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## MICROWAVE-ASSISTED EXTRACTION OF BREWERS' SPENT GRAIN ARABINOXYLANS

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**Abstract.** *Brewers' spent grain (BSG) is a by-product from beer industry that can be exploited as a source of arabinoxylo-oligosaccharides (AXOS) with prebiotic activity. In this study, microwave-assisted extractions were performed during 2 min at 140-210°C in order to evaluate the feasibility of this extraction technology for quantitative extraction of the arabinoxylans (AX) or AXOS from BSG. The AX yield increased with the increase of the temperature in the range used. The best condition of extraction of the AX was 210 °C during 2 min, allowing the extraction of 43% of total AX. These AX showed structural variability which allow to define specific types of compounds for different applications and uses depending on the extraction conditions used.*

**1. Introduction.** The brewers' spent grain (BSG) is a by-product resultant of the filtration of the liquid extracted from the mashing process during the brewing of beer. The BSG is composed exclusively by the material from barley husks, with 75% moisture, having a high percentage of polysaccharides, namely, arabinoxylans (AX, 28%), cellulose (16.8%) and small amounts of  $\beta$ -glucans<sup>1</sup>. It is estimated that Europe produces 3.5 million ton per year of BSG<sup>2</sup>. This by-product has been used for animal feed as a way to reduce the residue produced by these industries. The use of BSG as a source of polysaccharides with fiber properties and other biological activities that have been associated with AX and  $\beta$ -glucans, namely the prebiotic activity<sup>3</sup>, has an enormous potential. BSG AX are composed by a backbone of  $\beta$ -(1 $\rightarrow$ 4)-linked xylose residues containing single units of arabinose as side chains<sup>4</sup>. These polysaccharides are considered as dietary fibre due to their resistance to hydrolysis by digestive tract enzymes. Additionally, they can present immunomodulatory activity. The arabinoxylo-oligosaccharides (AXOS), obtained by partial hydrolysis of AX, have been described as prebiotics<sup>5</sup>, e.g., they promote the growth of beneficial bifidobacteria in the large bowel. Formulations combining AXOS with AX potentiate their prebiotic activity<sup>6</sup>.

The use of microwaves for AX extraction has been recently proposed for the residue of barley from the flour industry<sup>7</sup>. These polymers can be obtained directly in water, avoiding the use of alkali reagents. The aim of this work was to determine the changes in the structure and amount of AX extracted by microwave-assisted extraction from BSG under different temperatures.

## 2. Materials and Methods

**2.1 Raw material.** BSG provided by Unicer- Bebidas de Portugal in Leça do Balio, Portugal, was used as raw material.

**2.2 Extraction of AX by microwave-assisted hydrothermal treatment.** BSG (10 g) was suspended in 60 mL distilled water to a 100 mL Teflon-coated vessel at a liquid/solid ratio of 6:1 and the pH was measured before the microwave irradiation. The slurry was then treated in a microwave oven (MicroSYNTH Labstation for Synthesis). The microwave extraction was applied to BSG for extraction of the AX using temperatures of extraction from 140 to 210 °C during 2 min. The temperature and pressure were controlled with a thermocouple immersed in the slurry.

After microwave irradiation, the pH was measured. The reactor was cooled at room temperature and centrifuged at 24652g for 15 min at 1 °C and filtered to separate the water-soluble fractions from the cellulosic residues (CRs). The CRs were suspended in water, frozen, and freeze-dried.

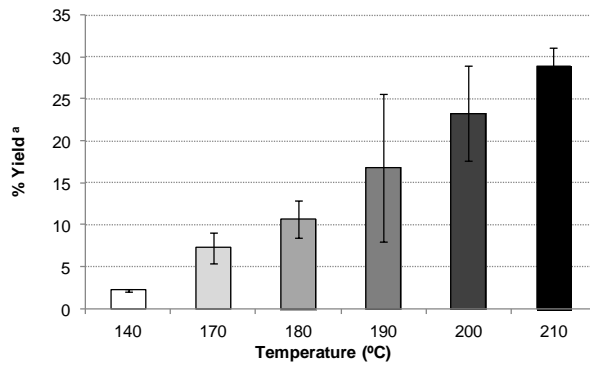
Absolute ethanol was added the water-soluble fractions to perform 70 % (v/v) ethanol solutions, assuming additive volumes. These solutions were stirred for 2 h at 4 °C until formation of precipitate and then were centrifuged to obtain a precipitate (Et70) that was removed from the supernatant solution (EtSn). To remove the ethanol completely, each precipitate and supernatant solution was dissolved in water, concentrated by rotary evaporation at 35 °C, frozen, and then freeze-dried.

**2.3. Neutral sugar analysis.** Neutral sugars were released by Saeman hydrolysis<sup>8</sup> and analyzed as their alditol acetates by gas-chromatography-flame ionization detection<sup>9,10</sup>. The hydrolysis of all fractions was performed in duplicate. Results used have less than 5% variability in the major component sugars.

