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# Autohydrolysis of agricultural by-products for the production of xylo-oligosaccharides

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**Abstract**

Six agricultural residues of different botanic origin, namely corncobs (CC), almond shells (AS), olive stones (OS), rice husks (RH), wheat straw (WS), and barley straw (BS), were tested as feedstocks for the production of xylo-oligosaccharides (XOs) by autohydrolysis at 179 °C for 23 min. The yield of XOs depended on the content of xylan and its accessibility, and was proportional to the acetyl content of the raw materials. It was higher for CC (60%) and AS (55%), while RH provided a low yield (30%) in accord with its lowest acetyl content. The compositional and GPC analyses of the hydrolysis products indicated that they contained partially acetylated oligomeric and polymeric xylan fragments, and some monosaccharides and degradation products. Combined 1D ($^{13}C$ and $^{1}H$) and 2D (HSQC) NMR spectra of the dialyzed XOs samples revealed that the acetyl groups were located in the xylose residues mainly in position 3 (between 60 and 67 mol%), whereas the occurrence of acetyl groups in position 2 and in both positions 2 and 3 was similar (19–30 mol% and 8–25 mol%, respectively). The NMR analyses showed the presence of 4-*O*-methylglucuronic acid (MeGA) residues in all XOs samples, as indicated by the MeGA/Xyl mole ratios ranging from 2.5:100 for CC up to 9.1:100 for OS. Regardless of the structural differences of the xylan-types present in the parent plant materials, all XOs showed structural features of a partially *O*-acetylated 4-*O*-methylglucuronoxylan.


*Keywords:* Corncobs; Almond shells; Olive stones; Wheat straw; Barley straw; Rice husks; Autohydrolysis; Acetylated xylo-oligosaccharides

## 1. Introduction

Xylo-oligosaccharides are xylose-based oligomers that may have variable proportions of substitute groups like acetyl, uronic, and phenolic acids, depending on the lignocellulosic from which they are extracted and the process of production. Substituted xylo-oligosaccharides have some specific characteristics that are driving research efforts to develop applications in fields related to the food and pharmaceutical industries. They may be used as soluble dietary fiber because of its low calorific value and acceptable organoleptic properties. Furthermore, they are non-carcinogenic and act as prebiotics promoting the growth of beneficial bifidobacteria in the colon (Crittenden & Playne, 1996; Yuan, Wang, & Yao, 2005), and are considered a possible ingredient in functional foods (Cummings, Edmond, & Magee, 2004; Gibson, 2004). Some studies point to the beneficial effect of xylo-oligosaccharides may have on reducing the risk of colon cancer (Wollowski, Rechkemmer, & Pool-Zobel, 2001; Hsu, Liao, Chung, Hsieh, & Chan, 2004). Recently, xylo-oligosaccharides extracted by autohydrolysis of bamboo have been found to posses a cytotoxic effect on human leukemia cells (Ando et al., 2004). Also xylo-oligosaccharides can be used as a source of xylose for the production of xylitol, a well-known low-calorie sweetener (Rivas, Domínguez, Domínguez, & Parajó, 2002). Ethers and esters prepared from xylan and xylo-oligosaccharides have been synthesized and used as thermoplastic compounds for biodegradable plastics, water soluble films, coatings, capsules,

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and tablets (Glasser, Jain, & Sjöstedt, 1995), also for the preparation of xylan-based hydrogels (Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000; Lindblad, Ranucci, & Albertsson, 2001), and of micro and nanoparticles (Garcia et al., 2001).

The composition and the structure of the xylo-oligosaccharides are influenced by the source of xylo-oligosaccharides and the production process. The processes used to isolate xylo-oligosaccharides from xylan-rich materials are essentially hydrolytic, either in basic or acidic media, or catalyzed by enzymes. By using strong alkali solutions, depolymerized xylan may be extracted from lignocellulosics, but the product obtained is completely deacetylated and has very limited solubility in water. Autohydrolysis, on the contrary, takes place in slightly acidic media due to the partial cleavage of acetyl groups, which liberates acetic acid. The xylo-oligosaccharides produced maintain a significant fraction of acetyl and uronic acid groups attached, and this gives them distinct characteristics like a very high solubility in water. Furthermore, autohydrolysis at mild temperature produces high-molar mass xylo-oligosaccharides without modifying cellulose and lignin substantially, allowing their recovery for further processing (Montané, Farriol, Salvadó, Jollez, & Chornet, 1998; Shimizu et al., 1998). By controlling the temperature and time of reaction it is possible to influence characteristics of the xylo-oligosaccharides such as the acetyl content and the molar mass distribution (Nabarlatz, Farriol, & Montané, 2004, 2005), but the nature of the raw material has also a significant role. In this work we explore the yield, molar mass distribution, chemical composition and structure of the xylo-oligosaccharides obtained by autohydrolysis of six agriculture residues: corncobs, almond shells, olive stones, rice husks, and straws of barley and wheat.

## 2. Experimental

### 2.1. Raw material

Samples of corncobs (CC), wheat straw (WS), barley straw (BS), rice husks (RH) and olive stones (OS) were collected in the northeast of Spain. Almond shells (AS) were purchased from MIMSA S.A. (Lleida, Spain). The samples of CC, WS, BS, and RH were milled to pass through a 1 mm screen, while OS and AS were milled and sieved to 300 μm.

