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Oligosaccharide formation during commercial pear juice processing

Jamie L. Willems, Nicholas H. Low *

Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A8, Canada

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ABSTRACT

The effect of enzyme treatment and processing on the oligosaccharide profile of commercial pear juice samples was examined by high performance anion exchange chromatography with pulsed amperometric detection and capillary gas chromatography with flame ionization detection. Industrial samples representing the major stages of processing produced with various commercial enzyme preparations were studied. Through the use of commercially available standards and laboratory scale enzymatic hydrolysis of pectin, starch and xyloglucan; galacturonic acid oligomers, glucose oligomers (e.g., maltose and cellotriose) and isoprimeverose were identified as being formed during pear juice production. It was found that the majority of polysaccharide hydrolysis and oligosaccharide formation occurred during enzymatic treatment at the pear mashing stage and that the remaining processing steps had minimal impact on the carbohydrate-based chromatographic profile of pear juice. Also, all commercial enzyme preparations and conditions (time and temperature) studied produced similar carbohydrate-based chromatographic profiles.

1. Introduction

Commercial pear juice production in the United States and South America mainly consists of three cultivars of the species *Pyrus communis* L., commonly referred to as European pear, namely Bartlett (or Williams), Beurre d'Anjou and Beurre Bosc (USDA, 2004). Other pear cultivars, such as Packham's Triumph, may also be used in juice processing based upon location and availability.

During pear juice production, the following common commercial processing steps are employed: (1) the fruit is washed to remove surface and chemical contaminants; (2) the washed fruit is converted into a mash by milling (e.g., hammer mill) and carbohydrases, including amylases, hemicellulases and pectinases are added; (3) the enzyme treated mash is pressed (belt and/or hydraulic press) and the remaining pomace particles are removed by screening; (4) the resulting juice is transferred to clarification tanks where, if required, additional carbohydrases are added followed by filtration; and (5) the resulting clarified juice is pasteurized (70–100 °C for 6–40 s) and packaged for sale or concentrated (multiple effect evaporation ranging from 45 to 100 °C) for transport and/or future use (Bates, Morris, & Crandall, 2001; Ceci & Lozano, 2010; Horváth-Kerkai & Stéger-Maté, 2012).

Pear fruit cell walls are comprised of a variety of polymers, including polysaccharides, proteins and lignin (Dongowski & Sembries, 2001; Schols, 1995). Of these, pectin is the major target of enzymatic treatment during pear juice production. Pectin is a polysaccharide comprised of galacturonic acid residues to which neutral carbohydrates, mainly L-arabinose, D-galactose, L-rhamnose and D-xylose are covalently bound (Kashyap, Vohra, Chopra, & Tewari, 2001). Pectin can be classified into three different categories based on structure. The simplest of these is homogalacturonan (HG), which is defined as a linear polymer of α-1,4 linked D-galacturonic acid residues. The second category is rhamnogalacturonan I (RGI) in which the disaccharide -1-[→4)-α-D-GalA-(1 → 2)-α-L-Rha-(1→]$_n$-4- repeats as the polysaccharide backbone, to which a variety of different oligosaccharides, primarily arabinans and galactans, are linked to the rhamnose residues at the O-4 or O-3 positions. Finally, rhamnogalacturonan II (RGII) consists of a HG backbone to which complex oligosaccharides may be attached to the galacturonic acid residues through a variety of different glycosidic linkages (Round, Rigby, MacDougall, & Morris, 2010; Willats, Knox, & Mikkelsen, 2006; Yapo, 2011). Further pectin structure changes also commonly exist in nature where some of the galacturonic acid residues are methyl esterified. As such, pectin structure in plants varies depending upon plant species, location within the cell wall and plant age (Round et al., 2010).

Due to both its high concentration and structural complexity in pear fruit, a variety of different pectinases are required for pectin hydrolysis so as to improve juice extraction and reduce product viscosity. Pectinases (both exo and endo) commonly used for these purposes include: (1) polygalacturonase (PG) and

* Corresponding author.
E-mail address: nicholas.low@usask.ca (N.H. Low).

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polymethylgalacturonase (PMG), which hydrolyze the α-1,4-glycosidic linkages of pectin; and (2) polygalacturonate lyases (PGLs) and polymethylgalacturonate lyases (PMGL), which break the α-1,4-glycosidic linkages of pectin by a trans-elimination mechanism, resulting in a site of unsaturation at the non-reducing end (Kashyap et al., 2001). In addition, pectinesterases (PE) may also be used, which hydrolyze the methyl-ester groups of pectin resulting in polygalacturonic acid and methanol formation (Horváth-Kerkai & Stéger-Maté, 2012; Kashyap et al., 2001). Typically, commercial pear/apple pectinase preparations contain many if not all of these activities so as to effectively hydrolyze pectin (Horváth-Kerkai & Stéger-Maté, 2012).

Along with pectin, starch and hemicellulose are also targeted by enzyme hydrolysis during juice production. The presence of colloidal starch and hemicelluloses can cause cloudiness and sedimentation, which is undesirable in pear juice (Carrín, Ceci, & Lozano, 2004; Sinh, 2012). Therefore, commercial juice enzyme preparations often contain amylases and/or hemicellulases, in addition to pectinases, in order to help clarify the juice. Along with clarification, the addition of hemicellulases also helps to break down the fruit cell wall improving juice extraction and filtration (Horváth-Kerkai & Stéger-Maté, 2012).

