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## 46. Hydrothermal processing of hardwoods and agro-industrial residues: evaluation of xylo-oligosaccharides production

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### Abstract

Autohydrolysis was used as a pre-treatment method for the selective hydrolysis of hemicelluloses of four lignocellulosic materials: eucalypt wood chips (EWC), corn cobs (CC), wheat straw (WS) and brewery's spent grain (BSG), and the process was optimized towards the production of xylo-oligosaccharides (XOS). The effects of temperature, both at isothermal (150–190°C), and non-isothermal (up to 240°C) conditions, and liquid-to-solid ratio (LSR), 8 or 10 g/g, on the composition of both the liquid and solid phases were studied. The operational conditions leading to the maximal recovery of XOS for each raw material were established and discussed based on the severity factor ( $\log R_0$ ). The higher yields obtained were in the range of 43–65% of the feedstock (arabino)xylan, with the highest value attained for CC for  $\log R_0=3.75$ . Under the optimised conditions for XOS production, glucan was only slightly solubilised, enabling an enrichment of the solid phase (up to 67% of glucan for EWC). Delignification was also not extensive reaching utmost 18%, in the case of CC.

### Introduction

The processing of hardwoods, agricultural and agro-industrial residues in aqueous media (autohydrolysis) has been demonstrated to be an effective method for the solubilisation of hemicelluloses [1-3], with the further advantages that cellulose and lignin can be recovered in the solid phase, only with minor losses, thus enabling full biomass utilization within the biorefinery framework. Hemicelluloses are mainly solubilised in the form of oligosaccharides (OS), specifically xylo-oligosaccharides (XOS) in the case of xylan-rich materials.

Xylo-oligosaccharides are a special interesting group of compounds presenting some beneficial health properties, namely the capability to stimulate the growth of probiotic bifidobacteria strains, which have been associated to the so-called bifidogenic effect [4,5]. Furthermore, they also present several technological advantages that distinguish them from other commercial OS, namely the long shelf life and high thermal stability and in a wide pH range. These properties are a function of their structural composition and depend on the biological origin of the raw material and on production processes used and their conditions. Currently, XOS are mainly used as food ingredients although cosmetic and pharmaceutical applications also hold a high potential promise. In this work, we study the operational conditions influencing the yield of XOS and explore the composition of liquors obtained by autohydrolysis of four lignocellulosic residues and by-products: eucalypt wood chips (EWC), corn cobs (CC), wheat straw (WS) and brewery's spent grain (BSG). The yield and chemical composition of the solid phase were also evaluated and the results are discussed based on the severity factor ( $\log R_0$ ).

## Experimental

### Raw materials

Brewery's spent grain (BSG), wheat straw (WS, Ardila cultivar), corn cobs (CC) and *Eucalyptus globulus* wood chips (EWC) were supplied by local producers. Both CC and WS were ground to particles < 1.5 mm, and EWC to particles < 6 mm. Brewery's spent grain was pre-treated (for residual starch removal) as previously described [6] and screened (> 0.5 mm retained). All materials were stored in vacuum-sealed bags.

### Autohydrolysis

The hydrothermal treatments (autohydrolysis) were performed in stainless steel reactors (Parr Instruments Company, USA) with a total volume of 0.6 l or 2 l. The reactors were heated by an external fabric mantle (0.6 l) or an electric heater (2 l) and cooled by cold water circulating through internal stainless steel loops. Temperature was controlled through Parr PID controllers (model 4842). The raw materials and water were mixed in the reactors in order to obtain the desired liquid-to-solid ratio (LSR) of 8 or 10 (w/w). The agitation speed was set at 150 rpm and the reactor heated to reach temperatures of 150°C, 170°C and 190°C for isothermal operation (BSG) and final temperatures between 150°C and 240°C for non-isothermal operation (CC, EWC, WS). The liquid and solid phases were recovered by filtration. Recovered solids were wash with twice the amount of water, filtered again and dried at 40°C. The yield and composition were determined as described below.

