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Okra pectin contains an unusual substitution of its rhamnosyl residues with acetyl and alpha-linked galactosyl groups

Nipaporn Sengkhamparn [a,b], Edwin J. Bakx [a], René Verhoef [a], Henk A. Schols [a], Tanaboon Sajjaanantakul [b], Alphons G. J. Voragen [a,*]

[a] Laboratory of Food Chemistry, Department of Agrotechnology and Food Sciences, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands
[b] Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Ladyao, Jatujak, Bangkok 10900, Thailand

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ABSTRACT

The okra plant, *Abelmoschus esculentus* (L.) Moench, a native plant from Africa, is now cultivated in many other areas such as Asia, Africa, Middle East, and the southern states of the USA. Okra pods are used as vegetables and as traditional medicines. Sequential extraction showed that the Hot Buffer Soluble Solids (HBSS) extract of okra consists of highly branched rhamnogalacturonan (RG) I containing high levels of acetyl groups and short galactose side chains. In contrast, the CHelating agent Soluble Solids (CHSS) extract contained pectin with less RG I regions and slightly longer galactose side chains. Both pectic populations were incubated with homogeneous and well characterized rhamnogalacturonan hydrolase (RGH), endo-polygalacturonase (PG), and endo-galactanase (endo-Gal), monitoring both high and low molecular weight fragments. RGH is able to degrade saponified HBSS and, to some extent, also non-saponified HBSS, while PG and endo-Gal are hardly able to degrade either HBSS or saponified HBSS. In contrast, PG is successful in degrading CHSS, while RGH and endo-Gal are hardly able to degrade the CHSS structure. These results point to a much higher homogalacturonan (HG) ratio for CHSS when compared to HBSS. In addition, the CHSS contained slightly longer galactan side chains within its RG I region than HBSS. Matrix-assisted laser desorption ionization-time of flight mass spectrometry indicated the presence of acetylated RG oligomers in the HBSS and CHSS enzyme digests and electron spray ionization-ion trap-mass spectrum showed that not only galacturonosyl residues but also rhamnosyl residues in RG I oligomers were O-acetylated. NMR spectroscopy showed that all rhamnose residues in a 20 kDa HBSS population were O-acetylated at position O-3. Surprisingly, the NMR data also showed that terminal α-linked galactosyl groups were present as neutral side chain substituents. Taken together, these results demonstrate that okra contained RG I structures which have not been reported before for pectic RG I.

1. Introduction

The okra plant, *Abelmoschus esculentus* (L.) Moench, family Malvaceae, is cultivated for its immature pods. The immature pod contains a thick and slimy mucilage. The okra pod is used as a vegetable and as a thickening agent for soups and stews.[1] In addition, it is used in traditional medicine as a dietary meal in the treatment of gastric irritations[2] and dental diseases[3] due to its high content of polysaccharides. Physiological studies showed that the okra polysaccharides (OKPs) had hypoglycemic properties and lower plasma cholesterol levels in rats.[2] In food applications, the OKP was a suitable egg-white substitute[4] and a fat substitute in cookies and in chocolate frozen dairy dessert.[5,6]

The OKP was an acidic polysaccharide which consists of galactose, rhamnose, and galacturonic acid.[7] The OKP has been reported to have a backbone repeating units of -4-α-Gal*p*A-(1,2)-α-L-Rha*p*-1- dimers and, on average, dimeric side chains of β-Gal*p*-(1,4)-β-Gal*p*-1.[8] The acetyl content was about 5.5% w/w.[8] Sequential extraction of okra cell wall material showed that okra contained different types of polysaccharides, that is, pectins, xyloglucans, xylans, and celluloses.[9] The Hot Buffer Soluble Solids (HBSS) fraction was the main fraction which contained mainly rhamnogalacturonan (RG) I with short galactose-containing side chains. The CHelating agent Soluble Solids (CHSS) fraction mainly contained homogalacturonan (HG) and slightly longer galactose-containing side chains connected to the RG I segments. In addition, the degree of acetylation of the galacturonic acid moieties for HBSS was relatively high. About 58 moles of acetyl groups were present for every 100 galacturonic acid moieties. NMR studies of HBSS polymer after incubation with polygalacturonase (PG) and pectin methyl esterase

* Corresponding author. Tel.: +31 317 482888; fax: +31 317 484893.
E-mail address: fons.voragen@wur.nl (A. G. J. Voragen).

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(PME) showed that the majority of the acetyl groups were not linked to galacturonosyl residues and substitution to other sugars such as rhamnosyl residues had to be considered.[9] In this study, we provide further structural information of the HBSS and CHSS fractions by degradation studies using homogeneous and well-characterized enzymes. Furthermore, NMR spectroscopy and mass spectrometry were used to indicate the position of the acetyl groups and details concerning the galactose side chains in both samples.

2. Results and discussion

The okra AIS was sequentially extracted with hot buffer and chelating agent. The sugar composition (Table 1) showed that the HBSS contained mainly rhamnogalacturonan (RG) I (85%) with short galactose-containing side chains and hardly any homogalacturonan (HG). The CHSS contained mainly HG and some RG I (24%) with more galactose and arabinose present in side chains.[9] Moreover, the configuration of all sugar compositions present in HBSS and CHSS was in D-configuration except of rhamnose, which was in the L-configuration.[9] The degree of acetylation (DA) was quite high in HBSS (58%) which is in agreement with levels found, example for apple RG I.[10] However, NMR studies of HBSS polymer showed that no acetyl groups substitution was present on the galacturonosyl residues of RG I backbone which is normally the case.[9] The DA of CHSS was relatively low and NMR studies of CHSS polymer showed that acetyl may be linked to galacturonic acid as well as to some other sugar residues.