### 3. Results and Discussion

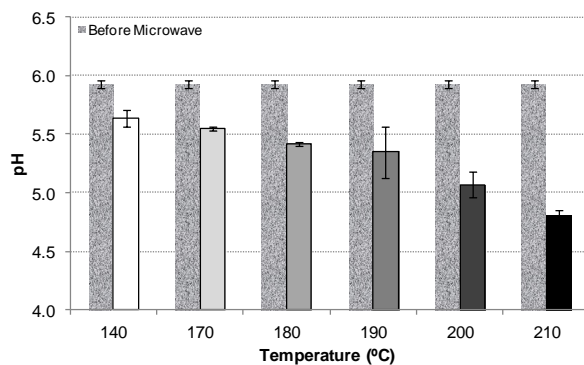
#### Influence of the temperature of microwave on the amount and chemical composition of the extracted carbohydrate material in the BSG.

**Figure 1** shows the results for the yield of the material extracted for the different temperatures applied in the microwave-assisted hydrothermal treatment. The amount of material extracted with hot water, on BSG dry basis, increased with the increase of temperature in the range of temperatures used. The extracts performed at 210 °C allowed an extraction of 28.8% of total soluble solids, those performed at 200 °C allowed an extraction of 23.3 %, and at 190 °C the extraction yield was 16.9 %.



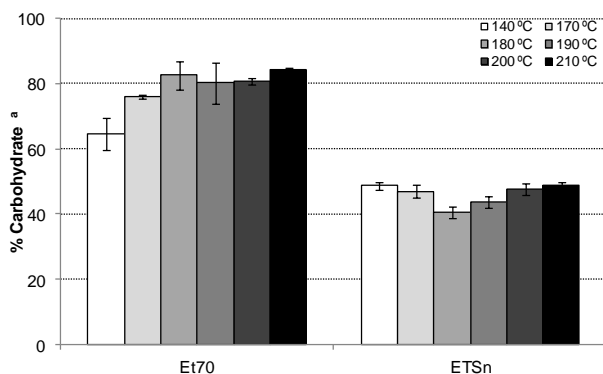
**Figure 1:** Yield of the material extracted from BSG by microwave-assisted hydrothermal treatment for the different temperatures. Bars represent the standard deviation. <sup>a</sup>In relation to the equivalent dry weight.

**Figure 2** shows the measurement of the pH of the BSG suspensions before and after the microwave-assisted hydrothermal treatment. The initial pH value, before the treatment, was 5.93. Values of the final pH of the aqueous extracts ranged from 5.64 to 4.81. It could be observed that the pH values decreased gradually with the increase of the temperature of microwave-assisted hydrothermal treatment. The decrease of pH was higher (-1.12 pH units) for the temperature of 210 °C, the temperature that extracted higher amount of material (**Figure 1**). This pH decrease and the release of insoluble material into solution seem to the occurrence of autohydrolysis of the insoluble BSG material upon microwave treatment<sup>11</sup>. The internal superheating of the cell water content, by absorbing the microwave radiation should enable the cell disruption allowing the solvent to access the compounds of interest, in this case, AX<sup>12</sup>. The pH decrease can be due to the formation of acids “*in situ*” by the hydrolysis of esters, namely those of AX with ferulic and acetic acids or by the thermal degradation of Ara residues that can be converted into formic and levulinic acids<sup>13</sup>.



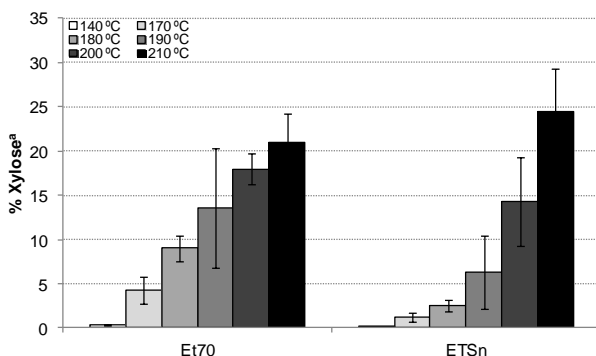
**Figure 2:** Representation pH of the BSG suspensions before and after microwave-assisted hydrothermal treatment for the different temperatures. The values shown are averages of duplicates for each experiment.

**Figure 3** shows amount of carbohydrates obtained in the Et70 and EtSn fractions. These results showed that the material that precipitated in solutions containing 70 % (v/v) ethanol (Et70 fractions) were those richest in sugars, containing a range between 64.6 and 84.2%. The material that remained soluble in 70 % (v/v) ethanol (ETSn fractions) were composed by 40.6 to 49.0% of sugars.



