Carbohydrate Polymers 146 (2016) 245–252

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Enzymatic production of pectic oligosaccharides from onion skins

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ARTICLE INFO

Article history:
Received 22 December 2015
Received in revised form 2 March 2016
Accepted 6 March 2016
Available online 14 March 2016

Keywords:
Pectic oligosaccharides
Onion skins
EPG-M2
Pectinase
Viscozyme
Methylated

ABSTRACT

Onion skins are evaluated as a new raw material for the enzymatic production of pectic oligosaccharides (POS) with a targeted degree of polymerization (DP). The process is based on a two-stage process consisting of a chelator-based crude pectin extraction followed by a controlled enzymatic hydrolysis. Treatment of the extracted crude onion skin's pectin with various enzymes (EPG-M2, Viscozyme and Pectinase) shows that EPG-M2 is the most appropriate enzyme for tailored POS production. The experiments reveal that the highest amount of DP2 and DP3 is obtained at a time scale of 75–90 min with an EPG-M2 concentration of 26 IU/mL. At these conditions the production amounts 2.5–3.0% (w/w) d.m for DP2 and 5.5–5.6% (w/w) d.m for DP3 respectively. In contrast, maximum DP4 production of 5.2–5.5% (w/w) d.m. is obtained with 5.2 IU/mL at a time scale of 15–30 min. Detailed LC–MS analysis reveals the presence of more methylated oligomers compared to acetylated forms in the digests.

1. Introduction

Oligosaccharides are carbohydrates that have sugars linked together with different degree of polymerization. In recent years, non-digestible oligosaccharides have found application in various fields, notably because of their specific prebiotic activities (Swennen, Courtin, & Delcour, 2006). It has been reported that prebiotic oligosaccharides benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (*Bifidobacteria* and *Lactobacilli*), suppressing the activity of pathogenic organisms (Garthoff et al., 2010). The fermentation of oligosaccharides in the colon results in the generation of short chain fatty acids, which exert a number of health effects *viz.* inhibition of pathogenic bacteria, relief of constipation, reduction in blood glucose level, improvement in mineral absorption, decreased incidence of colonic cancer and modulation of the immune system (Gullon et al., 2013). Studies that attempt to understand the real mode of action of prebiotics are still being conducted. To date, only a few types of oligosaccharides, like galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), are commercially available, but there is an increasing interest in higher performing and/or lower cost prebiotic ingredients. In this respect, pectin-derived oligosaccharides, also called pectic oligosaccharides, have been identified as emerging prebiotics (Olano-Martin, Gibson, & Rastall, 2002).

The major advantage of pectic oligosaccharides (POS) is that they are derived from the parent compound, "pectin," which is a polysaccharide widely present within the primary cell wall and intercellular regions of higher plants (Chen et al., 2013). More specifically, the POS is produced by tailoring the long chain pectin polysaccharides into smaller units of varying degree of polymerization. Another attractive property concerns their chemical identity, which is versatile and very different from FOS and GOS due to the different chemical natures of the starting material. Pectin can be comprised of different structural elements, such as homogalacturonan (HG), xylogalacturonan (XGA), rhamnogalacturonan-I (RG-I) and rhamnogalacturoanan-II (RG-II), arabinan and arabinogalactan. As a consequence, various types of POS, that is, rhamnogalacturonan-oligosaccharides, galacturonan-oligosaccharides, arabino-oligosaccharides, galacto-oligosaccharides, *xylo*-oligosaccharides, arabino-galactan oligosaccharides, can be produced, depending on the diverse structural elements present in pectin (Babbar, Dejonghe, Gatti, Sforza, & Elst, 2014). However, only limited information is available on this new class of molecules, especially in relation to their composition and prebiotic properties, requiring further research to assess their potential.

Waste valorization of pectin-rich agro-industrial residues into POS is an interesting way to use waste and by-product streams. Until now, a lot of work has been reported on the POS production from sugar beet pulp pectin, orange pectin and pure pectin (Bako, Eszterle, Kiss, Nemestithy, & Gubicza, 2007; Combo, Aguedo, Goffin, Wathelet, & Paquot, 2012; Iwasaki, Inove, & Matsubara,

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http://dx.doi.org/10.1016/j.carbpol.2016.03.011
0144-8617/

1998; Leijdekkers, Bink, Geuthes, Schols, & Gruppen, 2013; Olano, Mountzouris, Gibson, & Rastall, 2001; Yapo, Lerouge, Thibault, & Ralet, 2007). Some scant studies are available regarding POS production from potato pulp (Thomassen, Vigsnæs, Licht, Mikkelsen, & Meyer, 2011) and tomato processing waste (Suzuki et al., 2002). Nevertheless, the search for new resources and alternatives continues.

With a total production of 6.6×10^6 t (Faostat, 2011), onions are an important vegetable in the EU. Part of these onions are processed in the form of dried, whole, cut, sliced and broken pieces with a total sold volume of 3.5×10^4 t in the EU (Eurostat, 2011), thereby generating significant wastes including the skins. Onion skins are known to be very rich in pectin (Alexander & Salubele, 1973). In our previous work, onion skins were found to contain around 20% (w/w) d.m. of galacturonic acid (Babbar et al., 2015). Historically, onion skins had various applications. Being a thin, lightweight, strong and often translucent paper, it was used with carbon paper for typing duplicates in a typewriter. In addition, it was widely used for extracting pigment for dyeing cotton carpet and cloth (Bae, 2009). Nevertheless, the overall applicability has decreased, revealing the need of new valorization routes for these types of waste. Onion skins are, for example, currently being tested for their antioxidant effect (Albishi, John, Al-Khalifa, Shahidi, & Albishi, 2013; Urszula et al., 2013). However, given their high pectin content, they are expected to be a very suitable raw material for POS production, allowing for a more versatile application of the waste.