2.1. Enzymatic degradation of okra pectins

2.1.1. Polygalacturonase treatment

To characterize the HG segments within the extract sample, the samples were incubated with endo-polygalacturonase (PG) from *Aspergillus aculeatus*. This enzyme can cleave the α-1,4-D-galacturonosyl linkages of the HG-backbone by hydrolysis although PG action is hindered by the presence of methyl esters and acetyl groups.[11]

HPSEC of the PG digests of HBSS and saponified HBSS (sHBSS) (data not shown) showed a slight shift of the high Mw pectin population to lower Mw values. Some minor quantities of oligomers were released as well. Analyses by HPAEC showed the presence of monomers, dimers, and trimers of galacturonic acid (GalA) accounting only for about 0.1% (HBSS) and 3% (sHBSS) of all GalA residues present.

The HPSEC patterns of PG-treated CHSS (Fig. 1A) indicated that approximately 37% of CHSS remained as high Mw material, and 52% and 11% were found as intermediate and low Mw fragments, respectively. For sCHSS, about 50% of the polymer was present as low Mw fragments and no intermediate fragments were observed (Fig. 1B). The (limited) action of PG toward HBSS and CHSS confirms the relative abundance of HG segments in the samples and also reflects the methyl esterification in the CHSS sample.

2.1.2. Treatment with galactose releasing enzymes

To obtain more information about the length of the galactose-containing side chains, the samples were incubated with endo-Galactanase (endo-Gal) from *A. aculeatus*. This enzyme cleaves the 1,4 linkage between β-linked galactosyl residues within galactan chains and releases mono-galactose and galactose oligomers.[12]

The HPSEC patterns of HBSS and sHBSS after treatment with endo-Gal showed no shift of the polymer to low Mw material and no oligomers were released as indicated by HPAEC (results not shown). Only 5% of all galactose present was released by endo-Gal in HBSS and sHBSS. For CHSS and sCHSS, endo-Gal digest showed a minor shift to lower Mw values and HPAEC analysis showed a release of mono- and dimeric galactose representing about 10% (CHSS) and 15% (sCHSS) of all galactosyl residues present. These observations confirm our earlier indications for the presence of short galactan side chains within HBSS and CHSS,[9] mostly resistant against enzyme action.

Since endo-Gal showed only a limited action toward the HBSS fraction, we also studied the activity of α and β galactosidases for their ability to release galactose. Both enzymes were hardly able to remove galactosyl residues from the HBSS fraction.

2.1.3. Rhamnogalacturonan hydrolase (RGH) treatment

To gain information about the RG I structure, the samples were incubated with RGH from *A. aculeatus*. This enzyme acts exclusively on RG I[11] and is unable to split the RG I backbone in case of substitution with long galactose side chains and when acetyl groups are present in the backbone.[13]

The HPSEC patterns of the HBSS (Fig. 2) after incubation with RGH showed that about 20% of the HBSS polymer remained as high Mw material and 80% of the HBSS polymer was shifted to medium or low Mw material. The HPSEC pattern of sHBSS digests showed only 2 populations of which the <10 kDa Mw fraction was most dominant (66% of the sHBSS digest). The enzymatic degradation of sHBSS by RGH from sHBSS confirmed that the HBSS was indeed

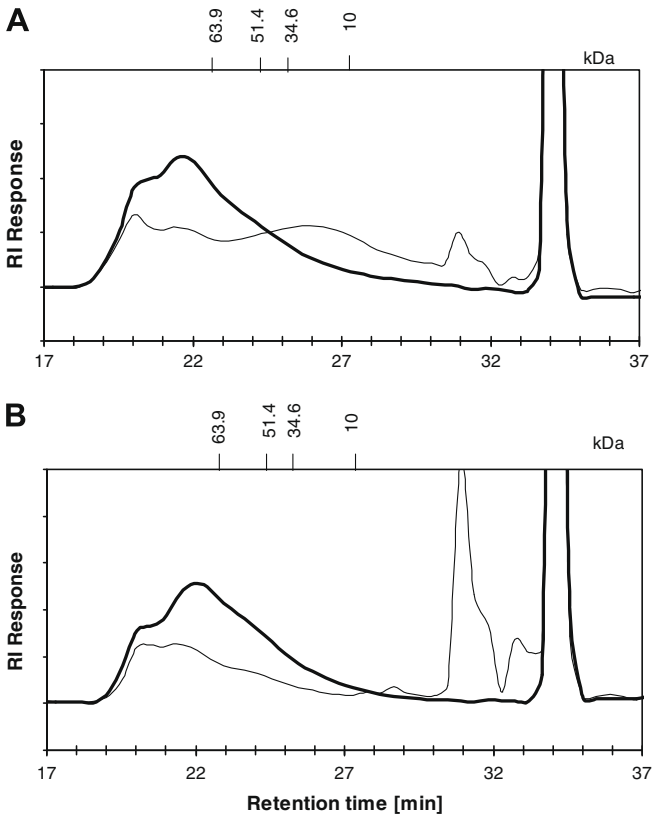

Figure 1. HPSEC elution patterns of CHSS (A) and sCHSS (B). Bold line: before incubation, thin line: after incubation with PG (the molecular weight indication is based on pectin standards).

Table 1
Sugar composition (mol %) of HBSS and CHSS fractions obtained from okra AIS[9]

	Rha	Ara	Gal	Glu	GalA	GlcA	DM[a] (%)	DA[a] (%)	Total sugar[b]
HBSS	26	0	34	1	35	3	24	58	90
CHSS	14	3	17	1	63	2	48	18	86

[a] Moles methanol or acetyl per 100 moles of galacturonic acid.
[b] Gram qualities per 100 g of fraction.