### 2.2. Hydrothermal processing

The experiments were developed using a 10 L stirred batch reactor that was constructed of ANSI 304- and 316-L stainless steel by EMMSA (Tarragona, Spain). The treatments of all samples were carried out in deionized water at 179 °C and 23 min of reaction. These conditions were chosen from previous experiments done at laboratory scale for the autohydrolysis of corncobs (Nabarlatz et al., 2004) and almond shells (Nabarlatz, Farriol, & Montané, 2005). The concentration of solids in the reactor was varied depending on the porosity and density of the different raw materials. For AS, OS, and RH the concentration was 14.3% (833 g of material per 5 L of water), for CC it was reduced to 11.1% (625 g of dry solid per 5 L of water), and for BS and WS to 5.7% (300 g of dry solid with 5 L of water). After the completion of the reaction the solid hydrolysis residue was separated from the liquid phase by filtration, washed with warm water and dried at room temperature. The final volume of the liquid product (LP) obtained by combining the liquid phase and washing water was measured and 100 mL were taken for analysis. The rest of the LP was treated by spray drying using a Büchi Mini Spray Dryer B-290 (Masso Analítica, Barcelona, Spain) to recover the xylo-oligosaccharides (SXOs), or stored in frozen state for further processing. A complete description of the reactor system and the operation procedures has been given in previous reports (Nabarlatz et al., 2004, 2005). The spray-dried XOs samples (SXOs) were purified by dialysis in order to prepare them for the NMR analysis. Solutions of SXOs in deionized water at concentrations 20 g/L with CC and AS and 40 g/L with OS, WS, BS, and RH, were exhaustively dialyzed using cellulose membrane (estimated MWCO of 12.4 kDa, Sigma Aldrich). The retentate obtained was recovered by lyophilization yielding samples of dialyzed xylo-oligosaccharides (DXOs), which were further analyzed by GPC and NMR as described below.

### 2.3. Analytical methods

The moisture content of the raw materials and hydrolysis residues was determined by drying three samples at 105 °C until constant weight. The ash content and organic extractives were determined by standard procedures ASTM D 1102–8 and ASTM D-1107–84, respectively. Klason lignin of both solids was determined by the two-step sulfuric acid hydrolysis according to ASTM D 1106–84.

High-pressure liquid chromatography (HPLC) analysis was used to quantify acetic acid, monosaccharides, furfural, and hydroxymethylfurfural (HMF). The analysis was done with an Agilent 1100 series chromatograph (Agilent, USA), using a Bio-Rad HPX87H column at 30 °C (Bio-Rad Laboratories, USA). The solvent was 0.005 M $H_2SO_4$ and flow rate 0.5 mL/min. An ultraviolet diode-array detector and a refractive index (RI) detector were connected in series. The UV detector was used to quantify furfural, and HMF in the samples that contained low concentrations of these compounds, while the RI detector was used for the samples with high concentrations. This detector was also used to quantify carbohydrates. The system was calibrated with glucose, xylose, arabinose, acetic acid, furfural, and HMF standards (Sigma–Aldrich). Before measurements, all the samples (2 mL) were filtered through 0.22 μm filter.

A sample of the liquid product (LP) was analyzed by HPLC directly in order to quantify the free monosaccharides, acetic acid, furfural, and HMF. Another sample of LP (5 mL) was mixed with 1 mL of 5 N $H_2SO_4$ and

hydrolyzed at 120 °C for 45 min to convert XOs to their constitutive monomers, and analyzed by HPLC to quantify the total amount of XOs in the LP. The analysis of the neutral sugar composition of the SXOs samples was performed by dissolving 3 g in 180 mL of 0.416 M $H_2SO_4$, followed by hydrolysis at 120 °C for 45 min, and then analyzed by HPLC. The lignin associated to the SXOs was measured gravimetrically as acid-resistant lignin, and acid-soluble lignin was estimated from the absorbance of the solution at 205 nm, following the Tappi T250 standard method. All analyses were performed in triplicate. The ash content in the SXOs samples was determined by standard procedure ASTM D 1102–8.

The molar mass distributions of SXOs, DXOs, and the XOs in the LP, were measured by Gel Permeation Chromatography (GPC). In the case of SXOs and DXOs, solutions (2 g/100 mL) were prepared by dissolving the dry sample in deionized water. In all the cases a sample of 1.2 mL of the filtered liquid was mixed with 0.2 mL of a solution 0.35 M $KNO_3$, containing 581 mg/L $NaN_3$. The GPC analysis was performed with a TSKGel G3000PWXL column (Toso Haas, Japan) at 25 °C using as solvent 0.05 M $KNO_3$, containing 83 mg/L $NaN_3$, at a flow rate 0.5 mL/min. The system was calibrated with narrow standards of oligomaltoses and dextrans (Fluka). Calculations of the molar mass distribution were performed using the Agilent Chemstation GPC add-on software.

The NMR analyses were performed on 10 mg of dry DXOs dissolved in 0.75 mL $D_2O$ (99.96%, Sigma–Aldrich). The $^1H$ spectra were obtained using a Varian Mercury VX400 spectrometer at 400 MHz, and the $^{13}C$NMR spectra were measured in a Varian Gémini 300 spectrometer at 300 MHz. The $^1H$ NMR spectra were recorded using a 75° pulse of 12 μs, a spectral width of 4500 Hz and a repetition time of 15 s. All spectra were acquired at ambient temperature. The proton-detected heteronuclear single quantum (HSQC) spectra were recorded over a spectral width ($t_1$) of 14,600 Hz and a width ($t_2$) of 3600 Hz, with a 2048 × 1024 matrix, and 0.125 transients per increment. The delay between transients was 15 s and the delay for polarization transfer was set to 140 Hz ($^1H$ $^{13}C$ coupling constant).

