peroxides (propagation) and thirdly a subsequent degradation of peroxides into secondary oxidation products (termination). These three reactions are described by three different rate constants, being k_1 , k_2 and k_3 , respectively (**Table 3**). Nevertheless, for the sake of studying the influence of addition of pectin with different molecular characteristics on the overall lipid

oxidation reaction, special focus will be put on determining differences in the ratio of estimated parameters k_3/k_2 , represented in **Fig. 3F** and **Table 3**.

Pectin addition to the LSSO emulsion led, in general, to a delayed lag phase of the lipid oxidation and overall reduction of lipid oxidation reaction depending on the concentration used and its structural characteristics (DM and DB_{abs}) (**Fig. 3**). The addition of M95 and C58 pectins to the emulsions did not retard the initiation and propagation phase, whereas in the emulsions with P60, P33 and P13, a delayed lag phase was observed. Thus, the addition of low or intermediate DM pectin, associated with high DB_{abs} , to emulsions led to a delay in the lipid oxidation reaction, even at low concentration (0.1% w/v). Moreover, in emulsions with high pectin concentration and/or low DM (particularly with high DB_{abs}) the maximum peroxide values was substantially reduced. Despite this, samples containing pectin presented higher k_1 values than the LSSO emulsion without pectin, implying a higher rate of the initiation phase (**Table 3**). Nevertheless, k_1 values ranged from 0.0002 ± 0.0001 to $0.0535 \pm 0.0322 \text{ days}^{-1}$, with a high uncertainty, up to 97%. Therefore, k_1 did not consistently explain differences in the initiation phase of lipid oxidation between the pectin samples with different DM or DB_{abs} .

More clear differences could be established when evaluating the ratio k_3/k_2 (**Table 3**), being the ratio between the estimated rate constants of the degradation and formation of lipid peroxides. A higher ratio is related with a higher antioxidant capacity as it implies a lower rate constant of lipid peroxides formation and/or a faster degradation of peroxides within the emulsion. However, this ratio has to be carefully interpreted in case of equal k_2 values. In that case, the ratio would only imply differences in k_3 and not being meaningful in comparing antioxidant capacities, as a higher k_3 value implies faster conversion to secondary oxidation products. Nevertheless, evaluating the ratio between k_3 and k_2 was found to be useful in describing the shape of the lipid peroxide curve as a function of time, and therefore was used

to describe the influence of pectin concentration and structure on its lipid antioxidant capacity. On the one hand, regardless the pectin DM, a higher pectin concentration was more effective in decreasing the lipid oxidation reaction. Emulsions containing 1% (w/v) pectin showed higher k_3/k_2 ratios compared to emulsions with 0.1% pectin (w/v), as can be seen in **Fig. 3F** and **Table 3**. Moreover, lower predicted maximum amounts of peroxide values in LSSO emulsions with 1% (w/v) pectin were observed compared to 0.1% (w/v), with a decrease up to 65% (**Fig. 3**). For instance, at 0.1% (w/v) M95 a maximum predicted amount of 14.5 mEq peroxide/L emulsions was found compared to 5.8 mEq peroxide/L emulsion for 1% (w/v) M95 and a decrease from 10.6 to 3.9 mEq peroxide/L emulsion was found for 0.1 and 1% (w/v) P33 respectively. On the other hand, and in agreement with the initial hypothesis, the DM influenced the lipid antioxidant capacity of pectin. High (M95) and intermediate (P60 and C58) DM pectin showed a remarkable antioxidant capacity at 1% (w/v), but rather limited at 0.1% (w/v), except for 0.1% (w/v) P60, where a substantial delay of the lipid oxidation was observed (**Fig. 3 A, B, C**). The k_3/k_2 ratios of emulsions containing 0.1% (w/v) pectin, independently of their DM, did not change remarkably from the LSSO emulsion without pectin, while this ratio increased from 129.0 ± 25.7 for the LSSO emulsion without pectin to 358.5 ± 226.6 for 1% (w/v) M95, to 211.4 ± 27.0 for 1% (w/v) P60 and to 292.2 ± 89.0 for 1% (w/v) C58 pectins (**Table 3**).

