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Pectin characterisation in vegetable waste streams: A starting point for waste valorisation in the food industry

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ABSTRACT

Vegetable waste streams, which are a major disposal issue in food industry, present a promising source of compounds which may be valorised because of their favourable functional features. In this context, the potential of five vegetable waste streams (rejected carrots, carrot steam peels, green beans cutting waste, leek cutting waste and celeriac steam peels) as a source for the extraction of pectin with interesting structural, and hence functional, properties was evaluated. Specifically, cell-wall components were extracted from the waste streams as alcohol-insoluble residue and subsequently fractionated into different (pectin) fractions based on their solubility. The pectic polysaccharides were characterised in terms of GalA content, neutral sugar content, linearity/branching, degree of methyl-esterification (DM), molar-mass distribution and the presence of bound protein. Pectin characterisation revealed considerable differences between the pectic polymers present in the investigated waste streams. For example, pectin in carrot steam peels showed a low DM, whereas the cutting waste of leek contained pectin with a very high DM. Furthermore, the level of protein bound to pectin was generally highest in carrot-derived waste streams. Depending on the intended pectin functionality, a deliberate choice for one of these vegetable waste streams as a source for pectin extraction can be made.

1. Introduction

Waste streams originating from vegetable processing present a major disposal issue for the food industry concerned. Nowadays, waste of plant materials is generally used for animal feed, as fertilizer or disposed as such. In addition, concepts such as composting and biogas production have been exploited for the conversion of waste (Laufenberg, Kunz, & Nystroem, 2003). Vegetable waste streams however present a promising source of compounds which may be valorised because of their favourable functional (e.g. rheological, nutritional, …) properties (Schieber, Stintzing, & Carle, 2001). Among the different compounds comprised in plant material waste, pectin represents an interesting polysaccharide, which can be used as functional food ingredient in many applications.

Pectin is a complex polysaccharide, rich in galacturonic acid (GalA), present in the cell wall of all higher plants. Structurally, three main pectin domains can be distinguished: homogalacturonan (HG), the linear region of pectin, and rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II), branched domains of pectin (Voragen, Coenen, Verhoef, & Schols, 2009). Specifically, HG is a homopolymer consisting of α-D-GalA residues in which some of the C-6 carboxyl groups are methyl-esterified. RG-I on the other hand is a family of highly branched pectic polymers that contain a backbone of the repeating disaccharide [→4)-α-D-GalA-(1 → 2)-α-L-Rha-(1→] of which the Rha residues can be substituted with side chains mainly consisting of galactosyl and/or arabinosyl residues. Finally, RG-II consists of a backbone of around nine GalA residues which is substituted by four hetero-oligomeric side chains with known and consistent composition and length (O'Neill et al., 1996). The nanostructural properties of pectin depend on the plant source as well as on the tissue part from which pectin was obtained. Specifically, pectic polymers can differ in their composition,

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solubility, degree of methyl-esterification (DM), degree of acetylation, distribution of ester groups, degree of branching and degree of polymerisation (molar mass) (Sila et al., 2009). Moreover, during food processing, several conversion reactions can take place modifying pectin's fine structure.

Extracted pectin (EU code E440) is widely used as a functional food ingredient in innumerable food products, whereby in all its applications as a food ingredient, the nanostructure of pectin profoundly affects its functionality. Pectin is primarily known as a gelling agent and is extensively applied in the production of jams and jellies, fruit juice, confectionary products and bakery fillings (Willats, Knox, & Mikkelsen, 2006). Depending on the DM of pectin, two different mechanisms of pectin gelation can be distinguished. High-methoxylated pectins (DM > 50%) on the one hand can form gels in the presence of co-solutes (typically sucrose at a concentration > 55%) and under acidic conditions (pH < 3.5) (Endress, Mattes, & Norz, 2006). Low-methoxylated pectins (DM < 50%) on the other hand form gels in the presence of divalent cations, particularly Ca^{2+}. Gelation is due to the formation of junction zones between HG regions of different pectin chains through calcium bridges between dissociated carboxyl groups (Fraeye, Duvetter, Doungla, Van Loey, & Hendrickx, 2010). Besides its use as a gelling agent, pectin is also often employed for the stabilisation of acidified milk drinks and yoghurts. Specifically, pectin adsorbs onto the casein micelles as a result of electrostatic interaction thereby preventing the flocculation of these milk proteins at acidic pH (Tromp, de Kruif, van Eijk, & Rolin, 2004). Finally, pectin can be applied as an emulsifying agent. This functionality of pectin has not been so widely exploited as its gelling and stabilizing features. The emulsion-stabilising potential of pectin has been related to the hydrophobic character of acetyl groups, ferulic acid groups and residual protein moieties present within the pectin as well as the molar mass of the pectic polymers (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Williams et al., 2005).

