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Two-step enzymatic fingerprinting of sugar beet pectin

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ABSTRACT

A two-step enzymatic fingerprinting method was introduced to analyze a highly methylesterified and acetylated sugar beet pectin having a degree of methylesterification (DM) of 62 and acetylation of 30. A cocktail of pectolytic enzymes, including endo-polygalacturonase II (endo-PGII) and pectin lyase (PL), was used for the first digestion. The endo-PGII and PL resistant pectin fragments were isolated and subjected to a second digestion using fungal pectin methylesterase and endo-PGII. After the two sequential digestions, 78% of the total GalA residues present in the parental pectin were recovered as mono- and oligomers, which were used to quantitatively describe the parental SBP. For this reason, the descriptive parameters degree of blockiness (DB_{abs}), degree of hydrolysis by PG (DH_{PG}) and degree of hydrolysis by PL (DH_{PL}) were established for both digestions. The first digestion revealed the presence of short blocks of nonesterified GalA residues and blocks of partly methylesterified and acetylated GalA residues in the parental SBP, in addition to blocks of highly methylesterified and acetylated GalA residues. The second digestion revealed the presence of blocks of methylesterified, partly methylesterified and/or acetylated GalA residues in a sequence not to be degradable by neither endo-PGII nor by PL. The acetyl groups were present in an blockwise manner. Application of the method to two differently prepared DM 50 SBPs showed that the two pectins differ in the ratio of blocks of nonesterified and blocks of partly methylesterified and acetylated GalA residues.

1. Introduction

Pectin isolated from apple pomace and lemon peel is widely used in the food industry as gelling, viscosifying, and stabilizing agent in food applications. Sugar beet is considered as a potential source of pectin. Sugar beet pectin (SBP) essentially consists of homogalacturonan (HG) and type I rhamnogalacturonan (RG-I) regions (Kravtchenko, Voragen, & Pilnik, 1992). About 90% of the total galacturonic acid (GalA) residues are present in the HG fragment of SBP and the remaining 10% GalA residues are present within the RG-I structural elements (Ralet & Thibault, 2009). RG-I is constructed of repeating units of α-(1 → 2)-linked rhamnosyl and α-(1 → 4)-linked GalA residues, in which the rhamnosyl unit can be substituted with neutral sugar side chains (Voragen, Coenen, Verhoef, & Schols, 2009). The GalA unit in both RG-I and HG can be acetylated at positions *O*-2 and/or *O*-3 (Quemener, Desire, Lahaye, Debrauwer, & Negroni, 2003). The level and distribution of esters have important commercial implications because of their effects on the functionality of the pectin (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Acid extracted commercial SBP may have a degree of acetylation (DA) and degree of methylesterification (DM) (Buchholt, Christensen, Fallesen, Ralet, & Thibault, 2004).

Revealing the ester distribution patterns in SBP is complex due to the fact that the HG is highly decorated with both methylesters and acetyl groups. An enzymatic fingerprinting method has been developed (Ralet, Crépeau, & Bonnin, 2008) for the elucidation of the distribution pattern of acetyl groups in SBP. Although the methylester distribution is highly important as well with respect to functionality of the pectin, it was not addressed in that study. Recently, the simultaneous use of endo-polygalacturonase (endo-PGII) and pectin lyase (PL) to degrade a highly methylesterified and acetylated parental sugar beet pectin (DM 62, DA 30) and the subsequent analysis of the digest using LC-HILIC coupled to online ELSD/MSn was reported (Remoroza, Buchholt, Gruppen, & Schols, 2014). This enzymatic degradation of SBP6230 resulted in 40% degradation of the HG region based on the amounts of GalA residues present in monomer and nonesterified, partially methylesterified/acetylated unsaturated GalA oligomer released. Quantification of these oligosaccharides was used for the determination of the pectin descriptive parameters absolute degree of blockiness (DB_{abs}), degree of hydrolysis by PG (DH_{PG}) and degree of hydrolysis by PL (DH_{PL}) (Remoroza et al., 2014). These parameters were used to describe the so-called blocks of (1) nonesterified, (2) partly methylesterified, acetylated and (3) highly substituted GalA

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Table 1
Chemical characteristics of pectin samples used in this study.

Mother pectin	De-esterification method	Pectin	GalA	Rha	Ara	Gal	DM[a] (%)	DA[a] (%)
			% (w/w)					
SBP6230		SBP6230	59	5	12	10	62	30
	p-PME	P5328	58	5	11	10	53	28
	f-PME	F5129	59	5	12	9	51	29

Sugar beet pectin (SBP); plant pectin methylesterase (p-PME); fungal pectin methylesterase (f-PME). Monosaccharide composition (Buchholt et al., 2004).
[a] Moles methanol or acetic acid per 100 moles of galacturonic acid.

residues in a pectin by the known modes of action of endo-PGII and PL. However, still the non-degradable part of the SBP, representing about 60% of the HG and being endo-PGII and PL resistant, was not included in the characterization.

In the present study, a two-step enzymatic fingerprinting approach is introduced to overcome the above mentioned drawback, making it possible to elucidate the distribution patterns in the highly methylesterified and acetylated pectins. In the first digestion, RG-I degrading enzymes, endo-PGII and PL are used to degrade the pectin, followed by the separation of the high molecular weight (Mw) fragments and the low Mw GalA mono- and oligomers. Subsequently, an enzyme mixture containing fungal pectin methylesterase (f-PME) and endo-PGII is used to further digest the high Mw pectin fragments. All diagnostic GalA oligomers generated were analyzed by HPLC-MS/ELSD. Using the pectin descriptive parameters the methylester and acetyl distribution over the SBP backbone is described.

