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

Miete Celus*, Laura Salvia-Trujillo, Clare Kyomugasho, Ine Maes, Ann M. Van Loey, Tara Grauwet, Marc E. Hendrickx

Laboratory of Food Technology, Leuven Food Science and Nutrition Research Centre (LFoRCe), Department of Microbial and Molecular Systems (M^2S), KU Leuven, Kasteelpark Arenberg 22, Box 2457, 3001 Leuven, Belgium

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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 (≤33%) exhibited a higher antioxidant capacity than high demethylesterified pectin (≥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 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.

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 a 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 & Decker, 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 peroxyl and alkoxyl radicals (McClements & Decker, 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 & Decker, 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 & Decker, 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

* Corresponding author at: Laboratory of Food Technology, Leuven Food Science and Nutrition Research Center (LFoRCe), Department of Microbial and Molecular Systems (M2S), KU Leuven, Kasteelpark Arenberg 22, Box 2457, 3001 Heverlee, Belgium.
E-mail addresses: miete.celus@kuleuven.be (M. Celus), laura.salviatrujillo@kuleuven.be (L. Salvia-Trujillo), clare.kyomugasho@kuleuven.be (C. Kyomugasho), ine-maes@hotmail.com (I. Maes), ann.vanloey@kuleuven.be (A.M. Van Loey), tara.grauwet@kuleuven.be (T. Grauwet), marceg.hendrickx@kuleuven.be (M.E. Hendrickx).

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of *clean label* products, which are more appealing to consumers (McClements & Decker, 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 *pKa* of pectin (3.38–4.10) thereby exhibiting the ability to interact with metal ions (Kohn, 1987; Kyomugasho, Willemsen, Christiaens, Van Loey, & Hendrickx, 2015). 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 et al., 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 et al., 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 PMEs, with an acidic pI, results in more random distributed demethylesterified GalA units (Cameron, Luzio, Goodner, & Williams, 2008; Limberg et al., 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 pectin application (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 MΩ cm resistance) was supplied by a Simplicity™ 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 ± 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