PECTIN AT THE OIL-WATER INTERFACE: RELATIONSHIP OF MOLECULAR COMPOSITION AND STRUCTURE TO FUNCTIONALITY

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Abstract

The present review examines how macromolecular structure and functional groups of pectin affect its functionality with particular focus on its interfacial activity. We venture into a description of the particularly complex pectin structure and describe the major building blocks and their properties. In the following section, the role of each structural parameter is discussed with particular attention to protein, degree of acetylation and methylation, molecular weight, and branching. Finally, we discuss how modification of the extraction conditions could be tailored to obtain pectin with the desired emulsification properties. It is proposed that pectin with protein content in the range of 3%, with degree of acetylation greater than 10%, molecular weight between 100 and 200 $x10^3$ g mol^{-1} and enriched in RG-I segments is more likely to perform well as an emulsifier. To tailor such a structure, an aqueous extraction protocol with low pH values (between 2.5-3.5) with a strong monoprotic acid (e.g., HCl) and one-step solvent precipitation should be selected. The proposed set of extraction conditions could be used as a first step towards rational design of pectin with desirable interfacial functionality.

Keywords: pectin, emulsions, interface, extraction, isolation

1. Introduction

Current food and pharmaceutical processes focus on several critical formulation aspects with the overall aim to improve human health (e.g., functional foods that lower cholesterol) or produce products with consumer-tailored specifications (e.g., products for vegetarians). The challenges arise from the increasing public interest in the availability of "natural" food ingredients where only naturally available materials such as carbohydrates or proteins should be used. For instance, replacement of gelatin or synthetic surfactants (e.g., Tweens) that have been utilized for structuring of foods or hard-shelled capsules are some examples of these demands. Therefore, the investigation of novel structures and sources that could replace existing ingredients is ongoing.

The technological performance as emulsifier of various polysaccharides is usually controlled by its molecular properties (e.g., conformation, polyelectrolyte nature, surface charge density, molecular weight etc.) and its intra- and inter- chain interactions. Several hydrocolloids (e.g., carrageenan, xanthan, Arabic gum) can be used as emulsifiers as they have the ability to rapidly adsorb to the interface, reduce the interfacial tension to facilitate droplet disruption and impede droplet aggregation. This is typically attributed to the presence of hydrophobic elements in biopolymer structure such as protein, ferulic acids, or acetyl groups (Bouyer, Mekhloufi, Rosilio, Grossiord & Agnely, 2012; McClements & Gumus, 2016; Petri, 2015). Pectin is a polysaccharide that is widely utilized across food and pharmaceutical industries as a gelling material, stabilizer or delivery agent. The structural diversity of pectin results in a multitude of functional properties and is considered as a potential multifunctional food and pharmaceutical ingredient. The aims of the present review are to embark on an exploration of how structure of pectin influences its interfacial properties and how

we can manipulate its structure with tailored extraction protocols to achieve optimum functionality.

2. Structural characteristics of pectin

Pectin belongs to family of covalently linked galacturonic acid-rich plant cell wall polysaccharides. They are found in primary cell walls of dicots and non-graminaceous monocots (~35%), in grasses and other commelinids (~2-10%), and in woody tissues (~5%) (Ridley, O'Neill, & Mohnen, 2001). Some pectin molecules are covalently bonded or tightly associated with other types of cell wall polysaccharides, such as hemicelluloses and cellulose (McCann & Roberts, 1991; Mohnen, 2008; Peng & She, 2014). The entire cellulose-hemicellulose network is embedded in a matrix of pectic polysaccharides, which form a hydrated and cross-linked three-dimensional network (Zandleven, et al., 2007). Early work on carbohydrate chemistry of plant cells used the umbrella term "pectic substances", which included pectin and other highly viscous polysaccharides such as xyloglucans (Sinnott, 2007). Current usage confines the word "pectin" to a group of heteropolysaccharides with backbone mainly composed of D-galacturonic acid units (D-Gal*p*A, ~65%) bonded with α-(1→4) glycosidic linkages. The diversity of pectin structures (e.g., length of neutral side chains, molecular weight, degree of polymerization, methyl- and acetyl- esterification, and branching of side chains) depends on the botanical source, plant ripening state and applied extraction conditions (Bagherian, Zokaee Ashtiani, Fouladitajar, & Mohtashamy, 2011; Guo, Zhao, Pang, Liao, Hu, & Wu, 2014; Müller-Maatsch, Bencivenni, Caligiani, Tedeschi, Bruggeman, Bosch, Petrusan, Van Droogenbroeck, Elst, & Sforza, 2016; Ng, Schröder, Sutherland, Hallett, Hall, Prakash, Smith, Melton, & Johnston, 2013; Paniagua, Pose, Morris, Kirby, Quesada, & Mercado, 2014).

In its simplest ideal description, pectin macromolecule is a diblock copolymer of two major structural classes. Homogalacturonan (HG) and rhamnogalacturonan I (RG-I), are found in most pectin assemblies and the intra- and inter- molecular interactions between these two segments control their functional properties. In the majority of the cases, other regions can be also distinguished depending on the source, namely, rhamnogalacturonan II (RG-II), xylogalacturonan (XGA), apiogalacturonan (AGA), arabinogalactan (AG-I, AG-II) and arabinan (Figure 1). Branches with distinct structure from the main backbone originate from the RG-I, RG-II and AG-I ("hairy" regions) making pectin essentially a graft copolymer of HG and RG-I. It should be stressed that extracted pectin is usually polydisperse consisting of complex mixtures of the previously mentioned segments.

Homogalacturonan is the most abundant polymeric segment of pectin, and plant cell walls consist of about 65% HG (Mohnen, 2008) (Figure 1). HG is composed of long chains of linear 1→4 linked α-D-Gal*p*A residues (~200 units) and some of the carboxyl groups are methyl-esterified at C-6 position and/or acetyl-esterified at *O*-2 and/or *O*-3 positions of Gal*p*A depending on plant species (Sinnott, 2007). *O*-Acetyl rich homogalacturonans have been also isolated from sugar beet, cacao pod husks and spinach (Perrone, Hewage, Thomson, Bailey, Sadler, & Fry, 2002; Ralet, Cabrera, Bonnin, Quemener, Hellin, & Thibault, 2005; Vriesmann, Teófilo, & Petkowicz, 2011). Conventionally, HGs with greater than 50% methyl-esterification of Gal*p*A residues are described as high methyl-esterified (HM) and those with lower than 50% are defined as low methyl-esterified (LM). The methyl esterification of linear HG units determines the industrial applicability of pectin (e.g., gelation), which depends not only on the amount of methyl-esterification, but also on

distribution of methyl groups on the HG backbone (Dominiak, Sondergaard, Wichmann, Vidal-Melgosa, Willats, Meyer, & Mikkelsen, 2014).