Moreover, low DM pectin (P33 and P13) exhibited an antioxidant capacity at both high (1% w/v) and low (0.1% w/v) concentration, although the capacity to slow down the lipid oxidation was more pronounced at 1% (w/v) (**Fig. 3 D, E**). In this sense, low DM pectin was more effective in decreasing the lipid oxidation reaction compared to high or intermediate DM, reflected by higher k_3/k_2 ratios (**Table 3** and **Fig. 3F**). In addition, the maximum predicted amounts of peroxide values in the LSSO emulsions with low DM pectin were clearly lower than in the emulsions containing high or intermediate DM pectin, with 2.2 mEq

peroxide/L emulsion for 1% (w/v) P13 compared to 9.8 mEq peroxide/L emulsion for 1% (w/v) P60 (**Fig. 3**). These results confirm the hypothesis that non-methylesterified GalA units in pectin can bind the pro-oxidative Fe^{2+} ions, being removed from the oil droplet surface at which lipid oxidation occurs. Therefore, low DM pectin, in particular with high DB_{abs} , contain a higher number of (successive) negative chargeable carboxylic groups, which can bind more pro-oxidant metal ions as previously reported by Celus et al. (2017), thus explaining the higher antioxidant capacity. The findings of the present study are in agreement with the observations of Chen et al. (2010) and Huang et al. (2011), who reported both that low methylesterified pectin was more effective in inhibiting lipid oxidation compared to high methylesterified pectin and which effect was more pronounced at higher pectin concentration. However, in both studies, pectins with different DM were obtained without specific control in changing only the DM without changing other molecular structures, such as the molecular weight, which might also determine the pectin cation binding capacity and therefore influence the lipid oxidation reaction. Therefore, the present work provides a novel mechanistic modeling insight of the antioxidant capacity of pectin with tailored molecular characteristics and similar molecular weight.

The influence of pectin DB_{abs} on lipid oxidation was studied by comparing the results of C58 and P60, both samples with similar DM but distinct DB_{abs} values (**Table 1**). No clear differences were observed in the k_3/k_2 ratio between the two pectin samples, for either 0.1 or 1% (w/v) (**Table 3** and **Fig. 3F**). In addition, the maximum predicted amount of peroxides were not largely different between P60 and C58 (**Fig. 3 B, C**), namely 14.3 and 17.4 mEq peroxide/L emulsion for 0.1% (w/v) and 9.8 and 7.2 mEq peroxide/L emulsion for 1% (w/v) P60 and C58, respectively. However, it is noteworthy that the lipid oxidation in the LSSO emulsion containing P60 showed a delayed lag phase compared to C58, suggesting that the distribution pattern of the negative chargeable carboxylic groups plays a slight role in the

pectin antioxidant capacity. These results are in accordance with the hypothesis as well, that pro-oxidant metal ions can bind more and/or stronger to successive negatively charged GalA units in the pectin chain. Nonetheless, pectin samples with intermediate DM presented a limited antioxidant capacity. In this sense, pectin samples with a lower DM but distinct DB_{abs} could have provided a clearer insight into the role of pectin DB_{abs} on the lipid antioxidant capacity of emulsions. Nevertheless, when further decreasing the pectin DM, the high number of non-methylesterified units leads to an almost completely demethylesterified pectin backbone, thus rendering the differences in the distribution pattern less clear.

