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Fe^{2+} adsorption on citrus pectin is influenced by the degree and pattern of methylesterification

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ABSTRACT

The present study aimed at gaining insight into the potential of citrus pectin to bind Fe^{2+}, a cation of great importance in several food products. In particular, the role of citrus pectin structural properties, namely the degree of methylesterification (DM) and the absolute degree of blockiness (DB_{abs} – ratio of non-methylesterified GalA units present in blocks to the total amount of GalA units) on the Fe^{2+} adsorption in aqueous solution was explored using adsorption isotherms. Demethylesterification of high DM citrus pectin enzymatically (using plant pectin methylesterase) or chemically (alkaline demethylesterification using NaOH) generated P- and C-pectins, respectively, characterized by comparable DMs but different distributions of non-methylesterified GalA units (DB_{abs}). Adsorption isotherms of P- and C-pectins in aqueous solutions of various Fe^{2+} concentrations revealed that both the DM and DB_{abs} influenced the pectin-Fe^{2+} interactions: the lower DM or higher DB_{abs}, the higher the Fe^{2+} binding capacity of citrus pectin. The Langmuir adsorption model was used to fit the experimental data for quantification of the maximum adsorption capacity (q^G_{max}) and the pectin-Fe^{2+} interaction energy (K_L) of the P- and C-pectins. It can be concluded that q^G_{max} (mol Fe^{2+}/mol GalA) was mainly determined by the DM and to a lesser extent by the DB_{abs} while the pectin-Fe^{2+} interaction energy was mainly influenced by the DB_{abs}. As a consequence, pectin modification to obtain targeted DM and DB_{abs} allows optimization of its binding capacity and therefore the associated functional properties.

1. Introduction

Pectin, a major functional ingredient in the food industry, is commonly extracted from by-products of the fruit juice industry, especially apple and citrus, for use as thickening or gelling agent in sauces and jams (May 1990; Petkowicz, Vriesmann, & Williams, 2017; Thakur, Singh, Handa, & Rao, 1997; Thibault & Ralet, 2003; Willats, Knox, & Mikkelsen, 2006). Pectin is also used in the pharmaceutical industry, as a carrier in colon-specific drug delivery systems (Liu, Fishman, & Hicks, 2006) or to reduce blood cholesterol levels (Wicker et al., 2014). These pectin functionalities, including its ability to bind divalent cations, are largely related to its structural diversity (Dronnet, Renard, Axelos, & Thibault, 1996; Jamsazzadeh Kermani, Shpigelman, Pham, Van Loey, & Hendrickx, 2015; Mohnen, 2008; Willats et al., 2006). Structurally, pectin is a complex cell wall heteropolysaccharide consisting of three covalently linked building blocks, homogalacturonan (HG), rhamnogalacturonan I and II (RG I and II). HG is the predominant and linear pectin domain with a backbone consisting of α-1,4-linked galacturonic acid (GalA) residues, while RG I and II are more branched pectin domains, composed of additional monosaccharides such as rhamnose, galactose or arabinose (Caffall & Mohnen, 2009; Voragen, Coenen, Verhoef, & Schols, 2009; Yapo, Lerouge, Thibault, & Ralet, 2007).

Of particular interest in the cation binding capacity of pectin are the structural properties of the HG pectin substructure. The degree of methylesterification (DM) of pectin, which is the percentage of GalA units of HG that are methylesterified at C6, is of great importance in determining its polyanionic nature and associated functional properties (Voragen et al., 2009). It is reported that the non-methylesterified GalA residues can be negatively charged at pH values higher than the *pKa* of pectin (3.38–4.10) and thereby possessing the ability to interact with divalent cations

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(Kyomugasho et al., 2017; Manrique & Lajolo, 2002; Sriamornsak, 2003; Thibault & Ralet, 2003). In addition to DM, the distribution pattern of non-methylesterified GalA units is hypothesized to have an important role in the cation binding capacity of pectin (Voragen et al., 2009). In fact, the DM and pattern of methylesterification have already been greatly explored in promoting pectin-Ca^{2+} interactions in context of gel formation, the more extensive pectin application (Fraeye et al., 2009; Lutz, Aserin, Wicker, & Garti, 2009; Löfgren, Guillotin, Evenbratt, Schols, & Hermansson, 2005; Ralet, Dronnet, Buchholt, & Thibault, 2001; Slavov et al., 2009; Ström et al., 2007; Tanhatan-Nasseri, Crépeau, Thibault, & Ralet, 2011; Willats et al., 2006). According to Löfgren et al. (2005) and Ralet et al. (2001), the lower the DM and the higher the number of successive de-esterified GalA units in HG, the more sensitive the pectin chains are to Ca^{2+} cross-linking, resulting in a formation of stiffer gels. Nonetheless, pectin interactions with divalent cations are not necessarily established via negatively charged carboxylic groups. Moreover, a recent study by Assifaoui et al. (2015) showed that the type of interaction between cations and pectin is also cation dependent. According to the aforementioned researchers, Zn^{2+} interacts with both the charged carboxylic group as well as with hydroxyl groups of GalA while Ca^{2+} binding occurs only via carboxylate groups. Thus, interactions between divalent cations and pectin, either with high DM, either at low pH, might occur as well via cross-linking mechanisms other than via negatively charged carboxylic groups (Assifaoui et al., 2015; Thibault & Ralet, 2003). However, the degree and pattern of methylesterification are reported to be of major interest.