Rhamnogalacturonan I (RG-I) represents around 20-35% of pectin in plant cell wall (Obro, Harholt, Scheller, & Orfila, 2004). Its backbone is composed of the repeating disaccharide galacturonic acid and rhamnose [α-(1→2)-D-Gal*p*A–α-(1→4)-L-Rha*p*]$_n$ where *n* can be greater than 100 (Figure 1). The RG-I backbone is partially substituted at *O*-4 and/or *O*-3 positions of α-L-Rha*p* residues with polymeric side-chains predominantly composed of α-(1→5)-L-arabinans and β-(1→4)-D-galactans, arabinogalactans-I (AG-I), arabinogalactans-II (AG-II) and galacto-arabinans (Mohnen, 2008) (Figure 1). The side-chains can be a single unit such as β-D-Gal*p*-(1→4), but also polymeric, such as arabinan and arabinogalactan-I (AG-I). The galactan and arabinan side-chains of RG-I are the most flexible parts of the pectin molecule with the higher degree of conformational freedom exhibited by arabinan (Sinnott, 2007). AG-I is composed of α-1→4 linked β-D-Gal*p* backbone and α-L-Ara*f* are attached to the *O*-3 position of galactosyl residues (Ridley, et al., 2001). The galactan chain of AG-I may have branches of one or more Ara*f* residues or a single terminal Ara*p* residue. Arabinogalactans-II (AG-II) are predominantly associated with proteins (arabinogalactan proteins or AGPs) (Vincken, 2003) (Figure 1). The proportion and distribution of branched Rha*p* residues typically varies in the range of 20-80% depending on the source of polysaccharide (Visser & Voragen, 1996). This also results in a heterogeneous structure of RG-I arabinan and galactan side-chains from source to source, something that has been observed for pectic polysaccharides from the walls of apple, sugar beet, soybean, persimmon, and potato (Duan, Wang, Dong, Fang, & Li, 2003; Huisman, Brüll, Thomas-Oates, Haverkamp, Schols, & Voragen, 2001; Obro, et al., 2004; Sakamoto & Sakai, 1995; Schols & Voragen,

1996). However, unbranched RG-I molecules have been also reported in seed mucilages (Western, Young, Dean, Tan, Samuels, & Haughn, 2004). The RG-I backbone can be acetylated at *O*-2 and/or *O*-3 positions of Gal*p*A or at *O*-3 position of Rha*p* residues depending on the plant species (Sengkhamparn, Bakx, Verhoef, Schols, Sajjaanantakul, & Voragen, 2009; Vincken, 2003; Voragen, Coenen, Verhoef, & Schols, 2009). Typically, carboxyl groups of *α*-D-Gal*p*A residue are not methyl-esterified in RG-I, however, methylated RG-I fractions has been reported in pectin isolates from apple, citrus peels, kidney beans and flax hypocotyls (Ridley, et al., 2001; Rihouey, Morvan, Borissova, Jauneau, Demarty, & Jarvis, 1995).

Rhamnogalacturonan II (RG-II) is a minor (~ 10%) pectic component of plant cell walls and represents about 0.5 to 8% in dicots, non-graminaceous, monocots, and gymnosperms, and less than 0.1% in primary walls of commelinid monocots (Jackson, Dreaden, Theobald, Tran, Beal, Eid, Gao, Shirley, Stoffel, Kumar, & Mohnen, 2007; Matsunaga, Ishii, Matsunamoto, Higuchi, Darvill, Albersheim, & O´Neill, 2004). RG-II has been detected in the cell walls of many tissues of edible plants including apple, kiwi, carrot, tomato, grape and pumpkin (Buffetto, Ropartz, Zhang, Gilbert, Guillon, & Ralet, 2014; Cui, 2005; Ishii, Matsunaga, & Hayashi, 2001). RG-II is typically described as a stretch of HG backbone, approximately seven to nine 1→4 linked *α*-D-Gal*p*A residues with four heteropolymeric side-chains attached (Caffall & Mohnen, 2009). The structure of RG-II is highly complex with twelve different types of sugars and over twenty different linkages. A number of uncommon sugars occur in RG-II structure including 2-keto-3-deoxy-D-manno octulosonic acid (Kdo), 2-keto-3-deoxy-D-*lyxo*-heptulosaric acid (Dha), apiose, 2-*O*-methyl xylose, 2-*O*-methyl fucose and aceric acid (Caffall, et al., 2009; Stevenson, Darvill, & Albersheim, 1988) (Figure 1). RG-II molecules are also known to self-

176 associate forming RG-II dimers *via* a boron diester bonds that was first demonstrated
177 in sugar beet pectin (SBP) (Caffall, et al., 2009; Ishii, et al., 2001).

178 AGA has been found in the walls of aquatic plants such duckweeds
179 (*Lemnaceae*) and marine seagrases (*Zosteraceae*) with D-apiose residues 2→3 linked
180 to HG (Caffall, et al., 2009). XGA has an HG backbone substituted by *β*-D-xylose at
181 the *O*-3 position and has been detected in cell walls of marine sea grasses, cotton
182 seeds, watermelons, peas, apples, and soybeans (Zandleven, Sørensen, Harholt,
183 Beldman, Schols, Scheller, & Voragen, 2007). Ferulic acid is another structural
184 element that is frequently observed in pectic polysaccharides. Pectin originating from
185 spinach, sugar beet, glasswort, quinoa and butternut are ester-linked to phenolic acids
186 and often referred to as "feruloylated pectins" (Fissore, Rojas, Gerschenson, &
187 Williams, 2013; Fry, 1982; Renard, Champenois, & Thibault, 1993; Renard, Wende,
188 & Booth, 1999; Rombouts & Thibault, 1986). In sugar beet and spinach cell walls,
189 ferulic acids are linked to L-Ara*f* residues of the main core of *α*-(1→5)-linked
190 arabinan chains at the *O*-2 position and to D-Gal*p* residues of the main core of *β*-
191 (1→4)-linked galactan chains at *O*-6 position (Ralet, et al., 2005). Recent enzymatic
192 digestion studies in sugar beet pectin showed that neutral sugar side-chains can be
193 also attached to proteins (Funami, Nakauma, Ishihara, Tanaka, Inoue, & Phillips,
194 2011). Generally, chemical analysis of pectin isolated from various sources reveals
195 the presence of proteins or its traces that are regularly considered as contaminants
196 originating from cell wall or as integral parts of the biopolymer (covalently linked).

