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Compositional and structural characterization of pectic material from Frozen Concentrated Orange Juice

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ABSTRACT

In citrus juices pectin is one of the major components of the suspended cloud material that imparts desirable appearance, texture and flavor. Citrus pectin is a structurally diverse polysaccharide with a backbone of α-(1,4)-galacturonic acid (GalA) residues, a variable proportion of which may be methyl-esterified, and interspersed with regions containing repeating rhamnogalacturonan dimers. We have acid extracted pectin from commercial Frozen Concentrated Orange Juice (FCOJ) and assessed its macromolecular properties, including soluble sugar composition and molecular weight as well as its degree (DM) and pattern (DB, DB_{abs}) of methyl-esterification. The extracted material was composed mainly of polysaccharides (82%) dominated by galacturonic acid (39.2%), arabinose (13.2%) and galactose (25.6%). Protein accounted for approximately 4% of the extracted material and included arabinogalactan protein (AGP). The Number Average Molecular Weight (M_n) of the extracted material as determined by MALLS-SEC was estimated at 1.462×10^6 Da indicating the presence of aggregates. The pectin DM was relatively high at 74.7% with very few contiguous demethylesterified GalA residues (DB = 15.92, DB_{abs} = 4.03). Treatment with various pectinolytic, hemicellulytic, or proteolytic enzymes allowed us to evaluate the contribution of various structural domains to the macrostructural architecture of the extracted material. Various enzyme treatments of the extract produced of a second, lower M_n peak and a loss of material in the larger M_n peak. The M_n varied according to the enzymatic digestion method employed, from 1.5×10^6 Da for the parent material, to 1.4×10^3 Da when treated with Rapidase Adex-P.
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1. Introduction

Among natural products that have been assayed, citrus fruits – in particular oranges, lemons, and grapefruit – contain the highest reported concentrations of pectin in their tissues (Campbell & Palmer, 1978). In citrus fruits, the majority of this pectin is contained within the peel, where it may constitute 20–30% of the albedo by dry weight (Braddock, 1999; Campbell & Palmer, 1978; Sinclair, 1961). Although the juice sacs within the orange fruit may contain some pectin, almost none is found within the juice itself (Sinclair, 1972). Yet in commercially prepared citrus juices, pectin accounts for approximately a third of the insoluble material that is found within the juice cloud (Baker & Bruemmer, 1969). Cloud is the general term for the material – primarily pectin, lipids, flavonoids, and protein – that forms a suspension in juice and contributes to its flavor and texture. It is hypothesized that pectin helps maintain the suspension of more highly charged proteins and flavonoids (Ellerbee & Wicker, 2011; Shomer, 1988), perhaps through the formation of carbohydrate shields as observed in pectin-stabilized acidified milk systems (Tromp, de Kruif, van Eijk, & Rolin, 2004). In the event of pectin demethylesterification in the presence of divalent cations, cloud stability is compromised and a clear, unappetizing serum results (Ackerley, Corredig, & Wicker, 2002; Krop, 1974).

Pectin, with associated arabinogalactan material, is a complex polysaccharide which is synthesized in plants and, along with cellulose and hemicelluloses, comprises one of three major components of the primary cell wall. While cellulose acts as the primary load-bearing component of the wall, pectin serves as a secondary load-bearing component and as a "glue" that spans the middle lamella and holds adjacent cells together (Christiaens et al., 2010;

Abbreviations: A1, α-L-arabinofuranosidase; A2, *endo*-arabinase; AGP, Arabinogalactan Proteins; C, Celluclast; DB, Degree of Blockiness; DB_{abs}, Degree of Absolute Blockiness; DM, Degree of Methylation; EPG, Endo Polygalacturonases M1 and M2; G, *endo*-1,4-β-galactanase; GalA, Galacturonic Acid; FCOJ, Frozen Concentrated Orange Juice; IPA, Isopropyl Alcohol; MALLS, Multi-Angle Laser Light Scattering; M_n, Number Average Molecular Weight; P, Pectinase; PME, Novoshape Pectin Methylesterase; R1, Rhamnogalacturonase; R2, Rhamnogalacturonan acetyl esterase; R!, Rapidase.

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Vincken et al., 2003). Pectin also appears to have a recently elucidated role in cell wall extensibility, with pectate-mediated expansion dependent upon hydration state, DM, and associated polygalacturonase activity (Peaucelle, Braybrook, & Hofte, 2012; Wolf & Greiner, 2012). Structurally, pectin is a very diverse molecule, as it may be composed of up to 17 different monosaccharides organized into several different domain architectures which are believed to be covalently linked (Atmodjo, Hao, & Mohnen, 2013). The simplest but most prevalent is homogalacturonan (HG), a chain of unsubstituted GalA residues between approximately 70 and 120 units in length (Thibault & Ralet, 2003; Yapo, Lerouge, Thibault, & Ralet, 2007). Yapo et al. (2007) in particular reports M_n values from ≈14,000 to 17,000 Da, which would correspond to a length of 79–96 GalA residues, for isolated HGs in acid extracted pectin from dried industrial citrus peels. More complex is the RG I domain, which is made up of 4)-α-D-Gal*p*A-(1,2)-α-L-Rha*p*-(1, repeats and which may be further substituted with arabinans, galactans, and mixed arabinogalactans. RG I domains make up approximately 20–35% of pectin in apples and a somewhat smaller percentage of pectin in citrus peels, and are believed to be interspersed between HG domains linking multiple HG domains together (Coenen, Bakx, Verhoef, Schols, & Voragen, 2007; Yapo et al., 2007). Estimates of over 100 residues in length for RG I domains, with side chains containing an average of 6 residues each have also been suggested (McNeil, Darvill, & Albersheim, 1980). The most complex domain is RG II, which has a 1,4-linked α-D-GalA backbone that is substituted at defined positions with one of four possible oligosaccharides. RG II domains are estimated to account for 1–8% of the material present in the cell walls of dicots (Atmodjo et al., 2013; Doco, Williams, Vidal, & Pellerin, 1997). To add to its complexity, the structure of pectin can be further modified through methylesterification at the C-6 position or O-acetylation at the C-2/C-3 position of GalA. In particular, the percentage and distribution of methylesterification has been well studied due to its defined structural–functional effects (Cameron, Luzio, Goodner, & Williams, 2008; Cameron, Luzio, Vasu, Savary, & Williams, 2011; Kim et al., 2013; Luzio & Cameron, 2008; Ralet et al., 2012; Tanhatan-Nasseri, Crepeau, Thibault, & Ralet, 2011).

