5430 J. Agric. Food Chem. 2006, 54, 5430–5436

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Membrane-Assisted Processing of Xylooligosaccharide-Containing Liquors

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Liquors from rice husk autohydrolyis, containing xylooligosaccharides, other saccharides, and nonsaccharide compounds, were subjected to two selected processing schemes to increase the proportion of substituted xylooligosaccharides in refined liquors. Nanofiltration through a ceramic membrane with a molecular mass cutoff of 1000 Da allowed simultaneous concentration and purification; this latter derived from the preferential removal of monosaccharides and nonsaccharide compounds. When liquors were nanofiltered to achieve a volume reduction factor of 5 operating at a transmembrane pressure of 14 bar, 58.6% of the nonsaccharide components and 20.9−46.9% of monosaccharides were kept in retentate, in comparison with 92% of xylooligosaccharides and glucooligosaccharides. When nanofiltered liquors were subjected to double ion-exchange processing, a final product with a nonsaccharide content near 9 kg/100 kg of nonvolatile components was obtained at a yield of 10.90 kg/100 kg oven dry rice husks. Alternatively, when nanofiltered liquors were subjected to ethyl acetate extraction and further double ion-exchange processing, a purified product with a nonsaccharide content of 5.66 kg/100 kg of nonvolatile components was obtained at a yield of 9.94 kg/100 kg oven dry rice husks. The nonsaccharide components remaining in the final concentrate were mainly made up of phenolic and nitrogen-containing compounds.

KEYWORDS: Extraction; ion exchange; nanofiltration; purification; xylooligosaccharides

INTRODUCTION

Xylans are plant polysaccharides that represent an immense resource of biopolymers for practical applications, accounting for 25−35% of the dry biomass of woody tissues of dicots and lignified tissues of monocots, and occur up to 50% in some tissues of cereal grains. The structures of xylans depend on the source considered. The most common xylans, including those of rice husks, are made up of a main backbone of xylose linked by β 1→4 bonds, where the structural units are often substituted at positions C2 or C3 with arabinofuranosyl, 4-O-methylglucuronic acid, acetyl, or phenolic substituents (*1*).

When the aqueous processing of xylan-containing lignocellulosic biomass (autohydrolysis) is carried out under suitable operational conditions, xylan is broken down to yield soluble fragments (here denoted xylooligosaccharides, XO) as major reaction products. As the reaction is not selective, a variety of other compounds (including monosaccharides coming from the hydrolytic degradation of the raw material polysaccharides, acetic acid coming from cleavage of acetyl groups, products derived from the extractive and acid-soluble lignin fractions, furfural generated from pentose dehydration, inorganic components, and protein-derived products) are also present in liquors (*2*, *3*). The molecular weight distribution depends on the substrate employed and on the reaction conditions. Treatments with increased severity lead to XO with decreased degrees of polymerization but also to increased decomposition of XO into xylose.

XOs have been manufactured by autohydrolysis of a variety of feedstocks, including hardwoods (*4*, *5*), softwoods (*6*), corn cobs (*7*, *8*), barley hulls and barley spent grains (*9*, *10*), brewery spent grains (*11*, *12*), almond shells (*13*), corn fiber (*14*, *15*), and rice hulls (*3*, *16*, *17*).

Considered as a raw material for autohydrolysis, rice husks present favorable features derived from their high xylan content and low content of nondesired products in autohydrolysis liquors (*9*). From a nutritional point of view, XO behave as nondigestible oligosaccharides (NDO), which are not degraded in the stomach and reach the large bowel intact, where they are degraded by the intestinal flora (*18*). The colonic fermentation of XO results in prebiotic effects related to the preferential growth of *Bifidobacteria* (*19*). Studies on the XO fermentability by probiotic bacteria have been reported recently (*20*, *21*).

Food applications require high purity XOs, which are already commercial products with market prices higher than other NDO

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10.1021/jf060525w CCC: $33.50 © 2006 American Chemical Society
Published on Web 07/04/2006