197 The fine structure of pectin has been widely investigated but the arrangement
198 of these structural elements in the macromolecule is still a matter of debate. Endo-
199 polygalacturonase (EPG) treatment of pectic polysaccharides demonstrated that HG,
200 RG-I, and RG-II backbones are covalently linked suggesting that the RG-I and RG-II

backbones are continuous with the HG backbone indicating the presence of a macromolecular structure with specific domains (di- or tri-block copolymers) (O'Neill, Warrenfeltz, Kates, Pellerin, Doco, Darvill, & Albersheim, 1996). The pectic network in the cell wall is structured with the aid of cross-linking including calcium and borate ester crosslinking, covalent linkages to phenolic and possibly other compounds (Caffall, et al., 2009). The HG segments of pectin may self-associate through calcium cross-linking depending on the degree of methyl-esterification. RG-I side-chains could be cross-linked to other wall components such as xylans, xyloglucans, lignins, and proteins. RG-II domains form crosslinks to other RG-II molecules through borate diester linkages. This extensive degree of cross-linking frequently leads to alternative models of macromolecular structure of pectin where HG is depicted as a side chain of RG-I (Vincken, 2003).

It is evident from the above discussion that pectin has a particularly complex molecular profile and straightforward structure-function relationships should be regarded with hesitation. However, there are some specific functional groups and moieties on the macromolecule that have a significant contribution to its interfacial functionality. In the next section, we single out and discuss the most important of the factors that control the arrangement of pectin at the oil-water interface.

3. Role of structural elements on the interfacial activity

3.1 The role of protein

The emulsifying capacity of pectin is typically associated with the chemical structure of biopolymer backbone such as the degree of methylation (DM) and acetylation (DA), the macromolecular characteristics of pectin chains (molecular weight, degree of branching of side-chains, hydrodynamic volume) and the presence of functional units such as protein and ferulic acids. The evaluation of dominance and

226 contribution of each of these structural parameters to the emulsification capacity of
227 pectin is in progress and still a matter of debate. It is widely suggested that the
228 interfacial activity of pectin is related to the presence of proteinaceous moieties acting
229 as hydrophobic anchors that facilitate adsorption of pectin chains at the interface thus
230 resulting in reduction of interfacial tension. The protein content in pectin depends on
231 the source, isolation conditions and detection methods with higher values typically
232 reported for sugar beet (up to ~9 %) and okra (~5%) as opposed to citrus or apple
233 pectin (e.g., ~3% and ~1%, respectively) (Alba, Laws, & Kontogiorgos, 2015; Chen,
234 Qiu, Gan, Liu, Zhu, & Yin, 2016a; Funami, et al., 2011; Schmidt, Schmidt, Kurz,
235 Endreß, & Schuchmann, 2015; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007a).
236 The contribution of proteinaceous moiety in emulsifying capacity of pectin has been
237 extensively investigated using enzyme treatments of sugar beet pectin (Funami,
238 Zhang, Hiroe, Noda, Nakauma, Asai, Cowman, Al-Assaf, & Phillips, 2007). It has
239 been shown that removal of protein from biopolymer structure results in reduction of
240 interfacial activity of sugar beet pectin as evidenced by higher droplet mean diameters
241 ($d_{3,2}$, $d_{4,3}$), broader droplet size distributions, and larger interfacial tension compared
242 to emulsions fabricated with non-modified pectin. The enzymatic treatment also
243 caused alterations in the macromolecular structure of modified pectins (e.g., reduction
244 in molecular weight and radius of gyration) thus restricting its steric stabilisation
245 efficiency. Although the proteinaceous component plays a dominant role in
246 emulsifying activity of sugar beet pectin, the long term emulsion stability has been
247 primarily attributed to the presence of neutral sugar side-chains (Funami, et al., 2011).
248 It has been also shown that the adsorbed fraction of sugar beet pectin or
249 depolymerised citrus pectin at the oil-water interface has greater concentration of
250 protein as opposed to that in the bulk (Akhtar, Dickinson, Mazoyer, & Langendorff,

2002; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Nakamura, Yoshida, Maeda, Furuta, & Corredig, 2004; Siew & Williams, 2008b; Yapo, et al., 2007a). However, it has been recently argued through sequenced enzymatic modification of sugar beet pectin that covalently-linked ferulic acid-arabinogalactan-protein complex has more notable impact on the interfacial activity, and emulsifying capacity of pectin than the protein alone (Chen, Fu, & Luo, 2016b). Furthermore, investigations of the effect of protein concentration on droplet size distributions ($d_{4,3}$) of SBP-stabilized emulsions proposed that ~3% protein content is needed for optimum surface activity of SBP (Chen, et al., 2016a). In conclusion, protein and ferulic acid seem to play crucial role to the emulsifying capacity (interfacial activity and emulsion stability) of sugar beet pectin agreeing with the earlier findings that fractions of SBP adsorbed at the o/w interface were abundant in protein and ferulic acid (Siew, et al., 2008b). Other schools of thought propose that neither high protein concentration nor the presence of protein ensure good emulsifying properties suggesting that the accessibility and chemical nature (e.g., composition of amino acids and conformation) of protein are foremost determinants. Several studies have shown that the chemical composition of protein in SBP was different and extensin was reported to be the main protein associated with sugar beet, cotton and hop pectin (Karnik, Jung, Hawking, & Wicker, 2016; Nuñez, Fishman, Fortis, Cooke, & Hotchkiss, 2009). It has been also shown that fractions of SBP separated using hydrophobic affinity chromatography with different proportions of protein (0.8% and 5.9%) result in formation of emulsions of comparable droplet sizes and stability (Williams, Sayers, Viebke, & Senan, 2005). In addition, the fractionation of SBP showed that hydroxyproline was a major amino acid. However, like total protein content, hydroxyproline-rich fractions did not demonstrate a good emulsifying

