Carbohydrate Polymers 86 (2011) 373–385

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Review

Pectic substances: From simple pectic polysaccharides to complex pectins—A new hypothetical model

Beda M. Yapo[a,b,*]

[a] Unit of Training and Research in Food Science and Technology (UFR-STA), University of Abobo-Adjamé, 02 BP 801 Abidjan 02, Côte d'Ivoire
[b] Unit of Pedagogy and Research in Biochemistry and Microbiology, Regional Unit of Higher Education (URES), BP 150 Daloa, Côte d'Ivoire

ARTICLE INFO

Article history:
Received 2 March 2011
Received in revised form 25 May 2011
Accepted 26 May 2011
Available online 24 June 2011

Keywords:
Pectic polysaccharides
Purification
Structural characteristics
Co-polymers
Pectin-complex models

ABSTRACT

Pectic substances are a group of polysaccharides in plant cell walls and some mucilages, which are endowed with multifunctional properties *in muro* as well as *out of muro*, such as the control of cell wall integrity and porosity, the protection of plants against phytopathogens, and gelling, emulsifying, stabilising, thickening and health benefit properties to name a few. The fine structures of distinct pectic elements and complex pectins formed from these are not, however, fully known. It is widely believed that three to four pectic element kinds, namely, homogalacturonan (HG), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II) and/or xylogalacturonan (XGA) are covalently inter-linked to form pectin-complex *in muro*, but the way different blocks of these pectic polysaccharides are positioned relative to one another in such a macromolecular pectin-complex is still a matter of controversy. This review aims at emphasizing on the depiction of the four possible structural elements of complex pectins and providing an update for them in the light of the latest findings. In addition, it pinpoints evidence for the insufficiency of the two main hypothetical models currently existing and the possibility that such a pectin composite could be more complicated as shown in a new hypothetical model.

Contents

* Correspondence address: Unit of Training and Research in Food Science and Technology (UFR-STA), University of Abobo-Adjamé, 02 BP 801 Abidjan 02, Côte d'Ivoire. Tel.: +225 48 73 61 30/32 78 10 88; fax: +225 32 78 75 70.
E-mail addresses: bedamarcel@yahoo.fr, yapobeda@uabobo.ci

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.05.065

1. Introduction

Among the polysaccharides which higher plant cell walls comprise, pectic substances are by far and undoubtedly the most complex and interesting with respect to structural organisation and functionality. The pectic substances (sometimes just termed pectins) were first discovered in the eighteenth century (Vauquelin, 1790) within tamarind fruit as 'a peculiar substance', but no such a specific name was given at that time until the nineteenth century, where a crude characterisation revealed that it gels, thereby becoming the 'active fruit component' responsible for gel formation (Braconnot, 1825). As a consequence, this worker suggested the word 'pectin', in reference to the Greek word 'pektikos', which means to 'congeal, solidify or curdle' (Nussinovitch, 1997), and also predicted that it would have important functions in all plants and many applications in the art of the 'confiseur'. On all points he was quite correct and the study of 'this remarkable macromolecule' has been pursued vigorously by both plant and food scientists ever since (Willats, Knox, & Mikkelsen, 2006). The chemistry of pectic substances actually began in 1917 when D-galacturonic acid (D-GalA), an isomer of D-glucuronic acid (D-GlcA), was discovered to be a basic constituent of all pectic substances so far examined with some of the D-GalA being partially esterified with methyl alcohol (Ehrlich, 1917). Since then, extensive structural and functional studies have been carried out on pectic substances, which have resulted in the discovery of other important functional properties *in vivo* as well as after extraction as predicted by Sir Hennri Braconnot. To date, pectins are thought to be composed of at least 17 kinds of monosaccharides, of which D-GalA is typically the most profuse, followed by D-galactose or L-arabinose (Vincken et al., 2003; Yapo, 2009a). *In planta*, pectins are principally structural components, fulfilling important biological functions such as the protection of plants against withering and drought, in the growth and development of cells and in the mechanical and physical properties of the cell wall. Non-extracted pectins in fruits and vegetables consumed daily are a part of dietary fibre, which may help prevent the occurrence of diseases such as diabetes and colecteral cancer (Yapo & Koffi, 2008a, 2008b). Extracted pectins from citrus peel and apple pomace are mainly used as gelling agents and secondarily as thickening agents, whereas those from sugar beet pulp are intended for oil-in-water (O/W) emulsification in different food and non-food formulations (Yapo, Wathelet, & Paquot, 2007). More recently, chemically altered (citrus) pectins, known under the generic term of 'modified citrus pectins' (MCPs), have been reported to possess health benefit properties, especially anti-metastatic properties, though the structural features of these bioactive macromolecules remain by far controversial (Glinsky & Raz, 2009). Despite this, the fine structures of pectic polysaccharides and complex pectins are neither plainly defined nor fully known, which unfortunately reduces their scope of application. Pectic substances were primarily thought to be a triad of homopolymers, viz. homogalacturonan (HG), arabinan and galactan (Hirst & Jones, 1939), before being shown to be heteropolysaccharides in which neutral sugars (NSs) such as L-rhamnose (L-Rha), L-arabinose (L-Ara), D-galactose (D-Gal), and D-xylose (D-Xyl) appeared to be incorporated in galacturonan macromolecules (McCready & Gee, 1960). Moreover, the reports of several pectic structural elements other than HG, namely xylogalacturonan (XGA), apiogalacturonan (A_pGA), rhamnogalacturonan-I (RG-I), rhamnogalacturonan-II (RG-II), galactogalacturonan (GGA), arabinogalacturonan (A_rGA) and galacturonogalacturonan (GaGA) (Aspinall & Fanous, 1984; Beck, 1967; Bouveng, 1965; Darvill, McNeil, & Albersheim, 1978; De Vries, den Uijl, Voragen, Rombouts, & Pilnik, 1983; Ovodova et al., 2006; Talmadge, Keegstra, Bauer, & Albersheim, 1973) from various plant materials allowed pectic substances to be viewed as a group of extremely complex and structurally diversified polysaccharides from all land plant cell walls and some mucilages (Aspinall, 1970; Ovodov, 2009; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995; Yapo, 2009a). Owing to a common galacturonan backbone, RG-II, XGA, A_pGA, GGA, A_rGA and GaGA are often gathered under the generic term of 'substituted galacturonans' (Ridley, O'Neill, & Mohnen, 2001; Yapo, 2009a). It is widely believed that HG, RG-I, and RG-II or XGA are covalently interconnected to one another, thereby forming complex pectin composites *in muro* (O'Neill & York, 2003; Schols & Voragen, 1996; Voragen et al., 1995). However, compelling evidence for supporting such a structure has been rather light and sufficiently rare until recently, where structural studies with modern techniques have enabled a group of workers to identify the connecting linkage between HG or XGA and RG-I in apple pectin (Coenen, Bakx, Verhoef, Schols, & Voragen, 2007). It remains a matter of debate as to the way in which the different structural blocks are arranged to form the pectin composite(s), inasmuch as the well-known model of alternating HG and NS-branched RG-I blocks, the so-called 'smooth' (SR) and 'hairy' (HR) regions of pectins, respectively (Schols & Voragen, 1996; Voragen et al., 1995), has recently been challenged by the proposal of a strikingly different hypothetic model (Vincken et al., 2003) that might be called 'the RG-I backbone model'. Furthermore, recent structural data, obtained from different new sources of pectins, suggest that both models may be a partial representation of complex pectin as it would natively exist *in muro*. The present review aims at focusing on the most recent and important work on pectic substances, dealing with the structural and functional features of the possible building blocks and hypothetic models of pectin-complex thereof proposed. Also examined is the possibility that the pectin-complex could be more composite than the existing two most striking models, which is expressed by the proposal of a new hypothetical model where both are accounted for.

2. Focus on the four different co-polymer blocks of complex pectins

2.1. Unsubstituted homogalacturonans

Unsubstituted homogalacturonans (HGs), also referred to as linear galacturonans, polygalacturonic acids, polygalacturonides or 'smooth regions (SRs)' of complex pectins are the first pectic polysaccharide type isolated from plant cell wall pectins. Structural studies, using methylation analysis after partial acid hydrolysis (PAH), anomeric configuration, and specific optical rotation allowed to delineate unsubstituted HGs as polymeric chains of (1 → 4)-linked-α-D-Gal*p*A residues, irrespective of plant origin. The GalA residues can be partially methyl-esterified at C-6 and acetyl-esterified at positions O-2 and/or O-3, depending on plant source. It is worth underlining that branched HGs, the so-called galacturonogalacturonans (GaGA; Yapo, 2009a) have also been purified from diverse cell walls (Bouveng, 1965; Ovodova et al., 2006; Round, Rigby, MacDougall, & Morris, 2010) and should be distinguished from unsubstituted (linear) HGs. It is widely believed that linear HGs are synthesised in a highly methyl-esterified (may be 100% methylated) form in the Golgi apparatus and then de-esterified enzymatically in the plant cell wall (Petersen, Meier, Duus, & Clausen, 2008). Linear HG blocks in which more than 50% of the α-D-Gal*Ap* units are esterified with methyl (or methoxyl) groups at the C-6 position, i.e., with a degree of methylesterification (DM) >50, are conventionally called high methyl-esterified HGs; otherwise they are referred to as low methyl-esterified HGs. Both the methyl- and acetyl-esterification of HGs are mainly accountable for the gelling behaviour of pectins (Voragen et al., 1995; Yapo & Koffi, 2006). Immunolocalisation studies have shown that low methyl-esterified HGs are localised to the middle lamella, at cell

Table 1
Proportion, glycosyl residue composition (mol%) and macromolecular parameters of unbranched homogalacturonans (HGs) from diverse cell wall pectins.

HG sources	PF	BP	YPFR	AP	SBP		CP					SW
					A	B	A	B	C	D	E	
Isolation procedure type	MAH	MAH	MAH	CAH	CAH	CAH	CAH	CAH	MAH	Enz	Enz	Enz
Proportion												
(% of pectic material)	58.6	67.3	82.3	83.8	66.4	55.8	89.5	88.3	90.2	79.8	80.0	53.5
(% of pectic material GalA)	88.5	91.3	97.3	92.7 95[a]	83.4	84.0	94.8	94.7	98.9	ND	ND	ND
Sugars (mol%)												
GalA	99.2	98.8	99	98.1	98.4	98.4	98.8	98.5	99.1	96.8	96.4	91.5
Rha	0.3	0.4	0.5	0.3	0.9	0.1	0.4	0.1	0.4	0.8	0.4	0.7
Ara	0.3	0.3	0.1	nd	0.1	0.3	nd	nd	0.2	0.4	0.6	4.1
Xyl	0.1	0.1	0.3	1.1	0.2	0.6	0.6	0.8	nd	0.0	0.2	nd
Gal	0.1	0.3	0.1	0.1	0.2	0.3	0.1	0.3	0.3	2.1	1.6	3.8
Glc	nd	nd	nd	0.2	nd	0.3	0.1	0.1	nd	0.0	0.6	nd
Total sugar	100	99.9	100	99.8	99.8	100	100	99.8	100	100.1	99.8	100.1
Macromolecular parameters[b]												
M_n (kDa)	10.4	11.8	16.5	12.7	16.0	15.9	20.0	15.3	18.1	ND	ND	ND
M_w (kDa)	11.2	12.3	18.5	21.2	19.0	18.8	24.2	18.8	19.3	22.0	ND	ND
DP_n	59	67	93	72	91	89	114	86	102	ND	ND	ND
DP_w	64	70	104	120	108	105	138	105	109	121	ND	ND