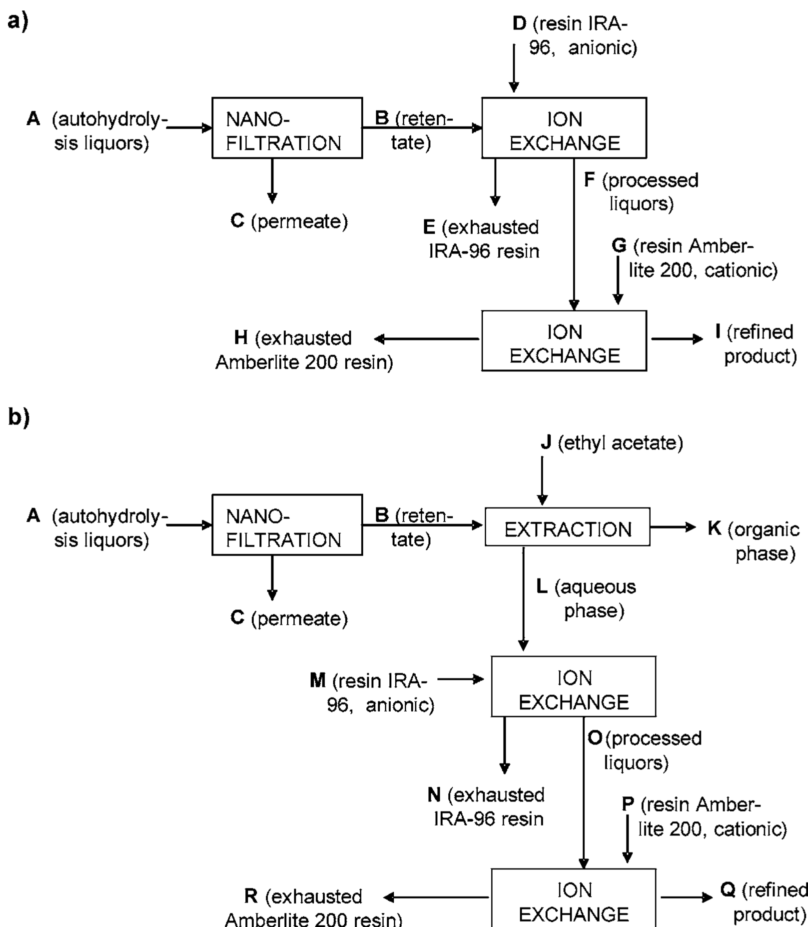

Figure 1. Processing schemes assayed in this work for XO purification.

(*22*). If the XO obtained by autohydrolysis is to be used for food purposes, the autohydrolysis liquors must be fractionated to remove nondesired compounds, a field in which membrane technologies have potential applications.

Little information exists on the utilization of membrane technologies for processing XO-containing solutions, and some of them deal with the processing of media obtained by a hydrolytic treatment followed by enzymatic reaction. In this field, Yuan et al. (*23*) reported on the manufacture of XO at the pilot plant scale by chemical−enzymatic processing of corn cobs (steaming followed by xylanase treatment) and further purification by flocculation, ion exchange, nanofiltration, charcoal adsorption, and vacuum evaporation. Izumi et al. (*24*, *25*) employed both reverse osmosis and ultrafiltration in the processing of XO-containing solutions coming from the xylanase treatment of pulp slurry, whereas Swennen et al. (*26*) compared ultrafiltration and ethanol precipitation for fractionating arabinose-substituted XO (arabinoxylooligosaccharides) obtained by enzymatic processing of wheat. Membrane reactors have been employed for the one-step DP reduction and fractionation of XO (*27*, *28*).

In related studies, data on the processing of other oligosaccharides different from XO, including pectic oligosaccharides (*29*, *30*), fructooligosaccharides (*31*), and soybean-derived fractions (*32*, *33*), have been reported. This work deals with the experimental evaluation of technologies for purification of XO-containing liquors obtained by hydrothermal processing of rice husk. Autohydrolysis liquors are processed by nanofiltration and ion exchange, with or without a sandwiched solvent extraction stage (see **Figure 1a**,**b**). Data on membrane performance and composition of process streams are reported.

MATERIALS AND METHODS

Raw Material. Rice husks were obtained in a local factory (Procesadora Gallega de Alimentos, Lalín, Pontevedra, Spain), air-dried, homogenized in a single lot to avoid compositional differences among aliquots, and stored until use.

Analysis of the Raw Material. Aliquots from stored samples were subjected to moisture determination (method ISO 638), to ash determination (method ISO 776), and to quantitative acid hydrolysis (method TAPPI T13m). The high-performance liquid chromatography (HPLC) analysis of liquors was carried out using a Bio-Rad column (HPX87H) and IR detection, to measure the concentrations of glucose, other monosaccharides, and acetic acid. These experimental results allowed the determination of the contents of cellulose, hemicellulosic polysaccharide constituents, and acetyl groups (*34*). The oven dry weight of the solid phase from quantitative hydrolysis measured the content of Klason lignin after correction for ashes. Uronic acids were determined by the method of Blumenkrantz and Asboe-Hansen (*35*) using galacturonic acid as a standard for quantification. The protein content of the raw material was estimated by elemental analysis (protein = 6.25 · *N*). Elemental analysis was also employed for measuring the melanoidin content of oligosaccharide concentrates, which was expressed as a "protein equivalent".

Autohydrolysis Processing of Rice Husks. Rice husks and water were mixed at the desired proportions (8 kg/kg oven dry solid) and reacted in a Parr reactor under nonisothermal conditions following the standard heating temperature profile up to reach the maximum xylooligomer concentration (*16*). At the end of treatments, liquors were recovered by filtration, analyzed, and processed as follows.

Analysis of Autohydrolysis Liquors. For analytical purposes, samples of liquors were filtered through 0.45 μm membranes and used for direct HPLC determination of glucose, xylose, arabinose, and acetic acid using the same method employed in the analysis of the raw material. A second sample of liquors was subjected to quantitative

posthydrolysis (treatment with 4% sulfuric acid at 121 °C for 30 min), and the reaction products were assayed by the same HPLC method. As oligosaccharides and acetyl groups were converted into monosaccharides and acetic acid, respectively, during the posthydrolysis treatment, the increase in the concentrations of monosaccharides and acetic acid caused by posthydrolysis provided a measure of the oligomer concentration and their degree of substitution by acetyl groups (*16*). Uronic acids were assayed as reported for the raw material. The content of raw or processed liquors in nonvolatile compounds (NVC) was measured by oven drying at 105 °C until constant weight. All determinations were made in triplicate.

Elemental Analysis. Elemental analysis of selected xylooligosaccharide concentrates was carried out using a Thermo Finnegan Flash EA 1112 Analyzer using 130 and 100 mL/min of He and O_2 and a oven temperature of 50 °C. Determinations were made in triplicate.

