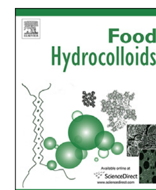
Food Hydrocolloids 44 (2015) 156–161

Contents lists available at ScienceDirect

Food Hydrocolloids

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



Multicatalytic enzyme preparations as effective alternative to acid in pectin extraction

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ARTICLE INFO

Article history:
Received 18 March 2014
Accepted 8 September 2014
Available online 20 September 2014

Keywords:
Apple pomace
Pectin extraction
Multicatalytic preparations
Optimization
Composition

ABSTRACT

Commercially available enzymatic preparations Celluclast, Econase and Viscoferm were applied to pectin isolation from apple pomace and compared to the conventional acid based extraction. All the enzymatic preparations were thoroughly characterized in respect of the glycosyl hydrolase activities: polygalacturonase, pectinesterase, cellulase, xylanase, 1,3-β-glucanase, arabanase, mannanase and rhamnogalacturonase. All the preparations effectively released pectin, even from nonhomogenized and nonbuffered material. The highest pectin yield was achieved for Celluclast (18.95%) and Viscoferm (17.86%), which was significantly higher than for acidic method (14.52%). The most important activities crucial for effective pectin extraction were xylanase and cellulase. Enzymatically isolated pectins had higher molecular weight (M_p), galacturonic acid, protein and ash content, lower water content and were comparably or less soluble than the pectin from acidic isolation. Enzymatic pectin extraction eliminates serious disadvantages of the acidic process, such as requirement for high temperatures, low pH, equipment corrosion and large volumes of sewage that needs neutralization. Therefore it represents a favorable alternative to the conventional methods.

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1. Introduction

Pectins are polysaccharides abundantly present (30–40% of dry mass) in the cell wall of higher mono- and dicotyledonous plants (Jayani, Saxena, & Gupta, 2005). Molecular structure of pectins is exceptionally complicated and polymorphic and varies depending on the plant species, type and age of the tissue. Nevertheless, the basic and common constituent is homogalactouronate, a linear polymer of galacturonic acid which is usually acetylated and methylated, as well as rhamnogalacturonate I which possesses a heteropolymeric backbone formed with repeated dimers of galacturonic acid and rhamnose moieties with side chains of arabinians and galactans. The third pectin constituent is a branched rhamnogalacturonate II which is a polygalacturonate polymer that contains nontypical side chain sugar moieties (Caffall & Mohnen, 2009; Wikiera & Mika, 2013). The mentioned pectin components are linked covalently into the pectin network in the plant primary cell wall and middle lamellae. Such a structure undergoes further modifications, mainly methylation, acetylation, xylosylation and apiosylation of galacturonic acid residues by the enzymes residing in the cell wall (Voragen, Coenen, Verhoef, & Schols, 2009). Physical, chemical and biological properties of pectin attract interest of researchers since pectin have become an important material utilized in various branches of industry and in recent years the demand for pectin has been increasing by 3–4% annually (Staunstrup, 2009; Yeoh, Shi, & Langrish, 2008). In the food industry pectins are used as gelifying agents in jams and jellies production, thickeners, emulsifiers and stabilizers in dairy products and margarines, mayonnaise and dressings (Dominiak et al., 2014; Willats, Knox, & Mikkelsen, 2006) or as fat substitutes in confectionery and ice creams (Kalapathy & Proctor, 2001), as well as to improve mechanical properties of protein films and coatings (Perez et al., 2013). Industrial resources for pectin extraction are mainly apple pomace and citrus peels that remain as waste after juice production. Currently, new technologies are being developed to utilize plant processing waste, such as soy husk or sunflower heads (fat industry), sugar beet press cake or cocoa seed husk (Iglesias & Lozano, 2004; Mollea, Chiampo, & Conti, 2008). Typical pectin isolation method employs hydrochloric, nitric, citric or sulfuric acids extraction in pH 1–3, for 1–12 h in the temperature of

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http://dx.doi.org/10.1016/j.foodhyd.2014.09.018
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70–100 °C (Shaha, Punichelvana, & Afandi, 2013; Srivastava & Malviya, 2011; Yapo, 2009). The more drastic conditions are applied, the lower degree of polymerization and esterification and lower neutral saccharides content in the obtained pectins (Garna et al., 2007). The main disadvantage of this industrial technology is a requirement of initial grinding of the material and repeated washing to remove sugars and color substances (Staunstrup, 2009). These steps hamper filtration process and produce huge amount of sewage and wastes that are hard to recycle. The very low pH and high temperatures used in the main process cause the risk of corrosion and rapid wearing out of the equipment.

Application of enzymatic preparations enables to overcome the mentioned drawbacks of the acid extraction, therefore it represents a favorable alternative for the latter method. Several pilot studies describing benefits of cellulase and protease application for pectin isolation from various materials, advocate for their broader use in the industry (Dominiak et al., 2014; Fissore et al., 2009; Lim, Yoo, Ko, & Lee, 2012; Zykwinska, Gaillard, Boiffard, Thibault, & Bonnin, 2009). In this paper we analyze the usefulness of commercially available multicatalytic enzyme preparations in the pectin isolation and define the enzyme activities that are crucially important for high yield.

2. Materials and methods

2.1. Materials

Dried apple pomace, a waste product of enzyme-free juice production was obtained from Pektowin S.A. company, Jaslo, Poland. Such a material is used in industrial pectin production. Prior to extraction, apple pomace was washed with distilled water (1/20 w/v), at 20 °C for 20 min with constant shaking (200 rpm). Washing was repeated 3 times, until refractometric concentration of sugars in water decreased below 1%. Next, the washed material was dried (60 °C, 24 h) and sieved through mesh no. 4 (4.75 mm). Some material was further ground until passing through mesh no. 40 (0.47 mm). Dry mass content in the pomace prepared according to described procedure was 94.77%.