## 3. Results and discussion

### 3.1. Autohydrolysis-induced compositional changes of the agricultural by-products

#### 3.1.1. Composition of the raw materials

Table 1 shows the chemical composition of the different raw materials we used in this study, namely corncobs (CC), wheat straw (WS), barley straw (BS), rice husks (RH), olive stones (OS), and almond shells (AS). Major differences were found in the ash content, which was low in CC, AS, and OS (1–2.8%). Higher amounts of ash (around 6%) were determined in WS and BS, and RH showed the highest content of about 15%, in agreement with the significant occurrence of silicates in these and related plant tissues (Theander & Åman, 1984). Organic extractives, including some phenolics, were higher in AS, OS, WS, and BS (5–6.5%) than in CC and RH (~2%). The degree of lignification estimated by the Klason lignin was about 16–19% in WS, BS, and CC, higher in RH and AS (24–27%), and particularly high in OS (31.3%). Due to the presence of ash, Klason lignin analysis gave an overestimation of the lignin content. Therefore, the obtained values were corrected by the ash content of the Klason lignin (Anglés et al., 1997). The cellulose content estimated from the amount of glucose in the Klason lignin hydrolysate varied between 24% for OS and 38.5% for CC, and was lower for the more lignified biomass species. However, it has to be noted that glucose might have released also from other cell wall components, such as hemicelluloses of the glucomannan, xyloglucan or mixed-linkage β-glucan types (Ebringerová, Hromadková, & Heinze, 2005). Their occurrence depends on the plant source, and they are not the dominant hemicelluloses in the materials covered in this study. The content of xylan was estimated from the amounts of xylose, arabinose, and acetyl groups. It was 36.8% and 32.5% for CC and AS, respectively, and was lower for the other species, up to 19.7% for RH. However, xylan also contains variable amounts of glucuronic acid and some phenolic acids, which were not quantified by the HPLC analytical procedure we used routinely. Table 1 also reports on the acetyl to Xyl mole ratios, which were

Table 1
Composition of the different raw materials (wt.%)[a]

| | CC | AS | OS | WS | BS | RH |
|---|---|---|---|---|---|---|
| Ash | 1.14 ± 0.03 | 2.83 ± 0.07 | 2.80 ± 0.04 | 6.39 ± 0.04 | 6.13 ± 0.05 | 15.21 ± 0.03 |
| Extractives | 2.10 ± 0.05 | 5.0 ± 0.4 | 6.4 ± 0.4 | 6.5 ± 0.8 | 5.9 ± 1.0 | 2.4 ± 0.3 |
| Klason lignin | 18.7 ± 0.4 | 27.4 ± 1.4 | 31.3 ± 0.1 | 15.9 ± 0.3 | 16.7 ± 0.3 | 24.6 ± 0.1 |
| Glucose[b] | 38.5 ± 0.3 | 26.8 ± 0.6 | 24.0 ± 0.2 | 31.5 ± 4.2 | 30.8 ± 4.3 | 29.7 ± 4.3 |
| Xylose[b] | 29.5 ± 0.7 | 26.1 ± 0.8 | 23.3 ± 0.1 | 19.8 ± 2.9 | 19.8 ± 2.8 | 16.5 ± 2.3 |
| Arabinose[b] | 3.3 ± 0.3 | 2.4 ± 0.3 | 1.4 ± 0.03 | 2.8 ± 0.3 | 3.0 ± 0.5 | 2.0 ± 0.3 |
| Acetyl groups | 4.0 ± 0.4 | 4.0 ± 0.9 | 3.1 ± 0.4 | 2.6 ± 0.9 | 2.5 ± 0.8 | 1.1 ± 0.1 |
| Others[c] | 6.2 | 5.5 | 7.8 | 14.7 | 15.2 | 8.4 |
| Acetyl/Xyl[d] | 0.29 | 0.34 | 0.28 | 0.29 | 0.28 | 0.15 |

[a] Related to dry material, with confidence interval ($\alpha = 0.025$).
[b] Expressed as anhydrous units.
[c] Calculated by difference.
[d] Ratio in mol/mol.

1:2.9 and 1:6.6 for AS and RH, respectively, and around 1:3.5 for the other lignocellulosics.

### 3.1.2. Cellulose component

The effect of the autohydrolysis reaction on the cellulose component is shown in Fig. 1, where the content of cellulose in the hydrolysis residue, and the gluco-oligosaccharides, glucose and hydroxymethylfurfural (HMF) in the liquid product, are shown as percentage of the glucose present in the raw materials. All of them were detected by HPLC, as described before. Essentially, cellulose remained in the hydrolysis residue and only a small part was depolymerized to oligomers and glucose and dissolved. Some of the gluco-oligosaccharides and glucose, however, were formed from minor hemicelluloses. OS and AS, the most lignified materials, showed lower formation of gluco-oligosaccharides and glucose. This might be caused by the more dense and lignified structure of their cell wall, which hinders the hydrolysis and solubilization of the non-crystalline segments of the cellulose chains.

### 3.1.3. Lignin component

Lignin was partially depolymerized during autohydrolysis and a small fraction of low molar mass phenolics dissolved. A part of lignin is known to be covalently bound to xylan, and phenolic acids, particularly ferulic acid, appears in side chains of heteroxylans from grasses and cereals, esterifying arabinose residues at position 5 (Ebringerová & Heinze, 2000). Therefore, some xylo-oligosaccharides might contain such phenolics. Fig. 2 shows the distribution of lignin between the hydrolysis residue and the liquid phase, expressed as percentage of the amount of lignin and organic extractives present in the original material. Organic extractives have been included because they are also solubilized partially during autohydrolysis and, depending on their characteristics, appear as Klason lignin in the hydrolysis residue or as acid-soluble lignin dissolved in the aqueous phase. The lower solubilization of lignin was observed

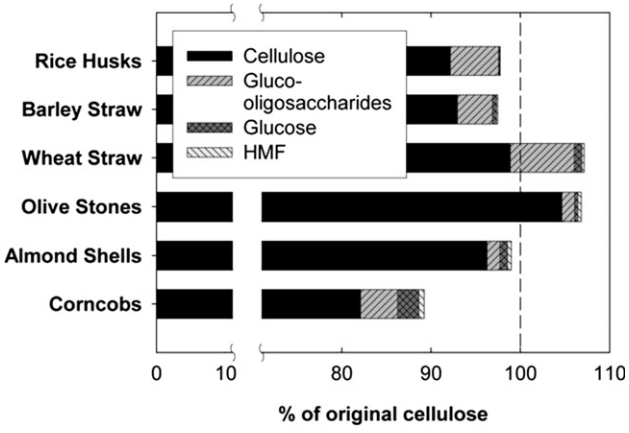


Fig. 1. Distribution of cellulose among reaction products: hydrolysis residue (cellulose) and liquid products (gluco-oligosaccharides, glucose, and HMF), expressed as percentage of original cellulose in the different raw materials.