### Analytical Methods

#### *Chemical characterisation of raw materials and processed solids*

The materials were ground in a knife mill to a particle size < 0.5 mm and the moisture content was determined by oven-drying at 105°C to constant weight. Protein was estimated by the Kjeldahl method using the N x 6.25 conversion factor. The samples were analysed for glucan, xylan, arabinan and acetyl groups after quantitative acid hydrolysis with 72% (w/w) H<sub>2</sub>SO<sub>4</sub> followed by 4% (w/w) H<sub>2</sub>SO<sub>4</sub> [7]. The acid insoluble residue was considered as Klason lignin, after correction for ash. The monosaccharides and acetic acid in the hydrolysates were analysed by HPLC as described below.

#### *Chemical characterisation of liquors and hydrolysates*

Glucose, xylose, arabinose, acetic acid, 5-hydroxymethyl furfural (HMF) and furfural were analysed by HPLC (Waters, USA) using an Aminex HPX-87H column (Bio-Rad, USA) as previously described [3]. A sample of the liquors was directly analysed by HPLC and another sample was hydrolysed with 4% (w/w) H<sub>2</sub>SO<sub>4</sub> [7]. Oligosaccharides concentrations were calculated from the increase in sugar monomers, after liquor hydrolysis. The term XOS has been used to name the hemicellulose-derived OS made up of xylose units and the possible arabinan-derived oligomers encountered have been included as XOS for the purpose of calculating yields and average compositions.

### Calculations

In order to have a simplified, empirical interpretation of the measured effects of both time and temperature, the severity factor  $\log R_0$  [8], was employed. It can be calculated using the equation:

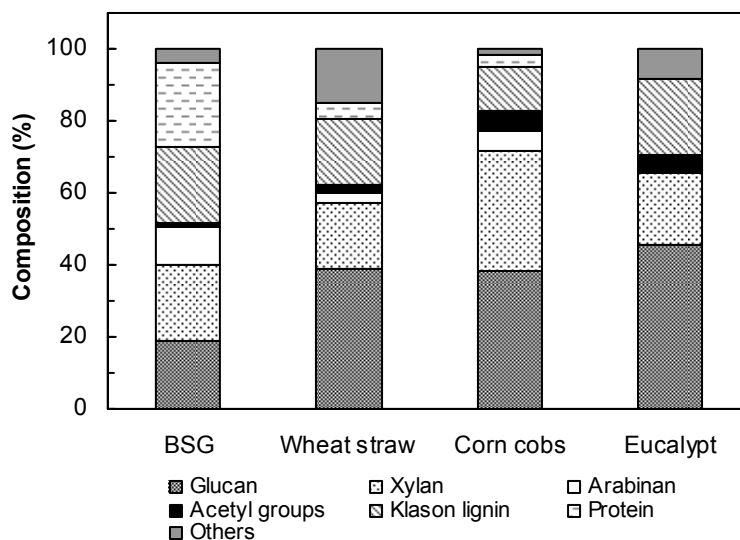
$$R_0 = \int_0^t \exp\left(\frac{T(t) - 100}{14.75}\right) dt$$

where the temperature ( $T$ ; °C) is a function of time ( $t$ , min), and 14.75 an empirical parameter related with the activation energy and temperature.

## Results and discussion

### Composition of the raw materials

Figure 1 shows the macromolecular composition of the raw materials used: brewery's spent grains (BSG), wheat straw (WS), corn cobs (CC) and eucalypt wood chips (EWC). All of them are xylan-rich materials but due to their different biological origin some differences in hemicellulose composition occur. Eucalypt hemicellulose, which belongs to the glucuronoxylan type, is almost devoid of arabinose and exhibited a relatively high content of acetyl groups. Compared to the other materials, BSG had the highest arabinose content (near half of xylose content) and was the less acetylated material.



**Figure 1** – Chemical composition (% of oven dry mass) of brewery's spent grain (BSG), wheat straw, corn cobs and eucalypt.

