Carbohydrate Polymers 142 (2016) 199–205

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Endo-xylanase and endo-cellulase-assisted extraction of pectin from apple pomace

Agnieszka Wikiera*, Magdalena Mika, Anna Starzyńska-Janiszewska, Bożena Stodolak

Department of Food Biotechnology, Faculty of Food Technology, Agricultural University of Cracow, ul. Balicka 122, 31-149 Kraków, Poland

ARTICLE INFO

Article history:
Received 4 September 2015
Received in revised form 5 January 2016
Accepted 27 January 2016
Available online 29 January 2016

Keywords:
Enzymatic extraction
Pectin composition
Cell wall polysaccharides
Byproducts

ABSTRACT

Pectins were extracted from apple pomace with monoactive preparation of endo-xylanase and endo-cellulase. The process was conducted for 10 h in conditions of pH 5.0 at 40 °C, with constant shaking. Endo-xylanase application resulted in the highest extraction efficiency of pectins (19.8%). The obtained polymer was characterised by a very high molecular mass, high level of neutral sugars – mainly arabinose, galactose and glucose, and very high DM (73.4). It also contained the highest level of protein and phenols. Pectin extracted with endo-cellulase had 1.5 fold lower molecular mass but contained significantly more GalA (70.5%) of a high degree of methylation (66.3%). The simultaneous application of both enzymatic preparations resulted in their cooperation, leading to a decrease of both the extraction efficiency and the molecular mass of pectin. However, this pectin was distinguished by the highest GalA (74.7%) and rhamnose contents.

1. Introduction

Pectins, together with cellulose and hemicellulose, are main polysaccharides of plant cell walls. Their molecules are characterised by a very complex structure that can contain three major fractions: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (Round, Rigby, MacDougall, & Morris, 2010; Wikiera & Mika, 2013). The way in which pectin fractions bind to the cellulose and hemicellulose filaments forming cell wall's scaffolding is still an object of numerous research (Gu & Catchmark, 2013; Popper & Fry, 2008; Wang, Zabotina, & Hong, 2012). It is established, however, that pectins may be released from these structures with the use of mineral acids. Hydrochloric, nitric, citric and sulfuric acids are most commonly applied on a commercial scale, together with a high-temperature treatment (70–100 °C) (Shaha, Punichelvana, & Afandi, 2013; Yapo, 2009). Acid-based technique resulted in 10–15% and 15–18% efficiency of pectin extraction from apples (Srivastava & Malviya, 2011) and citruses (Aina et al., 2012), respectively. Aforementioned methods may be improved, e.g. by pre-treatment of plant material with microwaves (Wang et al., 2007), ultrasound (Xu et al., 2014) or the addition of chelating agents such as EDTA, which weaken the bonds between pectin and bivalent cations (Yeoh, Shi, & Langrish, 2008) Another technique of pectin extraction from plant material is selective hydrolysis with the use of enzymes. Enzymatic extraction is regarded as an environmentally safe method because: (i) the effluents produced generally pose fewer problems with respect to treatment and disposal, as compared to acid hydrolysis; (ii) enzymes are organic materials; and (iii) low preparation concentrations are used during the treatment of food materials. The first attempts of enzymatic isolation of pectins were based on the application of microorganisms, which fermented pectin-rich plant materials, releasing the desired polymer. Sakai and Okushima (1980) obtained 2.6 g pectin from 100 g mandarin peels inoculated with *Trichosporon penicillatum SNO-3 strain.* The resulting polymer had higher molecular mass than one isolated with acid-based technique but contained significantly less polygalacturonic acid, which was the consequence of the strain capability to produce pectinases. Similar strategy was applied by Thibault, De Dreua, Gerades, and Tombouts (1988) who used crude extracts containing *endo*-arabinanase, *endo*-galactanase and residual *endo*-pectate lyase from *Bacillus subtilis* to isolate pectins from citrus peels, apple marks and sugar-beet pulps. In spite of the high effectivity of the process, the obtained polymers were also characterised by the considerably lower molecular mass, as compared to the ones obtained from the same materials by means of the conventional technique. It was concluded that pectins cannot be efficiently extracted with the aforementioned enzymes. Further attempts undertaken with the use of cell-free culture medium from *Bacillus polymyxa* (Matora, Shkodina, & Ptichkina, 1996), *Aspergillus awamori* and *Trichoderma*

