Production, purification and characterisation of oligosaccharides from olive tree pruning autohydrolysis

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ABSTRACT

The production of oligosaccharides (OS) by olive tree pruning autohydrolysis in the range 170–230 °C was studied. The best results in terms of maximum yield of OS along with a low amount of byproducts were obtained at 180 °C. After purification by preparative gel filtration chromatography a range of OS-fractions with average degree of polymerisation (DP) from 25 to 3 was selected for further characterisation. Gluco- and xylooligosaccharides were the predominant OS in these fractions. OS yields in the range 80–90% were obtained for fractions with average DP between 25 and 7, practically free of low molecular compounds. Both OS total yields and xylooligosaccharides proportion decreased for lower DP fractions while monosaccharides and other products concentrations increased. OS production and the recovery of other high value compounds can be envisaged as an interesting contribution to develop an olive-biomass biorefinery.

1. Introduction

Biomass obtained by pruning of olive trees constitutes an abundant and renewable agricultural residue in the Mediterranean countries for which no industrial applications were yet consistently envisaged. Nowadays, the normal practice is to dispose this biomass residue in the field, which poses environmental problems and represents an economic misuse. In the lack of alternatives, conversion into fuel ethanol has been proposed (Manzanares et al., 2011). This conversion process requires a pretreatment step to make cellulose susceptible for enzymatic saccharification (Hendriks and Zeeman, 2009). A number of processes have been assayed in our laboratories for fractionating olive tree residues, including steam explosion (Cara et al., 2008a; Ballesteros et al., 2011), dilute acid treatment (Cara et al., 2008b; Romero et al., 2007), alkaline delignification (Cara et al., 2006), organosolvolysis (Díaz et al., 2011) or liquid hot water (Cara et al., 2007), also called autohydrolysis.

Liquid fractions (hydrolysates) obtained after pretreatment contain a mixture of sugars whose conversion into ethanol is not as easy as that of glucose released from pretreated solids (Díaz et al., 2009). In the case of a hydrothermal pretreatment under conditions of moderate severity, hydrolysates contain the sugars mainly in the oligomeric form. These compounds possess economic interest as they can be used for several marketable applications (Mussatto and Mancilha, 2007; Patel and Goyal, 2011).

Obtaining valuable co-products coupled to ethanol production process is a characteristic feature of the biorefinery concept, which tries to integrate in a single facility the production of power, heat, chemical products and biofuels with lignocellulosic materials as a starting point in a similar way as oil refinery operates (Arato et al., 2005; Zhang et al., 2011). Industrial scale biorefineries can contribute greatly to make economically feasible second generation ethanol due to the simultaneous production of several other added-value products. For example, previous works have identified a number of compounds exhibiting antioxidant activity in the liquid fractions obtained after olive tree biomass hydrothermal pretreatment (Castro et al., 2008; Conde et al., 2009). The extraction of oligosaccharides from these fractions could also constitute an important step towards the development of the industrial biorefinery. Lama-Muñoz et al. (2012), in a work with alperujo (the olive oil by-product) concluded that this raw material represents a good

source not only of bioactive phenols but also poly- and oligosaccharides.

The interest in functional oligosaccharides (OS) is increasing worldwide because of their large scale application in foods, feed, pharmaceutical, cosmetics and agrochemistry industries (Patel and Goyal, 2011). OS can be obtained from natural sources (Courtois, 2009) or synthesised. Commonly employed production methods are hydrolysis of polysaccharides or synthesis from disaccharide substrates by enzymatic and chemical treatments (Mussatto and Mancilha, 2007). The selective fractionation of lignocellulosic residues by hydrothermal methods can be an economic interesting and environmental friendly alternative to obtain OS from sugar-rich materials (Garrote et al., 1999; Gullón et al., 2011a, 2011b; Nabarlatz et al., 2007).

The autohydrolysis resulting hydrolysates are thus a mixture of OS, monosaccharides, acetic acid and sugar-decomposition products. The concentration and formation rate of these compounds depend on the autohydrolysis conditions, which can be optimised for maximum OS production and/or controlled to produce OS mixtures with different ranges of molecular weight distribution (Carvalheiro et al., 2004; Garrote et al., 1999; Vázquez et al., 2006). The crude OS-rich hydrolysates can be then purified, *e.g.* using chromatographic techniques, for separating the OS in fractions with the desired degree of polymerisation (DP) range, and simultaneously excluding low molecular weight components, such as monosaccharides, and byproducts, such as furfural and hydroxymethylfurfural (HMF) (Moura et al., 2007).

This work addresses the fractionation and purification of oligosaccharides from olive tree biomass hydrolysates when the raw material is pretreated by autohydrolysis under conditions leading to the highest oligosaccharide yields. The goal is to obtain an interesting range of fractions for several applications, namely for the growth of probiotic bacteria. This is to our best knowledge the first time that olive tree pruning biomass has been reported as a suitable raw material for oligosaccharide production.