Nowadays, commercial pectin production is limited to a few sources, i.e. citrus peel and apple pomace (Endress et al., 2006). In this study, the potential of five vegetable waste streams (rejected carrots, carrot steam peels, green beans cutting waste, leek cutting waste and celeriac steam peels) as a source for the extraction of pectin with interesting structural, and hence functional, properties was evaluated. Specifically, cell-wall components were extracted from the waste streams as alcohol-insoluble residue and subsequently fractionated into different (pectin) fractions based on their solubility. By applying this type of procedure, insight into the native state of pectin in the plant cell wall can be obtained, hence revealing the full endogenous potential of pectin present in the different vegetable waste streams. Conversely, a direct pectin extraction would not have allowed for this information as, depending on the extraction conditions used, the structure of pectin is modified in terms of molar mass, DM, etc. Pectic polysaccharides were characterised in terms of GalA content, neutral sugar content, linearity/branching, DM and molar-mass distribution. Finally, by combining state-of-the-art techniques (size exclusion chromatography coupled with refractive index, multi angle laser light scattering and diode array detection), the level of protein bound to pectic polymers with a certain molar mass could be examined qualitatively.

2. Materials and methods

2.1. Vegetable waste streams

Five different vegetable waste streams were acquired from food companies in Belgium. Rejected carrots, carrot steam peels, celeriac steam peels and the cutting waste of green beans were obtained from a canning company, while the cutting waste of leek was provided by a leek producer. The rejected carrots and the cutting waste of leek were first frozen under liquid nitrogen and then packed in plastic bags. The other three waste streams were first vacuum-packed in plastic bags before freezing with liquid nitrogen. All samples were stored at −40 °C until further use.

2.2. Isolation of cell-wall components as alcohol insoluble residue (AIR)

The cell wall material of the different waste streams was isolated as alcohol-insoluble residue (AIR) using ethanol and aceton as described by Christiaens et al. (2011). The GalA content of AIR was determined as described in Section 2.4, while an estimation of the total pectin content of AIR was made based on the amounts of GalA and neutral sugars present in the pectin fractions (see Sections 2.4 and 2.5) divided by the fractionation yield (i.e. the amount of GalA retrieved in the different fractions compared to the level of GalA in the AIR).

2.3. Fractionation of AIR based on solubility

Cell-wall material, extracted as AIR, was fractionated into various polysaccharide fractions according to Houben, Jolie, Fraeye, Van Loey, and Hendrickx (2011). Specifically, water-soluble pectin (WSP), chelator-soluble pectin (CSP), sodium-carbonate-soluble pectin (NSP) and a hemicellulose fraction (HF) were obtained after subsequent extraction of the AIR with boiling water, 0.05 mol/l cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA) in 0.1 mol/l potassium acetate, 0.05 mol/l Na_2CO_3 containing 0.02 mol/l $NaBH_4$ and 4 mol/l KOH containing 0.02 mmol/l $NaBH_4$ and 35 g/l borate respectively. It is assumed that WSP consists of pectic polymers that are loosely bound to the cell wall through non-covalent and non-ionic bonds, while CSP mainly contains ionically cross-linked pectin and NSP is predominantly linked to cell wall polysaccharides through covalent ester bonds. HF on the other hand still contains some pectic polymers that are very strongly bound to cellulose or hemicelluloses. All polysaccharide fractions as well as the remaining residue were frozen with liquid nitrogen and stored at −40 °C. For certain analyses (determination of neutral sugar content and analysis of pectin fractions using high-performance size exclusion chromatography), WSP, CSP, NSP and HF were lyophilised using a freeze-dryer (Christ alpha 2–4 LSC, ice condenser temperature = −85 °C).

2.4. Determination of GalA content in AIR and all polysaccharide fractions

The GalA content in AIR as well as in the different polysaccharide fractions was determined spectrophotometrically as described by Christiaens et al. (2011). Specifically, samples were hydrolysed with concentrated sulfuric acid in a first step after which the concentration of GalA was determined with the m-hydroxydiphenyl method.

2.5. Determination of neutral sugar content in all polysaccharide fractions

Quantification of the neutral sugars (fucose, rhamnose, arabinose, galactose, glucose, xylose and mannose) in all polysaccharide fractions was performed via high-performance anion exchange chromatography according to Houben et al. (2011) after hydrolysis of the samples with trifluoroacetic acid. A Dionex Bio-LC system (DX600) equipped with a GS50 gradient pump, a CarboPac™ PA20 column (150 × 3 mm, pH range = 0–14), a CarboPac™ PA20 guard