Acid-Soluble Lignin. The acid-soluble lignin content of selected xylooligosaccharide concentrates was measured spectrophotometrically after hydrolysis with 4% sulfuric acid at 121 °C for 30 min using the method of Maekawa et al. (*36*). Determinations were made in triplicate.

Membrane Processing of Liquor. Raw autohydrolysis liquors were nanofiltered using a TiO_2/ZrO_2 Kerasep Nano membrane (Orelis, Miribel, France), with 0.25 m^2 and a cutoff value of 1000 Da, as given by the manufacturer. The maximum allowed transmembrane pressure was 20 bar. The membrane can withstand operating temperatures of up to 100 °C and pH values in the range 1−14. A small-scale pilot unit was used to carry out the nanofiltration experiments. A three-plunger pump was used to feed the liquors to the membrane module. Pressure was monitored at the entrance and exit of the membrane module, and a needle valve located after the membrane module was used to achieve the desired operating transmembrane pressure. The feed flow rate was measured using a rotameter. The temperature was monitored using a PT100 resistance thermometer and controlled by flushing tap water through a refrigeration coil placed in the 150 L feed tank. Preliminary experiments were carried out in full recycle mode operating at transmembrane pressures in the range 6−14 bar, and further experiments were carried out in concentration mode at the optimal transmembrane pressure. The retentate from the concentration run was subjected to further purification, as described below.

Solvent Extraction of Liquors. In selected experiments (see **Figure 1b**), aliquots of retentates from the membrane processing of raw autohydrolysis liquors were extracted with ethyl acetate in three sequential stages at a liquor:solvent mass ratio of 1:2 (kg/kg) per stage. The organic phases were mixed and vacuum-evaporated to dryness in a rotary evaporator to remove both solvent and volatile dissolved compounds, and the remaining solid phase was used to calculate the yield of the autohydrolysis−extraction process. The aqueous phases were assayed for composition using the same methods described for the autohydrolysis liquors.

Ion Exchange Processing of Liquors. Processed liquors were treated in sequential steps with Amberlite IRA 96 (a weak anion-exchange resin) and Amberlite 200 (an acidic cation-exchange resin). Liquors and resins were contacted for 24 h with gentle agitation at room temperature using 1 kg Amberlite IRA 96/15.3 kg liquors and 1 kg Amberlite 200/17.5 kg liquors. Samples were processed in triplicate, and both average values and standard deviations are presented.

RESULTS AND DISCUSSION

Characterization of Autohydrolysis Liquors and Purification Processes. Xylooligosaccharides obtained from autohydrolysis present a rich substitution pattern, conserving structural features of the native xylan (*37*, *38*). Data on the autohydrolysis conditions and rice husk conversion have been given in previous works (*3*, *9*, *16*). Autohydrolysis resulted in the solubilization of 24.2 kg/100 kg initial rice husks (oven dry basis). The composition of autohydrolysis liquors employed in this work, corresponding to stream A in **Figure 1a**,**b**, is given in **Table 1**.

Liquors were made up of volatile components (water and volatile reaction products) and NVCs. As the objective of this work was to refine NVC by increasing their XO content, the fate of volatile compounds through the process is not relevant. The relative amount of NVCs in liquors is characterized by the corresponding mass fraction (kg NVC/kg liquor). NVCs are made up of monosaccharides (xylose, arabinose, and glucose, which are undesired compounds for the purposes of this work), oligosaccharides (XO, glucooligosaccharides, and arabinosaccharides, expressed as monosaccharide equivalents), oligosaccharide substituents (acetyl and uronic groups, expressed as acetic acid and galacturonic acid equivalents, respectively), and other nonsaccharide, nonvolatile compounds (ONVC, which have to be removed). The composition of NVC is expressed in terms of mass fractions referred to NVC (kg component/kg NVC). The purification processes assayed in this work intend to selectively remove ONVC and monosaccharides, leading to a recovery yield of XO and XO substituents in the final product as high as possible (*3*, *10*).

Table 1. Amount[a] and Composition of Raw Autohydrolysis Liquors (Stream A in **Figure 1a**,**b**)

component	mass fraction (kg/kg NVC)	standard deviation
glucose	0.0084	0.0003
xylose	0.0252	0.0002
arabinose	0.0176	0.0014
glucooligosaccharides	0.1131	0.0022
xylooligosaccharides	0.4247	0.0073
arabinooligosaccharides	0.0289	0.0009
acetyl groups linked to oligosaccharides	0.0288	0.0033
uronic acids	0.0352	0.0011
ONVC	0.3181	0.0120

[a] Amount of NVC in stream A coming from the processing of 100 kg of oven dry rice husks, 21.02 kg. Mass fraction of NVC (kg NVC/kg A) = 0.0255 (standard deviation, 0.0001).

Reported studies on the refining of XO-containing solutions deal with multistage processes involving a variety of technologies, including two-stage hydrothermal treatments (*5*, *39*), concentration, solvent extraction (*3*, *5*, *10*), solvent precipitation (*3*, *5*, *10*), adsorption (*23*, *24*, *25*), flocculation (*23*), ion exchange (*3*, *10*, *23*, *39*), membrane processing (*23*, *24*), and chromatographic separation (*39*, *40*). In the following sections, the purification effects achieved by performing a first nanofiltration stage for concentration and partial purification of XO, followed by a double ion exchange treatment (with anionic and cationic resins) with or without previous extraction with ethyl acetate (see **Figure 1a**,**b**), are assessed.

