MAXIMIZATION OF MONOMERIC C5 SUGARS FROM WHEAT BRAN BY USING MESOPOROUS ORDERED SILICA CATALYSTS

Nuria Sánchez-Bastardo, Esther Alonso*

*Corresponding author: High Pressure Processes Group -Chemical Engineering and Environmental Technology Department, C/Dr. Mergelina s/n, University of Valladolid, 47011, Spain, +34 983 42 31 75, ealonso@iq.uva.es

Abstract

The hydrolysis process of real a fraction of arabinoxylans derived from wheat bran was studied using different mesoporous silica materials and the corresponding $RuCl_3$ catalysts in water. The influence of type and catalyst loading, reaction time and different metal cations were discussed in terms of the hydrolysis yield of arabinose and xylose oligomers as well as the formation of furfural as degradation product. A high yield of arabinoxylans into the corresponding monomeric sugars (96 and 94% from arabino- and xylo-oligosaccharides, respectively) was obtained at relatively high temperatures (180 ºC) and short reaction times (15 min) with a catalyst loading of 4.8 g of $RuCl_3$/Al-MCM-48 per g of initial carbon in hemicelluloses.

Keywords: arabinoxylan hydrolysis, wheat bran, biomass, heterogeneous catalysis, ruthenium catalysts.

Highlights:

- $RuCl_3$/Al-MCM-48 is an active catalyst for arabinoxylan hydrolysis.
- $RuCl_3$/Al-MCM-48 accelerates conversion of arabinoxylans into arabinose and xylose.
- $RuCl_3$/Al-MCM-48 catalysts inhibit further dehydration into furfural.
- Significant reduction in hydrolysis time from several hours to 15 min is achieved.
- A two-step process using $RuCl_3$/Al-MCM-48 maximizes C5 sugars from wheat bran.

1. Introduction

The current depletion of fossil resources is forcing society to seek renewable alternatives for energy and chemicals production. Biomass is considered a sustainable and renewable feedstock to substitute fossil-based fuels (Negahdar et al., 2016; Oh et al., 2015; Putro et al., 2016; Singhvi et al., 2014). Biomass accrues in large amounts all over the world as forestry and agricultural waste. Moreover, around 95% of this biomass consists of lignocellulosic material not edible for humans. Thus, its application to biofuels or chemicals synthesis does not compete with food production (Negahdar et al., 2016; Sahu and Dhepe, 2012).

Agricultural residues like straw, corn stover or wheat bran appear as interesting feedstocks to obtain high added-value products (Apprich et al., 2014). Wheat bran is a by-product of the wheat grain milling. About 150 million tons are produced per year worldwide and its main use is as a low value component in animal food (Prückler et al., 2014). The general composition of wheat bran is as follows: water (12.1%), proteins (13.2 – 18.4%), fats (3.5 – 3.9%), starch (13.8 – 24.9%), cellulose (11.0%), arabinoxylans (10.9 – 26.0%), β-glucans (2.1 – 2.5%), phenolic acids (0.02 – 1.5%) and ash (3.4 – 8.1%) (Apprich et al., 2014). Arabinoxylans (AXs) are a major component contained in the cell walls of wheat bran. AXs belong to the hemicellulosic part of biomass and are composed of a backbone of β-1,4 linked D-xylopyranosyl residues (Izydorczyk and Biliaderis, 2007). The abundance of arabinoxylans in wheat bran makes them susceptible to be extracted and converted into different platform molecules (furfural, succinic acid, xylitol, arabitol, among others) (Apprich et al., 2014).