PF, pineapple flesh; BP, banana peel; YPFR, yellow passion fruit rind; AP, apple pomace; SBP, sugar beet pulp; and CP, citrus peel pectins.
MAH: the Yapo (2009a) optimised mild-acid hydrolysis (MAH) procedure (0.2 M HNO_3, 100 °C, 45 min) on various pectins. All corresponding HG data are from the same reference.
CAH: the Thibault et al. (1993) controlled-acid hydrolysis (CAH) procedure (0.1 M HCl, 80 °C, 72 h) used on various commercial pectins. AP, SBP-A, and CP-A data are from the same reference, and SBP-B and CP-B are from Yapo (2007).
CP-D-Enz: the Hellín et al. (2005) polygalacturonase-free enzymatic procedure ([RG-ase + Endo-A + Endo-G + α-Ara-ase + β-Gal-ase], 0.5 nkat/mg enzyme, pH 4.5, 40 °C, 48 h) used on a commercial lime pectin.
CP-E-Enz: the Zhan et al. (1998) polygalacturonasic procedure (Endo-PG, 2830 nkat/100 μL soln., pH 4, 37 °C, 24 h) on a commercial citrus pectin.
SW-Enz: the Talmadge et al. (1973) polygalacturonasic procedure (Endo-PG, 6300 IU/200 mL soln., pH 5.2, 30 °C, 3 h) used on CWM from suspension-cultured sycamore cells.
ND, not determined.
nd, not detected.
[a] This value is from the De Vries, Voragen, Rombouts, and Pilnik (1986) enzymatic (pectate-lyase/pectin-lyase) degradation procedure of apple pectins.
[b] The macromolecular parameters were determined using pullulan and/or dextran standards and the so-called universal calibration technique.

corners, and around air spaces, whereas high methyl-esterified HGs are typically present throughout the cell wall (Ridley et al., 2001; Somerville et al., 2004). The middle lamella has been found to be enriched with HGs, in a gel-like state via Ca^{2+}-induced ionic cross-links, adjoining different cell walls abut. Thus, pure HGs and HG-rich pectins are thought to be involved, for example, in the control of the cell wall porosity, integrity, relative rigidity, and mechanical properties; intercellular adhesion and ionic environment (Ridley et al., 2001; Wolf, Mouille, & Pelloux, 2009). Different kinds of HG cross-links are important for strengthening the cell wall, intercellular adhesion, and normal growth in vascular plants (O'Neill, Ishii, Albersheim, & Darvill, 2004; Sila et al., 2009). Various methods, including mild alkali (NaOH) hydrolysis (Link & Nedden, 1931; Morell, Baur, & Link, 1934), mild acid (HCl, H_2SO_4 or HNO_3) hydrolysis (MAH) under different experimental conditions (Chambat & Joseleau, 1980; Jarvis, Forsyth, & Duncan, 1988; Thibault, Renard, Axelos, Roger, & Crépeau, 1993; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007; Yapo, 2009a), hydrogen fluoride (HF)-solvolysis at −23 °C for at least 30 min (Mort, 1983; Wiethölter et al., 2003; Zhang, Pierce, & Mort, 2007) and enzymatic procedures combining rhamnogalacturonan-hydrolase (RG-ase), α-(1 → 5)-L-endo-arabinanase (Endo-A), β-(1 → 4)-D-endo-galactanase (Endo-G), α-L-arabinofuranosidase (α-Ara-ase), and β-D-galactosidase (β-Gal-ase) (Hellín, Ralet, Bonnin, & Thibault, 2005; Nakamura, Furuta, Maeda, Takao, & Nagamatsu, 2002a; B. M. Yapo, unpublished results) have been reported to afford the isolation of HGs from extracted pectins or 'intact' CWMs. Other chemical methods such as 0.5% aqueous ammonium oxalate (AO)/oxalic acid followed by ultracentrifugation (Bhattacharjee & Timell, 1965) or 60% ethanol precipitation (Zitko & Bishop, 1966) have also been reported to afford HGs from the bark of amabilis fir and sunflower seed head residue, respectively. Linear HGs are the main structural element of cell wall pectins, representing approximately 55–70% of the total pectin amount (Jackson et al., 2007; Voragen, Coenen, Verhoef, & Schols, 2009; Wolf et al., 2009). They can, however, account for 50–90% (w/w) of most extracted pectins and encompass more than 80% of the pectin GalA residues (Table 1), thus highlighting their overwhelming majority in pectic substances. However, unbranched HGs have been found in low amounts in pectins from potato tubers (Øbro, Harholt, Scheller, & Orfila, 2004) and soybean cotyledons (Nakamura et al., 2002a), and almost absent in water-solubilised mucilages from *Arabidopsis thaliana* and *Linum usitatissimum* seeds (Deng, O'Neill, & York, 2006; Muralikrishna, Salimath, & Tharanathan, 1987; Naran, Chen, & Carpita, 2008). Unsubstituted HGs are likely to be homogenous in size within a given plant material (Fig. 1). The minimum mean degree of polymerisation ($\overline{DP}$) of HG, as determined by MAH/HPSEC methods, in contrast, appears variable (Table 1) and may range from ~60 (in pectins from commelinoid-related monocot cell walls) to about twice that in non-commelinoid monocots and dicot cell walls (Hellín et al., 2005; Thibault et al., 1993; Yapo, Lerouge, Thibault, & Ralet, 2007; Yapo, 2009a, 2010), though (polyGalA-branched) HGs some three-to-four times greater ($\overline{DP}$~320) have lately been isolated from cold aq. Na_2CO_3-solubilised pectins from mature green tomato fruit (Round et al., 2010). Therefore, they cannot be singularised as a unique macromolecule. The extraction conditions as well as the plant source can however influence the pectin HG amount and DP enough to be reflected in the gelling properties of pectins (Voragen et al., 1995; Yapo, 2009a, 2009b).

2.2. Rhamnogalacturonans-I

Unlike linear HGs, RGs-I are branched and compositionally heterogeneous, containing in addition to α-D-Gal*p*A, NS residues such as α-L-Rha*p*, α-L-Ara*f*, and β-D-Gal*p*. Also, the rare sugar L-Gal*p*, originally found in linseed mucilage (Anderson, 1933) and α-D-Gal*p* have respectively been found attached to O-3 and O-4 positions of Rha residues of RGs-I from water-extracted flaxseed

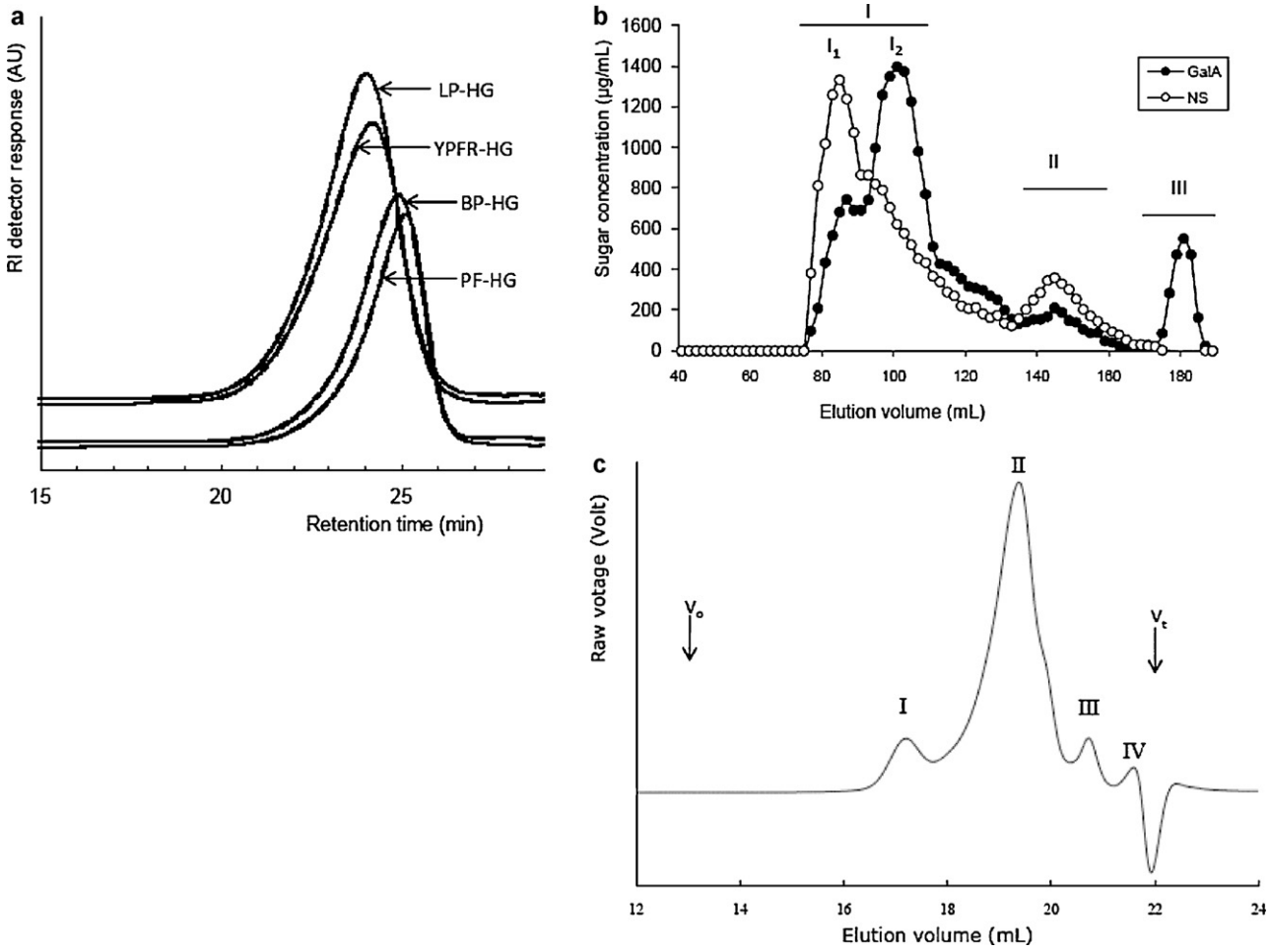

Fig. 1. Size-exclusion chromatography (SEC) elution profiles of (a) HGs from various cell wall pectins; LP, lemon peel; YPFR, yellow passion fruit rind; BP, banana peel; and PF, pineapple flesh (Yapo, 2009a), (b) Endo-PG-digested acid-extracted (deesterified) leek pectin, and (c) de-branched citrus RG-I (Yapo, 2007).

mucilage (Muralikrishna et al., 1987; Naran et al., 2008) and hot buffer-extracted pectins from okra cell walls (Sengkhamparn et al., 2009). The glycosyl residue composition of RGs-I depends on pectin source and isolation method. Although a relatively unbranched RG-I has been characterised from seedcoat mucilage of *A. thaliana* (Deng et al., 2006; Penfield, Meissner, Shoue, Carpita, & Bevan, 2001), RGs-I are generally highly ramified polysaccharides having a backbone made up of the repeating disaccharide $[\rightarrow 2)$-α-L-Rha*p*-$(1 \rightarrow 4)$-α-D-Gal*p*A-$(1\rightarrow]_{n \geq 1}$, partly substituted, at O-4 (mainly) and/or O-3 (scarcely) positions of α-L-Rha*p* residues, with single neutral glycosyl residues and polymeric side chains of different types, viz. $(1 \rightarrow 5)$-α-L-arabinans, $(1 \rightarrow 4)$-β-D-galactans, arabinogalactans-I (AG-I), arabinogalactans-II (AG-II), and possibly galacto-arabinans (Øbro et al., 2004; Tharanathan, Reddy, Muralikrishna, & Susheelamma, 1994; Yapo, 2007). The DPs of the cores of the polymeric side chains do not generally exceed 50 glycosyl residues, though a straight galactan with a $\overline{DP}$ up to 370 ($\overline{M_w}$~60 kDa) has been isolated from hot EDTA-solubilised pectins from tobacco cell walls (Eda & Katö, 1980). The relatively long and numerous (neutral) side chains make RGs-I be sometimes referred to as the 'hairy regions' (HRs) of pectins (De Vries, Rombouts, Voragen, & Pilnik, 1982). It is not known with certainty if AGs-II are an integral part of RGs-I side chains, considering that they are not structurally different from the glycan moiety of arabinogalactan-protein (AGPs) frequently found in the vicinity of pectins RGs-I (Immerzeel, Eppink, De Vries, Schols, & Voragen, 2006; Redgwell et al., 2011; Yapo, 2009a). The degree of substitution, by NS side-chains, of the α-L-Rha*p* of the RG-I backbone can fluctuate widely between ~20 and ~80%, depending on plant source and isolation method (Albersheim, Darvill, O'Neill, Schols, & Voragen, 1996; Ridley et al., 2001; Vincken et al., 2003). RGs-I can be acetyl-esterified at O-2 and/or O-3 positions of GalA residues of the backbone. Moreover, unusual acetylation at O-3 position of Rha*p* residues has recently been reported in RG-I regions from okra cell walls (Sengkhamparn et al., 2009). In contrast, there is no compelling evidence that the GalA residues are methyl-esterified, though methyl-esterification (up to ~100%) may occur in RGs-I from citrus peel, kidney bean cotyledon, apple fruit, mung bean and flax hypocotyls (O'Neill & York, 2003; Ridley et al., 2001; Yapo, 2007). Furthermore, both acid and neutral sugars are not typically attached to GalA residues of the RG-I backbone, though ~2% of GalA in beet RG-I were found substituted at O-3 position with single (terminal) β-D-GlcA (Renard, Crépeau, & Thibault, 1999). Feruloyl ester groups are attached to O-2/O-3/O-5 positions of α-L-Ara*f* residues of the arabinan side-chains and/or to the O-6 position of β-D-Gal*p* residues of the (arabino)galactan side-chains, and beet RG-I arabinan side chains may be cross-linked via dehydrodiferulates *in muro*. The fine structure of RGs-I is being continuously refined and may be limited by isolation procedures and/or analytical tools. RG-Is could possess diverse *in muro* functionalities. Immunocytochemistry suggests that the presence and location of the arabinan and galactan side chains of RGs-I are often correlated with stages of cell and/or tissue development. For example, the appearance of galactan and the diminution of arabinan in carrot

