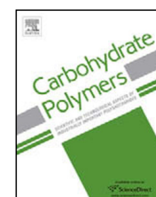
Carbohydrate Polymers 116 (2015) 215–222

Contents lists available at ScienceDirect

Carbohydrate Polymers

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



Valuation of brewers spent yeast polysaccharides: A structural characterization approach

Mariana Pinto[a], Elisabete Coelho[a,*], Alexandra Nunes[b], Tiago Brandão[c], Manuel A. Coimbra[a]

[a] QOPNA, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[b] Centro de Biologia Celular, Departamento de Biologia, Secção Autónoma de Ciências da Saúde, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[c] Unicer Bebidas, SA, Leça do Balio, 4466-955 S. Mamede de Infesta, Portugal

A R T I C L E I N F O

Article history:
Received 15 November 2013
Received in revised form 14 February 2014
Accepted 4 March 2014
Available online 14 March 2014

Keywords:
Saccharomyces
Glucans
Mannoproteins
Cellulose
Sequential extraction
Partial acid hydrolysis

A B S T R A C T

Brewers spent yeast (BSY) is a by-product from beer industry that can be exploited as source of glucans and mannoproteins, with potential biological activities. In order to solubilize these carbohydrate-rich polymeric materials, a sequential extraction with hot water and alkaline solutions (0.1–8 M KOH) was performed. Mannoproteins were mainly (85%) extracted with 4 M KOH whereas glucans were extracted with 8 M KOH and in an amount that accounted only for 34% of total glucose. Final residue still accounted for 34% of the initial glucans and contained 98% of glucose. Cellulase and α-amylase treatments showed the presence of both α- and β-(1→4)-Glc linkages. To promote total solubilization of these insoluble glucans, the final residue was submitted to a partial acid hydrolysis. This work is the first report showing that the most abundant polysaccharides in BSY are polymers that contain structural features similar to cellulose, thus justifying their resistance to alkaline extractions, acid hydrolysis, and insolubility in water.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Brewers Spent Yeast (BSY) represents 1.7–2.3 g/L of beer, used mainly in animal feed formulations. This by-product has, however, potential to be valued, particularly those which may result from carbohydrate polymers. BSY polysaccharides are mainly glucans and mannoproteins (Klis, Boorsma, & De Groot, 2006; Lipke & Ovalle, 1998), described as having immunostimulatory (Bohn & BeMiller, 1995; Chen & Seviour, 2007; Herre, Gordon, & Brown, 2004; Liu, Wang, & He, 2011), antioxidant (Jaehrig, Rohn, Kroh, Fleischer, & Kurz, 2007; Kogan et al., 2005; Križková, Ďuračková, Šandula, Sasinková, & Jánošík, 2001) and anti-tumoral activities (Križková et al., 2001; Lesage & Bussey, 2006; Liu, Wang, Cui, & Liu, 2008; Mantovani et al., 2008), and with emulsifying (Barriga, Cooper, Idziak, & Cameron, 1999; Cameron, Cooper, & Neufeld, 1988; Dikit, Maneerat, Musikasang, & H-kittikun, 2010) and prebiotic effects (Chen & Seviour, 2007; Laroche & Michaud, 2007). Consequently, they can be used as the basis of value-added products.

Brewing yeasts can be divided into two classes: top fermenting *ale* beer that uses *Saccharomyces cerevisiae* strains and bottom fermenting *lager* beer that uses *Saccharomyces pastorianus* or *S. carlsbergensis* strains, a natural allotetraploid hybrid from *S. cerevisiae* and *S. bayanus* (Ferreira, Pinho, Vieira, & Tavarela, 2010; Tamai, Momma, Yoshimoto, & Kaneko, 1998). Top- and bottom-fermenting brewing strains have similar surface ultrastructure, but different cell wall elasticity, degree of hydrophobicity, and polysaccharide properties. The surface of *S. pastorianus* is poor in proteins and much more hydrophilic than top fermenting species, explaining their tendency to sink, contrary to the floating properties of the *S. cerevisiae* due to their hydrophobic association with CO_2 bubbles (Alsteens et al., 2008; Dengis, Nélissen, & Rouxhet, 1995).