The reliability of experimental data concerning the composition of raw liquors and processed streams is of major importance for ensuring the coherence of the results derived from material balances. In this work, this point was confirmed by performing replicate assays, which allowed the calculation of the average values and the standard deviations of compositional data (see **Tables 1** and **3**−**8**).

Membrane Processing of Raw Autohydrolysis Liquors. Preliminary assays were carried out in full recycle mode at transmembrane pressures covering the whole experimental range (2−14 bar). The permeate flux, J, was measured at the selected transmembrane pressures (2, 4, 6, 8, 10, 12, or 14 bar) for 0.5−1 h until stable fluxes were reached, and then, the transmembrane pressure was increased to achieve a new stable permeate flux, and before proceeding to the next (higher) pressure, the pressure was decreased to its previous value to account for irreversible fouling (see **Figure 2**). Flux declines during membrane filtration because of concentration polarization and fouling phenomena.

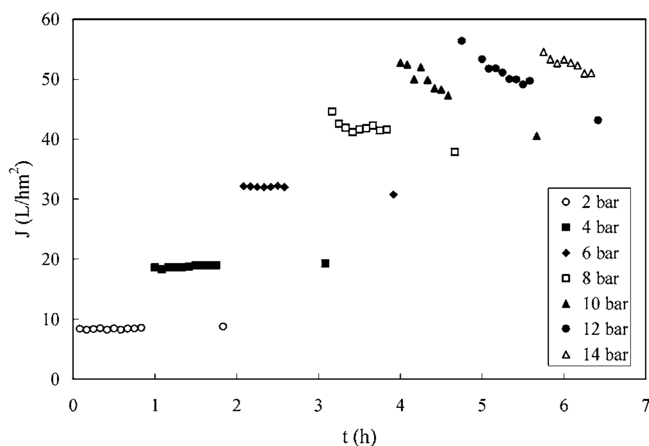

Figure 2. Time dependence of the permeate flux operating at the selected transmembrane pressures (feed flow velocity, 3 m/s; temperature, 26 ± 4 °C).

The former is inherent to the separation process, although it can be minimized as higher feed flow velocities are used, which result in a thinner boundary layer. Fouling includes all of the mechanisms (i.e., pore blocking, adsorption, and gel layer formation) that originate a decrease in membrane permeability (measured as pure water flux) even after replacing the liquors with pure water. For a potential implementation of nanofiltration, membrane performance (selectivity and flux) and membrane fouling (which will result in unit down periods for cleaning) have to be assessed. Therefore, pure water flux has been recorded before and after each filtration and increasing/decreasing pressure profiles have been used to qualitatively assess the effect of pressure on fouling. On the basis of both pure water flux reductions and flux data collected during the nanofiltration runs, it can be inferred that most of fouling took place as the membrane got in contact with the liquors, even at low pressures (about 40% pure water permeability is lost after filtering the liquors at 8 bar). Increasing the pressure above 8 bar resulted in a more pronounced flux decline, but that seems to be more related with concentration polarization effects, since the pure water permeability drop after filtering the liquors at 14 bar is only 10% lower than at 8 bar (i.e., more fouling takes place as pressure increases, but that solely cannot explain the observed leveling of flux).

Once the steady state flux was achieved at each transmembrane pressure assayed, samples of permeates were withdrawn and analyzed for NVC content and composition. The results in **Table 2** show that higher transmembrane pressures resulted in permeates with decreased contents of NVC and in better purification (confirmed by both decreased mass fraction of XO and increased mass fractions of monosaccharides and ONVC). Acetylated oligosaccharides were quantitatively retained in the concentrate effluent, whereas no significant fractionation effects were observed for XO-containing uronic substituents (mass fractions of uronic acids in permeates within the range of 0.0334−0.0370 in comparison with 0.0358 for the feed solution).

On the basis of both the better selectivity reached at high pressures and the relatively small differences in fouling rates, the pressure selected for concentration was 14 bar. **Figure 3** shows the dependence of the permeation flux on the volume reduction factor, VRF (the ratio between the initial volume and the concentrate volume), determined for the raw autohydrolysis liquors, whereas **Table 3** lists data on the composition of the retentate obtained at a VRF of 5 (5-fold reduction in volume, which corresponded to stream B in **Figure 1a**,**b**), as well as the corresponding standard deviations.

As it is often found in membrane filtration, the flux slowly decreased when VRF increased because of the increased concentration of solutes in retentate. On the basis of the different

Table 2. Composition of Permeates Obtained in Full Recycle Operation[a]

transmembrane pressure (bar)	6	8	10	12	14
mass fraction of NVC in permeate[b]	0.0067	0.0060	0.0053	0.0047	0.0042

	mass fraction of components in NVC (kg component/kg NVC) operating at				
	6 bar	8 bar	10 bar	12 bar	14 bar
glucose	0.0374	0.0381	0.0451	0.0496	0.0558
xylose	0.0794	0.0788	0.0857	0.0859	0.0904
arabinose	0.0587	0.0586	0.0625	0.0631	0.0738
glucooligosaccharides	0.0393	0.0357	0.0301	0.0218	0.0112
xylooligosaccharides	0.1886	0.1620	0.1400	0.1037	0.0725
arabinooligosaccharides	0.0189	0.0161	0.0144	0.0100	0
acetyl groups linked to oligosaccharides	0	0	0	0	0
uronic acids	0.0357	0.0348	0.0334	0.0354	0.0370
ONVC	0.5420	0.5759	0.5888	0.6305	0.6593

[a] Content of NVC (kg NVC/kg liquor) and composition of NVC in permeates. [b] Operating at stationary flux.