Although published work exists on cell wall changes that occur in pear during its ripening and storage (Ahmed & Labavitch, 1980; Dick & Labavitch, 1989; Hiwasa et al., 2004; Raffo, Ponce, Sozzi, Vicente, & Stortz, 2011), no literature reports are available on the role of juice processing enzymes on cell wall polysaccharides under industrial and laboratory scale pear juice production. Therefore, the aim of this work was to examine the formation (i.e., presence of new compounds) and changes (i.e., loss and/or increase in concentration) in pear juice carbohydrates (polysaccharide, oligosaccharide and monosaccharide) during the processing stages commonly employed in commercial pear juice production through high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) and capillary gas chromatography with flame ionization detection (CGC-FID).

2. Materials and methods

2.1. Samples

Samples collected at various stages of pear juice processing (mash to concentrate) were received from three different commercial producers representing the USA (Juice 1) and South America (Juices 2 and 3). Processing stage samples were immediately heated at 90 °C for 3 min in order to inactivate enzymes prior to shipping. Received commercial samples were stored at −30 °C until analyzed. The °Brix of each sample was measured using an Auto Abbe refractometer (Leica Inc., Buffalo, NY, USA).

Major commercial enzymes used for pear juice production were kindly provided by the same commercial companies along with dosage regimes used for pear juice production. These enzymes included: Pectinex Ultra Clear, Pectinex Ultra Mash, Amylase AG 300 L and Pectinex UF (Novozymes, Bagsvaerd, Denmark); SEBAmyl L and LiquiSEB APL (Specialty Enzymes, Chino, CA, USA); Pear Adex and Adex-d (DSM, Heerlen, Netherlands); and Natuzyme A and Natuzyme Extra (WeissBioTech, Ascheberg, Germany). All enzymes were stored 4 °C.

2.2. Chemicals

L-Arabinose, cellobiose (O-β-D-glucopyranosyl-(1 → 4)-O-D-glucopyranose), digalacturonic acid (O-α-D-galactopyranuronosyl-(1 → 4)-D-galacturonic acid), D-fructose, D-fucose, D-galacturonic acid, D-galactose, D-glucose, D-mannose, pectin from apple, L-rhamnose, sodium hydroxide (NaOH) solution (50% w/w), D-sorbitol, starch from wheat, D-sucrose, Sylon TP (TMSI + pyridine, 1:4), trigalacturonic acid (O-α-D-galactopyranuronosyl-(1 → 4)-O-α-D-galactopyranuronosyl-(1 → 4)-D-galacturonic acid) and D-xylose were purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). D-Galacturonic acid methyl ester was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Sodium acetate (NaOAc) was obtained from Fisher Scientific (Ottawa, ON, Canada). Cellotriose (O-β-D-glucopyranosyl-(1 → 4)-O-β-D-glucopyranosyl-(1 → 4)-O-D-glucopyranose), isoprimeverose (O-α-D-xylopyranosyl-(1 → 6)-O-D-glucopyranose) and xyloglucan from tamarind were obtained from Megazyme (Cedarlane, Burlington, ON, Canada). The water used throughout this study was obtained from a Millipore Milli-Q™ water system (Millipore Corp., Milford, MA, USA).

2.3. Laboratory scale polysaccharide sample preparation

A solution of 2.0% glucose, 6.0% fructose, 1.0% sucrose and 2.5% sorbitol (w/v) was prepared in water to approximate the major carbohydrate composition of pear juice (mock pear juice; Thavarajah & Low, 2006; Willems & Low, 2014). Samples containing 2.0% (w/w) pectin and 2.0% (w/w) pectin plus 0.5% (w/w) starch in water and in mock pear juice were prepared in order to approximate the average concentration of these polysaccharides in pear (Raffo et al., 2011; Whitaker, 1984). One set of the aforementioned polysaccharide samples was prepared at their natural pH of 3.2 ± 0.1 and one set was adjusted to pH 4.0 ± 0.1 with sodium hydroxide. In addition, aqueous 1.0% (w/w) xyloglucan (pH 4.0 ± 0.1) samples were prepared.

2.4. Enzymatic hydrolysis of laboratory scale polysaccharide samples

Each of the aforementioned samples was subjected to enzymatic treatment following four different dosage/time/temperature conditions as supplied by the commercial juice producers (Table 1). In brief, the samples were brought to 50.0 ± 2.0 °C in an Aqua-Therm shaking water bath (New Brunswick Scientific Co. Inc., Edison, NJ, USA) and pectinases were added at concentrations of 1.5 –2.6 mg/g pectin (commercial producer dosages ranged from 30 to 50 ppm mash, which converts to 1.5–2.6 mg enzyme/g pectin [assuming a level of 2.0% pectin]) and amylases at concentrations of 1.9 –3.5 mg/g pectin (commercial producer recommended dosages ranged from 40 to 70 ppm mash, which converts to 1.9 –3.5 mg enzyme/g pectin [assuming a level of 2.0% pectin]) depending upon the enzyme set used by the commercial producer

Table 1
Enzyme dosage, times and temperatures used for laboratory scale hydrolysis experiments.

	mg enzyme/ g pectin	Time	Temperature
Enzyme Set A			
Pectinex Ultra Mash	2.3	90 min, 90 min	21–23 °C, 50 °C
Pectinex Ultra Clear	2.5		
Amylase AG 300 L	2.0		
Enzyme Set B			
LiquiSEB APL	2.6	90 min	50 °C
SEBAmyl L	1.9		
Enzyme Set C			
Natuzyme Extra	1.5	180 min	50 °C
Natuzyme A	3.5		
Enzyme Set D			
Pear Adex	1.5	180 min	50 °C
Adex-d	2.3		
Natuzyme A	3.5		