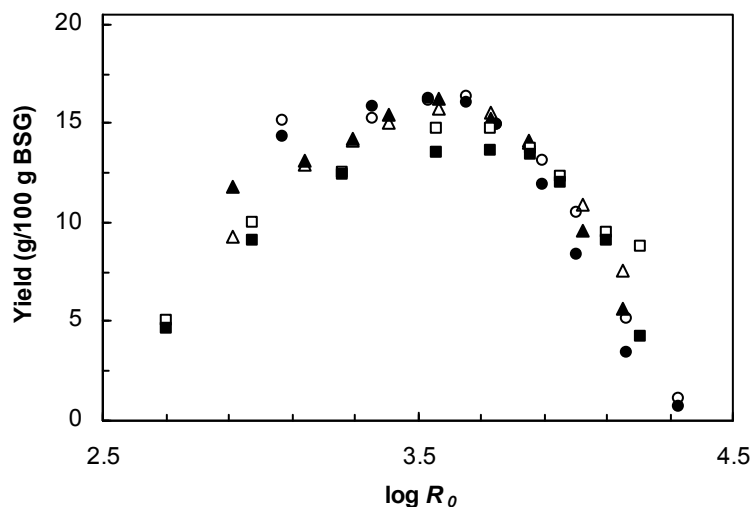
The cellulose content (measured as glucan) was the highest for EWC. The highest polysaccharide content was found for CC, which is also the less lignified raw material. The lowest content of polysaccharides plus lignin was exhibited by BSG, since this material also contains an important amount of protein [3], as it is a residue of the barley grain, and not a structural part of the plant.

### Effect of autohydrolysis on xylo-oligosaccharides production

For the optimisation of xylo-oligosaccharides (XOS) production, the variables studied were the temperature, the liquid-to-solid ratio (LSR) and reaction time. Figure 2 shows the yield of XOS as a function of the severity factor ( $\log R_0$ ), obtained for isothermal autohydrolysis of BSG at temperatures of 150°C, 170°C and 190°C and for the LSR of 8 and 10 g/g. For calculation purposes, XOS were considered as arabinose substituted xylooligomers as referred above. The maximum XOS yields were obtained for a severity of  $\log R_0=3.53$ , but in the range of  $\log R_0=3.35$ -3.73 there is not a significant decrease change and it varied between 13.6 and 16.3 g/100 g BSG, which correspond to an (arabino)xylan recovery as XOS of 43-52%.

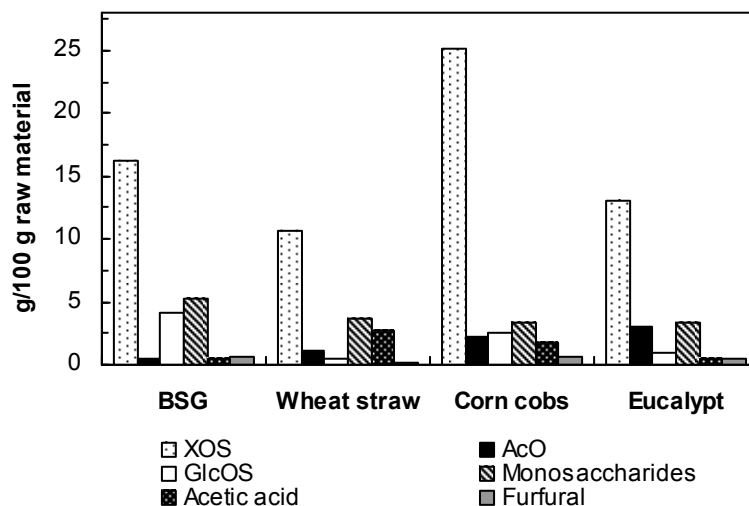
The yield seems to be only slightly affected by the LSR, and for the same severity, there seems to be a tendency to a slight increased recovery with the temperature. Nevertheless, the data fitting ( $R^2>0.95$ ) to an empirical model (quadratic in relation to  $\log R_0$  and linear in relation to the other variables,  $Y_{XOS} = \beta_0 + \beta_{11}(\log R_0)^2 + \beta_1(\log R_0) + \beta_2LSR +$

$\beta_3$ Temperature) demonstrated that both LSR and temperature coefficients were not statistically significant, even at  $p < 0.1$ . The maximum xylan conversion into XOS was within the range reported in literature for other xylan-containing raw materials of similar biological origin [9].