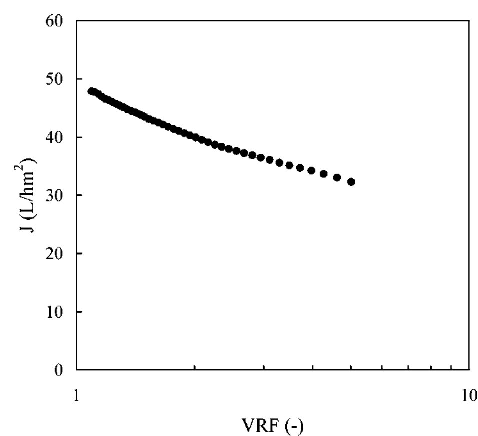

Figure 3. Dependence of the permeation flux, J, on the VRF operating at 14 bar (feed flow velocity, 3 m/s; temperature, 34 ± 5 °C; and transmembrane pressure, 14 bar).

Table 3. Amount[a] and Composition of Retentate after Concentration at a VRF = 5 (Stream B in **Figure 1a**,**b**)

component	mass fraction (kg/kg NVC)	standard deviation
glucose	0.0022	0.0001
xylose	0.0151	0.0002
arabinose	0.0089	0.0006
glucooligosaccharides	0.1323	0.0011
xylooligosaccharides	0.4994	0.0018
arabinooligosaccharides	0.0248	0.0003
acetyl groups linked to oligosaccharides	0.0357	0.0018
uronic acids	0.0432	0.0037
ONVC	0.2384	0.0180

[a] Amount of NVC in stream B coming from the processing of 100 kg of o.d. rice husks, 16.45 kg. Mass fraction of NVC (kg NVC/kg B) = 0.0976 (standard deviation, 0.001).

Table 4. Amount[a] and Composition of Stream F in **Figure 1a**

component	mass fraction (kg/kg NVC)	standard deviation
glucose	0.0025	0.0001
xylose	0.0180	0.0002
arabinose	0.0112	0.0002
glucooligosaccharides	0.1431	0.0009
xylooligosaccharides	0.5678	0.0027
arabinooligosaccharides	0.0323	0.0005
acetyl groups linked to oligosaccharides	0.0429	0.0024
uronic acids	0.0449	0.0001
ONVC	0.1373	0.0058

[a] Amount of NVC in stream F coming from the processing of 100 kg of o.d. rice husks, 12.42 kg. Mass fraction of NVC (kg NVC/kg F) = 0.0870 (standard deviation, 0.0004).

Table 5. Amount[a] and Composition of Stream I in **Figure 1a**

component	mass fraction (kg/kg NVC)	standard deviation
glucose	0.0027	0.0002
xylose	0.0185	0.0004
arabinose	0.0123	0.0003
glucooligosaccharides	0.1485	0.0019
xylooligosaccharides	0.6031	0.0048
arabinooligosaccharides	0.0348	0.0010
acetyl groups linked to oligosaccharides	0.0422	0.0010
uronic acids	0.0477	0.0007
ONVC	0.0902	0.0057

[a] Amount of NVC in stream I coming from the processing of 100 kg of o.d. rice husks, 10.90 kg. Mass fraction of NVC (kg NVC/kg I) = 0.0849 (standard deviation, 0.0004).

Table 6. Amount[a] and Composition of Stream L in **Figure 1b**

component	mass fraction (kg/kg NVC)	standard deviation
glucose	0.0024	0.0014
xylose	0.0161	0.0025
arabinose	0.0101	0.0010
glucooligosaccharides	0.1443	0.0058
xylooligosaccharides	0.5489	0.0168
arabinooligosaccharides	0.0285	0.0016
acetyl groups linked to oligosaccharides	0.0351	0.0005
uronic acids	0.0510	0.0008
ONVC	0.1636	0.0235

[a] Amount of NVC in stream L coming from the processing of 100 kg of o.d. rice husks, 13.88 kg. Mass fraction of NVC (kg NVC/kg L) = 0.1029 (standard deviation, 0.0014).

Table 7. Amount[a] and Composition of Stream O in **Figure 1b**

component	mass fraction (kg/kg NVC)	standard deviation
glucose	0.0029	0.0001
xylose	0.0203	0.0004
arabinose	0.0129	0.0005
glucooligosaccharides	0.1591	0.0018
xylooligosaccharides	0.6028	0.0062
arabinooligosaccharides	0.0348	0.0029
acetyl groups linked to oligosaccharides	0.0365	0.0003
uronic acids	0.0516	0.0045
ONVC	0.0791	0.0104

[a] Amount of NVC in stream O coming from the processing of 100 kg of o.d. rice husks, 10.76 kg. Mass fraction of NVC (kg NVC/kg O) = 0.0990 (standard deviation, 0.0010).

densities of raw autohydrolysis liquors and retentate, a VRF of 5 corresponded to a mass concentration ratio of 4.89. Material balances showed that the percentages of recovery in concentrates were 20.9−46.9% for monosaccharides, in comparison with 67.2% for arabinooligosaccharides and 92% for XO and glucooligosaccharides, with almost complete recovery of acetylated oligosaccharides. Interestingly, just 58.6% of the initial ONVC was kept in retentate, leading to a decreased ONVC mass fraction in stream B (0.2384) in comparison with stream A (0.3181). These data confirm that nanofiltration caused purification effects derived from the preferential removal of both monosaccharides and ONVC in permeate.