cell wall have been correlated with the transition from cell division to cell elongation (Ridley et al., 2001). It has recently been suggested that RGs-I function as a scaffold to which other pectic polysaccharides, such as HGs and RGs-II, are covalently attached as side chains (Somerville et al., 2004; Vincken et al., 2003). The relative elasticity and flexibility of the cell walls is likely to be determined by the backbone as well as by the arabinan side chains of RGs-I (Moore, Farrant, & Driouich, 2008). Changes in the firmness and tissue softening in ripening fruits are currently related to the degradation of pectins arabinan and galactan side chains. In sugar-acid-gels with normal sugar contents (55–65%), undesirable phenomena such as turbidity, syneresis and/or precipitation are thought to be avoided by junction zone-terminating structural elements present in the pectin chains along with the HG regions, i.e., rhamnose and neutral sugar-branched RG-I regions (Voragen et al., 1995). Because NS linkages are very sensitive to acids, the widely used isolation method is to treat pre-deesterified pectins or 'intact' CWMs with homogenous endo-polygalacturonases (Endo-PGs) in combination or not with exo-type polygalacturonases (Exo-PGs) to degrade the pectin HG regions, thereby releasing RGs-I, which are separated from the soluble products by size-exclusion chromatography (SEC), most often preceded by ion-exchange chromatography (IEC) as the highest $\overline{M_w}$ polysaccharide fraction (Fig. 1b-I). This method was originally used for the purification of RGs-I from purified 'intact' walls from suspension-cultured sycamore cells (McNeil, Darvill, & Albersheim, 1980; Talmadge et al., 1973). The RG-I backbone(s) can be purified by a step-wise degradation of the so-isolated RGs-I with a combination of NS side chain-degrading enzymes, notably [Endo-A + Endo-G + α-Ara-ase + β-Gal-ase], followed by fractionations of the end-products by IEC/SEC (Nakamura, Furuta, Maeda, Nagamatsu, & Yoshimoto, 2001; Yapo, Lerouge, et al., 2007). The latter methods also allow the isolation of the NS branches for the determination of the glycosyl linkage composition and DPs, though the NS side chains are generally concomitantly isolated with backbone oligomers by submitting 'intact' RGs-I to PAH. The RG-I amount may depend on the plant cell wall. For example, RGs-I account for ~36% of the mass of walls from potato tubers (Øbro et al., 2004) and only ~7% of the mass of walls isolated from suspension-cultured sycamore cells (McNeil et al., 1980; Talmadge et al., 1973). The RG-I content of (extracted) pectins generally varies from 5 to 48% (Jackson et al., 2007; Talmadge et al., 1973; Yapo, 2007), indicating that they are minor compared to linear HGs. However, RG-I appeared to be the dominant structural element of some pectic fractions (Nakamura et al., 2001), and almost the sole pectic component of some mucilages (Deng et al., 2006; Naran et al., 2008). Difficult to achieve is the assessment of the minimum length of the backbone(s) of RGs-I, because of a rather high heterogeneity (Nakamura et al., 2001; Schols, Voragen, & Colquhoun, 1994; Yapo, Lerouge, et al., 2007), as can be seen in Fig. 1c. Nevertheless, relatively homogenous backbone stretches of sycamore pectin RG-I may be composed of as many as 100–200 repeats of [→2)-α-L-Rha*p*-(1 → 4)-α-D-Gal*p*A-(1→] (Albersheim et al., 1996), those of soy bean pectin, 15–100 (Nakamura et al., 2001), whereas those of citrus pectin only 15–40 (Nakamura et al., 2001; Prade, Zhan, Ayoubi, & Mort, 1999; Yapo, Lerouge, et al., 2007). Since *in muro* probing of native RGs-I for structural characterisation is not possible so far and since no extractive method can solubilise them from the cell wall without causing any degradation, it may be that much remains to be known about these highly complex and diversified polysaccharides.

2.3. Rhamnogalacturonans-II

Among SGs, rhamnogalacturonans-II (RGs-II) are undoubtedly the most complex and probably the most widespread in plant cell walls. They have been reported to be components of the cell walls of some lower plants such as ferns, horstails, and lycopols (Matsunaga et al., 2004) and even of Pectinol AC (Spellman, McNeil, Darvill, & Albersheim, 1983), a commercial enzymatic preparation from the fungus *A. niger*, in addition to being present in the majority (if not in all) of higher plant cell walls hitherto examined. This pectic polysaccharide type was first isolated from sycamore cell walls (Darvill et al., 1978). RGs-II encompass 11–12 different glycosyl residues and about 28–36 individual sugars, interconnected by more than 20 different glycosidic linkages, which results in highly complex macromolecules with a (1 → 4)-linked α-D-galacturonan backbone, partially methyl-esterified at C-6 of GalA residues and branched with four oligosaccharide chains. RGs-II are, therefore, structurally different from RGs-I. Seven of these glycosyl residues, namely, Api (3-C-hydroxymethyl-β-D-erythrose), 2-O-Me-Fuc, 2-O-Me-Xyl (Darvill et al., 1978), aceric acid (AceA, 3-C-carboxy-5-deoxy-L-xylose) (Spellman et al., 1983), 2-keto-3-deoxy-D-*manno*-octulosonic acid (KdoA) (York, Darvill, McNeil, & Albersheim, 1985), 3-deoxy-D-*lyxo*-heptulosaric acid (DhA) (Stevenson, Darvill, & Albersheim, 1988) and α-L-Gal*p* are rarely observed in natural substances. However, only the former six can be viewed as the RG-II characteristic, specific, or diagnostic glycosyl residues, inasmuch as α-L-Gal*p* is also reported to be a constitutional sugar of RGs-I from flaxseed mucilages (Muralikrishna et al., 1987; Naran et al., 2008). AceA can be mono-O-acetylated/di-*O*-acetylated on O-3 position and 2-O-Me-Fuc on O-3/O-4 position, and Rha can be methylated on O-3 position in some pteridophyte and lycophyte RGs-II (Matsunaga et al., 2004). Since the early reports of borate-cross-linked RG-II dimers (dRG-II, B-dRG-II or dRG-II-B) in radish roots, sugar beet pulp, red wine, sycamore cells and etiolated pea stems (Albersheim et al., 1996), a widely held view is that RGs-II exist predominantly as dRG-II *in muro* rather than as RG-II monomers (mRG-II) (O'Neill et al., 2004; Pérez, Rodríguez-Carvajal, & Doco, 2003), which is corroborated by later work (Aboughe-Angone et al., 2009; Hilz, Williams, Doco, Schols, & Voragen, 2006; Yapo, 2007). RGs-II were originally purified from the soluble products of Endo-PG-digested sycamore CWM by IEC/SEC fractionations (Darvill et al., 1978). To date, Endo-PG/IEC-SEC methods are the most widely used to purify RGs-II from extracted pectins and intact CWMs. Exo-PGs have sometimes been combined with Endo-PGs (Prabasari, Filomena Pettolino, Ming-Long Liao, & Bacic, 2011; Reuhs et al., 2004) or alternatively, commercial crude enzymatic preparations such as Driselases, Pectinases, and Rapidases, which possess diverse pectinolytic and hemicellulase activities, have also been used (Doco, Williams, Vidal, & Pellerin, 1997; Matsunaga et al., 2004; Ortiz-Basurto, Williams, Belleville, & Doco, 2009). Pre-saponification of CWMs and non-alkali-extracted pectins before treatment with Endo-PGs (Thomas, Darvill, & Albersheim, 1989) or a combination of PMEs/PAEs to Endo-PGs may sometimes be required for RGs-II separation from RGs-I and/or HGs. On appropriate high-resolution size-exclusion chromatographic (HRSEC) columns such as Superdex-75 HR 10/30, Superose-12 HR 10/30, Sephacryl S-100 HR 16/60 and Sephacryl S-200 HR 16/90, RGs-II (Fig. 1b-II) are generally well-resolved concomitantly with RGs-I (Fig. 1b-I) and oligogalacturonides (from polygalacturonase-degraded linear HG polymers) (Fig. 1b-III). In case both dRG-II and mRG-II are present in the enzymatic digests, the SEC chromatograms show in M_w decreasing order; RG-I, dRG-II, mRGII and oligogalacturonide peaks (Edashige & Ishii, 1998; Yapo, 2007). The galacturonan backbone is usually obtained by PAH of RGs-II (e.g., 0.1 M TFA, 80 °C, 16 h; Whitcome, O'Neill, Steffan, Albersheim, & Darvill, 1995; and 0.1 M TFA, 40 °C, 16 h + 0.1 M TFA, 100 °C, 1 h; Ishii & Kaneko, 1998), followed by IEC/SEC fractionations of the acid-generated oligosaccharides. This hydrolytic method enables to the backbone to be purified in conjunction with the four oligosaccharide side chains, and can be made selective to (some of) them by modifying the hydrolytic conditions, particularly

temperature and time. Once purified, the RG-II backbone fragments are usually analysed by HPAEC-PAD and their DPs are estimated from co-chromatographying (comparison of elution times) with oligogalacturonide standards. The major disadvantage of PAH is the generation of RG-II backbone fragments of various DPs (3–15) instead of an 'intact' backbone, which renders rather demanding the estimation of the actual backbone length. Both mRG-II and dRG-II have the same (or very similar) glycosyl residue compositions, but differ from each other by the M_ws, which are ~5.0 and 10.0 kDa, respectively. A widely held view is that RGs-II are structurally conserved to such a point they are usually singularised, which is to be owed to the observations of very similar M_ws and oligosaccharide side-chains, almost irrespective of the origin (plant cell walls, fresh and fermented fruit juices, and Pectinol AC). Some intra-variations, such as differing backbone lengths (DP8–15) and DMs (Pellerin et al., 1996) as well as inter-variations (Edashige & Ishii, 1998) have, however, been reported, but it is clear that RGs-II are structurally more conserved than any other pectic polysaccharide type, notably the two afore-described. RGs-II can account for between 0.5% and 8% of the pectin-rich primary walls of dicots, nongraminaceous monocots, and gymnosperms, and less than 0.1% of the pectin poor primary walls of the commelinoid-related monocots (Doco et al., 1997; Matsunaga et al., 2004; O'Neill et al., 2004; Prabasari et al., 2011; Yapo, 2007), and hence represent quantitatively minor cell wall polysaccharides. Though reported to account for 10–11% of pectins (Jackson et al., 2007), the RG-II content of various pectins from several CWMs have recently been found to be less than 5% (Yapo, 2007). RG-II represent *ca.* 1.5% of Pectinol AC, and 20% of the alcohol-insoluble polysaccharides from red wine (Pellerin et al., 1996), thereby occurring as a dominant polysaccharide component of this fermented beverage. In spite of being quantitatively minor in plant cell walls, the ubiquitous character of RGs-II supports the widely held view that they must play specific role(s) *in planta*. For example, the formation of a three-dimensional pectic network *in muro*, which contributes to the mechanical properties of the primary wall, has a prerequisite of RG-II dimer formation. They are also required for normal plant growth and development in that changes in wall properties that result from decreased borate cross-linking of pectin may lead to many of the symptoms associated with boron deficiency in plants (Fleischer, O'Neill, & Ehwald, 1999; O'Neill et al., 2004). Furthermore, pollen tube growth and elongation in *A. thaliana* has been found conditioned by the synthesis of the RG-II diagnostic glycosyl residue KdoA (Delmas et al., 2008).