The information available relative to Saccharomyces cell wall polysaccharides concerns to *S. cerevisiae*. The β-glucans represent 50–60% of the cell wall composition, mannoproteins and chitin account for 35–40% and 1–3%, respectively (Klis et al., 2006; Lipke & Ovalle, 1998) and glycogen is between 1–23% (Aklujkar, Sankh, & Arvindekar, 2008; Arvindekar & Patil, 2002; Deshpande, Sankh, & Arvindekar, 2011; Northcote, 1953).

The β-glucans from *S. cerevisiae* are composed of β-(1→3)- and β-(1→6)-linked glucose residues (Bohn & BeMiller, 1995; Klis, Mol, Hellingwerf, & Brul, 2002; Lipke & Ovalle, 1998; Liu et al.,

* Corresponding author. Tel.: +351 234 370706; fax: +351 234 370084.
E-mail address: ecoelho@ua.pt (E. Coelho).

http://dx.doi.org/10.1016/j.carbpol.2014.03.010
0144-8617/© 2014 Elsevier Ltd. All rights reserved.

2008; Mantovani et al., 2008), in the proportion of 5:1 (Lipke & Ovalle, 1998; Liu et al., 2008). Glycogen, composed by α-(1→4)- and α-(1→4,6)-Glc linkages can be associated to β-glucans through covalent linkages, forming α,β-glucan complexes (Arvindekar & Patil, 2002; Kwiatkowski, Thielen, Glenney, & Moran, 2009). The *S. cerevisiae* mannoproteins are constituted mainly by mannose residues (89–96%) forming a highly branched and short chain structure, where most of the mannose residues are terminally linked and α-(1→2,6)-linked, together with (1→2)- and (1→3)-linked linear residues. Small amount of other sugar residues such as glucose, galactose, and xylose, linked to the protein moiety, are also present (Barriga et al., 1999; Jigami & Odani, 1999; Lipke & Ovalle, 1998).

The yield of extraction of *S. cerevisiae* carbohydrate polymers that can be calculated based on the data available in the bibliography is 14% for mannoproteins (Freimund, Sauter, Kappeli, & Dutler, 2003) and 22–26% for glucans (Freimund et al., 2003; Wang, Yao, & Wu, 2003). This allows to infer that the major part of these polymers remains insoluble in the cell wall matrix. To define the appropriate application for the valuation of this by-product, these structural features of this insoluble material should be solubilized and characterized.

In order to study the structural features of *S. pastorianus* glucans and carbohydrate moiety of its mannoproteins, a sequential extraction with water and alkali solutions with increasing concentrations (0.1 M, 0.5 M, 4 M, and 8 M KOH) was performed. In addition, the polysaccharides that remained unextracted upon this procedure were submitted to a partial acid hydrolysis and to α- and β-glucanase enzymatic hydrolysis. All fractions were analyzed concerning sugar and glycosidic linkage composition.

2. Materials and methods

2.1. Materials

BSY (*S. pastorianus*) was provided by the brewery Unicer Bebidas, SA, Portugal. The lot had 10% solids content, composed mainly by carbohydrates (49%) and proteins (27%) determined, respectively, as alditol acetates (section 2.6) and by the bicinchoninic acid method (Wrolstad et al., 2001). The carbohydrates were mainly glucose (71%) and mannose (19%), with lesser amounts of uronic acids (7%), arabinose (2%), xylose (1%), and galactose (<1%).

2.2. Sequential extraction of the BSY polysaccharides

The BSY polysaccharides were sequentially extracted with hot water and alkali solutions (Fleet & Manners, 1976; Liu et al., 2008). The suspension of BSY (64.5 g dry matter) polysaccharides were sequentially extracted with [1] hot water (1 L, 100 °C, 5 min); [2] 0.1 M KOH + 20 mM $NaBH_4$ (500 mL, 20 °C, 2 h); [3] 0.5 M KOH 20 mM + $NaBH_4$ (500 mL, 20 °C, 2 h); [4] 1 M KOH + 20 mM $NaBH_4$ (500 mL, 20 °C, 2 h); [5] 4 M KOH + 20 mM $NaBH_4$ (500 mL, 20 °C, 2 h); [6] 8 M KOH + 20 mM $NaBH_4$ (500 mL, 20 °C, 2 h). The alkali extractions were carried out with O_2-free solutions under nitrogen atmosphere. After each extraction, solubilized polymers were separated from the insoluble residue by centrifugation at 15,000 rpm, at 4 °C during 20 min. All extracts were acidified to pH 5 with acetic acid prior to dialysis (12–14 kDa cut off). The precipitates formed during dialysis of alkaline extracts were collected separately.