Processing of Retentates by Ion Exchange. According to **Figure 1a**, stream B from nanofiltration was treated with IRA-96 to yield stream F, which was treated with Amberlite 200 to give the refined product I. **Tables 4** and **5** list the data corresponding to the amounts of NVCs present in both streams, as well as their composition and standard deviations.

Both ion-exchange stages resulted in purification effects, with reduction in the mass fraction of ONVC (from 0.2384 to 0.01373 and to 0.0902). The final product was obtained at a yield of 10.90 kg/100 kg o.d. rice husks and contained a low proportion of monosaccharides (3.35 wt % of NVC) with an ONVC content near 9%, in the range reported for solvent extraction−ion exchange processing of autohydrolysis liquors of rice husks and an industrial waste made up of barley husks and barley spent grains (*3*, *10*), and compared well with other reported purification procedures dealing with two-stage hydrothermal treatments and further refining by solvent precipitation, freeze-drying extraction (*5*), or chromatographic separation and ion exchange (*39*).

Processing of Retentates by Ethyl Acetate Extraction and Ion Exchange. Extraction with ethyl acetate was introduced as a refining step after membrane concentration (see **Figure 1b**) because of its ability for removing nonsaccharide compounds (including waxes and low molecular phenolics), yielding an organic phase containing compounds with antioxidant activity (*41*, *42*) with potential applications as food ingredients, and in cosmetic manufacture.

Table 6 gives the amount and composition of stream L in **Figure 1b**, the aqueous phase resulting from the ethyl acetate extraction of retentates. As expected, the removal of lipophilic components and low molecular phenolics led to an ONVC content in stream L (0.1636) lower than in stream B (0.2384), with a corresponding increase in XO (from a mass fraction of 0.4994 in stream B to 0.5489 in stream L).

According to **Figure 1b**, further processing of stream L with anionic and cationic resins led to streams O and Q, whose compositions are given in **Tables 7** and **8**. A single treatment with resin IRA 96 improved the results obtained in the process depicted in **Figure 1a**, as can be seen from the lower content of ONVC in stream O with respect to stream I. Further ion exchange with Amberlite 200 (stream Q in **Figure 1b**) led to a further reduction in ONVC, yielding 9.94 kg of a solid containing 3.64 wt % of monosaccharides, 5.66 wt % of ONVC, and 90.70 wt % of oligosaccharides and oligosaccharide substituents, data that compare well with reported results (*3*, *5*, *10*, *39*).

According to literature data (*10*), phenolic acids chemically bonded to the xylan backbone and nitrogen-containing compounds are expected to make part of the ONVC fraction. These compounds are solubilized by treatment with 4% sulfuric acid

Table 8. Amount[a] and Composition of Stream Q in **Figure 1b**

component	mass fraction (kg/kg NVC)	standard deviation
glucose	0.0030	0.0001
xylose	0.0202	0.0003
arabinose	0.0132	0.0001
glucooligosaccharides	0.1629	0.0006
xylooligosaccharides	0.6210	0.0020
arabinooligosaccharides	0.0344	0.0001
acetyl groups linked to oligosaccharides	0.0366	0.0007
uronic acids	0.0521	0.0023
ONVC	0.0566	0.0060

[a] Amount of NVC in stream Q coming from the processing of 100 kg of o.d. rice husks, 9.94 kg. Mass fraction of NVC (kg NVC/kg Q) = 0.0987 (standard deviation, 0.0015).

and have been quantified as acid soluble lignin, which had a mass fraction of 0.0317 in stream Q (standard deviation, 0.0026).

Melanoidins (Maillard reaction products generated during the autohydrolysis step by reaction between amino acids and sugars) are also present in the final product. As melanoidins are complex compounds, generated by many simultaneous reactions and difficult to quantify, the sample was subjected to elemental analysis in order to assess its nitrogen content, to measure melanoidins as a "protein equivalent". The mass fraction of this "protein equivalent" was 0.02 in stream Q. According to the above data, the ONVC fraction is mainly made up of phenolic substituents and melanoidins, which could confer additional valuable features (antioxidant power, organoleptic properties) to the final product.

Conclusions. Xylooligosaccharide-containing liquors were obtained from rice husk autohydrolyis under selected operational conditions. Liquors also contained other saccharide components (such as oligosaccharides made up of glucose units and monosaccharides) and nonsaccharide compounds (measured by the ONVC fraction). Refining of xyloolo20ligosaccharides was assayed by two selected processing schemes (nanofiltration-double ion exchange and nanofiltration-ethyl acetate extraction-double ion exchange) to achieve purification effects, which were measured by the increase in the proportion of xylooligosaccharides in the refined products as well as by the decrease of the contents of ONVC and monosaccharides in the processed liquors. Nanofiltration through a TiO_2/ZrO_2 Kerasep Nano membrane with a molecular mass cutoff of 1000 Da allowed simultaneous concentration and purification; this latter was derived from the preferential removal of monosaccharides and nonsaccharide compounds. When liquors were nanofiltered to achieve a VRF of 5 operating at a transmembrane pressure of 14 bar, 58.6% of the nonsaccharide components and 20.9–46.9% of monosaccharides were kept in the retentate, in comparison with 92% of xylooligosaccharides and glucooligosaccharides. A further double ion-exchange stage led to a final product with a nonsaccharide content in the vicinity of 9 kg/100 kg NVC, which was obtained at a yield of 10.90 kg/100 kg oven dry rice husks. Stronger purification effects were achieved when nanofiltered liquors were subjected to ethyl acetate extraction before double ion-exchange processing. This operational procedure led to a purified product with a nonsaccharide content of 5.66 kg/100 kg NVC at a yield of 9.94 kg/100 kg oven dry rice husks. The nonsaccharide components remaining in the final concentrate were mainly made up of phenolic and nitrogen-containing compounds. The distribution of the nonvolatile components of liquors among streams is assessed by experimental data.