2.4. Xylogalacturonans

Xylogalacturonans (XGAs) are the second well-known SGs that have first been purified as distinct blocks of pectic substances from mountain pine pollen (Bouveng, 1965). Their general structure encompasses a linear interior chain of (1→4)-linked α-D-Gal*p*A units, partially substituted at O-3 position with single non-reducing β-D-Xyl*p* and/or with longer (DP2–8) 1→2/1→3/1→4/1→2,3/1→2,4/1→3,4-linked β-D-xylan chains. Xylose may also be linked to O-2 position of the backbone GalA residues, inasmuch as such a linkage was exclusively suggested in XGA fragments from zosterine, an AO-extracted pectin from marine sea grass plants (Ovodov, Ovodova, Bondarenko, & Krasikova, 1971), and together with the common β-D-Xyl*p*-(1→3)-α-D-Gal*p*A in pectin from cultured-carrot cells (Kikuchi, Edashige, Ishii, & Satoh, 1996). Hence, the Caffall and Mohnen (2009) report that the characterised XGA in pectic extracts of the Zosteraceae marine see grass, by Ovodov et al. (1971), consisted of HG substituted by the xylose disaccharide [Xyl*p*-(1→2)-Xyl*p*-(1→3)-Gal*p*A] is not a sufficiently correct information. The β-D-xylan side-chains of XGAs are often substituted with α-L-Ara*f*, α-L-Fuc*p* and β-D-Gal*p* and the latter two can be substituted at O-4 and O-4/O-6, respectively, with β-D-Glc*p*A residues (Ovodov, 1975), though pure XGA fragments have been reported from hot water-solubilised soy bean pectin (Nakamura, Furuta, Maeda, Takao, & Nagamatsu, 2002b). The degree of substitution of the galacturonan backbone with Xyl (i.e., Xyl/GalA molar ratio) and DM vary from ~20 to 100% and from ~40 to possibly 90%, respectively, depending on plant source. In contrast, it is not known with certainty if the XGA backbone is acetyl-esterified. The methods used for the isolation of XGAs include chemical procedures such as mild alkaline hydrolysis (Bouveng, 1965), MAH (Kikuchi & Sugimoto, 1976; Le Goff, Renard, Bonnin, & Thibault, 2001), as well as enzymatic methods using homogenous pectate lyase/pectin-lyase (De Vries et al., 1983), Endo-PGs (Yu & Mort, 1996), RG-ases (Schols, Bakx, Schipper, & Voragen, 1995), and the enzymatic combinations (α-Ara-ase + β-Gal-ase + Endo-A + Endo-G + Endo-PG; Nakamura et al., 2002b) and (Endo-A + Endo-G + α-Ara-ase + β-Gal-ase + RG-ase; B. M. Yapo, unpublished). The main drawback of acid procedures is that the β-xylan side chains as well as the galacturonan backbone of XGAs can be appreciably damaged, particularly under harsh temperature and time conditions (Beldman et al., 2001; Yu & Mort, 1996; B.M. Yapo unpublished). Endo-xylogalacturonan hydrolase, however, releases XGA oligomers of a wide range of lengths (Van der Vlugt-Bergmans, Meeuwsen, Voragen, & Van Ooyen, 2000; Zandleven et al., 2007). XGAs are generally susceptible to Exo-PGs, but seldom degraded by Endo-PGs, though an Endo-PG has recently been found to accommodate a xylosylated GalA residue of the backbone just following the hydrolysis site (Mort, Zheng, Qiu, Nimtz, & Bell-Eunice, 2008). Some other reported sources of XGAs are tragacanthic acid (of gum tragacanth from *Astralgus gummifer*, Leguminosae), kidney and red bean cotyledons, Japanese radish, pea hulls, onion bulbs, cotton seeds, potato tubers, chestnut bran, and grape, pear, apple, citrus and yellow passion fruit rinds (Cui, 2005; Gloaguen et al., 2008; Yapo, 2007, 2009a). XGAs are usually found in storage tissues of reproductive organs, and therefore may have specialised functions in these tissues (Albersheim et al., 1996), though they have been identified in non-reproductive organs (roots, stems, and leaves) of diverse plants (Pilarska, Czaplicki, & Konieczny, 2007; Xia, Liu, Zhang, & Luo, 2008; Zandleven et al., 2007), thus suggesting a larger array of functionalities. The $\overline{DP}$ of XGAs is not known with certainty, but some M_ws of ~20–30 kDa (Schols et al., 1995) and a backbone minimum $\overline{DP}$ in the range of 21–119 (Kikuchi & Sugimoto, 1976; Le Goff et al., 2001; Schols & Voragen, 1996; Yu & Mort, 1996, B. M. Yapo, unpublished) have been reported, suggesting that the backbone of native XGAs could be as long as unsubstituted HGs. This amply large range of minimum $\overline{DP}$ values may be partly caused by the used analytical methods, especially when the β-xylan side-chains of XGAs need to be removed by chemical procedures with limited selectivity such as PAH (e.g. 0.1–0.5 M TFA, 80–100 °C, 30–180 min) or HF-solvolysis at −12 °C for ~30 min (Kikuchi & Sugimoto, 1976; Yu & Mort, 1996) prior to estimating the backbone length.

3. On the *in muro* interconnections of pectic elements and pectin-complex formation

3.1. Pectins as a macromolecular complex of different structural elements

Pectins are generally viewed as polysaccharide composites, resulting from the covalent inter-linkage of linear HG, branched RG-I and RG-II and/or XGA elements (O'Neill, Albersheim, & Darvill, 1990; Schols & Voragen, 1996; Voragen et al., 1995) though there is not sufficiently compelling evidence showing, for instance, that

the first three structural elements are covalently inter-linked to form a macromolecular pectin-complex (Ishii & Matsunaga, 2001). The idea of the *in muro* existence of compositionally heterogeneous pectin-complex could be dated from around four decades earlier when non-uronides such as D-Gal, L-Ara, and L-Rha were shown to be incorporated into galacturonan macromolecules of pectic substances from most plant materials (McCready & Gee, 1960) and that repeats of α-D-Gal*p*A-(1 → 2)-α-L-Rha*p* and galacturonans other than linear HG such as XGA and A_pGA have been purified from various extracted pectins (Aspinall, 1970; Bouveng, 1965; Ovodov, 1975). It has then become common to view pectins as structurally composed of different polysaccharide elements interconnected to one another by linkages of non-identified nature.

3.1.1. Pectins as a composite of HG, RG-I, and RG-II

By hydrolysing homogenous primary walls, purified from suspension-cultured sycamore cells, it has been possible to separate HG, RG-I, and RG-II from the soluble products by SEC analysis (Albersheim et al., 1996). By similar enzymatic procedures, various studies (Edashige & Ishii, 1998; Ishii & Matsunaga, 2001; Prabasari et al., 2011; Yapo, 2007) showed that SEC fractionations of pectins, extracted under mild chemical conditions (e.g., cold water and aq. solutions of buffers and calcium-chelators) that did not apparently cleave glycosidic linkages, afforded separate peak materials of RG-I, RG-II and oligogalacturonide (from linear HG degradation) only after treatment with homogenous Endo-PGs or commercial Endo-PG-rich enzymatic preparations. To date, this is actually the fundamental basis of the apparently widely held view that these three structural blocks are covalently interconnected to one another, thus forming block copolymers (O'Neill & York, 2003; O'Neill et al., 2004; Ridley et al., 2001; Sila et al., 2009). Additionally advanced proofs to explain possible covalent linkage between at least HG and RG-II are the isolation of RGs-II of various backbone lengths from red wine (Pellerin et al., 1996), the difference that >95% of wall-bound dRG-II are converted to mRG-II by treatment with 50 mM CDTA, pH 6.5, compared to only 30–40% from pre-solubilised dRG-II (Fleischer et al., 1999), and the fact that aq. potassium phosphate-solubilised HMW-RG-II materials from *Chenopodium album* (>25 kDa) and *A. thaliana* (>100 kDa) are reduced to LMW-RG-II materials (5–10 kDa) by treating with Endo-PG/Exo-PG (Reuhs et al., 2004).

3.1.2. Pectins as a complex of HG, XGA, and RG-I

It is also assumed that XGA is covalently linked to RG-I, thus forming the so-called pectin HRs (Schols & Voragen, 1996), an assumption primarily based on the finding of XGA in pectate-lyase-/pectin-lyase-resistant apple pectin HRs (De Vries et al., 1983) and in Rapidase C600-generated apple MHRs (Schols et al., 1995), before being clearly ascertained by controlled-acid degradation of Rapidase Liq+-generated apple MHR followed by LC–MS and NMR analyses of the liberated products (Coenen et al., 2007). On the other hand, it has been suggested covalent linkages between XGA and HG, following the release of XGA fragments from watermelon (Yu & Mort, 1996) and soybean cotyledon (Nakamura et al., 2002b) pectins with Endo-PG and the enzymatic combination (Endo-A + Endo-G + α-Ara-ase + β-Gal-ase + Endo-PG), respectively. However, the fact that both HG and XGA appeared to be covalently linked to RG-I in apple pectin (Coenen et al., 2007) ruled out the latter assumption. It has also been found that the treatment of a highly (IEC/SEC-)purified alkali-extracted passion fruit pectin (AEPP) with the enzymatic combination (Endo-A + Endo-G + α-Ara-ase + β-Gal-ase + RG-ase) yielded on SEC analysis three well-resolved peak materials corresponding to XGA, HG, and RG-I oligomers (B. M. Yapo, unpublished), which strongly indicated that AEPP was a pectin-complex in which RG-I block was connected to both HG and XGA blocks, in good agreement with the report of Coenen et al. (2007). However, the relative position of each of the building blocks of pectin-complex was not known from all of these studies, and therefore remains a big challenge for future work, though some interesting hypothetical models have been proposed.

3.2. The key hypothetical models of pectin-complex

3.2.1. The precursory 'rhamnogalacturonan' model

The precursory hypothetic model of pectin-complex was the so-called 'rhamnogalacturonan' proposed for Endo-PG-solubilised HMW-pectic polymer from sycamore primary cell walls (Talmadge et al., 1973). In this model, the macromolecular complex was schematically represented to be composed of a HG sequence of a DP of ~8 alternating with the trisaccharide [Rha-(1 → 4)-GalA-(1 → 2)-Rha]. The macromolecule would have a zigzag shape, owing to the flexibility of (1 → 2)-Rha units in otherwise linear (1 → 4)-linked α-D-galacturonan chain, and the Rha formed a Y-shaped branch point via O-4 position substitution with a linear galactan side chains (Fig. 2).

3.2.2. The traditional model of alternating 'smooth' regions (SRs) and 'hairy' regions (HRs)

Degradations, with pectate-lyase/pectin-lyase preparations, of IEC/SEC-purified cold buffer, hot buffer, hot oxalate, and hot dilute acid-solubilised apple pectins allowed De Vries et al. (1982) to propose different models of pectin-complex, which were all characterised by an overall linear backbone consisting of SRs (or unbranched HG stretches) alternately linked to HRs (or branched RG-I stretches). One of these models, composed of successive stretches of HR and SR, was dominant in all the pectin extracts. In this model, the HRs were placed at regular intervals and close to the ends of the macromolecular complex to explain an observed inverse relationship between the NS content and the apparent M_w of some pectin fractions (De Vries et al., 1982). The NS strands, which formed the 'hairs', were found to be almost totally concentrated, as 'blocks' in the HRs, containing at most 5% of the total uronic acid of the whole macromolecule (De Vries et al., 1982), thus confirming previous assumption (Aspinall, 1970; Talmadge et al., 1973). In the course of running, this model of pectin-complex, as schematised in Fig. 3a, for which further refinement has lately been suggested (Coenen et al., 2007), has appeared to be the structural model of cell wall pectins, irrespective of plant source (Prade et al., 1999; Ralet, Bonnin, & Thibault, 2001; Schols & Voragen, 1996). However, since it cannot be ruled out that only part of the connecting points present in the apple pectin has been identified and not all unknown oligomers could be identified so far, the model of pectin-complex in which HG is positioned as a RG-I side chain could not be excluded irrevocably (Coenen et al., 2007).