Commercially pectin is often categorized as either high or low DM pectin. When the DM for a given pectin is low (≤50%), it can form a gel-like network through the formation of divalent-cation-mediated crosslinks. Conversely, when the DM of a pectin is high (≥50%), gelation can be achieved in the presence of high (≥55% w/w) concentrations of sugar (typically sucrose) (BeMiller, 1986). This gelation is further dependent upon the pattern of methylesterification; long blocks of 10 or more contiguous demethylesterified or methylesterified GalA residues are more conducive to the formation of cation-mediated crosslinks and hydrophobic interactions, respectively, relative to shorter more interspersed blocks (Haminiuk, 2006; Luzio & Cameron, 2008; Ralet, Dronnet, Buchholt, & Thibault, 2001). In the case of either a high or low DM pectin, gelation results in a flexible gel-like material that may exhibit diverse physiochemical properties, including thermoreversibility and varied gel strength, depending on the mode of formation. While the formation of cation-mediated crosslinks can present problems for the preservation of juice cloud, the same physical properties outlined above have given pectin a prominent place in the food industry as a thickening or suspension agent in the production of jams, jellies, and beverages.

Despite its critical role in determining product quality and consumer acceptance by maintaining juice cloud integrity, very few structural details are known about the pectin and hemicellulosic material that is found in commercial citrus juices. Here we report, for the first time, on the extraction and structural characterization of pectic and arabinogalactan material from *Citrus sinensis* Frozen Concentrated Orange Juice (FCOJ), and provide details of its composition, macromolecular properties and nanostructural architecture.

2. Materials and methods

2.1. Materials

FCOJ was provided by Nestlé Professional Vitality (Tampa, FL). α-L-arabinofuranosidase (A1), *endo*-arabinase (A2), *endo*-1,4-β-galactanase (G), and polygalacturonases (EPG-M1, EPG-M2) were purchased from Megazyme (Wicklow, Ireland). Pectinase (P) (P2166) and proteinase K (Pk) were purchased from Sigma Aldrich (St. Louis, MO). Novoshape pectin methylesterase (PME) and Celluclast (C) were purchased from Novozymes (Hellerup, Denmark). Rapidase ADEX-P was purchased from DSM Food Specialties (Delft, The Netherlands). β-glucosyl-Yariv, α-galactosyl-Yariv, and gum arabic were purchased from Biosupplies (Bundoora, Australia). Rhamnogalacturonase (RGase)(R1) and rhamnogalacturonan acetyl esterase (RGAE)(R2) were a gift from M.-C. Ralet, INRA, Nantes, France.

2.2. Methods

2.2.1. Pectin extraction

The extraction was carried out using a modified version of a commercial procedure for separating pectin from citrus solids (Joye & Luzio, 2000; May, 1990). To 2.5 L of juice concentrate, an equal volume of distilled water, as well as lithium azide to 0.02% (w/v) and potassium meta-bisulfate to 0.43% (w/v) were first added. This solution was defined as being 1 volume for the purposes of this protocol. The solution was brought to 70 °C in a water-jacketed vessel (Wilmad-LabGlass, Vineland, NJ) and adjusted to a pH of 1.8 with concentrated HNO_3. After 3 h of continuous stirring at temperature, the pH was further adjusted to 2.2 with concentrated NaOH, and the solution was drained through a 1 mm filter (to remove residual pulp) into 3 volumes of chilled isopropyl alcohol (IPA). After 16 h at 4 °C, the solution was centrifuged at 12,000 × *g* for 30 min at 4 °C; the supernatant was then discarded, and the pellet washed twice with one volume of IPA and centrifuged each time as above. Next, the pellet was solubilized into one volume of distilled water, and again centrifuged as above. The pellet contained no pectin and was discarded; the supernatant was dialyzed (Spectra/Por MWCO: 6000–8000 Da) against 2 volumes of distilled water for 24 h with 4 solution changes throughout. At the conclusion of dialysis, 3 volumes of chilled IPA were added to the supernatant. After 16 h at 4 °C, the solution was again centrifuged

Table 1
Reactions conditions for enzymes used.

Enzyme	Reaction temperature (°C)	Units per mg Pectin
α-L-arabinofuranosidase (A1)	30	0.032
endo-arabinase (A2)	30	0.0125
polygalacturonase M1	30	2.1
polygalacturonase M2	30	5
PME	30	10
proteinase K (Pk)	30	0.020 mg[a]
endo-1,4-β-galactanase (G)	40	0.047
RGase (R1)	40	0.4 mg[a]
RGAE (R2)	40	0.1 mg[a]
Celluclast (C)	45	0.36 μL[b]
Rapidase ADEX-P (R!)	45	1 μL[b]
Pectinase (P)	45	1 μL[b]

[a] Enzyme Activity has not been quantified.
[b] Commercial Enzyme Mixture. Units per mg unknown.