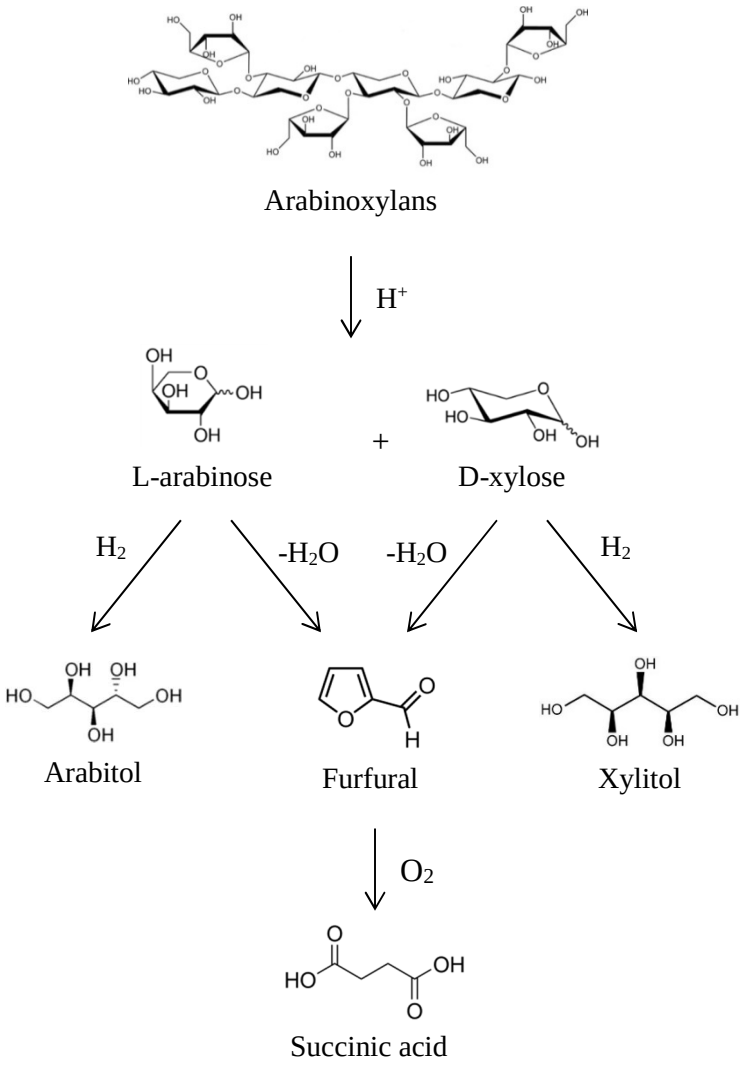


Fig. 1. Conversion of arabinoxylans into high added-value products (Choudhary et al., 2013; Kobayashi et al., 2011; Tathod et al., 2014; Zhang et al., 2012).

In general, hemicelluloses are partly solubilized and hydrolyzed during biomass fractionation, resulting in an aqueous fraction enriched in hemicellulosic poly/oligosaccharides and in a solid fraction with a high content in cellulose and lignin. Although hemicellulosic poly/oligosaccharides have important applications in pharmaceutical and food industries, the potential as platform molecules is larger for monomeric sugars. The reaction routes for the production of different chemicals from arabinoxylans are shown in Fig. 1. Arabitol, xylitol and succinic acid are found among the top 12 value-added products derived from biomass included in the report published by the US Department of Energy (DOE). For example, arabitol and xylitol are used as sugar substituents due to their low calorie content and also they are well

known for their anticariogenic properties (Koganti and Ju, 2013). Succinic acid has its main application as a C4 building block for fuel additives, solvents and biopolymers, among others (Choudhary et al., 2013).