The final residue (FR1) that remains after the last alkali extraction (8 M KOH + 20 mM $NaBH_4$) was suspended in 400 mL of distilled water and the solution was acidified to pH 5 and dialyzed. The suspension was centrifuged and the supernatant solution (Sn-FR) was collected separately by centrifugation from the final residue (FR2). All extracts collected after dialyses were freeze-dried (Fig. 1).

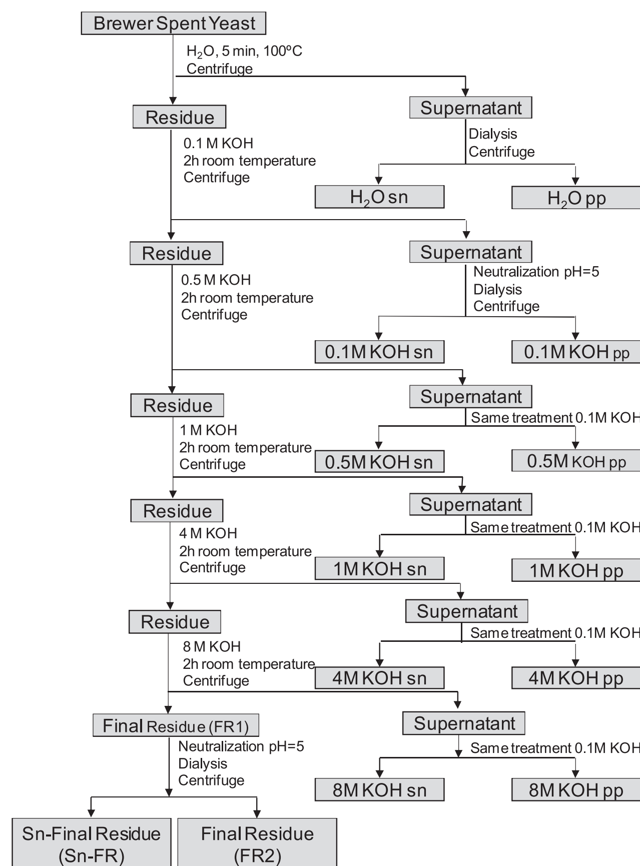


Fig. 1. Schematic representation of sequential extraction from brewer spent yeast polysaccharides.

2.3. Partial acid hydrolysis

The final residue was submitted to a partial acid hydrolysis according to the procedure described by (Nunes, Reis, Silva, Domingues, & Coimbra, 2008). The sample (10 mg) was suspended in 2 mL of TFA 250 mM, at 70 °C, during 30 min. The residue was then separated from the supernatant solution by centrifugation at 4000 rpm, during 20 min. The residue obtained was resuspended in TFA 250 mM and a new TFA treatment under the same conditions was performed. These steps were repeated until the complete solubilization of the material. For this propose seven cycles of partial acid hydrolysis were necessary. In each cycle the supernatant obtained was collected and evaporated under reduced pressure at 30 °C. The compounds obtained in each supernatant were fractionated by size exclusion chromatography on Biogel P-30 and submitted to methylation analysis.

2.4. Size exclusion chromatography

Preparative size exclusion chromatography was performed on a Pharmacia Biotech XK 26 chromatography column (42.5 cm × 1.6 cm column) containing Biogel P-30, using a flow rate of 0.08 mL/min. Exclusion and total volume were calibrated with blue dextran and glucose, respectively.

The freeze-dried material was dissolved in 1 mL of 50 mM sodium–phosphate buffer, pH 6.5, and loaded on the column previously equilibrated with the loading buffer. Fractions (2 mL) were collected and assayed for sugars with the phenol–H_2SO_4 method (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956).

The fractions of each chromatographic peak were pooled and rotary evaporated, dialyzed and then freeze-dried.

Download English Version:

https://daneshyari.com/en/article/1385685

Download Persian Version:

https://daneshyari.com/article/1385685

Daneshyari.com