276 capacity and therefore were not directly associated with the emulsifying activity of
277 SBP. Further investigations did not identify a direct relationship between the protein
278 type and content, and emulsifying capacity of SBP or okra pectin obtained by
279 different isolation methods (Alba, Sagis, & Kontogiorgos, 2016; Yapo, et al., 2007a).
280 Therefore, it has been proposed that accessibility of the proteinaceous component to
281 the interface is hindered by the bulky carbohydrate chains thus restricting the
282 emulsifying capacity of pectin (Castellani, Al-Assaf, Axelos, Phillips, & Anton,
283 2010). The association of protein with pectin has been also evidenced by atomic force
284 microscopy (AFM) describing SBP as "tadpoles" (protein-carbohydrate complex) and
285 citrus pectin as a network of "rods and spheres" with embedded (co-eluted)
286 proteinaceous structures (Fishman, Chau, Qi, Hotchkiss, Garcia, & Cooke, 2015;
287 Kirby, Macdougall, & Morris, 2008).

288 *3.2 The role of acetyl and methyl groups*

289 Several studies reported that acetyl groups, similarly to ferulic groups, could
290 enhance interfacial activity of pectin resulting in smaller droplets during
291 emulsification (Akhtar, et al., 2002; Dea & Madden, 1986; Leroux, et al., 2003; Siew
292 & Williams, 2008a). De-acetylation studies in pectin revealed that the presence of
293 acetyl groups does not contribute to the emulsion-forming capacity to a great extent
294 (Leroux, et al., 2003). However, the de-acetylation of sugar beet pectin in the above
295 mentioned study was performed and compared with citrus pectin disregarding the fact
296 that the latter had lower protein content compared to the SBP. Recent studies
297 demonstrate that acetyl groups (minimum DA=10%) improve considerably the
298 emulsifying properties of pectin, particularly at low protein contents (Chen, et al.,
299 2016b; Schmidt, Koch, Rentschler, Kurz, Endreß, & Schuchmann, 2014). Moreover,
300 alkylated citrus pectins with different alkyl chain length and degree of alkyl

substitution demonstrated improved emulsifying activity as evidenced by smaller droplet mean diameters (~7 μm) of emulsions stabilized with modified pectin than those stabilized with non-alkylated (~ 60 μm) (Liang, Wang, Chen, Liu, & Liu, 2015).

In addition to the acetyl groups, interfacial activity is also related to the presence of methyl groups. Some authors have demonstrated a direct relationship between the DM and emulsifying capacity of citrus pectin by increasing the DM from ~70% to ~80% (Schmidt, et al., 2014). Interestingly, it has been also shown that increase of DM beyond 80% did not result in further reduction of droplet size something that has been attributed to the self-association of citrus pectin and, therefore, decrease in the accessibility of hydrophobic groups to the oil-water interface. In contrast, other authors investigated citrus pectin with DM ranging from 22 to 73% and concluded that the content of methyl esters is of minor importance for the emulsifying properties pectin (Akhtar, et al., 2002). The de-methylesterification of SBP with methyl-esterase from DM of 67 to ~7% resulted in a minor increase of droplet size and interfacial tension further corroborating the aforementioned results (Chen, et al., 2016b). An attempt to study the impact of block-wise distribution of carboxylic acid groups (at comparable DM=63.0%) on interfacial properties of pectin has been also performed but negligible differences in interfacial tension were observed for commercial apple pectin (6.3 mN m^{-1}), modified highly-ordered pectin (5.6 mN m^{-1}) and modified less-ordered pectin (6.3 mN m^{-1}) (Lutz, Aserin, Wicker, & Garti, 2009).

3.3 The role of molecular weight and side chains

The accessibility of protein (or other surface active components such as ferulic acids and acetyl groups) is determined by biopolymer entanglement and its molecular weight. The impact of molecular weight on emulsifying properties of pectin has been

326 widely reviewed in the past and results are inconsistent. Early reports suggest that low
327 molecular weight (e.g., 35-90 $\times 10^3$ g mol^{-1}) favours emulsifying activity of pectin,
328 possibly due to the better accessibility of surface-active groups caused by
329 depolymerisation or increased adsorption kinetics. However, pectin fractions of very
330 low molecular weight result in lower interfacial activity and coarser emulsions due to
331 the inability of short, disentangled polymer chains to provide efficient steric
332 stabilisation (Akhtar, et al., 2002; Leroux, et al., 2003; Yapo, et al., 2007a; Yapo,
333 Wathelet, & Paquot, 2007b). Similar results were obtained for SBP of high Mw
334 showing that pectins with Mw of 306, 470 and 562 $\times 10^3$ g mol^{-1} result in formation of
335 emulsions with larger droplet mean diameters ($d_{4,3}$) compared with those stabilized
336 with low Mw pectins (153, 155, 283 $\times$ 10^3 g mol^{-1}) (Williams, et al., 2005). However,
337 some recent studies did not demonstrate a direct relationship between Mw of citrus
338 pectin and its emulsifying capacity, particularly after adjusting the viscosity ratio of
339 emulsions using various amounts of sucrose (Schmidt, et al., 2014). It has been also
340 shown that reduction of Mw from 76 $\times 10^3$ to 47 $\times 10^3$ g mol^{-1} did not result in
341 improved emulsifying properties of citrus pectin (at low protein content) as indicated
342 by the droplet mean diameters ($d_{3,2}$). Increase of Mw of SBP *via* cross-linking of
343 ferulic acid groups showed that emulsions fabricated with cross-linked biopolymer
344 (Mw ~ 1860 $\times 10^3$ g mol^{-1}) have smaller droplet mean diameters and improved long
345 term stability compared to those stabilized with non cross-linked pectin (Mw ~ 780
346 $\times 10^3$ g mol^{-1}) (Zhang, Shi, Shangguan, Fang, Nishinari, Phillips, & Jiang, 2015). The
347 lack of the consensus on the impact of molecular weight on the emulsifying capacity
348 of pectin also suggests that the other structural characteristics discussed earlier (DA,
349 DM and the presence of ferulic acids) cannot be disregarded.