3.2.3. The 'RG-I backbone' model

Recently, however, another model of pectin-complex, here referred to as the 'RG-I backbone model' (Fig. 4a) in which HG is arranged to be a side chain of RG-I core has been proposed by Vincken et al. (2003), though it is not known with certainty if HG is fully a RG-I side chain and/or 'grafted' to NS side chains of RG-I. The striking differences between the traditional (or conventional) model and this model are (i) that the backbone of the pectin-complex is exclusively of a RG-I type and (ii) linear HG and XGA appeared to be side chains of the RG-I backbone. Thus, the macromolecular complex is completely of a 'hairy type'; the 'hairs' being composed of strands of NSs (arabinan, galactan, AG-I and possibly AG-II), HG and XGA. Unknown are how HG and XGA blocks will be oriented over the RG-I backbone, the side chain kinds and their distribution patterns. Therefore, it may be more realistic to draw all the side chains, perpendicularly to the RG-I backbone, in various

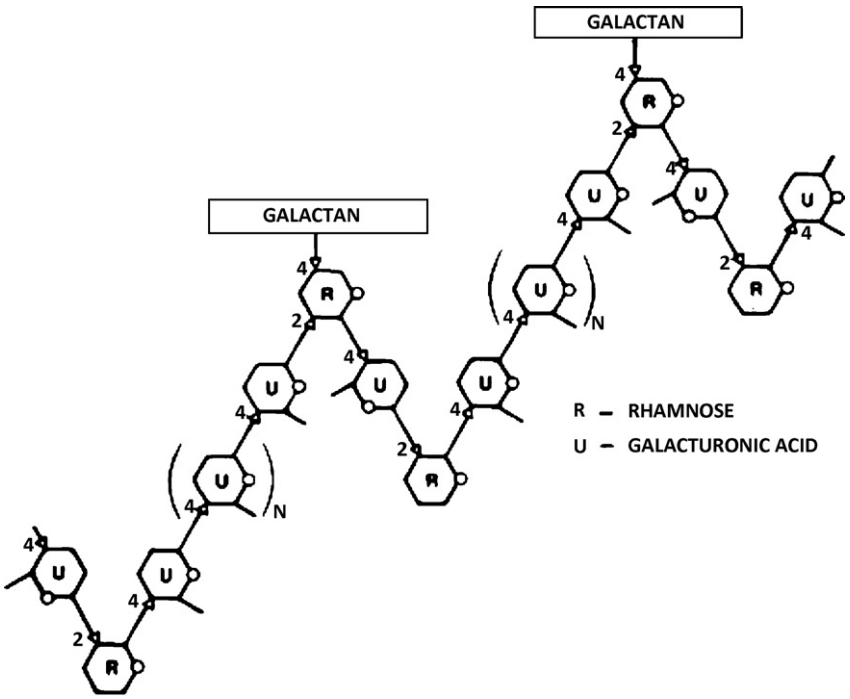

Fig. 2. A proposed structure for the rhamnogalacturonan. The sugar residues in the figure are designated as R = rhamnose and U = galacturonic acid. *N* = an undetermined number, probably between 4 and 10. Reproduced with permission from Talmadge et al. (1973).

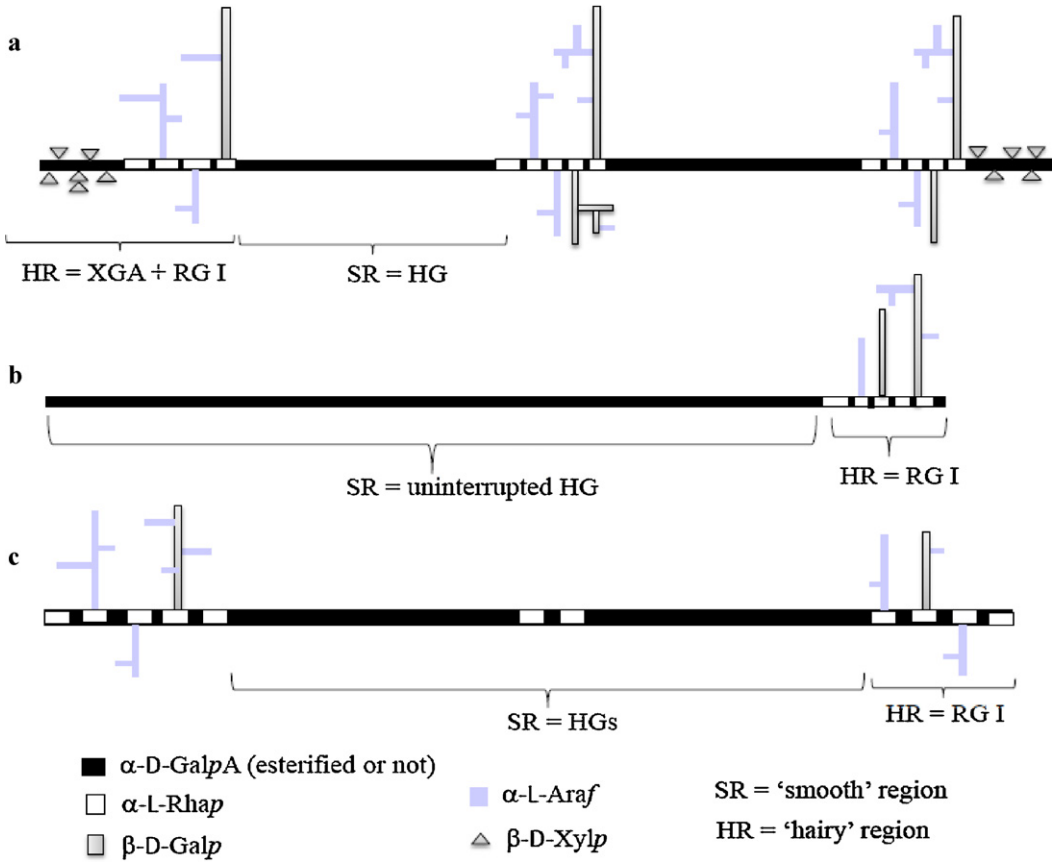

Fig. 3. Traditional models of pectin-complex showing an overall linear backbone made up of unbranched HG and the core of neutral sugar-branched RG-I, and the galacturonan core of XGA (if present) (a) based on structural features on various apple pectins according to Voragen et al. (1995) and refined, regarding the position of XGA in the HR region, by taking into account the recent work of Coenen et al. (2007), (b) based on structural features of commercial citrus pectin (Zhan et al., 1998), and (c) based on structural characteristics of (acid-extracted) sugar beet and hop pectins (Oosterveld et al., 2002).

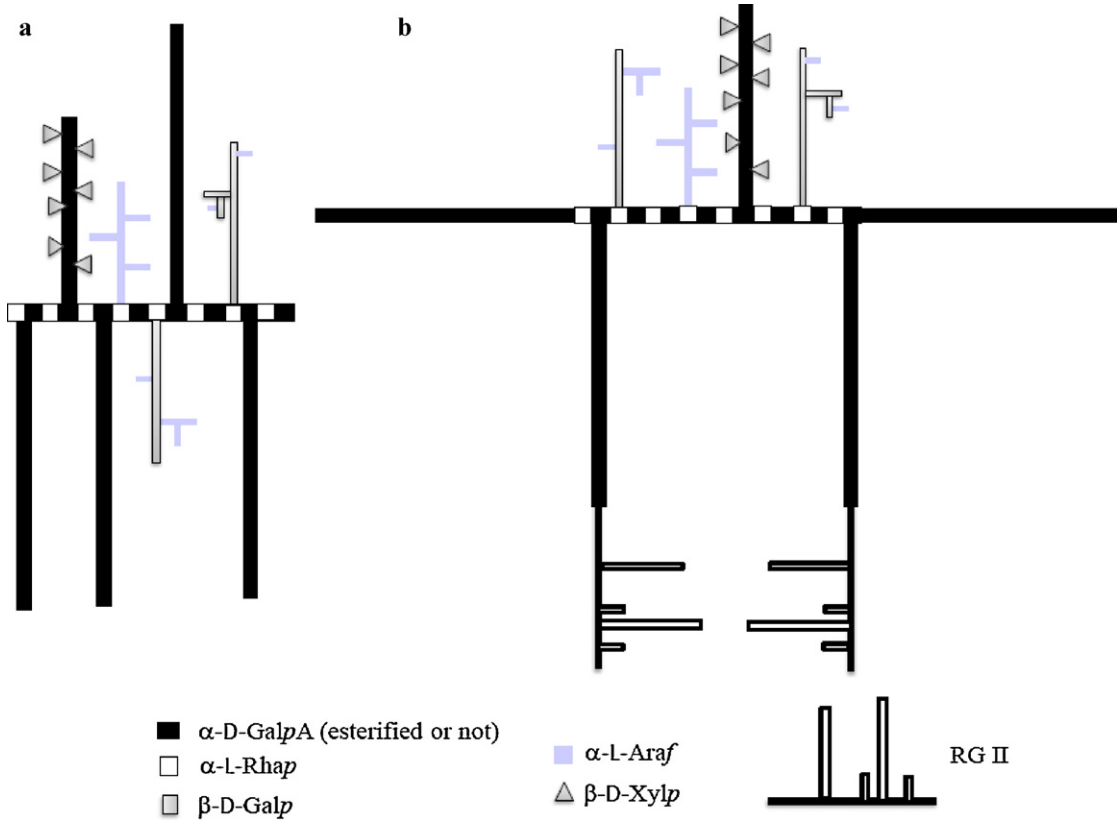

Fig. 4. Non-traditional pectin-complex models: (a) the 'RG-I backbone' model after Vincken et al. (2003) showing an exclusive RG-I backbone and the positioning of HG and XGA as side chains of RG-I together with the neutral sugar (arabinan, arabinogalactan I and possibly arabinogalacatn II) side chains. The orientation as well as the position of the side chains on the RG-I backbone is arbitrary; (b) the 'living thing-like' model, here proposed on the basis of structural and macromolecular data obtained by mild-acid hydrolysis (MAH) and two different enzymatic (polygalacturonase and rhamnogalacturonase) degradations of mild alkali-extracted IEC-and-SEC-purified pectin (AEPP) from CWM of yellow passion fruit rind. In the latter model, the overall backbone of the pectin complex is thought to be composed of two unbranched HG blocks per one RG-I core which are alternately connected to one another. The two HG elements in the main chain may take a horizontal as well as a vertical position (upwards and downwards) owing to the flexibility of (1 → 2)-linked α-L-Rha units via which they are attached to the RG-I core. The other HG blocks would be positioned as (downwards) side chains of the RG-I core, whereas the neutral polymers (arabinan, galactan and/or arabinogalactan) and (if present) SGs other than RG-II (e.g., XGA) would be positioned as (upwards) side chains. It could not be ruled out that some of the side chain HGs may be linked the main chain HGs, thereby giving rise to branched HGs as recently reported from various pectins. The branching points of the side chains to the RG-I core as well as their orientation are not known with certainty, though it might be as shown. Two distinct RG-II blocks would be linked to the reducing ends of two distinct HG blocks and orientated in such a way that dRG-II-B formation may be promoted easily.

directions (Vincken et al., 2003). The structural differences in the two model types (Figs. 3a and 4a) should give rise to differences in pectin hydrodynamic properties as well as in recalcitrance towards pectinolytic enzymes, which might impact on the mechanical properties and integrity of the cell wall. For example, the RG-I backbone model should display a smaller hydrodynamic volume but a greater resistance to pectinases than the traditional model. The proposal of this model has been motivated not only by the possible length periodicity of linear HG (Thibault et al., 1993) but also by several more recent reports likely to refute the traditional model such as the lack of an expected single Rha residue in Endo-PG-generated GalA oligomers from commercial citrus pectin (Zhan, Janssen, & Mort, 1998) as shown in Fig. 3b, the suggestion that XGA might be a side chain of RG-I in cellulose-associated apple pectins (Oechslin, Lutz, & Amadò, 2003) and the observation, by atomic force microscopy (AFM), of long polyGalA-branched pectin-complex in tomato pectin fractions (Round, Rigby, MacDougall, Ring, & Morris, 2001), to which could be added the report, by Oosterveld, Voragen, and Schols (2002), that (arabinogalactan-)branched RG-I backbones were predominantly located at the extremities of acid-extracted sugar beet and hop pectins, in contradiction with the general assumption that RG-I stretches are present throughout the pectin complex (Schols & Voragen, 1996). The latter data suggested that if the two extracted pectins comprised more than one linear HG block (assuming a length periodicity), they would be linked to one another via single/few Rha residues, as lately reported in comaruman and cirsiuman, two aq. AO-extracted pectins from the aerial part of the phanerogam of the marsh cinquefoil *Comarum palustre* (Ovodova et al., 2006) and the stems of the ground thistle *Cirsium esculentum* (Khramova et al., 2011) or short GalA-Rha repeats to form a large (linear) HG domain bordered by two NS-ramified RG-I domains at the two ends of each macromolecule (Fig. 3c). However, recent structural studies of pectins from YPF rind (AEPP) have indicated that pectin structural arrangement could be more complex than the previous two, which allows the proposal of a new model.