Conclusion

This study explored the influence of citrus pectin structural properties, particularly the degree of methylesterification (DM) and absolute degree of blockiness (DB_{abs}), on the physicochemical and oxidative stability of linseed/sunflower oil (LSSO) emulsions, previously stabilized by Tween 80. Pectin was located in the aqueous phase of LSSO emulsions and caused physicochemical instability, which was concentration dependent. The addition of pectin to the emulsion systems caused the flocculation of oil droplets and subsequent creaming, which led to coalescence when a high concentration of pectin (1% w/v) was added due to stronger osmotic attractions between the oil droplets. The DM of pectin, which determines its anionic nature, did not play a role in the physicochemical stability of the LSSO emulsions. Nevertheless, pectin DM clearly influenced the oxidative stability of LSSO emulsions. Low DM pectin ($\leq 33\%$) exhibited a higher lipid antioxidant capacity compared to high or intermediate DM pectin ($\geq 58\%$), which was concentration dependent. The pectin lipid antioxidant activity can be mainly attributed to its capacity to chelate the pro-oxidant Fe^{2+} , which can be established by ionic interaction with the negatively chargeable carboxylic groups. The DB_{abs} , as a measure of the distribution pattern of non-methylesterified carboxylic groups, did only slightly influence the lipid antioxidant capacity of pectin at a given DM, by

delaying the lipid oxidation. The results of this study suggest that citrus pectin can be useful as a natural alternative to artificial antioxidants. Moreover, targeted pectin modification can direct its binding with cations and therefore its lipid antioxidant capacity. However, since pectin may cause emulsion instability, food formulation should be carried out carefully to obtain stable emulsion-based food products, in which pectin functionalities such as a natural lipid antioxidant can be exhibited optimally.

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Declaration of interests

The authors of the present work declare no conflict of interests

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Figure captions

Figure 1: ζ -potential (mV) $\pm$ standard deviation at day 0 of storage of the pectin solutions (A-C) and linseed/sunflower oil (LSSO) emulsions with (0.1 and 1% w/v) and without demethylesterified pectin samples (D-F), with and without Fe^{2+} (0.1 mM).

Figure 2: Micrographs at day 0, 7 and 14 of storage of the linseed/sunflower oil (LSSO) emulsions without (A-C) and with (1% w/v) (D-L) demethylesterified pectin samples, with Fe^{2+} (0.1 mM). Scale bar represents 50 μm .

Figure 3: Peroxide concentration (mEq peroxide/L emulsion) as a function of storage time (days) (A-E) and ratio of the estimated rate constant k_3/k_2 (F) of the linseed/sunflower (LSSO) oil emulsions with (0.1 and 1% w/v) and without demethylesterified pectin samples, with Fe^{2+} (0.1 mM). Symbols indicate the experimental data $\pm$ standard deviation. Black (—), dotted (.....) and dashed (-----) curves represent the modeled peroxide concentrations in the emulsions with 0, 0.1 and 1% (w/v) pectin, respectively.

Fig. 1

Fig. 2

Fig. 3

Table 1

Sample code	DM (%)	DB _{abs} (%)	GalA (g GalA/g pectin)	M _w (kDa)	Intrinsic Fe concentration (µg/g pectin)
M95	95.1 ± 1.8 ^a	< 0.74 ^e	0.69 ± 0.01 ^c	56.1 ± 1.3 ^{bc}	17.1
P60	59.8 ± 1.4 ^b	29.0 ± 1.3 ^c	0.76 ± 0.02 ^{ab}	64.4 ± 3.0 ^{ab}	36.8
P33	33.0 ± 2.1 ^c	57.1 ± 2.5 ^b	0.70 ± 0.03 ^c	61.7 ± 2.4 ^{ab}	18.4
P13	13.0 ± 1.3 ^d	82.4 ± 1.7 ^a	0.72 ± 0.03 ^{bc}	66.2 ± 0.1 ^a	11.0
C58	58.3 ± 1.2 ^b	6.8 ± 0.3 ^d	0.76 ± 0.04 ^a	48.1 ± 2.2 ^c	21.6

Table 1: Overview of structural characteristics of the demethylesterified pectin samples, with P the enzymatic demethylesterified pectins, C the chemical demethylesterified pectin and M95 refers to the mother pectin. DM (%) is the degree of methylesterification ± standard deviation, DB_{abs} (%) is the absolute degree of blockiness ± standard deviation, GalA (g GalA/g pectin) is the galacturonic acid content ± standard deviation and M_w (kDa) refers to the weight-average molar mass ± standard deviation. Different letters (a-e) indicate significant ($p < 0.05$) differences between the pectin samples for a structural characteristic.