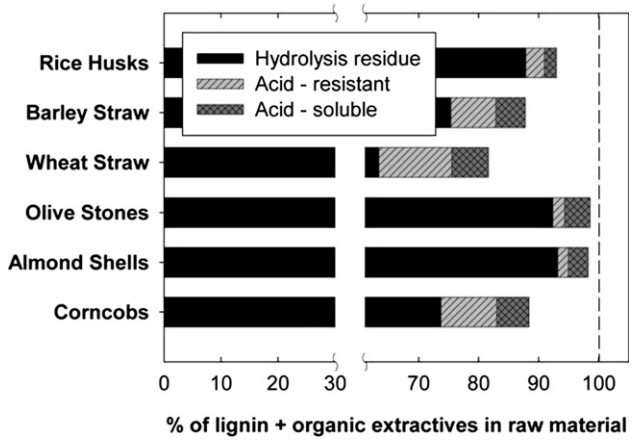


Fig. 2. Distribution of lignin among reaction products: hydrolysis residue (Klason Lignin), and liquid products (acid-resistant and acid-soluble lignin), expressed as percentage of original Klason lignin + organic extractives in the different raw materials.

for OS and AS, regardless of their higher lignin content, which is coherent with a dense and closed cell wall structure and a highly condensed lignin. On the contrary, lignin in the less lignified materials was more accessible towards hydrolysis and solubilization, especially for WS. The concentrations of lignin-derived products in the liquid phase at the end of the reaction were 0.30, 0.28, 0.20, 0.18, 0.17, and 0.12% w/w for CC, OS, AS, RH, WS, and BS, respectively. The presence of lignin-derived species in the liquid product calls for the inclusion of additional purification treatments to isolate xylo-oligosaccharides with adequate purity and well-defined and repetitive composition and molar mass. However, the lower the concentration of lignin products relative to that of xylo-oligosaccharides is in the autohydrolysis liquid, the simpler the purification sequence and the higher its effectiveness should be. Extraction with organic solvents, followed by a secondary treatment like ion exchange, lead the best results for the purification of xylo-oligosaccharides from rice husks, and from solid residues coming from the malting industry (Vegas, Alonso, Domínguez, & Parajó, 2004; Vegas, Alonso, Domínguez, & Parajó, 2005). Adsorption onto activated carbons (Montané, Nabarlatz, Martorell, Torné-Fernández, & Fierro, 2006), and ultrafiltration using polymeric membranes (Nabarlatz, Torras, García-Valls, & Montané, 2006), are promising methods for the separation of lignin-derived compounds from the xylo-oligosaccharides presents in the autohydrolysis liquor of almond shells.

### 3.1.4. Xylan component

Hemicelluloses are readily depolymerized and dissolved during autohydrolysis of lignocellulosics at the temperature that we used in this study. Xylan is the main component in the hemicelluloses of the six species we tested. Xylan from monocotyl plants (grasses and cereals) contains arabinose, acetyl groups, and glucuronic acid as side chains, but in dicotyl plants (hardwoods) xylan consists only of partially acetylated 4-*O*-methylglucuronoxylan

chains (Ebringerová & Heinze, 2000). Therefore, the arabinose found in the hydrolyzates of hemicelluloses extracted from almond shells and olive stones was probably originated from minor pectic polysaccharides comprising arabinan and/or arabinogalactan side chains. However, the term xylo-oligosaccharides (XOs) has been used to name the hemicellulose-derived oligosaccharides from autohydrolysis along this paper, and the possible arabinan-derived oligomers encountered in the case of AS and OS have been included as XOs for the purpose of calculating yields and average compositions.

Fig. 3 shows the distribution of the main constituents of the hemicelluloses (xylose, arabinose, and acetic acid) in the raw material, in the hydrolysis residue and in the liquid phase, for the six lignocellulosic species. Products from the hemicelluloses in the liquid phase were present in form of XOs, as free monomers, acetic acid and as furfural, the main decomposition product of pentoses. The hemicelluloses remaining in the hydrolysis residue were about 33–35% of the original for CC, AS, and OS, and shows that around 65% were solubilized. The depolymerization of WS and BS was not so effective, and about 45% and 55%, respectively, of the hemicelluloses remained in the hydrolysis residue. In the case of RH, the percentage was much higher, indicating that less than 30% was solubilized. These differences are closely related to the amount of acetyl groups that are present in the lignocellulosics, as shown in Table 1. The larger the amount of acetyl groups that were present in the raw material, the more acetic acid was liberated by cleavage of the acetyl groups and available to catalyze the depolymerization of hemicelluloses into XOs. This was corroborated with the final pH of the liquid product collected from the reactor, which was between 3.5 and 3.9 for the experiments with CC, AS, and OS, and around 4.2 for WS, BS, and RH. As seen in Fig. 3, the composition of the hemicelluloses remaining in the hydrolysis residue was similar for all substrates, with xylose as the main component. In fact, the remaining hemicelluloses (expressed as the sum of xylose, arabinose, and acetyl groups) were richer in xylose (85–91%) than the native (78–84%), showing that autohydrolysis preferentially released XOs that were rich in arabinose and acetyl groups (Carvalheiro, Esteves, Parajó, Pereira, & Gírio, 2004). The XOs recovered in the liquid product had a xylose content of 83–87%, which is below the value of the hemicellulose remaining in the hydrolysis residue, but higher than in native hemicelluloses.