3.2.4. *A new hypothetical model*

Different recent studies permit us to put forward that the traditional model is less and less compelling as the suitable model for all pectins, irrespective of the origin, but the 'RG-I backbone model' may also reflect only a partial representation of pectin-complex as it would exist *in muro*. It has indeed been found that neither chemical (0.1 M TFA) nor enzymatic (RG-ase + Endo-G) hydrolysis of a galactan-rich RG-I from bast fibre peels of developing flax stem yielded oligoGalA residues, indicating that RG-I blocks were not interrupted by (linear) HG regions (Gur'janov, Gorshkova, Kabel, Schols, & van Dam, 2007). Furthermore, an overwhelming concentration of the pectin GalA residues (more than 80% of total uronic acid) is usually found in the HG regions of various pectins (see for e.g., Table 1), suggesting that HG regions are predominant

over RG-I regions in those pectins as also found in pectins from CWMs of many other plant sources, such as *Arabidopsis thalania*, tea (*Camellia sinensis*), *Basella rubra*, *C. esculentum*, mangosteen, plantain, pawpaw, cashew apple, garden egg, avocado pear, and cacao (Dong, Hayashi, Mizukoshi, Lee, & Hayashi, 2011; Ele-Ekouna, Pau-Roblot, Courtois, & Courtois, 2011; Khramova et al., 2011; Vriesmann, Teófilo, & Petkowicz, 2011; Nwanekezi, Alawuba, & Mkpolulu, 1994; Ralet et al., 2008; Yapo, 2010). Besides, the number of GalA residues contained in the HG regions was estimated to be 98% of total sugar (GalA + Rha) in lemon pectins (Nakamura et al., 2001). Thus, assuming a length periodicity for HG, it appears virtually untenable that the backbone of pectin-complex would consist of consecutive stretches of HG and RG-I (Yapo, Lerouge, et al., 2007). Therefore, the idea that unsubstituted HG is otherwise than linearly connected to the RG-I core in the constitution (or construction) of pectin-complex is plausible, even if strict evidence is being searched for. Cell wall-rich pumpkin (*Cucumis moschata* Duch.) digestion with cellulase released pectin macromolecules which included linear HG side chains, the RG-I core and RG-II, in conjunction with XGA (Fissore, Ponce, Stortz, Rojas, & Gerschenson, 2007). Lately, polyGalA-branched HG (GaGA) complexes have been shown to be an integral part of Na_2CO_3-extracted tomato pectins (Round et al., 2010). All these data suggest an *in muro* existence of various pectin-complexes, including the two models afore-mentioned or a more elaborate macromolecular complex from which both may be derived following partial degradation by endogenous enzymes. Thus, having also recently observed that the enzymatic combination [RG-ase + Endo-A + Endo-G + α-Ara-ase + β-Gal-ase] afforded the separation of HG, XGA and RG-I oligomers from AEPP, whereas no separate XGA material was concomitantly obtained with separate materials of RG-I, RG-II-like, and oligoGalAs by Endo-PG treatment on SEC fractionations, it has been concluded, in combination of the occurring changes in the pectin macromolecular parameters (intrinsic viscosity and M_w) following enzymatic treatments, that neither the traditional model nor the 'RG-I backbone' model is amply representative of the purified pectin (B. M. Yapo, unpublished). On the basis of all these latest findings, a new hypothetical model of pectin-complex has here been proposed (Fig. 4b) for those pectins encompassing dominant blocks of HG, RG-I, XGA, and RG-II. In the present model, which sounds like a 'living thing', both the traditional and 'RG-I backbone' models are accounted for. In this 'living thing-like' model, the backbone of the pectin-complex is alternately composed of two linear HG elements and one RG-I core. The latter is decorated with NS (arabinan, galactan, arabino-galactan) and XGA strands as side chains, which may include other SGs (when present) except for RG-II. The two HG blocks in the main chain can take a horizontal as well as a vertical position (upwards and downwards) owing to the flexibility of (1 → 2)-linked α-L-Rha units via which they are attached to the RG-I core. The trunk is composed of blocks of HG perpendicularly linked to the RG-I core and may be oriented downwards. Finally, blocks of RG-II are linearly connected to these HG blocks as the 'roots' of the pectin-complex. By such a hypothetical model, the observed overwhelming majority of HG over RG-I, the liberation of homogenous linear HG stretches by MAH, the release of XGA together with HG and RG-I oligomers by the enzymic combination [Endo-A + Endo-G + α-Ara-ase + β-Gal-ase + RG-ase] but not together with RG-I, RG-II, and oligoGalAs by homogenous Endo-PG, and the possible dimerisation of mRGs-II could be explained.

4. Concluding remarks

Pectic substances represent an outstanding family of the cell wall polysaccharides, which are extraordinary versatile but not yet fully known on a structural as well as a functional point of view. This group comprises at least eight different pectic polysaccharide types, of which homogalacturonan, types I and II rhamnogalacturonans, and to lesser extent xylogalacturonan are the most common. However, the unavailability of analytical tools capable of directly exploring pectins *in muro* appears to be a great limitation to gaining full knowledge of the fine structure(s) as well as functionalities of most of them, especially rhamnogalacturonan-I and the substituted galacturonans. Owing to differences in sensitivity and susceptibility of the glycosidic linkages that cell wall pectins are composed of, extractive methods, whether chemical or enzymatic, engender degradations to different degrees so that *out of muro* described structures may not completely reflect *in muro* would be structures. Therefore, the development of new approaches, including chemical and enzymatic analyses using enzymes of specificity and direct *in muro* probing with anti-pectic polysaccharide antibodies should be given priority. That the different pectic polysaccharides are present in cell walls as distinct structural blocks of complex pectins or as separate (but closely associated) pectic polymers or both is not known with certainty. Nevertheless, numerous solid data allow to conclude that a part of them is likely present as macromolecular pectin-composite(s) which framework is not yet totally unravelled. Our newly proposed hypothetical model of pectin-complex could fill the gap between the conventional and the 'RG-I backbone' models which have been so far the two most striking hypothetical models of pectin-complex. Nevertheless, there is still a long way to go to comprehensively elucidate the enigmatic fine structure of cell wall pectins as well as their extraordinary multifunctional properties, of which some are to be discovered. That much is known about the structural characteristics of pectic substances is obviously certain, but what remains to be known may be bigger and more promising in relation to functional properties.

Acknowledgement

The author is profoundly indebted to Yeshua Ewrade for his assistance and precious help with this review.

References

Aboughe-Angone, S., Bardor, M., Nguema-Ona, E., Rihouey, C., Ishii, T., Lerouge, P., et al. (2009). Structural characterization of cell wall polysaccharides from two plant species endemic to central Africa, *Fleurya aestuans* and *Phragmenthera capitata*. *Carbohydrate Polymers*, *75*, 104–109.

Albersheim, P., Darvill, A. G., O'Neill, M. A., Schols, H. A., & Voragen, A. G. J. (1996). An hypothesis: The same six polysaccharides are components of the primary cell walls of all higher plants. In J. Visser, & A. G. J. Voragen (Eds.), *Pectins and pectinases, progress in biotechnology* (pp. 47–55). Amsterdam: Elsevier Science.

Anderson, E. (1933). The preparation of L-galactose from flaxseed mucilage. *The Journal of the Biological Chemistry*, *100*, 249–253.

Aspinall, G. O. (1970). Gums and mucilages. *Advances in Carbohydrate Chemistry and Biochemistry*, *24*, 333–379.

Aspinall, G. O., & Fanous, H. K. (1984). Structural investigations on the non-starchy polysaccharides of apples. *Carbohydrate Polymers*, *4*, 193–214.

Bhattacharjee, S. S., & Timell, T. E. (1965). A study of the pectin present in the bark of Ambilis fir (*Abies amabilis*). *Canadian Journal of Chemistry*, *43*, 758–765.

Beck, E. (1967). Isolation and characterization of an apiogalacturonan from the cell wall of *Lemna minor*. *Zeitschrift für Pflanzenphysiologie*, *57*, 444–461.

Beldman, G., Vincken, J.-P., Schols, H. A., Meeuwsen, P. J. A., Herweijer, M., & Voragen, A. G. J. (2001). Degradation of differently substituted xylogalacturonans by endoxylogalacturonan hydrolase and endopolygalacturonases. *Biocatalysis and Biotransformation*, *21*, 189–198.

Bouveng, H. O. (1965). Polysaccharides in pollen. II. The xylogalacturonan from mountain pine (*Pinus mugo* Turra) pollen. *Acta Chemica Scandinavica*, *19*(4), 953–963.

Braconnot, H. (1825). Recherches sur un nouvel acide universellement répandu dans tous les végétaux. *Annales de Chimie et de Physique*, *28*(2), 173–178.

Caffall, K. H., & Mohnen, D. (2009). The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. *Carbohydrate Research*, *344*, 1879–1900.

Chambat, G., & Joseleau, J. P. (1980). Isolation and characterization of a homogalacturonan in the primary walls of *Rosa* cells cultures *in vitro*. *Carbohydrate Research*, *85*, C10–C12.

Coenen, G. J., Bakx, E. J., Verhoef, R. P., Schols, H. A., & Voragen, A. G. J. (2007). Identification of the connecting linkage between homo- or xylogalacturonan and rhamnogalacturonan type I. *Carbohydrate Polymers*, *70*, 224–235.

Cui, S. W. (2005). *Food carbohydrates: Chemistry, physical properties, and applications*. Boca Raton, FL: CRC Press (Taylor & Francis Group).

Darvill, A. G., McNeil, M., & Albersheim, P. (1978). Structure of plant cell walls. VIII. A new pectic polysaccharide. *Plant Physiology*, *62*, 418–422.

Delmas, F., Séveno, M., Northey, J. G. B., Hernould, M., Lerouge, P., McCourt, P., et al. (2008). The synthesis of the rhamnogalacturonan-II component 3-deoxy-D-manno-2-octulosonic acid (Kdo) is required for pollen tube growth and elongation. *Journal of Experimental Botany*, *59*, 2639–2647.

Deng, C., O'Neill, M. A., & York, W. S. (2006). Selective chemical depolymerization of rhamnogalacturonans. *Carbohydrate Research*, *341*, 474–484.

De Vries, J. A., den Uijl, C. H., Voragen, A. G. J., Rombouts, F. M., & Pilnik, W. (1983). Structural features of the neutral sugar side chains of apple pectic substances. *Carbohydrate Polymers*, *3*, 193–205.

De Vries, J. A., Rombouts, F. M., Voragen, A. G. J., & Pilnik, W. (1982). Enzymic degradation of apple pectins. *Carbohydrate Polymers*, *2*, 25–33.

De Vries, J. A., Voragen, A. G. J., Rombouts, F. M., & Pilnik, W. (1986). Structural studies of apple pectins with pectolytic enzymes. In M. F. Fishman, & J. J. Jen (Eds.), *Chemistry and function of pectins* (pp. 38–48). Washington DC: ACS Symposium Series, American Chemical Society.

Doco, T., Williams, P., Vidal, S., & Pellerin, P. (1997). Rhamnogalaturonan II, a dominant polysaccharide in juices produced by enzymic liquefaction of fruits and vegetables. *Carbohydrate Research*, *297*, 181–186.

Dong, C.-X., Hayashi, K., Mizukoshi, Y., Lee, J.-B., & Hayashi, T. (2011). Structures of acidic polysaccharides from *Basella rubra* L. and their antiviral effects. *Carbohydrate Polymers*, *84*, 1084–1092.

Eda, S., & Katö, K. (1980). Pectin isolated from the midrib of leaves of *Nicotiana tabacum*. *Agricultural and Biological Chemistry*, *44*(12), 2793–2801.