2. Materials and methods

2.1. Raw material

Olive tree pruning biomass, locally collected after fruit-harvesting, was formed by thin branches (usually <5 cm diameter) and leaves. It was chopped in the field and, once in the laboratory, air-dried at room temperature to equilibrium moisture content of about 10%, milled using a laboratory hammer mill (Retsch) to a particle size smaller than 10 mm, homogenised and stored until used.

Two different lots (A and B) of raw material were employed. The sugar content of lot A was 225 g cellulose, 98 g xylan, 21 g arabinan, 14 g galactan and 7 g mannan per kg olive tree pruning biomass (dry basis). Other components per kg accounted for 314 g extractives (including 79 g glucose of which 32 g in monomeric form), 166 g Klason lignin, 22 g acid-soluble lignin, 25 g acetyl groups and 34 g ash (Cara et al., 2007). Lot B contained 227 g cellulose, 114 g xylan, 19 g arabinan, 16 g galactan and 11 g mannan per kg olive tree pruning biomass (dry basis). Other components per kg accounted for 233 g extractives (including 76 g glucose of which 22.8 g in monomeric form), 71 g other glucans as starch, 166 g Klason lignin, 25 g acid-soluble lignin, 25 g acetyl groups and 34 g ash (Ballesteros et al., 2011). The differences between both material lots, especially concerning glucan and extractives, are ascribed mainly to the heterogeneity of this biomass, which can present a variable proportion of leaves as well as different branch diameters. One special feature of this material is the relatively high glucose content in the extractives. This is due to the presence of non structural glucans

such as oleuropein, a glucoside present in the leaves (Guinda et al., 2002), that is readily soluble and can be easily recovered in a water extraction process (Ballesteros et al., 2011).

2.2. Autohydrolysis

Autohydrolysis was performed in a laboratory scale stirred Parr reactor. The reactor had a total volume of 2 L, with an electric heater. The temperature/speed controller is a combination of furnace power control and motor speed control with tachometer. Cooling water was circulated through a serpentine coil to cool the reactor content at the end of each run. The amount of raw material loaded was 200 g (dry basis). Both water (1 L) and raw material were initially at room temperature. Agitation was set at 300 rpm. The average heating rate was 3 °C/min. Once the selected temperature (in a range 170–230 °C) was reached, this temperature was kept during 10 min (10 min of isothermal treatment). After treatment, the reactor was cooled using cold water circulating through the serpentine coil; the reactor was removed from the heating jacket and the vessel was introduced in an ice bath. The content of the reactor cooled down to 80 °C in approx 5 min. The reactor was kept sealed, and the slurry agitated until the reactor was cooled to about 40 °C. Then the wet material was filtered for solid and liquid recovery. Liquid fraction (hydrolysate) issued from autohydrolysis was analysed for sugars, acetic acid and sugar-degradation products.

2.3. Purification of the olive tree pruning hydrolysates

The liquid phases obtained from the autohydrolysis treatments (at selected conditions) were pooled together for separation of OS in a preparative gel filtration chromatography (GFC) column BPG 100/950 mm (Amersham Pharmacia Biotech, Uppsala, Sweden) with a bed volume of 4.2 L. The column was filled with Superdex 30 TM (fractionation range up to 1×10^4 Da), using deionized water as eluent at a flow rate of 0.025 L/min. The system was equipped with a refractive index detector (K-2401 Knauer, Berlin, Germany). 400 mL hydrolysate samples were purified for a total elution time of 250 min. In the elution volume ranging from 1255 to 4755, 125 mL fractions were separated and collected into plastic bottles using a Super-frac TM collector (Amersham Pharmacia Biotech). The fraction were subsequently freeze-dried (Labconco, Freezone 6, Missouri, USA) and weighted for dry matter determination.

2.4. Analytical methods

Hydrolysates obtained from autohydrolysis were centrifuged and filtered through 0.45 μm membranes (Gelman Sciences, Michigan, USA) and analysed by HPLC for quantitative carbohydrate analysis. The HPLC system (Waters, Milford, USA) was equipped with a refractive index detector (Waters). A Transgenomic CHO-682 carbohydrate analysis column operating at 80 °C with ultrapure water as a mobile-phase (0.4 mL/min) was used for the monomeric sugars (glucose, xylose, galactose, arabinose, mannose and fructose) and mannitol determinations. The oligosaccharides (OS) were measured by an indirect method based on quantitative acid hydrolysis (QAH) of the hydrolysates with 3% v/v of H_2SO_4 at 121 °C for 30 min. The OS concentration was expressed as the increase in sugar monomers, as analysed by HPLC, after QAH. Acidic samples were neutralized with $CaCO_3$ before being analysed in the CHO-682 column. Other compounds (acetic acid, formic acid, furfural and hidroximethylfurfural (HMF)) were determined using the same HPLC system with a Bio-Rad HPX-87H column operating at 65 °C with 5 mM H_2SO_4 as a mobile-phase (0.5 mL/min). Acetyl groups (AcO) bound to OS were determined by analysing acetic acid liberated during the same QAH procedure as described above for OS quantification. A portion of freeze-dried samples was