350 It has been also shown that pectin fractions adsorbed at the oil-water interface
351 were enriched in neutral sugars (e.g., arabinose and galactose) suggesting that RG-I
352 containing pectin could have improved emulsifying properties as opposed to pectins
353 with linear backbone (Siew, et al., 2008a). These results were further supported by the
354 enzymatic degradation of sugar beet pectin using arabinase and galactase (Chen, et
355 al., 2016b). The investigation revealed a decrease of interfacial and stabilizing
356 properties of enzymatically modified pectin in comparison to non-modified in terms
357 of interfacial tension values (17.5 and 38.6 dyne cm^{-1} for non-modified and modified
358 SBP, respectively) and droplet mean diameters ($d_{3,2}$ ~ 0.4 to 1.9 μm for non-modified
359 and modified SBP, respectively). The impact of side-chains on emulsion-forming
360 properties of pectin could be attributed to the interfacial activity of protein and ferulic
361 acid that are attached to the side-chains and act as anchors. In addition, the presence
362 of neutral sugar side-chains contributes to the long-term emulsion stability due to the
363 formation of thick interfacial layers thus providing effective steric stabilisation that
364 impedes emulsion coarsening (Funami, et al., 2011).
365 It has been also reported that multilayer adsorption of sugar beet pectin at the
366 interface is possible and originates from electrostatic interactions between positively
367 charged protein moieties (~10%) within the SBP chains and the negatively charged
368 galacturonic acid residues (Chee, Williams, Cui, & Wang, 2008). The ability of pectin
369 to stabilise sterically oil droplets is attributed to the RG-I domains, whereas
370 electrostatic stabilisation originates from HG-domains due to the ionisation of
371 carboxylic groups. Emulsions stabilized with pectin are pH- and ionic strength-
372 sensitive and changes in these factors result in alterations of its emulsifying capacity.
373 At pH values greater than ~3.5 carboxyl groups of pectin are ionized and the
374 biopolymer chains are extended due to the electrostatic repulsions between the

carboxylate anions. The number and distribution of negative charges is determined by the degree of methyl esterification and degree of blockiness (DB) of methyl groups. The ionization of carboxylic groups decreases with pH (pH < pK_a) and consequently promotes self-association of the chains. It has been shown that okra and sugar beet pectin stabilize o/w interfaces at low pH values, where biopolymers adopt highly compact conformations resulting in the formation of thick interfacial layers thus providing effective steric stabilization (Figure 2) (Alba, et al., 2016; Castellani, et al., 2010). It becomes apparent that modification of conformational characteristics of pectin with the aid of environmental conditions (e.g., pH, ionic strength) could result in improved emulsifying capacity, enhanced steric stabilization and long-term emulsion stability. Taking everything into account, Figure 2 summarizes the arrangement of pectin at the interface at pH values below and above the pK_a of Gal*p*A. At low pH values, chains attain compact conformations due to protonation of Gal*p*A, as described above allowing hydrophobic groups to come into close proximity with the oil interface and adsorb. Furthermore, the compact arrangement results in effective steric stabilisation preventing coalescence of droplets. The interface is depicted as complex with a large number of groups being able to anchor at the interface. On the other hand, at high pH values, pectin attains an extended conformation with fewer groups attached at the oil-water interface. Desorption can occur easily leaving the droplet surface exposed and subject to coalescence.

From the above discussion it is difficult to pinpoint relationships between structure and function that result in optimum emulsification performance. This is partially due to the large number of protocols that can be used to extract pectin that control the outcome of the structure. It emerges, however, that some critical parameters (e.g, protein, Gal*p*A, DM, Mw, etc.) can be manipulated and some general

guidelines can be drawn in an effort to rationally design pectin with optimum emulsification properties.

4. Influence of isolation procedures on pectin structure

Recently, several extraction methods of pectic polysaccharides have been introduced including isolation using enzymes (e.g., polymethylgalacturonases, polygalacturonases, polygalacturonate lyases), electromagnetic induction heating, and microwave- or ultrasound-assisted extractions (Bagherian, et al., 2011; Kashyap, Vohra, Chopra, & Tewari, 2001a; Kashyap, Vohra, Chopra, & Tewari, 2001b; Wang, Chen, Wu, Wang, Liao, & Hu, 2007). It has been shown that microwave heating could effectively decrease depolymerisation and maintain high Mw and intrinsic viscosity of sugar beet pectin compared to the standard thermal extraction methods (Fishman, Chau, Cooke, & Hotchkiss Jr, 2008). In a separate investigation, no appreciable differences in DM, Gal*p*A and protein contents of pectin were found using high hydrostatic pressure (HHP), high-speed shearing homogenizer (HSSH) or standard thermal extraction (TT) (Guo, et al., 2014). However, it has been also demonstrated that utilization of HHP extraction results in isolation of pectin with higher Mw and better emulsifying properties contrasting those extracted with HSSH and TT. Several studies have reported that enzymatic extractions (e.g., from chicory roots, cauliflower and gold kiwifruit) result in isolation of higher yields of pectin rich in Gal*p*A and DM comparable to those extracted with conventional methods (e.g., acidic aqueous extraction). However, enzymatic extractions may lead to alterations in functional properties due to the low Mw and viscosity (Panouille, Thibault, & Bonnin, 2006; Ptichkina, Markina, & Rumyantseva, 2008; Yuliarti, Matia-Merino, Goh, Mawson, Williams, & Brennan, 2015). In contrast, enzymatically isolated lime pectin

has been also reported with higher DM (~ 82%) and without losses in functionality compared to the acid-extracted (67–74%) (Dominiak, et al., 2014).