**Figure 2** – Effect of severity and liquid-to-solid ratio on XOS yield during isothermal autohydrolysis of BSG. Squares: 150°C, triangles: 170°C, circles: 190°C. Closed symbols: LSR=8 g/g; open symbols: LSR=10 g/g

Since the yield of XOS obtained for autohydrolysis of BSG was almost not affected by LSR, only the highest solids concentration condition (LSR=8 g/g) was used in the subsequent experiments, now carried out under non-isothermal conditions. A similar quadratic trend was obtained for WS and CC [7,10] as well as for EWC (data not shown). Figure 3 presents the recoveries of OS, monosaccharides and by-products obtained for the different raw materials, under the conditions leading to the maximum recovery of XOS for BSG ( $\log R_0=3.53$ ), CC ( $\log R_0=3.75$ ), EWC ( $\log R_0=3.73$ ) and WS ( $\log R_0=3.96$ ). The maximum XOS yield for the studied raw materials decreased as follows: CC>BSG>EWC>WS and can be related to the amount of xylan in the raw material. The XOS yield obtained for CC (25.1 g/100 g) corresponds to 65% recovery of the (arabino)xylan of the raw material. Autohydrolysis of EWC and WS yield lower amounts of XOS, respectively 13.0 g/100 g and 10.6 g/100 g of the corresponding raw material. These values corresponds to 60% and 50% recovery of the (arabino)xylan of the EWC and WS, respectively. The maximum XOS yield obtained for BSG (16.3 g/100 g) correspond to 52% recovery of the initial (arabino)xylan. The differences in the recoveries obtained could be mainly explained by the relation between the (arabino)xylan content and higher acetyl groups content of CC and EWC as compared to BSG and WS, given that these groups favour the hydrolytic process. Comparing with similar XOS production approaches, XOS yield obtained for WS and CC were higher or in the range previously reported for the autohydrolysis of those materials [2,9] and the yield obtained for EWC XOS are in the range of the values reported by Garrote et al. [1].



**Figure 3** – Yield of oligosaccharides and by-products obtained from autohydrolysis of BSG, CC, EWC and WS in the optimised conditions for XOS production. AcO - acetyl groups linked to oligosaccharides; BSG - brewery's spent grain; GlcOS - gluco-oligosaccharides.

Besides XOS, the hydrolysates obtained were also characterised for other OS, namely, OS made up of glucose (gluco-oligosaccharides, GlcOS), acetyl groups both linked to OS (AcO) and acetic acid, monosaccharides, furfural and HMF. During autohydrolysis treatment, the progressive hydrolysis of acetyl groups from the raw material also results in the generation of acetic acid although an important amount can remain linked to the OS (AcO). This was the case of EWC liquors where AcO was the highest, corresponding to 3.0% of the raw material. This is in agreement with previous studies that shown that OS from eucalypt mainly consist in highly acetylated XOS [11]. In contrast, acetyls in WS liquors are mainly present as free acetic acid. The OS with a higher acetylation degree were obtained from highly acetylated raw materials, such as the case of EWC and CC. This can be an important aspect since XOS which maintain a significant fraction of acetyl and uronic groups attached may have distinct characteristics like a very high solubility in water [9].