The yield of XOs was around 60% of the original xylan content for CC, in agreement with previous studies done at laboratory scale (Garrote, Domínguez, & Parajó, 2002; Nabarlatz et al., 2004). The same agreement in the yields (55%) was observed with AS (Nabarlatz et al., 2005). The yields for OS, WS, and BS were about 43% of the original xylan, while for RH it was only about 30%. The composition of the xylo-oligosaccharides was close to that of the hemicelluloses that remained in the hydrolysis residue,

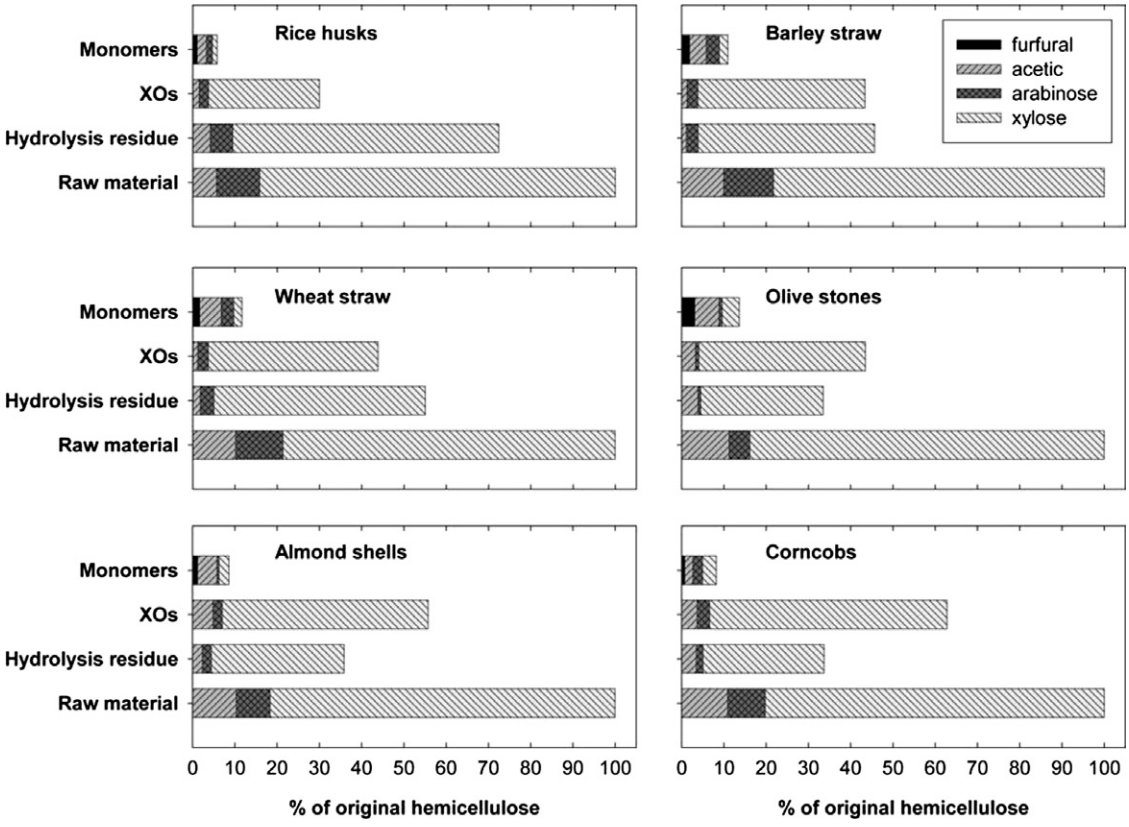


Fig. 3. Hemicellulose in the raw material and its distribution between the hydrolysis residue and the non-dried liquid products (XOs, monosaccharides, acetic acid, and furfural), expressed as percentage of original hemicellulose in the different raw materials.

Table 2
Composition of the spray-dried liquid product (SXOs)

| Composition (wt.%)[a] | CC | AS | OS | WS | BS | RH |
|---|---|---|---|---|---|---|
| Gluco-oligosaccharides | 5.2 | 4.1 | 2.4 | 13.2 | 6.3 | 15.3 |
| Xylo-oligosaccharides | 59.4 | 61.2[b] | 53.5[b] | 41.2 | 47.1 | 42.8 |
| Xylose | 51.7 | 53.9 | 46.3 | 34.3 | 39.3 | 37.3 |
| Arabinose | 3.3 | – | – | 4.4 | 4.8 | 3.5 |
| Acetyl groups | 4.4 | 7.4 | 7.2 | 2.5 | 3.0 | 1.9 |
| Arabinans | – | 3.9 | 1.2 | – | – | – |
| Monosaccharides | | | | | | |
| Glucose | 2.4 | 0.8 | 0.3 | 0.8 | 0.5 | 0.5 |
| Xylose | 2.9 | 2.6 | 4.2 | 0.9 | 1.4 | 2.0 |
| Arabinose | 2.5 | 1.6 | 1.4 | 1.9 | 2.6 | 2.9 |
| Acetic acid | 0.4 | 1.7 | 0.5 | 0.6 | 0.9 | 2.2 |
| Degradation Products | | | | | | |
| HMF | 0.5 | 0.3 | 0.2 | 0.2 | 0.1 | 0.1 |
| Furfural | 0.0 | 0.1 | 0.1 | 0.0 | 0.0 | 0.1 |
| Acid-resistant lignin | 5.6 | 1.8 | 2.3 | 8.4 | 6.3 | 5.9 |
| Acid-soluble lignin | 3.3 | 3.5 | 5.1 | 4.2 | 4.1 | 3.9 |
| Ash | 4.0 | 7.0 | 7.3 | 14.2 | 15.4 | 13.6 |
| Others[c] | 13.7 | 11.2 | 21.3 | 15.3 | 14.4 | 10.6 |

[a] Related to dry material.
[b] Excluding anhydrous arabinose.
[c] Calculated by difference.

accordingly with the preferential cleavage of acetyl groups and/or arabinose from the backbone of the xylan chains. The yields of arabinose and xylose in the liquid product were not high because they readily decomposed into furfural at the reaction conditions.