The nanostructure of pectin can be modified enzymatically or chemically to direct pectin functionality, in this context its cation binding capacity. On the one hand, enzymatic demethylesterification can be achieved by the action of pectin methylesterase (PME), which catalyzes the hydrolysis of methylesters at C-6 of GalA units, resulting in negatively chargeable carboxylic groups. PMEs with an alkaline (mostly plant PME) or acidic (microbial PME) pI are distinguished. Plant PMEs are known to hydrolyse methylesters in a blockwise manner, via a single chain or multiple attack mechanism. Single chain mechanism involves hydrolysis of the methylesters until the end of the pectin chain or a blocking residue is reached prior to dissociation of the enzyme-substrate complex, whereas multiple attack mechanism describes hydrolysis of a limited average number of subsequent methylesters per enzyme attack. Demethylesterification by microbial PMEs results in more randomly distributed demethylesterified GalA units (Cameron, Luzio, Goodner, & Williams, 2008; Daas, Meyer-Hansen, Schols, Ruiter, & Voragen, 1999; Limberg et al., 2000; Ralet et al., 2001; Willats et al., 2001). On the other hand, chemical demethylesterification, which includes a treatment of pectin with an alkali (sodium hydroxide), generates randomly distributed non-methylesterified GalA units (Limberg et al., 2000). Enzymatic and chemical demethylesterification consequently result in different distributions of non-methylesterified GalA units, quantified by the degree of blockiness (DB), a concept first defined by Daas et al. (1999). The DB was established by treatment of pectin with endo-polygalacturonase followed by determination of the ratio of the amount of non-methylesterified mono-, di- and trimers released by the enzyme to the total number of non-methylesterified GalA units. Demethylesterification by plant PME results in pectins with a higher DB than alkaline demethylesterified pectins for a given DM. In addition to the DB, the absolute degree of blockiness (DB_{abs}), which is the ratio of the amount of non-methylesterified mono-, di- and trimers released by the enzyme to the total amount of GalA units, is commonly used and expresses the absolute number of blocks of non-methylesterified GalA in pectin (Guillotin et al., 2005). In this study, the DB_{abs} is used, given that a number of authors suggested that DB_{abs} is more informative than DB (Guillotin et al., 2005; Slavov et al., 2009; Ström et al., 2007).

Although pectin DM and DB are extensively explored in the context of gelation with Ca^{2+}, pectin has also been a subject of adsorption studies with cations other than Ca^{2+}. For instance, pectin has been explored for use in removing toxic metal ions from humans (Braudo et al., 1996; Eliaz, Hotchkiss, Fishman, & Rode, 2006; Zhao et al., 2008) or heavy metals from wastewater (Dronnet et al., 1996; Kartel, Kupchik, & Veisov, 1999; Khotimchenko, Kolenchenko, & Khotimchenko, 2008). In addition, few studies have explored the interaction between pectin and Fe^{2+}, mainly in the context of bio-accessibility (Debon & Tester, 2001; Kim, 1998; Kyomugasho et al., 2017; Miyada, Nakajima, & Ebihara, 2012). This is interesting, given that Fe^{2+} can promote lipid oxidation in a variety of lipid-based food products and that Fe^{2+} adsorption by pectin might effectively reduce lipid oxidation (Chen, McClements, & Decker, 2010; Huang, Lu, Wang, & Wu, 2011). Moreover, pectin is a dietary fiber which meets current consumer demand for natural additives (Varela & Fiszman, 2013). Fundamental insight into adsorption of Fe^{2+} by pectin could contribute to this innovative aspect. However, to the best of our knowledge, no quantitative analysis has been performed on the influence of pectin most studied structural properties, DM and DB_{abs}, on the Fe^{2+} adsorption by using adsorption isotherms.

Therefore, the present work aims at investigating the role and extent to which the DM and DB_{abs} influence the Fe^{2+} binding capacity of pectin. Given that the DM and DB_{abs} are hypothesized to influence the pectin-Fe^{2+} interaction, targeted modification of these pectin structural properties, followed by determination of the adsorption isotherms could be of great importance in exploring its Fe^{2+} binding capacity and related pectin functionalities. A better understanding of the influence of these structural factors on the Fe^{2+} adsorption could provide more insight into optimization of pectin-Fe^{2+} interactions in view of its functionalities.

2. Material and methods

2.1. Materials

High methylesterified citrus pectin, with a DM of 95%, from Sigma-Aldrich Belgium, was used as starting material for the production of pectin samples with different degrees and patterns of methylesterification. Carrots (*Daucus carota* cv. Nerac) and kiwis (*Actinidia deliciosa* cv. Hayward) were purchased from a local shop. The carrots were peeled, cut into 0.2 cm slices, frozen in liquid nitrogen and stored at −40 °C until extraction of PME. Kiwis were stored at room temperature to ripen, followed by extraction of PME inhibitor (PMEI). All chemicals used were of analytical grade. Ultrapure water (organic free, 18.2 MΩ cm resistance) was supplied by a Simplicity™ water purification system (Millipore, Billerica, USA) and used for the adsorption as well as analytical experiments.

2.2. Preparation of pectin samples with different degrees and patterns of methylesterification

Commercial citrus pectin, mother pectin with a DM of 95.1 ± 1.8% (M95) was demethylesterified to produce pectin samples with different DMs. In addition, distinct patterns of methylesterification were achieved by applying different methods of demethylesterification. To this extent, enzymatic or chemical demethylesterification of M95 was performed to obtain blockwise or randomly distributed methylesterified GalA units, respectively (Ngouémazong et al., 2011).

Enzymatic demethylesterification with purified plant PME was performed as described by Ngouémazong et al. (2011). First, PMEI