Therefore, the fractionation and the complete hydrolysis of hemicellulosic poly/oligosaccharides into monomers are critical for an integrated biorefinery. Fractionation implies the selectively release of hemicelluloses from the biomass structure to the liquid medium. In a previous work, a hydrothermal fractionation assisted by heterogeneous catalysts was used, and their conditions were optimized (Sánchez-Bastardo et al., 2017). Hydrolysis of hemicelluloses is commonly carried out by two different methods: using mineral acids (hydrolysis yield: 50-89%) (Hilpmann et al., 2016; Kim et al., 2013; Kusema et al., 2013; Li et al., 2016; Nakasu et al., 2016) or enzymes (hydrolysis yield: 6-84%) (Jia et al., 2016; Lee et al., 2013; Li, Wang et al., 2016; Li, Xue et al., 2016; Lou et al., 2016; Moreira and Filho, 2016). Chemical hydrolysis using mineral acids is not an environmentally-friendly process. Although the acids themselves are such a cheap reagent, this process involves an increase in capital costs, as it requires corrosion resistant materials. Moreover, a precipitation with calcium ions to remove the acid is needed, what produces lime as a side product (Negahdar et al., 2016). The use of enzymes is a green process. Nevertheless, the long time required (several hours and even days), the nonexistence of recovery methods, the extremely high prize of enzymes and the critical control of reactions make necessary to look for other alternatives (Aden et al., 2002; Cará et al., 2013; Hendriks and Zeeman, 2009; Ormsby et al., 2012). Hydrolysis of hemicelluloses using solid acid catalysts appears as a green alternative to these methods. Operation times can be reduced and consequently the formation of degradation products and energy consumption. In the last 6 years, the development of solid acid catalysts applied to hydrolysis processes of hemicelluloses has attracted the interest of several authors (Cará et al., 2013; Dhepe and Sahu, 2010; Kusema et al, 2011; Sahu and Dhepe, 2012; Salmi et al., 2014; Vilcocq et al., 2014; Zhou et al., 2013). Hydrolysis of polysaccharides over solid acid catalysts is a sequence of three first order reactions: 1) hydrolysis of polysaccharides into oligosaccharides, 2) hydrolysis of

oligosaccharides into monosaccharides, 3) depending on the reaction conditions, dehydration of monosaccharides into products such as furfural (Vilcocq et al., 2014). Different solid acid catalysts have been tested for hemicelluloses hydrolysis: zeolites, sulfonated resins, mesoporous silica materials, etc. Cará et al. (2013) reported a maximum hydrolysis yield of xylose + arabinose of 80% using Amberlyst 35 as solid catalyst and commercial beechwood xylan as raw material (120 ºC, 10 bar argon, 4 hours). Dhepe et al. (2010) achieved a hydrolysis yield (xylose + arabinose) equal to 54% from oat spelt using zeolite HUSY (Si/Al=15) (170 ºC, 2 h, 50 bar nitrogen). Kusema et al. (2011) used two different sulfonated resins to study the hydrolysis of commercial arabinogalactan. The highest yield they reported was 95% (monomeric arabinose) with Smopex-101 (pH=2) at 90 ºC after 3 hours. Sahu et al. (2012) studied the effect of different solid acid catalysts on the oat spelt hydrolysis. They got a hydrolysis yield (xylose + arabinose) of 41% using zeolite HUSY (Si/Al=15) (170 ºC, 3 h, 50 bar nitrogen). All these studies use commercial hemicelluloses as model compounds of real hemicelluloses contained in different types of biomass. However, only few studies are focused on the hydrolysis of hemicelluloses extracted directly from biomass (Vilcocq et al., 2014). The process starts with the fractionation of biomass to recover selectively the hemicellulosic fraction and complete the hydrolysis subsequently. When hemicelluloses are extracted from real biomass, the purity of the extracts is limited. Other compounds such as extractives, sugars, proteins and ash are also obtained together with the hemicelluloses. All these compounds can interfere in the efficiency of the hydrolysis process resulting in some limitations. Optimizing the hydrolysis step of a real mixture enriched in hemicelluloses is an issue of utmost importance for a concept of a biorefinery.

In this work, the heterogeneous catalytic hydrolysis of an extract enriched in arabinoxylans obtained from destarched wheat bran has been studied. Different experimental conditions, such as type and amount of catalyst and reaction time, have been tested in order to maximize the yield into monomers (xylose and arabinose) and minimize their further degradation into furfural.