The composition of the XOs obtained after spray drying (SXOs) is shown in Table 2. The raw material that gave the highest content of SXOs in the powder product was AS (61% of the product), followed by CC (59%), OS (54%), BS (47%), WS, and RH (~42%). The latter two products, showed a higher content of gluco-oligosaccharides, namely 13% and 15%, respectively. In the case of dicotyledonous plants, to whom almond and olive trees belong, arabinose was not considered as part of the xylo-oligosaccharides (Ebringerová et al., 2005), and it was expressed as arabinan with a content of 3.9% and 1.2% for AS and OS, respectively. The levels of acetic acid, furfural, and HMF were negligible because they were evaporated during the spray drying process. The products contained phenolics, determined as acid-resistant and acid-soluble lignin in amounts ranging from 1.8% and 3.5% in AS, to 8.4% and 4.2% in WS. As we mentioned before, the low lignin levels in the liquid products after the reaction are

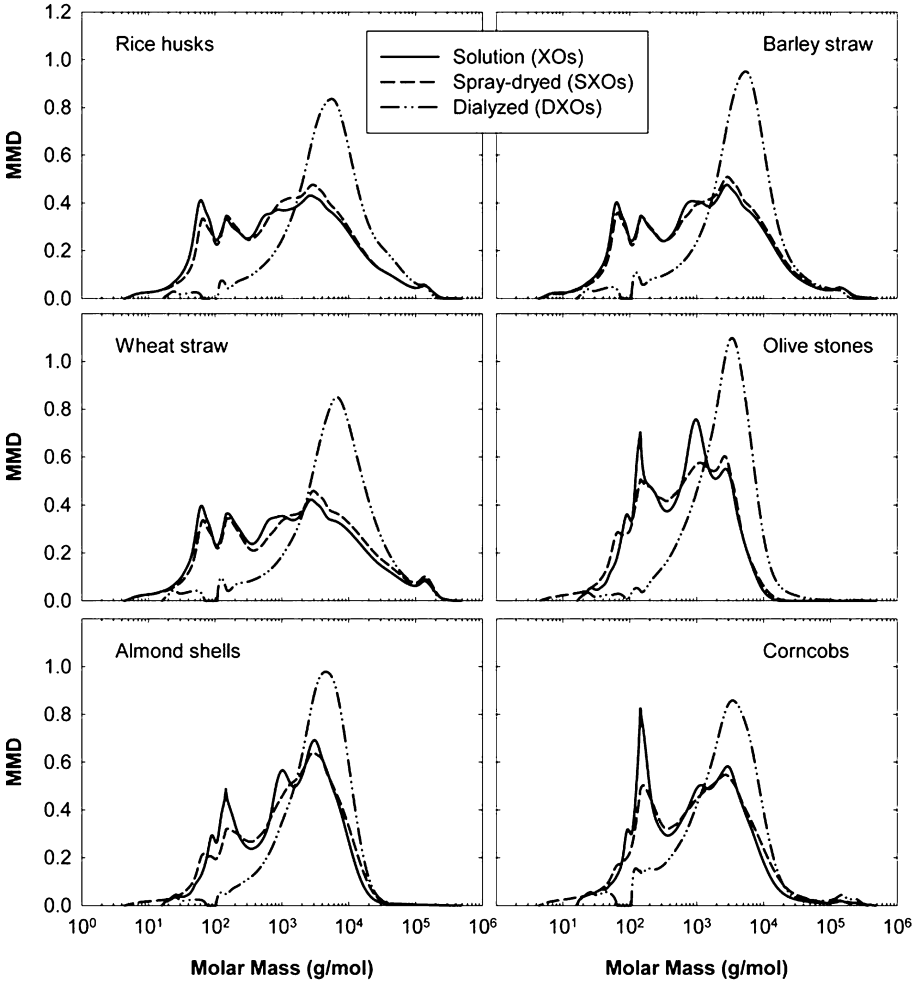


Fig. 4. Molar mass distribution of the xylo-oligosaccharides in the non-dried liquid product (XOs), obtained after spray drying (SXOs) and obtained after dialysis (DXOs), for the six raw materials studied.

beneficial for the subsequent purification steps. The ash content of the powder products was in accord with that of the raw materials (Table 1).

### 3.2. Molar mass distribution of the xylo-oligosaccharides

The molar mass distribution (MMD) obtained by GPC of the hydrolysis liquor (Fig. 4) was similar for all XOs, showing several molecular peaks ($M_p$) in various proportions. About 1/3 of the products were of very small molar mass ($M_p \sim 60$ and 300 g/mol) including degradation products, and mono- and disaccharides. The rest comprises oligosaccharides ($M_p \sim 1000$ and 3000 g/mol) and smaller proportions of polymeric xylan fragments with a broad range of molar masses from 6000 to 60,000 g/mol, which were most pronounced in WS, BS, and RH. OS and CC gave a higher proportion of low molecular weight products than AS, in agreement with the higher concentration of acetic acid in the reaction media. The spray-dried liquid products (SXOs) exhibited about the same MMD as the non-dried product. The main difference was a slight reduction of species with low molar masses (from 60 to 150 g/mol), due to the evaporation of furfural, acetic acid, and other volatile compounds during drying. Fig. 4 shows also that dialysis of SXOs effectively removed most of the low molar mass material. The resulting dialyzed xylo-oligosaccharides (DXOs) exhibited a low molecular mass with a narrower MMD, which show a single characteristic peak at −3500 g/mol for CC and OS, −5000 g/mol for AS, −5500 g/mol for RH and BS, and −6500 g/mol for WS, indicating a mixture of xylan oligomers and polymers.

