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Valuation of brewer's spent grain using a fully recyclable integrated process for extraction of proteins and arabinoxylans

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ABSTRACT

The objective of this work was to design an integrated process to valuate brewer's spent grain (BSG) proteins and arabinoxylans (AX) to be used as food ingredients. For this purpose, a sequential extraction of proteins and AX from BSG with increasing alkali (KOH or NaOH) concentrations of 0.1 M, 0.5 M, and 4 M, was optimized. A ratio of 1:2 (w/v) (weight of BSG by volume of alkali solution) at room temperature for 24 h was preferred to minimize reagents and energy consumption. To fully integrate the process, alkaline extracts were acidified to pH 3 with citric acid, to obtain the protein-rich fractions. The AX were recovered by ethanol precipitation and citric acid and ethanol were recycled. This integrated extraction process allowed a yield of 82–85% of the BSG total proteins and 66–73% of total AX with formation of a cellulose rich residue almost devoid of nitrogen.

1. Introduction

Brewer's spent grain (BSG) is the main by-product generated during the beer production process. Around 15–20 kg of BSG is obtained per hectolitre of beer produced. BSG has been used, almost exclusively, for animal feed, particularly cattle. However, it is a by-product of great interest, given its nutritional and functional characteristics for the sectors of biotechnology, food, and pharmaceutical industry (Mussatto et al., 2006, 2007; Aliyu and Bala, 2011). BSG proteins and its hydrolysates can be used as food texture enhancers due to their emulsifying properties (Celus et al., 2009) and may present immunomodulatory effects (McCarthy et al., 2013) and antimicrobial activity (Kotlar et al., 2013). BSG is also considered a major biomass resource for the production of second generation biofuels, it can be used for ethanol production using a bioconversion process (Xiros et al., 2008, 2011).

The spent grains are separated from beer wort by filtration after the mashing phase. They correspond to the insoluble fraction of the wort, essentially composed by a lignocellulosic material containing protein (~30% on a dry weight basis), lignin (~28%), hemicelluloses (~25%), and cellulose (~17%) (Celus et al., 2006; Treimo et al., 2009). The major proteins of the BSG are hordeins (A, B, and C), constituting over 50% of the total amount of proteins, followed by glutenins. The less abundant protein fraction includes the albumins (about 2%). The major hemicelluloses of BSG are the arabinoxylans (AX), which are composed by a backbone of β-(1 → 4)-linked xylopyranose, in part substituted with single units of arabinofuranose at positions 2, 3, or both (Viëtor et al., 1992), which can be extracted under strong alkali solutions (Mandalari et al., 2005). The AX are considered as dietary fiber due to their resistance to hydrolysis by digestive tract enzymes. They can present immunomodulatory activity and the arabinoxylo-oligosaccharides (AXOS), obtained by partial hydrolysis of AX, have been described as prebiotics (Van Craeyveld et al., 2008; Delcour et al., 2008). Formulations combining AXOS with AX have been shown to potentiate their prebiotic activity (Broekaert et al., 2010).

BSG contains 70–80% moisture, thus, a considerable amount of energy is necessary for its drying. Separation of spent grain components can be performed by physical or chemical procedures. Physical processes, such as pressing and sieving of wet BSG, can be performed when a suspension in hot water (80 °C) is prepared and passed through a sieve to obtain two fractions: a proteic fraction (rich in protein and fat and low in fiber) and a fiber fraction (low in protein and rich in AX (Schwencke, 2006)). Chemical extractions of BSG proteic fraction in alkaline medium are also described, namely the alkaline extraction of BSG at pH 11–12 and 104–121 °C

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where the proteic fraction is then obtained by isoelectric precipitation (Sohtaroh et al., 1992). However, this process involves decomposition of proteins due to the drastic conditions of the high temperature used, resulting in a low yield of proteins and deterioration of product quality. Furthermore, the process requires high amount of energy for the high temperatures of extraction. The preparation of protein concentrates after alkaline extraction of BSG (17%, w/v) with 0.1 M NaOH at 60 °C was also proposed (Celus et al., 2007). After 60 min of extraction, samples were filtered and the proteins precipitated by acidification at pH 4 using 2 M citric acid.

The AX of BSG are usually extracted by a well established sequentially procedure using solutions of KOH with increasing concentration of 0.5 M, 1 M, and 4 M (Mandalari et al., 2005). Before the sequential extraction, the BSG was pre-treated with a solution of 80% ethanol (v/v) under reflux, followed by two water extractions, and a pronase treatment (Mandalari et al., 2005).

The goal of this work is the valuation of BSG by the development of an integrated process of simultaneous extraction of proteins and AX through the use of alkaline reagents directly from the BSG, without any pre-treatment. Selective precipitation of proteins by acidification of the medium and addition of ethanol for AX recovery was performed, carrying out a total recycling of the reagents. These compounds (proteins and AX) can be used as ingredients in the food industry, in dietetic and/or pharmaceuticals products.

2. Experimental

2.1. Materials

Five lots of 1 kg of brewer's spent grain (BSG) from different grists of malt/wheat were supplied weekly by a brewery industry (Unicer Bebidas SA, Leça do Balio, Portugal), coded from 1 to 5. The spent grains in a wet-form were used for BSG characterization and for integrated extraction of proteins and arabinoxylans (AX).