Edashige, Y., & Ishii, T. (1998). Rhamnogalacturonan-II from cell walls of *Cryptomeria japonica*. *Phytochemistry*, *49*, 681–690.

Ehrlich, F. (1917). Die pektinstoffe, ihre konstitution und bedeutung. *Chemiker Zeitung*, *41*, 197–200.

Ele-Ekouna, J.-P., Pau-Roblot, C., Courtois, C., & Courtois, J. (2011). Chemical characterization of pectin from green tea (*Camellia sinensis*). *Carbohydrate Polymers*, *83*(3), 1232–1239.

Fissore, E. N., Ponce, N. M., Stortz, C. A., Rojas, A. M., & Gerschenson, L. N. (2007). Characterisation of fiber obtained from pumpkin (*Cucumis moschata* Duch.) mesocarp through enzymatic treatment. *Food Science and Technology International*, *13*(2), 141–151.

Fleischer, A., O'Neill, M. A., & Ehwald, R. (1999). The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan-II. *Plant Physiology*, *121*, 829–838.

Glinsky, V. V., & Raz, A. (2009). Modified citrus pectin anti-metastatic properties: One bullet, multiple targets. *Carbohydrate Research*, *344*, 1788–1791.

Gloaguen, V., Krausz, P., Brudieux, V., Closs, B., Leroy, Y., & Guerardel, Y. (2008). Structural patterns of rhamnogalacturonans modulating Hsp-27 expression in cultured human keratinocytes. *Molecules*, *13*, 1207–1218.

Gur'janov, O. P., Gorshkova, T. A., Kabel, M., Schols, H. A., & van Dam, J. E. G. (2007). MALDI-TOF MS evidence for the linking of flax bast fibre galactan to rhamnogalacturonan backbone. *Carbohydrate Polymers*, *67*, 86–96.

Hellín, P., Ralet, M. C., Bonnin, E., & Thibault, J. F. (2005). Homogalacturonans from lime pectins exhibit homogeneous charge density and molar mass distributions. *Carbohydrate Polymers*, *60*, 307–317.

Hilz, H., Williams, P., Doco, T., Schols, H. A., & Voragen, A. G. J. (2006). The pectic polysaccharide rhamnogalacturonan-II is present as a dimer in pectic populations of bilberries and black currants *in muro* and in juice. *Carbohydrate Polymers*, *65*, 521–528.

Hirst, E. L., & Jones, J. K. N. (1939). Pectic substances. Part III. Composition of apple pectin and the molecular structure of the araban component of apple pectin. *Journal of the Chemical Society*, *103*, 454–460.

Immerzeel, P., Eppink, M. M., De Vries, S. C., Schols, H. A., & Voragen, A. G. J. (2006). Carrot arabinogalactan proteins are interlinked with pectins. *Physiologia Plantarum*, *128*, 18–28.

Ishii, T., & Kaneko, S. (1998). Oligosaccharides generated by partial hydrolysis of the borate-rhamnogalacturonan-II complex from sugar beet. *Phytochemistry*, *49*(5), 1195–1202.

Ishii, T., & Matsunaga, T. (2001). Pectic polysaccharide rhamnogalacturonan-II is covalently linked to homogalacturonan. *Phytochemistry*, *57*, 969–974.

Jackson, C. L., Dreaden, T. M., Theobald, L. K., Tran, N. M., Beal, T. L., Eid, M., et al. (2007). Pectin induces apoptosis in human prostate cancer cells: Correlation of apoptotic function with pectin structure. *Glycobiology*, *17*, 805–819.

Jarvis, M. C., Forsyth, W., & Duncan, H. J. (1988). A survey of the pectic content of non-lignified monocot cell walls. *Plant Physiology*, *88*, 309–314.

Khramova, D. S., Golovchenko, V. V., Shashkov, A. S., Otgonbayar, D., Chimidsogzol, A., & Ovodov, Yu. S. (2011). Chemical composition and immunomodulatory activity of a pectic polysaccharide from the ground thistle *Cirsium esculentum* Siev. *Food Chemistry*, *126*, 870–877.

Kikuchi, A., Edashige, J., Ishii, T., & Satoh, S. (1996). A xylogalacturonan whose level is dependent on the size of cell clusters is present in the pectin from cultured carrot cells. *Planta*, *200*, 369–372.

Kikuchi, T., & Sugimoto, H. (1976). Detailed structure of an acidic polysaccharide in soy sauce, confirmed by use of two kinds of purified pectinases. *Agricultural and Biological Chemistry*, *40*, 87–92.

Le Goff, A., Renard, C. M. G. C., Bonnin, E., & Thibault, J. F. (2001). Extraction, purification, and chemical characterisation of xylogalacturonans from pea hulls. *Carbohydrate Polymers*, *45*, 325–334.

Link, K. P., & Nedden, R. J. (1931). Improvements in the preparation of D-galacturonic acid. *The Journal of Biological Chemistry*, *94*, 307–314.

Matsunaga, T., Ishii, T., Matsumoto, S., Higuchi, M., Darvill, A., Albersheim, P., et al. (2004). Occurrence of the primary cell wall polysaccharide rhamnogalacturonan-II in pteridophytes, lycophytes, and bryophytes. Implications for the evolution of vascular plants. *Plant Physiology*, *134*, 339–351.

McCready, R. M., & Gee, M. (1960). Determination of pectic substances by paper chromatography. *Journal of Agriculture and Food Chemistry*, *8*, 510–513.

McNeil, M., Darvill, A. G., & Albersheim, P. (1980). Structure of plant cell walls: X. Rhamnogalacturonan-I, a structurally complex pectic polysaccharide in the walls of suspension-cultured sycamore cells. *Plant Physiology*, *66*, 1128–1134.

Moore, J. P., Farrant, J. M., & Driouich, A. (2008). A role for pectin-associated arabinans in maintaining the flexibility of the plant cell wall during water deficit stress. *Plant Signaling & Behavior*, *3*, 102–104.

Morell, S., Baur, L., & Link, K. P. (1934). The methylglycosides of the naturally occurring hexuronic acids. III. Polygalacturonic acid-methylglycosides derived from pectin. *The Journal of Biological Chemistry*, *105*, 1–13.

Mort, A. J. (1983). An apparatus for safe and convenient handling of anhydrous liquid hydrogen fluoride at controlled temperatures and reactions times. Application to the generation of oligosaccharides from polysaccharides. *Carbohydrate Research*, *122*, 315–321.

Mort, A., Zheng, Y., Qiu, F., Nimtz, M., & Bell-Eunice, G. (2008). Structure of xylogalacturonan fragments from watermelon cell-wall pectin. Endopolygalacturonase can accommodate a xylosyl residue on the galacturonic acid just following the hydrolysis site. *Carbohydrate Research*, *343*, 1212–1221.

Muralikrishna, G., Salimath, P. V., & Tharanathan, R. N. (1987). Structural features of an arabinoxylan and a rhamnogalacturonan derived from linseed mucilage. *Carbohydrate Research*, *161*, 265–271.

Nakamura, A., Furuta, H., Maeda, H., Nagamatsu, Y., & Yoshimoto, A. (2001). Analysis of structural components and molecular construction of soybean soluble polysaccharides by stepwise enzymatic degradation. *Bioscience, Biotechnology and Biochemistry*, *65*, 2249–2258.

Nakamura, A., Furuta, H., Maeda, H., Takao, T., & Nagamatsu, Y. (2002a). Structural studies by stepwise enzymatic degradation of the main backbone of soybean soluble polysaccharides consisting of galacturonan and rhamnogalacturonan. *Bioscience, Biotechnology and Biochemistry*, *66*, 1301–1313.

Nakamura, A., Furuta, H., Maeda, H., Takao, T., & Nagamatsu, Y. (2002b). Analysis of the molecular construction of xylogalacturonan isolated from soluble soybean polysaccharides. *Bioscience, Biotechnology and Biochemistry*, *66*, 1155–1158.

Naran, R., Chen, G., & Carpita, N. C. (2008). Novel rhamnogalacturonan-I and arabinoxylan polysaccharides of flax seed mucilage. *Plant Physiology*, *148*, 132–141.

Nussinovitch, A. (1997). *Hydrocolloid applications: Gum technology in the food and other industries*. New York: Blackie Academic & Professional.

Nwanekezi, E. C., Alawuba, O. C. G., & Mkpolulu, C. C. M. (1994). Characterization of pectic substances from selected tropical fruits. *Journal of Food Science and Technology*, *31*, 159–161.

Øbro, J., Harholt, J., Scheller, H. V., & Orfila, C. (2004). Rhamnogalacturonan-I in *Solanum tuberosum* tubers contains complex arabinogalactan structures. *Phytochemistry*, *65*, 1429–1438.

Oechslin, R., Lutz, M. V., & Amadò, R. (2003). Pectic substances isolated from apple cellulosic residue: Structural characterisation of a new type of rhamnogalacturonan-I. *Carbohydrate Polymers*, *51*, 301–310.

O'Neill, M. A., Albersheim, P., & Darvill, A. (1990). The pectic polysaccharides of primary cell walls. In P. M. Dey (Ed.), *Methods in plant biochemistry, carbohydrates* (1st ed., pp. 415–441). London: Academic Press.

O'Neill, M. A., Ishii, T., Albersheim, P., & Darvill, A. G. (2004). Rhamnogalacturonan-II: Structure and function of a borate cross-linked cell wall pectic polysaccharide. *Annual Review of Plant Biology*, *55*, 109–139.

O'Neill, M. A., & York, W. S. (2003). The composition and structure of plant primary cell walls. In J. K. C. Rose (Ed.), *The plant cell wall* (pp. 1–54). Boca Raton, FL: Blackwell/CRC.

Oosterveld, A., Voragen, A. G. J., & Schols, H. A. (2002). Characterization of hop pectins shows the presence of an arabinogalactan-protein. *Carbohydrate Polymers*, *49*, 407–413.

Ortiz-Basurto, R. I., Williams, P., Belleville, M. P., & Doco, T. (2009). Presence of rhamnogalacturonan-II in the juices produced by enzymatic liquefaction of Agave pulquero stem (*Agave mapisaga*). *Carbohydrate Polymers*, *77*, 870–875.

Ovodov, Yu. S. (1975). Structural chemistry of plant glycuronoglycans. *Pure Applied Chemistry*, *42*(3), 351–369.

Ovodov, Yu. S. (2009). Current views on pectin substances. *Russian Journal of Bioorganic Chemistry*, *35*, 269–284.

Ovodov, Yu. S., Ovodova, R. G., Bondarenko, O. D., & Krasikova, I. N. (1971). The pectic substances of Zosteraceae. Part IV. Pectinase digestion of zosterine. *Carbohydrate Research*, *18*, 311–318.

Ovodova, R. G., Popov, S. V., Bushneva, O. A., Golovchenko, V. V., Chizhov, A. O., Klinov, D. V., et al. (2006). Branching of the galacturonan backbone of comaruman, a pectin from the marsh cinquefoil *Comarum palustre* L. *Biochemistry (Moscow)*, *71*, 538–542.

Pellerin, P., Doco, T., Vidal, S., Williams, P., Brillouet, J. M., & O'Neill, M. A. (1996). Structural characterization of red wine rhamnogalacturonan-II. *Carbohydrate Research*, *290*, 183–197.

Penfield, S., Meissner, R. C., Shoue, D. A., Carpita, N. C., & Bevan, M. W. (2001). *MYB61* is required for mucilage deposition and extrusion in the Arabidopsis seed coat. *The Plant Cell*, *13*, 2777–2791.

Pérez, S., Rodríguez-Carvajal, M. A., & Doco, T. (2003). A complex plant cell wall polysaccharide: Rhamnogalacturonan-II. A structure in quest of a function. *Biochimie*, *85*, 109–121.

Petersen, O. B., Meier, S., Duus, J. Ø., & Clausen, M. H. (2008). Structural characterization of homogalacturonan by NMR spectroscopy—Assignment of reference compounds. *Carbohydrate Research*, *343*, 2830–2833.

Pilarska, M., Czaplicki, A. Z., & Konieczny, R. (2007). Patterns of pectin epitope expression during shoot and root regeneration in androgenic cultures of two wheat cultivars. *Acta Biologica Cracoviensia Series Botanica*, *49*, 69–72.