LITERATURE CITED

(1) Ebringerova, A.; Heinze, T. Xylan and xylan derivatives-biopolymers with valuable properties. 1. Naturally occurring xylan structures, isolation procedures and properties. *Macromol. Rapid Commun.* **2000**, *21*, 542–556.

(2) Garrote, G.; Domínguez, H.; Parajó, J. C. Production of substituted oligosaccharides by hydrolytic processing of barley husks. *Ind. Eng. Chem. Res.* **2004**, *43*, 1608–1614.

(3) Vegas, R.; Alonso, J. L.; Domínguez, H.; Parajó, J. C. Processing of rice husk autohydrolysis liquors for obtaining food ingredients. *J. Agric. Food Chem.* **2004**, *52*, 7311–7317.

(4) Garrote, G.; Parajó, J. C. Nonisothermal autohydrolysis of *Eucalyptus* wood. *Wood Sci. Technol.* **2002**, *36*, 111–123.

(5) Vázquez, M. J.; Garrote, G.; Alonso, J. L.; Domínguez, H.; Parajó, J. C. Refining of autohydrolysis liquors for manufacturing xylooligosaccharides: Evaluation of operational strategies. *Bioresour. Technol.* **2005**, *96*, 889–896.

(6) Palm, M.; Zacchi, G. Extraction of hemicellulosic oligosaccharides from spruce using microwave oven or steam treatment. *Biomacromolecules* **2003**, *4*, 617–623.

(7) Garrote, G.; Domínguez, H.; Parajó, J. C. Autohydrolysis of corncob: Study of nonisothermal operation for xylooligosaccharide production. *J. Food Eng.* **2002**, *52*, 211–218.

(8) Nabarlatz, D.; Farriol, X.; Montané, D. Kinetic modeling of the autohydrolysis of lignocellulosic biomass for the production of hemicellulose-derived oligosaccharides. *Ind. Eng. Chem. Res.* **2004**, *43*, 4124–4131.

(9) Garrote, G.; Cruz, J. M.; Domínguez, H.; Parajó, J. C. Production of xylooligosaccharides by autohydrolysis of lignocellulosic materials. *Trends Food Sci. Technol.* **2004**, *15*, 115–120.

(10) Vegas, R.; Alonso, J. L.; Domínguez, H.; Parajó, J. C. Manufacture and refining of oligosaccharides from industrial solid wastes. *Ind. Eng. Chem. Res.* **2005**, *44*, 614–620.

(11) Carvalheiro, F.; Esteves, M. P.; Parajó, J. C.; Pereira, H.; Girio, F. M. Production of oligosaccharides by autohydrolysis of brewery's spent grain. *Bioresour. Technol.* **2004**, *91*, 93–100.

(12) Carvalheiro, F.; Garrote, G.; Parajó, J. C.; Pereira, H.; Girio, F. M. Kinetic modeling of brewery's spent grain autohydrolysis. *Biotechnol. Prog.* **2005**, *21*, 233–243.

(13) Nabarlatz, D.; Farriol, X.; Montané, D. Autohydrolysis of almond shells for the production of xylo-oligosaccharides: Product characteristics and reaction kinetics. *Ind. Eng. Chem. Res.* **2005**, *44*, 7746–7755.

(14) Mosier, N. S.; Hendrickson, R.; Brewer, M.; Ho, N.; Sedlak, M.; Dreshel, R.; Welch, G.; Dien, B. S.; Aden, A.; Ladisch, M. R. Industrial scale-up of pH-controlled liquid hot water pretreatment of corn fiber for fuel ethanol production. *Appl. Biochem. Biotechnol.* **2005**, *125*, 77–97.

(15) Kim, Y.; Hendrickson, R.; Mosier, N.; Ladisch, M. R. Plug-flow reactor for continuous hydrolysis of glucans and xylans from pretreated corn fiber. *Energy Fuels* **2005**, *19*, 2189–2200.

(16) Vila, C.; Garrote, G.; Domínguez, H.; Parajó, J. C. Hydrolytic processing of rice husks in aqueous media: A kinetic assessment. *Collect. Czech. Chem. Commun.* **2002**, *67*, 509–529.

(17) Kumagai, S.; Hayashi, N.; Sakai, T.; Nakada, M.; Shibata, M. Fractionation and saccharification of cellulose and hemicellulose in rice hull by hot-compressed-water treatment with two-step heating. *J. Jpn. Inst. Energy* **2004**, *83*, 776–781.

(18) Okazaki, M.; Fujikawa, S.; Matsumoto, N. Effect of xylooligosaccharide on the growth of *Bifidobacteria*. *Bifidobacteria Microflora* **1990**, *9*, 77–86.

(19) Vázquez, M. J.; Alonso, J. L.; Domínguez, H.; Parajó, J. C. Xylooligosaccharides: Manufacture and applications. *Trends Food Sci. Technol.* **2001**, *11*, 387–393.

(20) Van Laere, K.; Hartemink, R.; Bosveld, M.; Schols, H.; Voragen, A. Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. *J. Agric. Food Chem.* **2000**, *48*, 1644–1652.