The main goal of this work is to explore the use of onion skins for the production of pectic oligosaccharides. To the authors' knowledge, this is the first study to report on the production and characterization of pectic oligosaccharides (POS) from this new raw material. The study is therefore taken with an objective of optimizing process parameters to tailor and maximize the POS production. The research follows a two-stage process: (i) extraction of crude pectin followed by (ii) enzymatic tailoring of extracted pectin to POS.

2. Material and methods

2.1. Raw material and chemicals

Onion skins with a dry matter content of 95.6% (w/w) were provided by the Institut für Getreideverarbeitung (IGV, GmbH), Germany. The skins were milled with a laboratory blender and screened on its particle size (<1 mm) and stored in ziplock bags at room temperature until use. Celluclast 1.5 L (C-2730), predominantly (containing cellulase), Viscozyme L (V-2010) (a multienzyme complex) and Pectinase were obtained from Sigma-Aldrich (St. Louis, MO, USA). Endo-polygalacturonase M2 (EPG-M2) and polygalacturonic acid were purchased from Megazyme, Ireland. Standards for rhamnose, arabinose, galactose, xylose, glucose, fructose and galacturonic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Germany). The standards of di-galacturonic acid and tri-galacturonic acid were obtained from Sigma-Aldrich and the standard of tetra-galacturonic acid from Elicityl Oligotech (France). The galacturonan oligosaccharide mixture DP1–DP10 was kindly provided by B. Whatelet and M. Paquot from Gembloux, Agro-Bio Tech (Belgium).

2.2. Total pectic sugar composition and extraction of crude pectin from the onion skins

The total pectic sugars present in onion hulls were estimated by following the protocol optimized in our previous study (Babbar et al., 2015). Based on our preliminary tests, sodium hexametaphosphate was selected as an extractant for onion skins (Babbar et al., 2015). Sodium hexametophosphate is already commonly used in food industry, though for other applications (Shirashoji, Jaegggi, & Lucey, 2010). Onion skins (1 g) were pretreated with 2% sodium hexametaphosphate at 95 °C for 0.5 h in a hot water bath. The biomass was then centrifuged at 5000*g* for 10 min. The supernatant containing the crude pectin was collected and analyzed for its free monosaccharide as well as total saccharide composition. The latter was then taken as a measure for the polysaccharide content. The analysis was performed on HPAEC-PAD, as described elsewhere in the article.

2.3. Enzyme activity measurements

The *endo*-polygalacturonase activity of three enzymes was assessed on the substrate polygalacturonic acid following the protocol provided by Megazyme International, Ireland. Briefly, the method consisted of mixing 0.2 mL of a preincubated enzyme solution (suitably diluted) and 0.5 mL of preincubated substrate solution (1% w/v) in glass test tubes while vigorously mixing. The mixtures were incubated for 3, 6, 9 and 12 min at 45 °C and measured photospectrometrically at 520 nm using the Nelson-Samogvi method (McCleary & McGeough, 2015). The analyses of samples and standard solutions containing galacturonic acid (50 μgrams *i.e.* 0.2 mL of 250 μg/mL in 0.2% benzoic acid) were performed on a spectrophotometer (UV-1650 PC, Shimadzu, Koyto, Japan) against a reaction blank). One unit of *endo*-polygalacturonase activity is defined as the amount of enzyme required to release 1 μmol of galacturonic acid per minute from the polygalacturonic acid.

The activity of Viscozyme, Pectinase and EPG M2 expressed as EPG units was determined to be 4135, 2612 and 2600 U/mL.

2.4. Enzymatic pectic oligosaccharides (POS) production from the crude pectic extract from onion skins

Hydrolysis of pectin obtained from onion skins was done with three different enzymes so as to study the distribution of oligomers and monomers. Three different enzymes *i.e.* Viscozyme, Pectinase and EPG-M2 were used. All the enzymes used here are known for their diverse pectinase activity (Combo et al., 2012). All the commercial enzyme solutions were diluted 50 times (accounting to 82.7, 52.2 and 5.2 U/mL). The hydrolysis was then conducted at 10% (v/v) of the diluted enzyme/pectic solution. The hydrolysis was done for a period for 2 h at 45 °C, 150 rpm and the samples were collected at a regular interval of 15 min until 120 min. The enzymes were inactivated by thermal treatment at 100 °C for 5 min and further analyzed on HPAEC-PAD as stated elsewhere in the paper.

Based on the results obtained a further optimization of the selected enzyme (EPG-M2) was done. In the initial study, the EPG-M2 was added at 52 IU/mL, whereas in the optimization study the concentration was extended to 26 IU/mL, 5.2 IU/mL and 2.6 IU/mL. The hydrolysis was conducted at 10% (v/v) of the enzyme/pectic solution. The hydrolysis was done at 45 °C and samples were withdrawn every 5 min until 90 min. The enzyme was inactivated by thermal treatment at 100 °C for 5 min further analyzed on HPAEC-PAD.

2.5. Analysis of the free monosaccharides by HPAEC-PAD

The samples were adequately diluted and injected into high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) for the analysis of galacturonic acid and other neutral sugars. The HPAEC-PAD used for analytical purpose is a Dionex ICS-5000 model (Thermo Scientific, Inc., USA) equipped with ED-5000 electrochemical detector. Separation of monosaccharides was carried out with Carbopac PA-1 (4 mm × 50 mm) column coupled to a Carbopac PA-1