Prabasari, I., Filomena Pettolino, F., Ming-Long Liao, M.-L., & Bacic, A. (2011). Pectic polysaccharides from mature orange (*Citrus sinensis*) fruit albedo cell walls: Sequential extraction and chemical characterization. *Carbohydrate Polymers*, *84*, 484–494.

Prade, R. A., Zhan, D., Ayoubi, P., & Mort, A. J. (1999). Pectins, pectinases and plant–microbe interactions. *Biotechnology and Genetic Engineering Reviews*, *16*, 361–391.

Ralet, M. C., Bonnin, E., & Thibault, J. F. (2001). Pectins. In E. Vandamme (Ed.), *Biopolymers* (pp. 345–380). Weinheim, Germany: Wiley-VCH Verlag.

Ralet, M.-C., Crépeau, M.-J., Lefèbvre, J., Mouille, G., Höfte, H., & Thibault, J.-F. (2008). Reduced number of homogalacturonan domains in pectins of an Arabidopsis mutant enhances the flexibility of the polymer. *Biomacromolecules*, *9*, 1454–1460.

Redgwell, R. J., Curti, D., Wang, J., Dobruchowska, J. M., Gerwig, G. J., Kamerling, J. P., et al. (2011). Cell wall polysaccharides of Chinese Wolfberry (*Lycium barbarum*): Part 2. Characterisation of arabinogalactan-proteins. *Carbohydrate Polymers*, *84*, 1075–1083.

Renard, C. M. G. C., Crépeau, M. J., & Thibault, J. F. (1999). Glucuronic acid directly linked to galacturonic acid in the rhamnogalacturonan backbone of beet pectins. *European Journal of Biochemistry*, *266*, 566–574.

Reuhs, B. L., Glenn, J., Stephens, S. B., Kim, J. S., Christie, D. B., Glushka, J. G., et al. (2004). L-Galactose replaces L-fucose in the pectic polysaccharide rhamnogalacturonan-II synthesized by the L-fucose-deficient mur1 Arabidopsis mutant. *Planta*, *219*, 147–157.

Ridley, B. L., O'Neill, M. A., & Mohnen, D. (2001). Pectins: Structure, biosynthesis, and oligogalacturonide-related signaling. *Phytochemistry*, *57*, 929–967.

Round, A. N., Rigby, N. M., MacDougall, A. J., & Morris, V. J. (2010). A new view of pectin structure revealed by acid hydrolysis and atomic force microscopy. *Carbohydrate Research*, *345*, 487–497.

Round, A. N., Rigby, N. M., MacDougall, A. J., Ring, S. G., & Morris, V. J. (2001). Investigating the nature of branching in pectin by atomic force microscopy and carbohydrate analysis. *Carbohydrate Research*, *331*, 337–342.

Schols, H. A., Bakx, E. J., Schipper, D., & Voragen, A. G. J. (1995). A xylogalacturonan subunit present in the modified hairy regions of apple pectin. *Carbohydrate Research*, *279*, 265–279.

Schols, H. A., & Voragen, A. G. J. (1996). Complex pectins: Structure elucidation using enzymes. In J. Visser, & A. G. J. Voragen (Eds.), *Pectins and pectinases, progress in biotechnology* (pp. 3–19). Amsterdam: Elsevier Science.

Schols, H. A., Voragen, A. G. J., & Colquhoun, I. J. (1994). Isolation and characterization of rhamnogalacturonan oligomers, liberated during degradation of pectic hairy regions by rhamnogalacturonase. *Carbohydrate Research*, *256*, 97–111.

Sengkhamparn, N., Bakx, E. J., Verhoef, R., Schols, H. A., Sajjaanantakul, T., & Voragen, A. G. J. (2009). Okra pectin contains an unusual substitution of its rhamnosyl residues with acetyl and alpha-linked galactosyl groups. *Carbohydrate Research*, *344*, 1842–1851.

Sila, D. N., Van Buggenhout, S., Duvetter, T., Fraeye, I., De Roeck, A., Van Loey, A., et al. (2009). Pectins in processed fruits and vegetables: Part II—Structure–function relationships. *Comprehensive Review of Food Science and Food Safety*, *8*, 86–104.

Somerville, C., Bauer, S., Brininstool, G., Facette, M., Hamann, T., Milne, J., et al. (2004). Toward a systems approach to understanding plant cell walls. *Science*, *306*, 2206–2211.

Spellman, M. W., McNeil, M., Darvill, A. G., & Albersheim, P. (1983). Isolation and characterization of 3-*C*-carboxyl-5-deoxy-L-xylose, a naturally occurring, branched-chain, acidic monosaccharide. *Carbohydrate Research*, *122*, 115–129.

Stevenson, T. T., Darvill, A. G., & Albersheim, P. (1988). 3-Deoxy-D-*lyxo*-2-heptulosaric acid, a component of the plant cell-wall polysaccharide rhamnogalacturonan-II. *Carbohydrate Research*, *179*, 269–288.

Talmadge, K. W., Keegstra, K., Bauer, W. D., & Albersheim, P. (1973). Structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. *Plant Physiology*, *51*, 158–173.

Tharanathan, R. N., Reddy, G. C., Muralikrishna, G., & Susheelamma, N. S. (1994). Structure of a galactoarabinan-rich pectic polysaccharide of native and fermented blackgram (*Phaseolus mungo*). *Carbohydrate Polymers*, *23*, 121–127.

Thibault, J. F., Renard, C. M. G. C., Axelos, M. A. V., Roger, P., & Crépeau, M. J. (1993). Studies of the length of homogalacturonic regions in pectins by acid hydrolysis. *Carbohydrate Research*, *238*, 271–286.

Thomas, J. R., Darvill, A. G., & Albersheim, P. (1989). Isolation and structural characterization of the pectic polysaccharide rhamnogalacturonan-II from walls of suspension-cultured rice cells. *Carbohydrate Research*, *185*, 261–277.

Van der Vlugt-Bergmans, C. J. B., Meeuwsen, P. J. A., Voragen, A. G. J., & Van Ooyen, A. J. J. (2000). Endo-xylogalacturonan hydrolase, a novel pectinolytic enzyme. *Applied and Environmental Microbiology*, *66*, 36–41.

Vauquelin, M. (1790). Analyse du tamarin. *Annales de Chimie*, *5*, 92–106.

Vincken, J. P., Schols, H. A., Oomen, R. J. F. J., McCann, M. C., Ulvskov, P., Voragen, A. G. J., et al. (2003). If homogalacturonan were a side chain of rhamnogalaturonan I. Implications for cell wall architecture. *Plant Physiology*, *132*, 1781–1789.

Voragen, A. G. J., Coenen, G.-J., Verhoef, R. P., & Schols, H. A. (2009). Pectin, a versatile polysaccharide present in plant cell walls. *Structural Chemistry*, *20*, 263–275.

Voragen, A. G. J., Pilnik, W., Thibault, J.-F., Axelos, M. A. V., & Renard, C. M. G. C. (1995). Pectins. In A. M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 287–339). New York: Marcel Dekker.

Vriesmann, L. C., Teófilo, R. F., & Petkowicz, C. L. O. (2011). Optimization of nitric acid-mediated extraction of pectin from cacao pod huks (*Theobroma cacao* L.) using response surface methodology. *Carbohydrate Polymers*, *84*, 1230–1236.

Whitcome, A. J., O'Neill, M. A., Steffan, W., Albersheim, P., & Darvill, A. G. (1995). Structural characterization of the pectic polysaccharide, rhamnogalacturonan-II. *Carbohydrate Research*, *271*, 15–29.

Wiethölter, N., Graeßner, B., Mierau, M., Willats, W. G. T., Knox, J. P., Bruno, M., et al. (2003). Isolation and characterisation of the homogalacturonan from type II cell walls of the commelinoid monocot wheat using HF-solvolysis. *Carbohydrate Research*, *338*, 423–431.

Willats, W. G. T., Knox, J. P., & Mikkelsen, J. D. (2006). Pect: New insights into an old polymer are starting to gel. *Trends in Food Science and Technology*, *17*, 97–104.

Wolf, S., Mouille, G., & Pelloux, J. (2009). Homogalacturonan methyl-esterification and plant development. *Molecular Plant*, *2*, 851–860.

Xia, W., Liu, S.-Q., Zhang, W.-Q., & Luo, G.-A. (2008). Structural features of a pectic polysaccharide from mulberry leaves. *Journal of Asian Natural Products Research*, *10*, 857–865.

Yapo, B. M. (2007). *Etude de la variabilité structurale des pectines (Study of the structural variability of pectins)* (163 pp.). Ph.D. Thesis. Nantes, France: Université de Nantes.

Yapo, B. M. (2009a). Pineapple and banana pectins comprise fewer homogalacturonan building blocks with a smaller degree of polymerization as compared with yellow passion fruit and lemon pectins: Implication for gelling properties. *Biomacromolecules*, *10*(4), 717–721.

Yapo, B. M. (2009b). Biochemical characteristics and gelling capacity of pectin from yellow passion fruit rind as affected by acid extractant nature. *Journal of Agriculture and Food Chemistry*, *57*(4), 1572–1578.

Yapo, B. M. (2010). Improvement of the compositional quality of monocot pectin extracts contaminated with glucuronic acid-containing components using a step-wise purification procedure. *Food and Bioproducts Processing*, *88*(2–3), 283–290.

Yapo, B. M., & Koffi, K. L. (2008a). The polysaccharide composition of yellow passion fruit rind cell wall: Chemical and macromolecular features of extracted pectins and hemicellulosic polysaccharides. *Journal of the Science of Food and Agriculture*, *88*(12), 2125–2133.

Yapo, B. M., & Koffi, K. L. (2008b). Dietary fiber components in yellow passion fruit rinds—A potential fiber source. *Journal of Agriculture and Food Chemistry*, *56*(14), 5880–5883.

Yapo, B. M., & Koffi, K. L. (2006). Yellow passion fruit rind—A potential source of low-methoxyl pectin. *Journal of Agriculture and Food Chemistry*, *54*(7), 2738–2744.

Yapo, B. M., Lerouge, P., Thibault, J.-F., & Ralet, M.-C. (2007). Pectins from citrus peel cell walls contain homogalacturonans homogenous with respect to molar mass, rhamnogalacturonan-I and rhamnogalacturonan-II. *Carbohydrate Polymers*, *69*(3), 426–435.

Yapo, B. M., Robert, C., Etienne, I., Wathelet, B., & Paquot, M. (2007). Effect of extraction conditions on the yield, purity and surface properties of sugar beet pulp pectin extracts. *Food Chemistry*, *100*(4), 1356–1364.

Yapo, B. M., Wathelet, B., & Paquot, M. (2007). Comparison of alcohol precipitation and membrane filtration effects on sugar beet pulp pectin chemical features and surface properties. *Food Hydrocolloids*, *21*(2), 245–255.

York, W. S., Darvill, A. G., McNeil, M., & Albersheim, P. (1985). 3-Deoxy-D-*manno*-2-octulosonic acid (Kdo) is a component of rhamnogalacturonan-II, a pectic polysaccharide in the primary cell walls of plants. *Carbohydrate Research*, *138*, 109–126.

Yu, L., & Mort, A. J. (1996). Partial characterization of xylogalacturonans from cell walls of ripe watermelon fruit: Inhibition of endopolygalacturonase activity by xylosylation. In J. Visser, & A. G. J. Voragen (Eds.), *Pectins and pectinases, progress in biotechnology* (pp. 79–88). Amsterdam: Elsevier Science.

Zandleven, J., Sørensen, S. O., Harholt, J., Beldman, G., Schols, H. A., Scheller, H. V., et al. (2007). Xylogalacturonan exists in cell walls from various tissues of *Arabidopsis thaliana*. *Phytochemistry*, *68*, 1219–1226.

Zhan, D., Janssen, P., & Mort, A. J. (1998). Scarcity or complete lack of single rhamnose residues interspersed within the homogalacturonan regions of citrus pectin. *Carbohydrate Research*, *308*, 373–380.

Zhang, Z., Pierce, M. L., & Mort, A. J. (2007). Changes in homogalacturonans and enzymes degrading them during cotton cotyledon expansion. *Phytochemistry*, *68*, 1094–1103.

Zitko, V., & Bishop, C. T. (1966). Structure of a galacturonan from sunflower pectic acid. *Canadian Journal of Chemistry*, *44*, 1275–1282.