(21) Kabel, M.; Kortenoeven, L.; Schols, H.; Voragen, A. *In vitro* fermentability of differently substituted xylo-oligosaccharides. *J. Agric. Food Chem.* **2002**, *50*, 6205−6210.
(22) Taniguchi, H. Carbohydrate research and Industry in Japan and the Japanese Society of Applied Glycoscience. *Starch* **2004**, *56*, 1−5.
(23) Yuan, Q. P.; Zhang, H.; Qian, Z. M.; Yang, X. J. Pilot-plant production of xylo-oligosaccharides from corncob by steaming, enzymatic hydrolysis and nanofiltration. *J. Chem. Technol. Biotechnol.* **2004**, *79*, 1073−1079.
(24) Izumi, Y.; Azumi, N.; Kido, Y.; Nakabo, Y. Oral preparations for atopic dermatitis containing acidic xylooligosaccharides. Patent JP 2004210666, 2004.
(25) Izumi, Y.; Ikemizu, S.; Shizuka, F. Intestinal environment improving agents containing acidic xylooligosaccharides. Patent JP 2004182609, 2004.
(26) Swennen, K.; Courtina, C. M.; Van der Bruggen, B.; Vandecasteele, C.; Delcour, J. A. Ultrafiltration and ethanol precipitation for isolation of arabino-xylooligosaccharides with different structures. *Carbohydr. Polym.* **2005**, *62*, 283−292.
(27) Freixo, M. R.; Norberta de Pinho, M. Enzymatic hydrolysis of beechwood xylan in a membrane reactor. *Desalination* **2002**, *149*, 237−242.
(28) Yang, F.; Fang, Z.; Xu, Y.; Yao, C.; Yu, S.; Zhu, Q. Separation of xylo-oligosaccharides from enzymatic hydrolytes using membrane reactor. *J. Cent. South Univ. Technol.* **2003**, *10*, 122−125.
(29) Olano-Martin, E.; Mountzouris, K. C.; Gibson, G. R.; Rastall, R. A. Continuous production of pectic oligosaccharides in an enzyme membrane reactor. *J. Food Sci.* **2001**, *66*, 966−971.
(30) Iwasaki, K. I.; Matsubara, Y. Purification of pectate oligosaccharides showing root-growth-promoting activity in lettuce using ultrafiltration and nanofiltration membranes. *J. Biosci. Bioeng.* **2000**, *89*, 495−497.
(31) Li, W.; Li, J.; Chen, T.; Chen, C. Study on nanofiltration for purifying fructo-oligosaccharides. *J. Membr. Sci.* **2004**, *245*, 123−129.
(32) Matsubara, Y.; Iwasaki, K.; Nakajima, M.; Nabetani, H.; Nakao, S. Recovery of oligosaccharides from steamed soybean wastewater in tofu processing by reverse osmosis and nanofiltration membranes. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 421−428.
(33) Kim, S.; Kim, W.; Hwang, I. K. Optimization of the extraction and purification of oligosaccharides from defatted soybean meal. *Int. J. Food Sci. Technol.* **2003**, *38*, 337−342.
(34) Garrote, G,: Domínguez, H.; Parajó, J. C. Mild autohydrolysis: An environmentally friendly technology for xylooligosaccharide production from wood. *J. Chem. Technol. Biotechnol.* **1999**, *74*, 1101−1109.
(35) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484−489.
(36) Maekawa, E.; Ichizawa, T.; Koshijima, T. An evaluation of the acid-soluble lignin determination in analyses by the sulphuric acid method. *J. Wood Chem. Technol.* **1989**, *9*, 549−567.
(37) Kabel, M. A.; Carvalheiro, F.; Garrote, G.; Avgerinos, E.; Koukios, E.; Parajó, J. C.; Gírio, F. M.; Schols, H. A.; Voragen, A. G. J. Hydrothermally treated xylan rich byproducts yield different classes of xylo-oligosaccharides. *Carbohydr. Polym.* **2002**, *50*, 47−52.
(38) Kabel, M. A.; Schols, H. A.; Voragen, A. G. J. Complex xylo-oligosaccharides identified from hydrothermally treated *Eucalyptus* wood and brewery's spent grain. *Carbohydr. Polym.* **2002**, *50*, 191−200.
(39) Endo, M.; Kuroda, Y. Production method of xylose and xylooligosaccharides from natural compounds containing xylan by hot water pretreatment. JP Patent 2000236899, 2000.
(40) Ohsaki, S.; Tamura, M.; Yamaura, T. Method for purification of saccharide. U.S. Patent 2000-693373 20001019, 2003.
(41) Cruz, J. M.; Domínguez, H.; Parajó, J. C. Antioxidant activity of isolates from acid hydrolysates of *Eucalyptus globulus* wood. *Food Chem.* **2005**, *90*, 503−511.
(42) Moure, A.; Domínguez, H.; Parajó, J. C. Antioxidant activity of liquors from aqueous treatments of *Pinus radiata* wood. *Wood Sci. Technol.* **2005**, *39*, 129−139.

Received for review February 22, 2006. Revised manuscript received May 29, 2006. Accepted June 2, 2006. We are grateful to the Spanish Ministry of Science of Technology for the financial support of this work (in the framework of the Research Project "Development of Technologies for the Integral Benefit of Industrial Byproducts", reference CTQ2005-00745/PPQ, which had partial financial support from the FEDER funds of the European Union).

JF060525W