2. Materials and Methods

2.1 Support and catalyst preparation

Synthesis of two mesoporous silica supports, MCM-48 and Al-MCM-48, was carried out using the procedure described by Romero et al. in a previous work (Romero et al., 2016). 2.0 g of n-hexadecyltrimethylammonium bromide (for molecular biology, ≥ 99%; Sigma-Aldrich) were dissolved in 42 mL of distilled water, 18 mL of absolute ethanol (Panreac AppliChem) and 13 mL of an aqueous ammonia solution (20% $w \cdot w^{-1}$) (Panreac AppliChem). After 15 minutes stirring, 0.077 g of sodium aluminate (technical, anhydrous; Sigma-Aldrich) were incorporated only for the Al-MCM-48 synthesis. Then, 4 mL of tetraethyl orthosilicate (≥ 99.0% (GC); Sigma-Aldrich) were added dropwise and the solution was further stirred for 18 h. A white precipitate was formed and recovered by filtration while washing with distilled water. This precipitate was dried at 60 ºC overnight. After drying, the samples were calcined to eliminate the surfactant applying a heating rate of 2 $ºC \cdot min^{-1}$ from 80 to 550 ºC and maintained at 550 ºC overnight.

Ruthenium or iron chloride catalysts were synthetized by wetness impregnation method using the so prepared MCM-48 or Al-MCM-48 as silica supports (Romero et al., 2016). Ruthenium (III) chloride (anhydrous; Strem Chemicals Inc.) or iron (III) chloride (reagent grade, 97%; Sigma-Aldrich) together with the corresponding support were suspended in water and sonicated for 10 minutes. This mixture was heated up from 30 to 80 ºC with a heating rate of 1 $ºC \cdot min^{-1}$ and constant stirring. When water was evaporated, the resulting catalyst was dried overnight at 105 ºC to eliminate the remaining water.

2.2 Support and catalyst characterization

Nitrogen adsorption-desorption isotherms were performed with ASAP 2020 (Micromeritics, USA) to determine surface and pore properties of the catalysts. Before analysis, the samples were outgassed overnight at 350 ºC. The multipoint BET method at $P/P_0 \leq 0.3$ was used to calculate the total specific surface area. The total specific pore volume was evaluated from N_2

uptake at $P/P_0 \geq 0.99$ and the pore diameter was determined by BJH adsorption average ($4 \cdot V \cdot A^{-1}$, nm).

The ruthenium amount of the $RuCl_3$-based catalysts was determined by atomic absorption (AA) spectrophotometry (SPECTRA 220FS analyser) after a digestion of the samples with HCl, H_2O_2 and HF using microwave at 250 ºC.

The acidity of the different supports and metal catalysts was estimated by titration with NaOH. This method is based on procedures already reported by several authors (Hu et al., 2015; Hu et al., 2016; Liu et al., 2013; Wang et al., 2011; Zheng et al., 2014).

2.3 Recovery of arabinoxylan fraction from wheat bran

A destarched wheat bran suspension (30 $g \cdot L^{-1}$) together with 500 mg of $RuCl_3$/Al-MCM-48 was located in an extractor of 170 mL volume, made of AISI 304 stainless steel. The extraction was performed at 180 ºC and autogenous pressure for 10 minutes under hot compressed water conditions. The optimization of these fractionation conditions is described in a previous work (Sánchez-Bastardo et al., 2017). After cooling, the final mixture was filtered to separate the remaining solid and the liquid extract enriched in arabinoxylans. This arabinoxylan extract, composed mainly by oligomers, was further subjected to the hydrolysis process itself. The composition of this extract that is indeed the raw material for the hydrolysis study is presented in Table 1.

2.4 Hydrolysis experiments

Hydrolysis experiments of the extract enriched in arabinoxylans were performed in a commercial stainless steel high pressure reactor (30 mL, Berghof BR-25) with a PID controller. First, the corresponding solid catalyst was placed inside the reactor. Once the reactor was closed, it was vented three times with nitrogen in order to eliminate the oxygen and heated up to the operating temperature (180 ºC). When desired temperature was reached, the arabinoxylan solution preheated at 50 ºC was pumped using a HPLC pump (PU-2080 Plus; Jasco). After pumping, temperature inside the reactor dropped to approximately 170 ºC and initial time (0 min) was considered when temperature reached again 180 ºC (this lasts approximately 3 min).

At this moment, nitrogen pressure was adjusted to the operating pressure (50 bar). At the end of the experiments, the reaction was quenched by introducing the reactor in an ice-bath. The catalyst is recovered by filtration and the liquid was used for further analysis of monomeric sugars, degradation products and by-products. The amount of catalyst in each experiment is given as g of catalyst per g of C in initial hemicelluloses. However, in order to shorten, only $g \cdot g\ C^{-1}$ appears along the text.