Conventional methods of pectin extraction are by means of cold or hot aqueous buffers (e.g., sodium acetate buffer), use of chelating agents (e.g., potassium-oxalate or EDTA), dilute acids (e.g., HCl or HNO_3) or dilute sodium hydroxide solutions. Previous works have reported the remarkable effect of pH, extraction time-temperature combinations, type of acid, number of extraction cycles, water-to-raw-material ratio and volume of organic solvent on the yield and chemical composition of pectin from various plant sources, such as apple and peach pomace, mango peel, okra pods and passion fruit (Kliemann, de Simas, Amante, Prudêncio, Teófilo, Ferreira, & Amboni, 2009; Kumar & Chauhan, 2010; Pagan, Ibarz, Llorca, & Coll, 1999; Samavati, 2013; Sudhakar & Maini, 2000). The isolation of pectin is mainly performed using hot acid treatment in combination with high temperatures between 60 and 100 °C. The pH varies between 1.0 and 3.5 and the time of extraction between 20 and 360 min depending on raw material (i.e., efficiency of protopectin release) and desired chemical composition of pectin. Most pectic polysaccharides isolated using conventional extraction methods are polydisperse with high molecular weight (> 200 g mol^{-1}) and represent complex mixtures of HG, RG-I and RG-II rather than one uniform polysaccharide species. Generally, the abundance of “smooth” and “hairy” pectic polysaccharides, and distribution and length of side-chains varies considerably from species to species and is controlled by the extraction conditions. It has been reported that pectic polysaccharides isolated from apple, citrus and sugar beet are primarily composed of HG, whereas those isolated from soybean, linseed extracts, green tea leaves, and okra contain large amounts of RG-I (Alba, et al., 2015; Ele-Ekouna, Pau-Roblot, Courtois, & Courtois, 2011; Leroux, et al., 2003; Muralikrishna,

449 Salimath, & Tharanathan, 1987; Nakamura, Furuta, Maeda, Nagamatsu, &
450 Yoshimoto, 2001).

451 It has been reported that pectin extraction from sugar beet, pomelo and banana
452 peels at low pH (< 2.5) results in isolation of biopolymers rich in galacturonic acid
453 that were only moderately affected by selection of the time and temperature of the
454 extraction (Methacanon, Krongsin, & Gamonpilas, 2014; Oliveira, Rosa, Cavalcante,
455 Pereira, Moates, Wellner, Mazzetto, Waldron, & Azeredo, 2016; Yapo, et al., 2007a).
456 Other studies attributed the high Gal*p*A contents in pectin fractions isolated from
457 sugar beet, pomelo and peach pomace to the depolymerisation followed by hydrolysis
458 of biopolymer backbone as evidenced by a reduction in Mw, intrinsic viscosity,
459 neutral sugars and ferulic acids (Methacanon, et al., 2014; Pagan, Ibarz, Llorca,
460 Pagan, & Barbosa-Canovas, 2001; Yapo, et al., 2007a). Furthermore, other authors
461 observed a more pronounced effect of temperature on Gal*p*A content in pectin
462 isolated from apple pomace and cocoa husk, and reported a decrease in Gal*p*A at very
463 low pH (~1.5) (Chan & Choo, 2013; Garna, Mabon, Robert, Cornet, Nott, Legros,
464 Wathelet, & Paquot, 2007; Happi Emaga, Garna, Paquot, & Deleu, 2012). The
465 authors suggested that harsh acidic conditions resulted in isolation of pectin with
466 diminished purity (expressed as Gal*p*A) due to the hydrolysis of pectin backbone and
467 co-extraction of non-pectic substances. In contrast, protein content in pectin fractions
468 isolated at very low pH values (~1.5) was lower as opposed to those isolated using
469 milder conditions (pH 2.0) (Yapo, et al., 2007a).

470 The modification of pH of extraction also results in isolation of pectin with
471 different degrees of methyl-esterification (HM or LM) and therefore, could be utilized
472 for tuning its functional properties. Acidic extractions yield pectin of high DM,
473 whereas high pH values typically result in isolation of pectin with low DM due to the

474 saponification of the ester groups. Several studies also showed that pectin isolated
475 from apple pomace, pomelo, durian rinds, banana and pomegranate peels at very low
476 pH values (1.5 – 2.0) had lower DM and DA than their counterparts extracted at
477 milder pH (2.5 – 3.2) and the impact of pH was even further amplified by higher
478 temperatures and times of extraction (Garna, et al., 2007; Methacanon, et al., 2014;
479 Oliveira, et al., 2016; Pereira, Oliveira, Rosa, Cavalcante, Moates, Wellner, Waldron,
480 & Azeredo, 2016; Wai, Alkarkhi, & Easa, 2010). This was ascribed to the de-
481 esterification of pectin backbone and co-extraction of strongly bound pectin (e.g.,
482 chelator-soluble type of pectin) that are typically of lower DM.

483 Moreover, the chemical composition of isolated pectin varies with respect to
484 the type of acid used (e.g., hydrochloric, nitric, sulfuric, oxalic and citric acid). It has
485 been shown (Visser, et al., 1996) that pectin isolated from various plant sources (e.g.,
486 leek, pineapple, sugar beet, cucumber, lemon, fennel) appears to be rich in HG when
487 isolated with mild agents (e.g., K-oxalate) and becomes considerably richer in RG-I
488 when extracted by stronger agents (e.g., HCl, NaOH). The effect of acid type on the
489 structural and macromolecular characteristics of citrus pectin has been also explored
490 and it was shown that oxalic acid-extracted pectin has higher Mw and intrinsic
491 viscosity as opposed to those isolated with nitric acid due to the preservation of side
492 chains, RG-I backbone and impediment of hydrolysis of non-methylesterified
493 segments of HG (Kaya, Sousa, Crepeau, Sorensen, & Ralet, 2014). In addition, pectin
494 of high Mw has also been isolated from pomelo fruit using HCl (Methacanon, et al.,
495 2014).