Table 2

Emulsion	Day 0		Day 7		Day 14		Creaming index (%)
	d ₄₃ (μm)	d ₃₂ (μm)	d ₄₃ (μm)	d ₃₂ (μm)	d ₄₃ (μm)	d ₃₂ (μm)	Day 14
LSSO	0.91 ± 0.05 ^{Af}	0.52 ± 0.01 ^{Ae}	0.88 ± 0.01 ^{Af}	0.51 ± 0.04 ^{Ae}	1.19 ± 0.48 ^{AcD}	0.45 ± 0.01 ^{Ag}	0
LSSO + Fe	0.90 ± 0.04 ^{Cf}	0.51 ± 0.01 ^{Ae}	5.37 ± 0.07 ^{Cb}	0.49 ± 0.01 ^{Ae}	3.60 ± 0.05 ^{Bcd}	0.52 ± 0.04 ^{Ag}	0
LSSO + 0.1% M95	1.20 ± 0.01 ^{Ae}	0.87 ± 0.01 ^{AcD}	1.20 ± 0.02 ^{Af}	0.87 ± 0.01 ^{Ad}	1.13 ± 0.02 ^{Bd}	0.85 ± 0.01 ^{Bf}	20.0
LSSO + 0.1% M95 + Fe	1.17 ± 0.01 ^{Be}	0.86 ± 0.01 ^{Bd}	1.20 ± 0.03 ^{ABf}	0.86 ± 0.01 ^{Bd}	1.25 ± 0.05 ^{Ad}	0.88 ± 0.01 ^{Af}	14.0
LSSO + 1% M95	1.76 ± 0.10 ^{Cbc}	1.18 ± 0.07 ^{Cb}	2.20 ± 0.03 ^{Be}	1.44 ± 0.03 ^{Bc}	3.81 ± 0.23 ^{AcD}	1.83 ± 0.07 ^{Ae}	28.4
LSSO + 1% M95 + Fe	1.62 ± 0.02 ^{Bc}	1.13 ± 0.01 ^{Cb}	2.25 ± 0.20 ^{Be}	1.47 ± 0.09 ^{Bc}	21.45 ± 0.68 ^{Aa}	2.28 ± 0.02 ^{Ac}	22.5
LSSO + 0.1% P60	1.14 ± 0.04 ^{Ae}	0.85 ± 0.01 ^{Ad}	1.11 ± 0.03 ^{Af}	0.84 ± 0.01 ^{Ad}	1.18 ± 0.05 ^{Ad}	0.87 ± 0.02 ^{Af}	19.4
LSSO + 0.1% P60 + Fe	1.08 ± 0.04 ^{Ae}	0.83 ± 0.01 ^{Ad}	1.12 ± 0.02 ^{Af}	0.84 ± 0.01 ^{Ad}	1.05 ± 0.06 ^{Ad}	0.83 ± 0.02 ^{Af}	12.7
LSSO + 1% P60	1.78 ± 0.06 ^{Bab}	1.16 ± 0.03 ^{Cb}	3.07 ± 0.49 ^{Bd}	1.72 ± 0.11 ^{Bb}	15.20 ± 2.33 ^{Ab}	2.88 ± 0.13 ^{Ab}	23.8
LSSO + 1% P60 + Fe	1.92 ± 0.05 ^{Ba}	1.26 ± 0.02 ^{Ca}	7.38 ± 0.11 ^{Ba}	1.92 ± 0.09 ^{Ba}	24.73 ± 5.34 ^{Aa}	3.34 ± 0.27 ^{Aa}	21.6
LSSO + 0.1% P13	1.14 ± 0.04 ^{Ae}	0.88 ± 0.02 ^{AcD}	1.07 ± 0.03 ^{Bf}	0.84 ± 0.01 ^{Bd}	1.08 ± 0.01 ^{Bd}	0.85 ± 0.01 ^{Bf}	18.4
LSSO + 0.1% P13 + Fe	1.09 ± 0.06 ^{Ae}	0.86 ± 0.02 ^{Ad}	1.09 ± 0.07 ^{Af}	0.87 ± 0.01 ^{Ad}	1.11 ± 0.03 ^{AcD}	0.86 ± 0.01 ^{Af}	15.1
LSSO + 1% P13	1.14 ± 0.08 ^{Ce}	0.83 ± 0.01 ^{Cd}	3.25 ± 0.06 ^{Bd}	1.72 ± 0.01 ^{Bb}	5.84 ± 0.59 ^{Ac}	2.02 ± 0.04 ^{Ad}	16.0
LSSO + 1% P13 + Fe	1.39 ± 0.09 ^{Cd}	0.93 ± 0.03 ^{Cc}	3.73 ± 0.12 ^{Bc}	1.87 ± 0.02 ^{Ba}	4.13 ± 0.13 ^{AcD}	2.07 ± 0.02 ^{AcD}	18.3