2. Materials and methods

2.1. Pectin samples

Commercially extracted SBPs, SBP6230 (DM 62, DA 30), P5328 and F5129 were provided by Danisco (Brabrand, Denmark). The chemical characteristics are described in Table 1.

2.2. Enzymes and enzymatic hydrolysis

Purified and well characterized RG-I and HG degrading enzymes were used to hydrolyze sugar beet pectins. The enzymes used in this study were *Aspergillus aculeatus* endo-galactanase (EC 3.2.1.89) (Schols, Posthumus, & Voragen, 1990), endo-arabinase (EC 3.2.1.99) (Beldman, Searle-van Leeuwen, De Ruiter, Siliha, & Voragen, 1993), RG-hydrolase (EC 3.2.1.B9) (Mutter, Renard, Beldman, Schols, & Voragen, 1998), *Chrysosporium lucknowense* (C1) exo-arabinase (EC 3.2.1.1) (Kühnel et al., 2010), *Aspergillus niger* fungal pectin methyl esterase (fungal PME) (EC 3.1.1.11) (Van Alebeek, Van Scherpenzeel, Beldman, Schols, & Voragen, 2003), pectin lyase (EC 4.2.2.10) (Schols et al., 1990) and endo-polygalacturonase II (EC 3.2.1.15) (Limberg et al., 2000b). Fig. 1 shows the schematic diagram of the two-step enzymatic fingerprinting approach for the SBPs.

2.3. First digestion

SBP solution (10 mg/2 ml) in 50 mM sodium citrate buffer (pH 5.0) was digested at 40 °C for 24 h by RG-I (endo-galactanase + endo/exo arabinase + RG hydrolase) and HG (endo-PGII + PL) degrading enzymes as described elsewhere (Remoroza et al., 2014). The reaction was stopped by heating at 100 °C for 6 min. The total digest after the first digestion was freeze-dried.

2.4. Second digestion

The degradation products obtained after the first digestion of SBP were fractionated into high Mw fragments and low Mw oligomers by size exclusion chromatography (see below). For the second digestion, the high Mw material was dissolved in 2 ml 50 mM sodium acetate buffer (pH 5.0) and digested using *A. niger* f-PME (20 U/ml) and endo-PGII (10 U/ml) at 40 °C for 24 h. Inactivation of enzymes was performed by heating at 100 °C for 6 min. This digest was denoted as pool I digest.

Freeze-dried pectin digest (10 mg pectin) after the first enzymatic digestion was dissolved into 100 μl 50 mM sodium citrate buffer (pH 5.0) and applied onto a PD-10 column with packed bed size of 1.45 × 5.0 cm (8.3 ml) containing *Sephadex G-25 Medium* (GE Healthcare Bio-sciences Uppsala, Sweden). The PD-10 column was equilibrated with 25 ml of 50 mM sodium citrate buffer (pH 5.0) at room temperature. Subsequently, the digest was eluted with 4.90 ml of 50 mM sodium citrate buffer (pH 5.0). The fractionation allows the separation of high Mw fragments from low Mw fragments. Fractions (0.5 ml) were collected and analyzed by HPSEC, followed by pooling on the basis of the Mw of the fragments. Two pools were obtained; pool I (fractions 1–5;

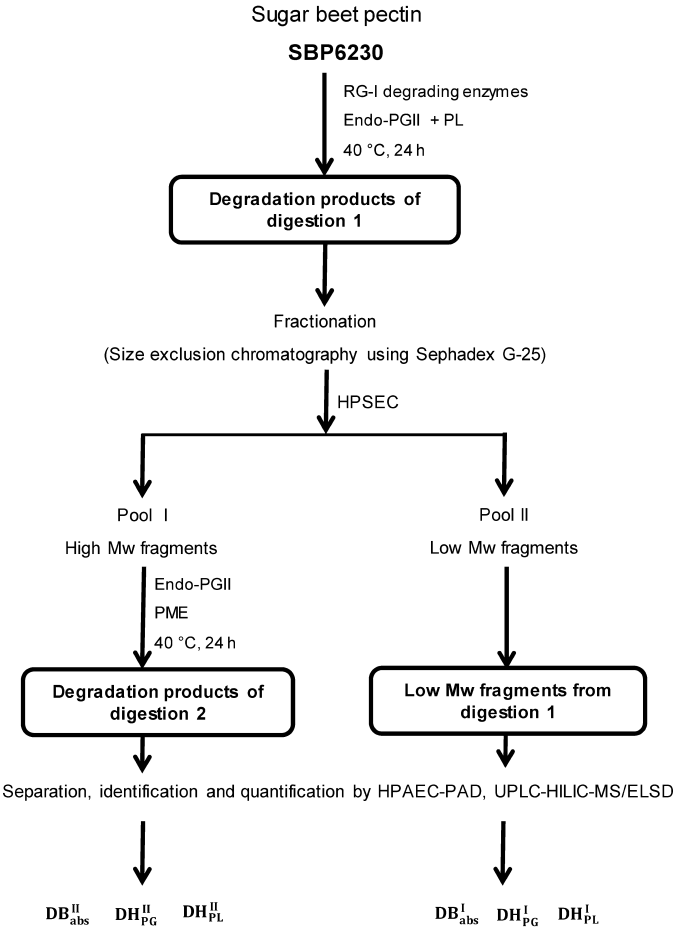

Fig. 1. Schematic diagram of the two-step enzymatic fingerprinting method to study the fine structure of sugar beet pectin.