All chemicals were from Sigma–Aldrich and were of, at least, analytical grade. Eluents used for the chromatographic separations were ultra-pure water (obtained from a Seral – Seralpur Pro 90 CN water purifying system) and LiChrosolv acetonitrile (Merck, Darmstradt, Germany). Trifluoroacetic acid (TFA) (Fluka, Seelze, Germany) was added to both eluents.

2.2. Extraction of proteins and AX from BSG

2.2.1. Optimization of the sequential extraction of proteins and arabinoxylans

BSG without pre-treatment (100 g) was added to 500 mL of a 0.1 M KOH solution (ratio 1:5, w/v). Sodium metabisulfite (5 mM) was added as antioxidant. Two different time and temperature conditions were tested concerning contact of the sample with alkaline reagent. Test conditions were 24 h, room temperature, and occasional shaking (coded as Test A) and contact of the sample with alkaline reagent for 2 h, 40 °C, with continuous shaking (coded as Test B). As summarized in Fig. 1, Residue 1 and the Extract 1were obtained by sedimentation and filtration of the 0.1 M KOH extract. Each Residue 1 was extracted with 500 mL of a 0.5 M KOH with 5 mM sodium metabisulfite, according to the conditions established for Tests A and B. The separation of the residues from the 0.5 M KOH extracts was performed by decantation and filtration that were designated, respectively, as Residue 2 and Extract 2.

The Residue 2 was extracted with 500 mL of a 4 M KOH with 5 mM sodium metabisulfite, using the respective Tests A and B conditions. Each residue was separated from the respective 4 M KOH extract by decantation and filtration that were designated, respectively, as Residue 3 and Extract 3.

The Extracts 1, 2, and 3 were separately acidified to pH 3 with a saturated solution of citric acid. This value of pH allows the precipitation of BSG proteins (mostly hordeins and glutenins) that are separated by decantation and filtration, giving proteic fractions PF1, PF2, and PF3 fractions, respectively (Fig. 1). The fractions soluble in citric acid, which contain the AX, were further acidified with 37% HCl until a pH below 2 was achieved. The AX were recovered by precipitation with 70% (v/v) aqueous ethanol solutions. The citric acid present, due to its full protonation at a pH below 2, remained soluble in this solution. The AX were separated by decantation and washed with ethanol. The AX fractions obtained were designated, respectively, by AX1, AX2, and AX3 (Fig. 1). A final wash with water was done to the Residue 3, obtaining the last fraction of AX coded as AX4.

A Test C was performed using similar conditions of Test A except the ratio of BSG to KOH aqueous solution that was 1:2 (w/v), e.g. 100 g of BSG and 200 mL of alkali solvent.

2.2.2. Sequential extraction of proteins and arabinoxylans and reagents recycling

Two tests of four sequential and consecutive extractions using NaOH or KOH reagents were also performed using Test C conditions, using a ratio of BSG to alkali solution of 1:2 (w/v), at room temperature, overnight.

Another set of extractions was performed including the recycling of ethanol and citric acid. The ethanol was recovered by distillation of the ethanolic solution resultant from the precipitation of polysaccharides, leaving an aqueous solution containing citric acid and NaCl dissolved. The NaCl was partially removed as a precipitate upon concentration of the solution, allowing to obtain an aqueous solution saturated in citric acid. An extraction with ethanol was also performed to the precipitated NaCl to recover co-precipitated citric acid. The ethanol was recovered by evaporation and the two aqueous solutions containing the citric acid were combined and reused as a saturated citric acid solution.

2.3. Analytical methods

Dry matter was evaluated using an oven at 105 °C until constant weight. Nitrogen content was estimated by the Kjeldahl method (AOAC, 1975). The protein content was calculated from nitrogen using a conversion factor of 6.25. Fat was extracted from dried BSG (2 g) by a soxhlet extractor with 150 mL of *n*-hexane during 5 h. The ash was determined gravimetrically by incineration of BSG at 650 °C during 24 h in a muffle furnace. Carbohydrate content was determined by sugar analysis derivatized as alditol acetates. Monosaccharides were released from polysaccharides by a pre-hydrolysis in 0.2 mL of 72% H_2SO_4 (w/w) for 3 h at room temperature followed by 2.5 h hydrolysis in 1 M H_2SO_4 at 100 °C (Selvendran et al., 1979). Neutral sugars were analyzed as their alditol acetates by gas-chromatography-flame ionization detection (Blakeney et al., 1983; Harris et al., 1988). The hydrolysis was performed in duplicate.

Chromatographic analyses of proteins present in BSG and Residues 1, 2 and 3 require extraction under reducing conditions (Celus et al., 2006). For this purpose, the procedure described by Schmitt et al. (1989) was used. FP1, FP2 and FP3 fractions were dissolved in water containing 40% acetonitrile (0.01 g in 10 mL), acidic extracts were injected directly. The reversed phase high performance liquid chromatography (RP-HPLC) analyses were carried out using a HPLC unit (Jasco, Tokyo, Japan) composed of a low pressure quaternary pump (Jasco PU-1580 intelligent HPLC pump), a degasification unit (Jasco DG-1580-54 4-line degasser), a type 7981 Jones Chromatography column heater (Jones Chromatography, Hesperia, CA, USA), a type 7725i Rheodyne injector (Rheodyne, Rohnert Park, CA, USA), and a UV/VIS detector (Jasco UV-970