* Corresponding author.
E-mail address: a.wikiera@ur.krakow.pl (A. Wikiera).

http://dx.doi.org/10.1016/j.carbpol.2016.01.063
0144-8617/

viride (Ptichkina, Markina, & Rumyantseva, 2008), containing cellulase, xylanase, β-glucosidase and endopolygalacturonase, were also not fully successful. The efficiency of the process was satisfactory, but the resulting polymers had lower molecular mass and contained less galacturonic acid than the ones obtained by the acidic extraction. Later studies focused mainly on the application of cheap, commercial multicatalytic preparations. Their authors assume that the efficient release of pectins from plant tissues must be performed together with the disintegration of non-pectic major cell wall components, such as cellulose, hemicellulose and proteins. Therefore, attempts are being made concerning the application of multicatalytic preparations which are readily available on the market, with dominant cellulolytic (Celluclast, Cellulyve, Cytolase CL, GC220, GC880), xylanolytic (Pentopan) and proteolytic (Alcalase, Neutrale, Papaina) activities (Dominiak et al., 2014; Jeong et al., 2014; Naghshineh, Olsen, & Georgiou, 2013; Panouillé, Thibault, & Bonnin, 2006; Wikiera, Mika, & Grabacka, 2015; Yuliarti, Matia-Merino, Goh, Mawson, & Brennan, 2012; Zykwinska, Boiffard, Kontkanen, Thibault, & Bonnin, 2008). The level of pectolytic activity in these preparations is usually very low, which allows obtaining pectins which contain the satisfactory amounts of galacturonic acid, even higher than in the case of acidic extraction (Wikiera et al., 2015a). These polymers are also characterised by the interesting rheological (Dominiak et al., 2014) and biological properties, such as antioxidant and anticancer ones (Wikiera, Mika, Starzyńska-Janiszewska, & Stodolak, 2015). Moreover, the efficiency of the extraction is often higher, as compared to the acidic treatment. However, the impact of individual enzymatic activities present in preparations on the course of an extraction process and the quality of obtained polymers is still not known.

In the presented study, the pectin extraction from dried apple pomace was performed with monoactive preparations endo-cellulase (endo-β-1,4-glucanase, EC 3.2.1.4) and endo-β-1,4-xylanase (EC 3.2.1.8), which allowed determining the importance of each enzyme and the strength of their possible cooperation during the process. The detailed characterisation of obtained polymers demonstrating the effect of the applied activities on the composition, and thus the quality of isolated pectins.

2. Materials and methods

2.1. Chemicals and reagents

Dextran standards, monosaccharides standards: L-(+)-arabinose 99%, D-(+)-fucose 99%, D-(+)-galactose 99%, D-(+)-glucose 99,5%, L-(−)-mannose 99%, L-(−)-rhamnose monohydrate 99%, D-(−)-xylose 99%, and polygalacturonic acid 98% were obtained from Sigma/Aldrich Chemical Co. Trifluoroacetic acid (TFA) 99% was from Merck (Germany), NaOH standard solution HPLC grade was purchased from Fluka/Sigma–Aldrich Chemical Co. Commercial apple pectin ($P_{commercial}$) (pectin isolated with sulfuric acid) was obtained from Pektowin S.A., Poland, and used for comparative purposes.