496 Following the hot extraction step, recovery of pectin from extraction liquor is
497 most commonly performed with organic solvent precipitation (e.g., methanol, ethanol
498 or isopropanol) (Garna, et al., 2007). Typically, precipitation of pectin is performed

499 with alcohol-to-extract ratio 2:1 and ethanol concentration between 70 and 80%. It
500 has been widely shown that the ethanol precipitation step has considerable impact on
501 the yield and physico-chemical properties of isolated pectin and therefore could be
502 utilized for "fine" tuning the pectin structure and functional properties (Xu, Yue, Liu,
503 Ho, Yi, Chen, & Han, 2014). Previous studies reported a decrease in DM of pectin
504 with increase in ethanol volume and this effect was attributed to the interactions
505 between water molecules, carboxylic groups of pectin, and hydroxyl groups of
506 ethanol (Faravash & Ashtiani, 2007). Recently, it has been shown that
507 polysaccharides with different structural and macromolecular properties could be
508 fractionated by precipitation of the extract in various concentrations of ethanol (Guo,
509 Meng, Zhu, Tang, Pan, & Yu, 2016). Stepwise precipitation of sugar beet pectin with
510 incremental ethanol increase from 50-80% results in isolation of pectin fractions of
511 smaller molecular weights (~46 $x10^3$ g mol^{-1}) with lower DM, protein and acetyl
512 contents as opposed to these obtained in one-step precipitation. Comparable results
513 were obtained for SBP fractionated with isopropanol, but an increase was observed in
514 DM and Mw in the precipitation stages with high volume fractions of isopropanol
515 (Karnik, et al., 2016). Furthermore, some authors observed a relationship between the
516 yield of SBP and pH at which ethanol precipitation was performed with the highest
517 yields reported for pH at around 3.0 (Guo, Meng, Tang, Pan, Zhu, & Yu, 2016). Table
518 1 summarizes the influence of the extraction variables and the major impact that each
519 individual variable has on the structural characteristics of pectin. It should be noted
520 that the interplay among the variables would ultimately determine the fine structure of
521 the extracted polymer. Furthermore, the botanical origin (i.e., the starting material)
522 will also influence the final composition, as the extraction it is a system-dependent
523 process.

5. Conclusions

The structural components that influence the emulsification properties of pectin extracted from a range of sources have been reviewed with the aim to identify potential guidelines towards rational design of pectin as emulsifier. The structural complexities of pectin architecture do not allow for unambiguous correlation between structure and function. It is possible, however, to glean information from the literature on the qualities that the "ideal" pectin should possess in order to effectively arrange at the interface and to provide efficient emulsification and long-term stabilization. It becomes apparent that pectin should contain protein in the range of 3% with a minimum 10% degree of acetylation whereas the degree of methylation should not be one of the aims of the isolation process. Generally, the molecular weight should remain between 100-200 $x10^3$ g mol^{-1} and the pectin should be preferably rich in RG-I and ferulic acid. Higher Mw values restrict accessibility of protein at the interface and the particularly high viscosity impedes fast adsorption and organization at the interface. These particular structural characteristics can be achieved by selecting an aqueous extraction with pH values between 2.5-3.5 using a strong monoprotic acid (e.g., HCl) followed by one step alcohol precipitation. It should be noted that the botanical source should not be disregarded, as for instance sugar beet and okra pectin provide better starting material for tailoring the emulsification properties.

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FIGURE CAPTIONS

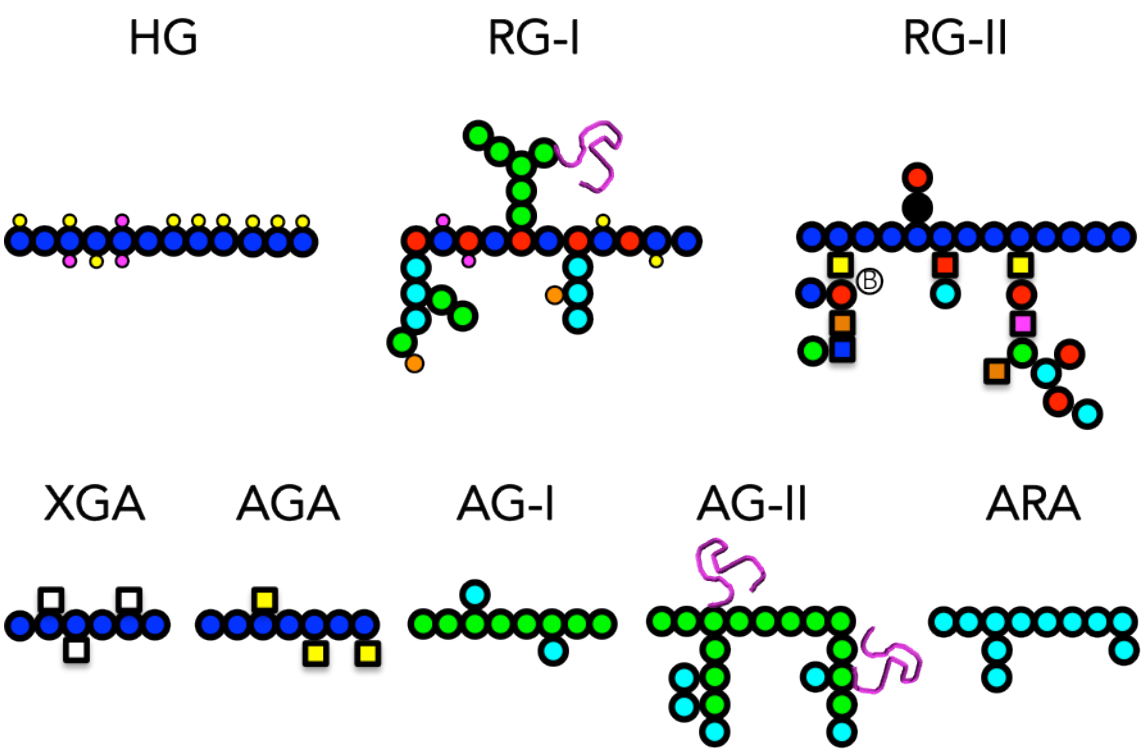
Figure 1: Schematic of the major building blocks encountered in pectin from various botanical sources. HG: homogalacturonan, RG-I: rhamnogalacturonan-I, RG-II: rhamnogalacturonan-II, XGA: xylogalacturonan, AGA: apiogalacturonan, AG-I: arabinogalacturonan-I, AG-II: arabinogalacturonan-II, and ARA: arabinan. Protein can be found on RG-I and AG-II and contribute to interfacial activity.

Figure 2: Schematic of pectin adsorption at the oil-water interface. Protein anchors the chains at the interface supported by acetyl and methyl groups and, when available, ferulic acid; a) The pH of the continuous phase is below the pK_a of galacturonic acid usually lower than ~3.5. Protonation of the carboxylate anion results in compact conformation of the chains. The globular conformation allows hydrophobic groups to come into close proximity with the oil interface and adsorb resulting in effective steric stabilisation; b) at high pH values (> ~4.5), pectin attains extended conformation due to electrostatic repulsions between galacturonic acid residues with fewer groups being able to attach at the oil-water interface. Desorption can occur easily leaving the droplet surface exposed and subject to coalescence.