The formation of GlcOS was most significant in BSG hydrolysates. Although in the preparation of this material a previous pre-treatment was performed in order to remove the residual starch [6] it is possible that some starch still remain in the material contributing to an increase formation of these compounds. During autohydrolysis the monosaccharides were also produced via hydrolytic cleavage of OS. The lowest yield of monosaccharides was obtained for EWC whereas BSG presented the highest, accounting for close to 20% of the hydrolysate soluble matter (data not shown). The by-products resulting from the dehydration reactions of monosaccharides, i.e., furfural and HMF were always low in all conditions, especially HMF. Actually, HMF does not rise above 0.1 g/100 g raw material (for EWC) and was omitted from Figure 3. The highest production of furfural was obtained for BSG, and the lowest for WS, and corresponds to 0.64% and 0.23% of the raw material, respectively. From a purification point of view, the presence of furfural in such concentrations is not problematic as it can easily be removed from the hydrolysates by evaporation.

### Effect of autohydrolysis on the solid composition

Table 1 shows the recoveries of glucan, xylan, arabinan and Klason lignin and the composition of the processed solids for the severity conditions leading to the highest recovery of XOS for each raw material. Glucan solubilisation ranged between 4.6% (for CC) and

21.3% (for BSG). As occurred for CC, EWC glucan was almost not affected by the hydrolytic treatment, leading to a solid residue with increased glucan (up to 67%). The results obtained for glucan yield in BSG are consistent with the formation of glucose and GlcOS found in BSG hydrolysates (Figure 3). As it is not expected that BSG can still contain  $\beta$ -glucans as occur in barley [12], it is possible that cellulose hydrolysis of this material was easier or even the presence of some residual starch also may contribute to these results.

**Table 1** – Composition of the solid phase and polymers recovery obtained for BSG, WS, CC and EWC in the optimised conditions for XOS production.

	BSG	Wheat straw	Corn cobs	Eucalypt
Optimal condition <sup>a</sup>	3.53	3.96	3.75	3.73
Solids composition (%) <sup>b</sup>				
Glucan	25.4	56.1	60.8	66.9
Xylan	12.2	12.2	16.6	9.0
Arabinan	2.9	0.8	2.0	0.?
Klason lignin	*45.8	24.9	17.6	29.9
Recoveries (solids) <sup>c</sup>				
Glucan	78.7	89.5	95.4	94.1
Xylan	33.4	41.6	30.3	28.5
Arabinan	16.2	17.1	21.0	0
Klason lignin	*122.9	85.4	82.0	90.4

<sup>a</sup> log  $R_0$ ; <sup>b</sup> g/100 g of processed solids; <sup>c</sup> g/100 g of polymer in the raw material; \* also includes protein.

In general, the results obtained for glucan are in good agreement with the previous reported for similar raw materials [1,2]. Klason lignin solubilisation ranged between 9.6 and 18.0%, for CC, EWC and WS, with the highest value obtained for the less lignified material (CC). The low solubilisation of lignin demonstrated that hydrothermal treatment does not significantly interact with lignin. The values obtained were similar or even lower than the reported for the autohydrolysis of CC and EWC under similar operational conditions [1,2]. In BSG, a recovery of Klason lignin higher than 100% was obtained. This can be explained by the significant content of protein in this raw material which is only partially solubilised during autohydrolysis (under these conditions protein recovery in the solid residue was 95.8%, data not shown) and that remains in the acid insoluble residue after quantitative acid hydrolysis as experimentally confirmed by FTIR and Kjeldahl protein determination (unpublished data). For the operational conditions leading to the highest recovery of XOS, xylan removal can reach up to 71.5% in the case of EWC, whereas the hydrolysis of arabinose substituents, expressed as arabinan, was always higher, generally above 80%.

## Conclusions

Autohydrolysis enables the selective solubilisation of BSG, CC, EWC and WS hemicelluloses with a high recovery of pentoses, mainly XOS, which can be used as potential prebiotic ingredients, in a high added-value application. The solids obtained are rich in cellulose and (sulfur-free) lignin and therefore can be easily used for further processing, e.g., for bioethanol production and lignin upgrade.

Thus, it can be stated that autohydrolysis is an effective pre-treatment method for the selective biomass fractionation leading to integrated valuable co-upgrade solutions in a biorefinery framework.

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