2.2. Enzymes

Pectin extraction from apple pomace was performed with cellulase (endo-β-1,4-glucanase, EC 3.2.1.4) produced by filamentous fungus *Trichoderma viride* (Sigma/Aldrich Chemical Co., Cat. No. C9422) and xylanase (endo-β-1,4-xylanase, EC 3.2.1.8) from *Trichoderma viride* (Sigma/Aldrich Chemical Co., Cat. No. X3876).

Cellulase and xylanase activity was determined according to Anthony, Chandra Raj, Rajendran, & Gunasekaran (2003), using carboxymethylcellulose (Sigma–Aldrich Chemie, Germany) and oat spelt xylan (Sigma–Aldrich Chemie, Germany) as substrates. The units of cellulase and xylanase activity were defined as the quantities of enzyme preparation necessary to release 1 μmol of glucose or xylose during 1 min at pH 5.0 and 40 °C. Cellulase activity was 10 U/mg, xylanase 229 U/mg.

In order to extract pectin from apple pomace both enzymatic preparations were applied at 50 U/g of dried apples. Those doses of preparations were characterised according to the level of foreign activities, as presented in Table 1.

2.3. Apple pomace

Apple pomace (Pektowin S.A., Jasło, Poland) is the solid remains after pressing for juice without the use of enzymes, utilised in the commercial production of pectin. The pomace was dried to constant mass (60 °C, 24 h) and its moistness was determined. Before the extraction, the pomace was ground to particles passing through a 40-mesh sieve (0.47 mm).

2.4. Pectin extraction

Enzymatic extraction of pectin from apple pomace (solid/liquid ratio 1 g/15 ml) was performed with the use of endo-cellulase, endo-xylanase and the mixture of both preparations, at a dose of 50 U/g each. The process was conducted for 10 h in conditions of pH 5.0 at 40 °C, with constant shaking (200 rpm). All extractions were performed in 5 replicates.

For the acidic extraction, apple pomace was treated with a H_2SO_4 solution of pH 2.0 (20 ml per 1 g of material), at constant shaking (200 rpm) at 85 °C for 3 h. The applied conditions are commonly used in the laboratory and industry practice (Garna et al., 2007; Srivastava & Malviya, 2011). The extraction was performed in 5 replicates.

After the enzymatic or acidic extraction, the samples were cooled down to 20 °C and centrifuged (4100 rpm, 10 min, 4 °C). The supernatants were filtered through blotting paper and the pellets were washed with distilled water, centrifuged again and the second supernatants were filtered as well. Next, cooled (4 °C) 96% ethanol was added to supernatants. Ethanol volume was calculated to reach the final concentration of 70%. The precipitated pectin was collected by centrifugation (4000 rpm, 20 min), washed with 70% ethanol, centrifuged as previously and the pellets were dried at 60 °C for 24 h until the constant weight was achieved. The resulting pectins: P_{acid} (pectin extracted with acid), P_{cel} (pectin extracted with cellulase), P_{xyl} (pectin extracted with xylanase) and $P_{cel+xyl}$ (pectin extracted with cellulase and xylanase) were ground to a particle passing through a 60-mesh screen (0.251 mm).

2.5. Analytical methods

2.5.1. Extraction efficiency

The pectin extraction efficiency (% w/w) was calculated as follows:

Pectin yield (%) = (weight (g) of dried pectin/weight (g) of dried apple pomace used in the extraction) × 100.

Water content in the pectin preparations was calculated as a decrease of mass after 3 h drying in 105 °C.

2.5.2. Molecular mass (M_w) determination

The molar mass distribution of differently extracted pectins was studied using high performance size exclusion chromatography. This was performed using a Dionex Ultimate 3000 HPLC system equipped with a column of TSK gel GMPWxl (30 cm × 7.8 mm × 13 μm, Tosoh Biosence, Germany) in combination with a TSKguard column. Solutions of pectin (1 mg/ml) were filtered through 0.45 μm nylon filters, and 30 μl filtrates were injected on a column. Isocratic elution was carried on with phosphate buffer (0.05 M, pH 7.2) with 0.87% NaCl, with a flow