As a reference material for molecular weight and insoluble fraction, ash and water content, a commercial apple pectin (Pektowin SA, Poland) was used.

2.2. Enzymes and enzyme activity assays

Three multicatalytic enzymatic preparations intended for plant tissue break down were tested: Celluclast® 1.5L, Viscoferm® (both from Novozymes Corp., Norway) and Econase® CE (AB Enzymes Corp., UK). Enzymatic activity profile of each preparation was assessed in pH 4.5, 50 °C according to standard procedures. Each type of activity was determined in replicates ($n = 5$). General pectinolytic activity was determined according to Patil and Dayanand (2006), and the activity unit [ºPM] was defined as the percentage by which the viscosity of a 1 percent pectin solution is decreased by 1 ml of an enzymatic preparation diluted 5 times. Polygalacturonase activity was determined according to Castillho, Medronho, and Alves (2000) with polygalacturonic acid as a substrate. The activity unit [PGU] was defined as a quantity of enzyme preparation that in standard condition releases 1 μmol of D-galacturonic acid in 1 min. Pectinesterase activity was assessed by potentiometric titration of carboxylic groups released from highly methylated apple pectin during the hydrolysis. The pectinesterase activity unit [PEU] was defined as an enzyme preparation quantity equivalent to 0.1 ml 0.01 M NaOH that was used to keep the constant pH of reaction mixture for 1 min. General celullolytic and xylanolytic activity was determined according to Anthony, Chandra Raj, Rajendran, and Gunasekaran (2003), using carboxymethyl cellulose (Sigma–Aldrich Chemie, Germany) and oat spelt xylan (Sigma–Aldrich Chemie, Germany) as substrates. The units of cellulolytic [CU] and xylanolytic [XU] activity were defined as the quantities of enzyme preparation necessary to release 1 μmol of glucose or xylose during 1 min in standard conditions. Activities of 1,3-β-glucanase, arabanase and mannanase were evaluated with the following substrates: AZCL-curdlan, AZCL-arabinan and AZCL-galactomannan 0.22% (w/v) suspensions in 0.05 M acetate buffer, pH 4.5, respectively (all were purchased from Megazyme, Ireland). Nine hundred (900) μl of such suspensions were mixed with 100 μl diluted enzyme preparations and incubated at 50 °C for 30 min with constant shaking. Reactions were stopped by 5-minute boiling, and next the samples were centrifuged at 15,000 rpm for 5 min. Absorbance of supernatants was recorded at 660 nm using a microplate reader (BioRad, USA). Completely hydrolyzed substrate solutions (incubated with endo-(1-3)-β-glucanase (Sigma–Aldrich Chemie, Germany), arabanase and mannanase (Megazyme, Ireland), respectively, for 60 min at 50 °C with shaking) were used as standards. Endo-(1-3)-β-glucanase [GlU], arabanase [AU] and mannanase [MU] activity unites were defined as the quantity of enzyme preparation that liquefied 1 μg of substrate in 1 min in standard conditions. Rhamnogalacturonase activity was assessed according to Salvador, Suganuma, Kitahara, Fukushige and Tanuoe (2002) using 2% AZ-rhamnogalacturonate (Megazyme, Ireland). The rhamnogalacturonase activity [RGU] unit was defined as the enzyme preparation quantity sufficient for liquefaction of 1 μg of substrate in 1 min in standard conditions.

2.3. Acidic extraction of pectins

Pectin was extracted from disintegrated apple pomace (4 and 40 mesh) with H_2SO_4 (dried substrate to extractant ratio 1/5, 1/8, 1/10, 1/15, 1/20 and 1/25 g/ml) at 85 °C, pH 2.0 for 3 h with constant shaking (200 rpm). The parameters of extraction were chosen to match the conditions frequently used in industry. Six independent extractions were performed.

2.4. Enzymatic extraction of pectins

Enzymatic extraction parameters were optimized in several steps. First, pH was optimized at the constant temperature (40 °C), time (3 h) and enzyme preparation dose (50 μl/g of dried substrate), with shaking (200 rpm). Equal portions (0.5 g) of washed apple pomace, sieved through 4 mesh, were mixed with distilled water of pH 4.0, 4.5, 5.0 and 5.5. Extraction solution volume (with enzymatic preparation added) was kept constant (1:8 solid to liquid ratio). Extractions were performed in quadruplicate. This procedure allowed to determine the optimal pH for each enzyme preparation. Subsequently, we analyzed the influence of material grinding on the pectin yield. The material was ground to 4 or 40 mesh. Each extraction was performed in the previously assessed pH, optimal for a given enzyme preparation, in 40 °C, using the dose of 50 μl/g and 1:8 solid to liquid ratio. Further the volumes of 5, 8, 10, 15, 20 and 25 ml per 1 g of the optimally ground material were tested. Extraction was carried on for 3 h at 40 °C, optimal pH and enzyme doses 50 μl/g. Next steps involved determining temperature, extraction time, and enzyme preparation doses necessary to obtain the maximal pectin yield. The experiments were performed according to the three-state complete design with 4 replicates. Initial parameters comprised 3 temperatures (40, 50, 60 °C), 3 extraction durations (6, 10, 18 h) and 3 enzyme preparation doses (10, 25, 50 μl/g of apple pomace). Each

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