### 3.3. Structural features of the xylo-oligosaccharides

1D ($^{13}$C and $^{1}$H) and 2D (HSQC) NMR spectra of the DXOs were collected in order to elucidate the structural features. The signals for $^{13}$C and $^{1}$H were assigned based on the HSQC spectra following published NMR data for xylo-oligosaccharides and glucuronoxylans (Ebringerová, Hromádková, & Hribalová, 1995; Kardošová, Matulová, & Malovíková, 1998; Teleman, Lundqvist, Tjerneld, Stålbrand, & Dahlman, 2000; Teleman, Tenkanen, Jacobs, & Dahlman, 2002). As seen in Fig. 5, the $^{13}$C NMR spectral patterns of all six DXOs are the same, differing only in the intensity of the signals. The dominating five signals gave HSQC $^{13}$C/$^{1}$H cross peaks at δ 102.57/4.45, 73.6/3.28, 74.6/3.54, 77.3/3.76, and 63.9/4.04 + 3.40, corresponding to C-1–C-5 of 4-linked β-Xyl*p* residues. The cross peaks at δ 98.8/5.28 (C-1) and 83.1/3.19 (C-4) indicate the presence of α-linked 4-*O*-methylglucuronic acid (MeGA) residues. In spite of the occurrence of low amounts of arabinose in all SXOs (Table 2), no signals were detected in the spectra corresponding to α-Ara*f* residues. These signals would appear in the downfield region at ~107–110 ppm, similarly as reported for the arabinose-containing heteroxylans (Ebringerová et al., 2005). Very probably, due to the low amount and variety of positions of α-Ara*f* residues in the DXOs as well as in contaminating fragments of pectic arabinans and arabinogalactans, released by the hydrothermal treatment, the arabinose signals were not detectable. In contrast to the DXOs from OS and AS, which are tissues of dicotyl plants containing 4-*O*-methylglucuronoxylans, α-Ara*f* residues could be expected in DXOs from CC, RH,

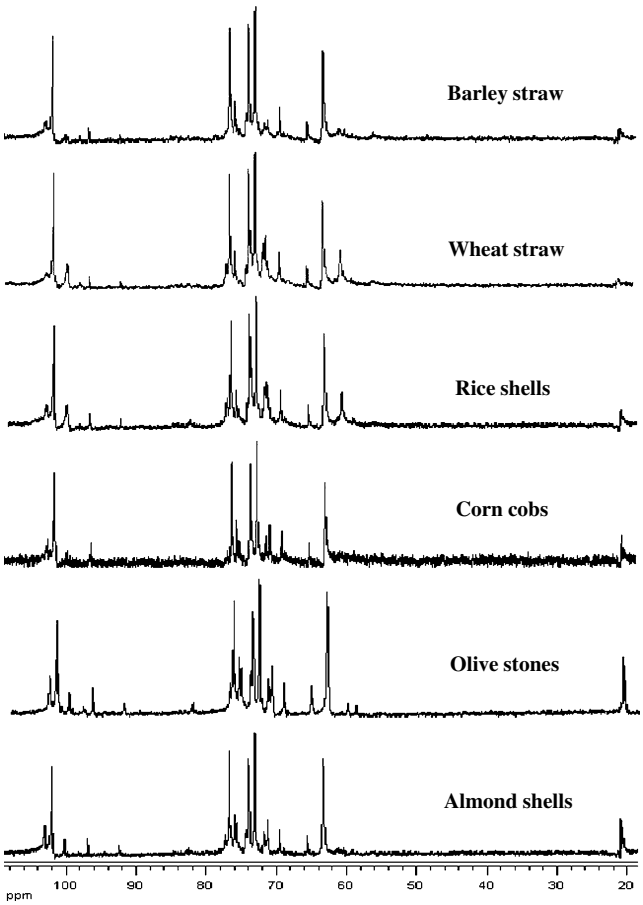


Fig. 5. $^{13}$C NMR spectra (in $D_2O$) of DXOs from various plant sources.

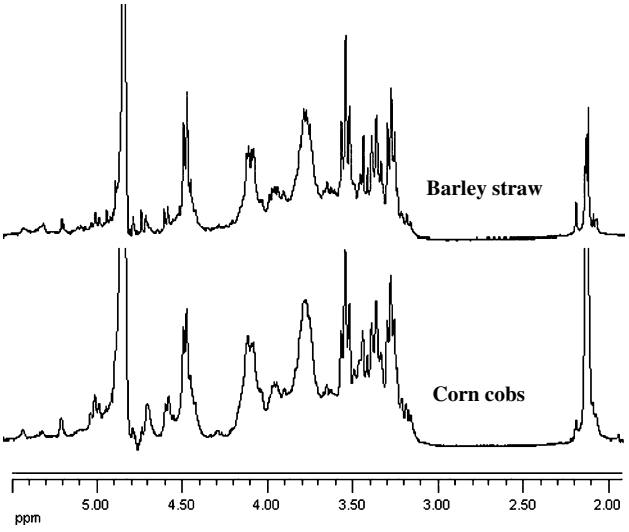


Fig. 6. $^{1}$H NMR spectra of XOs from barley straw and corncobs.

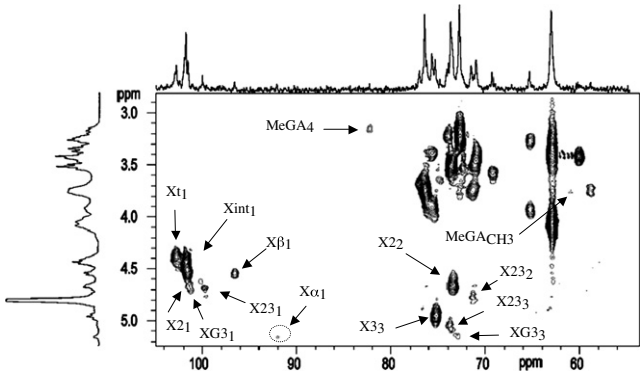


Fig. 7. The 2D HSQC spectrum of DXOs from olive stones, illustrating MeGA residues and various Xyl*p* residues. The following designations are used: X3: 3-*O*-acetylated Xyl*p*, X2: 2-*O*-acetylated Xyl*p*, X23: 2,3-*O*-diacetylated Xyl*p*, XG3: MeGA 2-*O*-substituted and 3-*O*-acetylated Xyl*p*, MeGA: 4-*O*-methylglucopyranosyl uronic acid, Xint, Xt, Xα, and Xβ: non-acetylated internal, non-reducing terminal, and reducing terminal Xyl*p* residues. The subscript number corresponds to the H- and C-atom.