Table 2: Volume-weighted (d₄₃) and surface-weighted (d₃₂) particle sizes (μm) ± standard deviation, measured at day 0, 7, 14 of storage and the creaming index (%) at day 14 of storage of the linseed/sunflower (LSSO) emulsions with (0.1 and 1% w/v) and without demethylesterified pectin samples, with and without Fe²⁺ (0.1 mM). Different capital letters (A-C) indicate significant differences (p < 0.05) in d₄₃ or d₃₂ between storage times of the same sample. Different lower case letters (a-g) indicate significant differences (p < 0.05) in d₄₃ or d₃₂ between samples of the same storage day.

Table 3

Emulsion	k_1 (days ⁻¹)	k_2 (days ⁻¹)	k_3 (days ⁻¹)	k_3/k_2
LSSO + Fe	0.0026 ± 0.0021	0.0070 ± 0.0011	0.897 ± 0.109	129.0 ± 25.7
LSSO + 0.1% M95 + Fe	0.0085 ± 0.0054	0.0059 ± 0.0010	0.800 ± 0.110	136.2 ± 29.8
LSSO + 1% M95 + Fe	0.0535 ± 0.0322	0.0026 ± 0.0012	0.937 ± 0.404	358.5 ± 226.6
LSSO + 0.1% P60 + Fe	0.0002 ± 0.0001	0.0072 ± 0.0003	1.000 ± 0.028	139.0 ± 6.6
LSSO + 1% P60 + Fe	0.0007 ± 0.0004	0.0068 ± 0.0007	1.438 ± 0.119	211.4 ± 27.0
LSSO + 0.1% C58 + Fe	0.0018 ± 0.0006	0.0069 ± 0.0005	0.763 ± 0.025	110.5 ± 9.0
LSSO + 1% C58 + Fe	0.0066 ± 0.0064	0.0080 ± 0.0019	2.327 ± 0.429	292.2 ± 89.0
LSSO + 0.1% P33 + Fe	0.0047 ± 0.0013	0.0038 ± 0.0003	0.732 ± 0.032	193.9 ± 17.3
LSSO + 1% P33 + Fe	0.0210 ± 0.0115	0.0013 ± 0.0007	0.718 ± 0.345	541.8 ± 390.6
LSSO + 0.1% P13 + Fe	0.0077 ± 0.0057	0.0033 ± 0.0009	1.163 ± 0.246	357.9 ± 124.5
LSSO + 1% P13 + Fe	0.0401 ± 0.0169	0.0023 ± 0.0009	2.254 ± 0.765	989.9 ± 509.7

Table 3: Parameter estimates of rate constants k_1 , k_2 and k_3 (days⁻¹) ± standard deviation and the ratio k_3/k_2 ± standard deviation describing the peroxide concentration as a function of storage time (days) of the linseed/sunflower oil (LSSO) emulsions with (0.1 and 1% w/v) and without demethylesterified pectin samples, with Fe^{2+} (0.1 mM).

- Pectin samples with distinct nanostructures were produced as a starting point.
- All pectins exhibited an antioxidant capacity in linseed/sunflower oil emulsions.
- The degree of methylesterification mainly determined the antioxidant capacity.
- Low demethylesterified pectin showed the highest antioxidant capacity.
- Pectin (0.1 and 1% w/v) caused instability of linseed/sunflower oil emulsions.