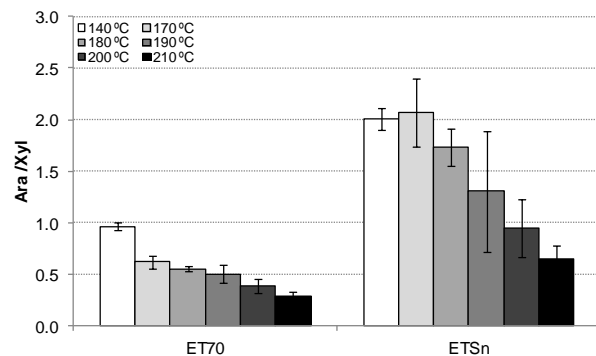
**Figure 3:** Amount of carbohydrate extracted from BSG by microwave-assisted extraction for different temperatures. Bars represent the standard deviation. <sup>a</sup>Grams of anhydrosugar per 100 g of fraction.

**Figure 4** shows the amount of xylose extracted from BSG by microwave-assisted extraction for different temperatures and recovered in the Et70 and EtSn fractions. The analysis of the Et70 fractions shows the increase of the amount of xylose with the increase of the temperature. The same observation is made for EtSn fractions. Amount of xylose in the Et70 fractions ranged between 0.34 and 21.0 %, whereas for EtSn the amount of xylose ranged from 0.15 to 24.4 %. The majority of the xylose-rich compounds were present in Et70, with the exception of those recovered in the experiment at 210 °C. At this temperature, the amount of xylose in the supernatant exceeded the amount of xylose in Et70 fraction. This shows that for this temperature the size of the AX were too small to precipitate in the ethanol solutions. The maximum extraction of xylose, considering both Et70 and EtSn fractions, was 45.3 %, occurring at 210 °C.



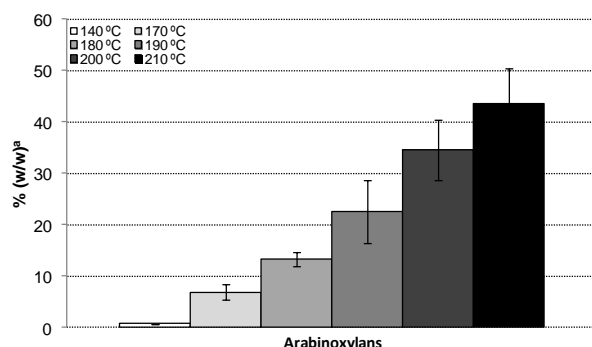
**Figure 4:** Amount of xylose extracted from BSG by microwave-assisted extraction for different temperatures in the Et70 and EtSn fractions. Bars represent the standard deviation. <sup>a</sup>Grams of anhydrosugar per 100 g of fraction.

The Ara/Xyl ratios of AX extracted at different temperatures are shown in **Figure 5**. This ratio allows to infer the degree of branching of the AX. The Ara/Xyl ratio data showed that Et70 fractions ranged from 0.29 to 0.96 while the EtSn fraction varied between 0.65 and 2.07, indicating a low degree of arabinose substitution in Et70. These AX showed structural variability, mainly due to the degree of substitution (estimated by Ara/Xyl ratio) and degree of polymerization (inferred by the different solubility in the ethanol solutions). Due to the different temperatures applied in the microwave-assisted extraction and purification process by ethanol precipitation, it is possible to provide fractions with different AX characteristics.



**Figure 5:** Arabinose to xylose (Ara/Xyl) ratios of two fractions (Et70 and EtSn) AX extracted from BSG by microwave-assisted extraction for different temperatures. Bars represent the standard deviation.

Based on the data presented, **Figure 6** shows the amount of AX extracted from BSG by microwave-assisted hydrothermal treatment for the temperatures between 140 and 210 °C. The amount of AX extracted increase with the increase of temperature. However, temperatures of the microwave from 140 to 180 °C extracted only small amount of AX (0.69, 6.86 and 13.3%, respectively). The best extraction conditions were achieved using higher temperatures 190, 200 and 210 °C, yielding 22.6, 34.5 and 43.4 % of AX, respectively.



**Figure 6:** Amount of AX extracted from BSG by microwave-assisted extraction. The values shown are averages of duplicates for each experiment. <sup>2</sup>Grams of anhydrosugar per 100 g of fraction.

#### 4. Conclusion.

This work is the first investigation on isolation of arabinoxylans (AX) by microwave-assisted extraction from brewers' spent grain (BSG). The best conditions of extraction, at 210 °C during 2 min, allows to obtain 43% of BSG AX. Depending on the extraction conditions used, AX present structural variability, as the higher temperatures promotes depolymerization and debranching. The compounds formed are highly soluble in water and even in ethanol solutions, allowing their use in aqueous and alcoholic matrices. The results of this work open several opportunities for future valorization BSG under study as potential source of nutraceuticals with potential prebiotic effects in the fractions obtained.

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