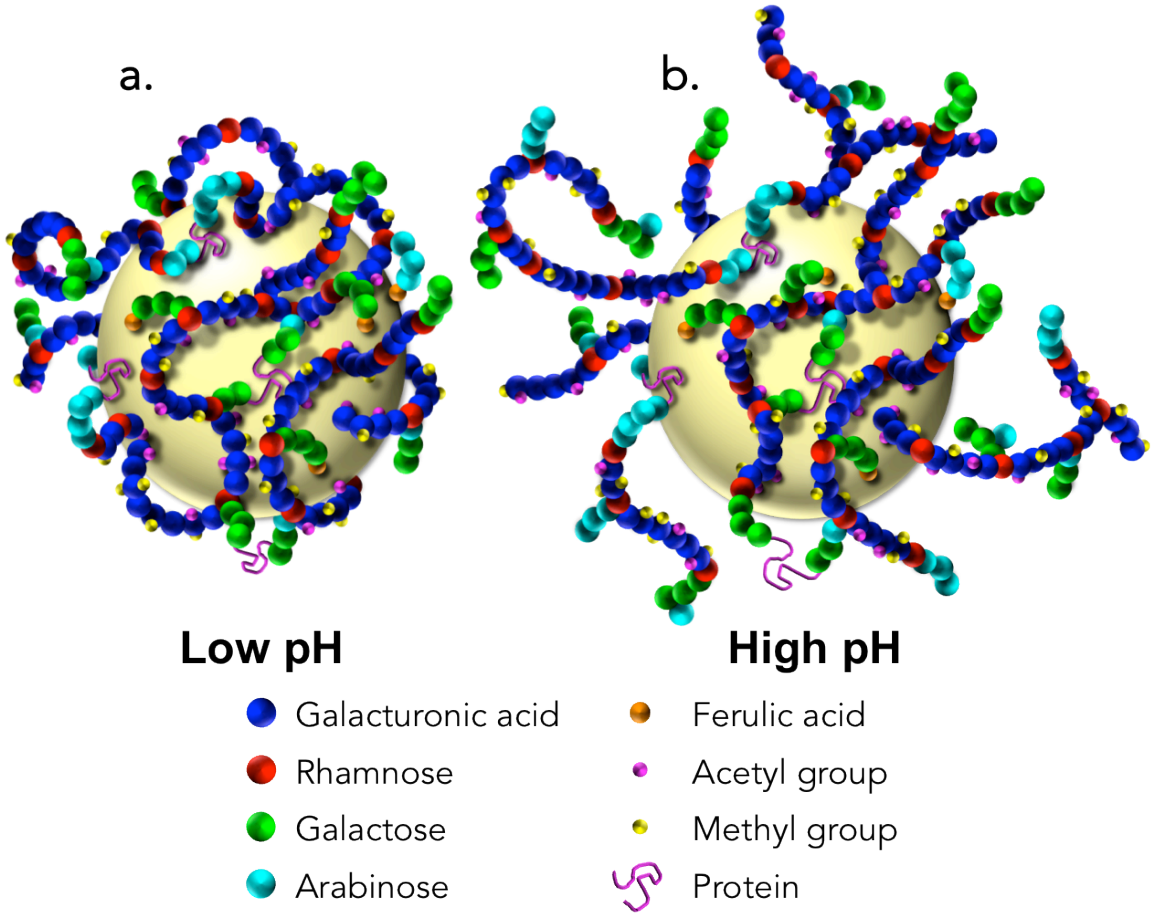
TABLES

Table 1: Extraction variables and typical effects on structural properties of pectin.

Extraction variable	Affected structural characteristics	References
pH	High pH increases GalA High pH decreases DM and DA Low pH decreases protein Low pH decreases Mw and η Low pH removes neutral sugars from backbone and ferulic acids	Alba, et al., 2015; Ele-Ekouna, et al., 2011; Leroux, et al., 2003; Muralikrishna, et al., 1987; Nakamura, et al., 2001; Yapo, et al., 2007; Garna et al., 2007; Methacanon, et al., 2014; Oliveira et al., 2016; Pereira et al., 2016; Wai, et al., 2010
Time-Temperature	High temperature and long times lower Mw and protein Variable effects on GalA content	Kliemann et al., 2009; Kumar & Chauhan, 2010; Pagan, et al., 1999; Samavati, 2013; Sudhakar & Maini, 2000; Methacanon, et al., 2014; Oliveira et al., 2016; Yapo, et al., 2007; Pagan, et al., 2001; Yapo, et al., 2007; Chan & Choo, 2013; Garna et al., 2007; Happi et al., 2012
Type of acid	Mild acids promote HG Strong acids promote RG-I Ratio of RG-I/HG can be manipulated	Visser & Voragen, 1996; Kaya, et al., 2014; Methacanon, et al., 2014
Alcohol volume	High volume of ethanol decreases DM	Garna et al., 2007; Xu et al., 2014; Faravash & Ashtiani, 2007; Guo, Meng, Zhu, et al., 2016; Karnik, et al., 2016; Guo, Meng, Tang, et al., 2016
Extraction process	Variable effects on Mw, η, DM, GalA	Bagherian, et al., 2011; Kashyap, et al., 2001a; Kashyap, et al., 2001b; Wang, et al., 2007; Fishman, et al., 2008; Guo, et al., 2014; Panouille, et al., 2006; Ptichkina, et al., 2008; Yuliarti, et al., 2015; Dominiak et al., 2014



- Galacturonic acid
- Rhamnose
- Galactose
- Arabinose
- Methyl ester
- *O*-Acetyl
- Glucuronic acid
- Apiose
- Dha
- Aceric acid
- Fucose
- Xylose
- Ferulic acid
- Kdo
- Ⓑ Borate
- Protein


a.
b.
Low pH
High pH
Galacturonic acid
Rhamnose
Galactose
Arabinose
Ferulic acid
Acetyl group
Methyl group
Protein