BS, and WS. The last are tissues of monocotyl plants, where arabinose represents a sugar constituent of the heteroxylan component (Ebringerová & Heinze, 2000). The cross peaks at δ 100.6/5.38 and 61.0/3.80 + 3.73 were not assigned because of lack of further information. They might correspond to α-linked hexopyranose residues such as galactose, originating from side chains of the heteroxylan component and/or of contaminating cell wall hexoglycans (Ebringerová et al., 2005).

The pattern of $^{1}$H NMR of all DXOs spectra was similar (shown for OS and BS in Fig. 6). The present acetyl groups gave HSQC $^{13}$C/$^{1}$H cross peaks at δ 22–20.7/2.22–2.10. The average degree of substitution of the DXOs was calculated from the $^{1}$H NMR spectra by dividing the area of the acetyl group signals by the cumulative signal of all carbohydrates (Teleman et al., 2002; Gröndahl, Teleman, & Gatenholm, 2003).

The proportion of Xyl*p* and MeGA residues as well as the location of the acetyl groups was elucidated by means of proton assignments in the anomeric region of the $^{1}$H NMR HSQC spectra (demonstrated for OS in Fig. 7). The obtained chemical shifts were in accord with values already published for hardwood 4-*O*-methylglucuronoxylans (Teleman et al., 2000, 2002). The relative proportions of Xyl*p* residues, free and *O*-acetylated, were determined according to Teleman et al. (2000) by integrating the areas of the H-1 signals of internal, non-reducing and reducing terminal Xyl*p* (Xint, Xt, Xα, Xβ), the H-2 signal from 2-*O*-acetylated Xylp (X2), the H-3 signals from 3-*O*-acetylated Xyl*p* (X3) and 2,3-*O*-diacetylated Xyl*p* (X23), and Xyl*p* bearing the 2-linked MeGA (XG3). The proportion of MeGA was derived from its H-1 signal area.

The values of average degree of acetylation estimated from the $^{1}$H NMR spectral data and those obtained by quantitative HPLC analysis of the acetic acid liberated by the acid hydrolysis of SXOs are summarized in Table 3. The chemical analysis revealed that the SXOs from OS had the highest degree of acetylation (35%), followed by AS (30%) and CC (19%), whereas SXOs from WS and BS had similar values (around 17%), and from RH were the less acetylated SXOs (12%). These values agree reasonably well with the average degree of acetylation determined by $^{1}$H NMR spectroscopy for OS (41%), AS (31%), CC (21%), and RH (11%) and less in the case of BS (8%) and WS (9%). As shown in Table 3, the DXOs were acetylated mainly in position 3 (between 60 and 67 mol%), whereas the occurrence of acetyl groups in position 2 and in both positions 2 and 3 was similar (19–30 and 8–25 mol%, respectively).

Estimation of the MeGA content from the $^{1}$H NMR spectra, expressed in mol MeGA per 100 mol Xyl, revealed a very low value for XOs from CC (2.5:100), higher values for those from AS, RH, BS, and WS ranging from 4.3:100 to 7.4:100. The highest content was found for XOs from OS, having 9 of 100 Xylp residues on the average substituted by MeGA residues, what is close to the average MeGA/Xyl ratio (1:10) found in 4-*O*-methylglucuronoxylans from hardwoods (Ebringerová & Heinze, 2000).

## 4. Conclusion

All lignocellulosic substrates, independently on their structurally different heteroxylan components, generated xylo-oligomers typical of partially acetylated 4-*O*-methylglucuronoxylan, however, with different degrees of substitution for the acetyl and the 4-*O*-methylglucuronic acid groups. A comparative assessment of the results shows that the

Table 3
Relative amounts of monosaccharides and acetyl groups, and distribution of acetyl groups among xylose residues in the DXOs from different raw materials

| Raw material | MeGA/Xyl mole ratio | Degree of acetylation (acetyl/Xyl) | | Acetylated positions in Xyl (% of the total) | | |
|---|---|---|---|---|---|---|
| | NMR[a] | NMR[b] | HPLC[c] | 2 | 3 | 2,3 |
| Corncobs | 2.5:100 | 0.21 | 0.19 | 19.3 | 67.1 | 13.6 |
| Almond shells | 4.4:100 | 0.31 | 0.30 | 21.6 | 59.4 | 16.0 |
| Olive stones | 9.1:100 | 0.41 | 0.35 | 23.3 | 62.7 | 21.0 |
| Rice husks | 4.3:100 | 0.11 | 0.12 | 29.8 | 62.7 | 7.5 |
| Wheat straw | 7.4:100 | 0.08 | 0.16 | 22.1 | 51.9 | 26.0 |
| Barley straw | 6.0:100 | 0.09 | 0.17 | 19.1 | 66.9 | 14.0 |

[a] Approximate values determined by integration of signals in the fingerprint region of the $^{1}$H NMR spectra.
[b] Approximate values determined by integration of the H-1 signals of acetyl groups and of all carbohydrate signals (according to Teleman et al., 2002).
[c] See experimental.

characteristics of the raw material determined the yield and composition of the XOs. Their yield depended not only on the content of xylan in the raw material, but was proportional to the content of acetyl groups, since their cleavage liberated acetic acid, which catalyzed the depolymerization of xylan into xylo-oligosaccharides and xylan polymers of low mass. However, the density of the substrate played a major role in the process. AS and OS had very lignified and dense cell wall structures that prevented the liberation of degraded lignin and the formation of gluco-oligosaccharides during autohydrolysis, and they yielded the purer XOs. From our results, AS are the best substrate for XOs preparation, because it allows processing at a high concentration of xylan in the reactor, offers an elevated yield (more than 55% of the original xylan), and the XOs have a relatively low lignin content.

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