2.5 Initial and final products analysis

The identification and quantification of sugars and degradation products were done by High Performance Liquid Chromatography (HPLC).

The monosaccharides and degradation products were directly analyzed in the liquid samples. However, for the total sugars determination in the initial solution (monomers and oligomers) an acid hydrolysis pretreatment was used according to the Laboratory Analytical Procedure (LAP) by NREL described by Sluiter et al. (2008). This method consisted of adding 0.8 mL of sulfuric acid (72%) to 20 mL of the initial liquid sample. After the sample was autoclaved at 121 ºC for 1 hour, calcium carbonate was added to get a pH between 5 and 6. An aliquot of 10 mL was filtered (pore size 0.22 μm, Diameter 25 mm, Nylon; FILTER-LAB) and treated with 1 g of mixed bed ion exchange resin (Dowex® Monosphere® MR-450 UPW; Sigma-Aldrich) to remove all the ions before the HPLC analysis. All the samples were analyzed using a chromatography system consisting of an isocratic pump (Waters 1515; Waters Corporation) and an automatic injector (Waters 717; Waters Corporation). Two different HPLC columns were used for the identification and quantification of the different products in the liquid samples: 1) Supelcogel Pb (Supelco) for sugars (milli-Q water as mobile phase, 0.5 $mL \cdot min^{-1}$ as flow rate and 85 ºC as temperature) and 2) Sugar SH-1011 (Shodex) for degradation products (sulfuric acid 0.01 N as mobile phase, 0.8 $mL \cdot min^{-1}$ as flow rate and 50 ºC as temperature). Sugars and acids were identified using an IR detector (Waters 2414; Waters Corporation). 5-hidroxymethylfurfural (5-HMF) and furfural were determined with an UV-Vis detector (Waters 2487; Waters Corporation) at a wavelength of 254 and 260 nm, respectively. The standards

193 employed for the HPLC analysis were: cellobiose (98%), glucose (99%), xylose (99%),
194 galactose (99%), arabinose (99%), mannose (99%), fructose (99%), glyceraldehyde (95%),
195 glycolaldehyde (99%), lactic acid (85%), formic acid (98%), acetic acid (99%), levulinic acid
196 (98%), acrylic acid (99%), pyruvaldehyde (40%), 5-hydroxymethilfurfural (99%) and furfural
197 (99%).All these chemicals were purchased from Sigma-Aldrich (Spain) and used as received.
198 The hydrolysis yield of arabinose and xylose oligomers into arabinose and xylose, respectively,
199 and the total arabinoxylan hydrolysis yield were calculated as follows (Eq. 1-3):

$$\%\ \text{Ara hydrolysis yield} = \frac{\text{Ara as monomeric sugar in hydrolyzed liquid (g)}}{\text{Ara as total sugars}^{a}\text{ in initial liquid extract (g)}} \times 100 \qquad \text{(Eq. 1)}$$

$$\%\ \text{Xyl hydrolysis yield} = \frac{\text{Xyl as monomeric sugar in hydrolyzed liquid (g)}}{\text{Xyl as total sugars}^{a}\text{ in initial liquid extract (g)}} \times 100 \qquad \text{(Eq. 2)}$$

$$\%\ \text{AX hydrolysis yield} = \frac{\text{(Ara+Xyl) as monomeric sugar in hydrolyzed liquid (g)}}{\text{(Ara+Xyl) as total sugars}^{a}\text{ in initial liquid extract (g)}} \times 100 \qquad \text{(Eq. 3)}$$

200 Ara: Arabinose, Xyl: Xylose
201 [a] Total sugars refer to the sum of the corresponding sugars (arabinose and/or xylose) as monomers plus
202 oligomers

203 ## 3. Results and Discussion

204 3.1 Composition of initial arabinoxylan extract

205 The composition of the enriched arabinoxylan extract obtained from wheat bran and used in the
206 hydrolysis experiments is given in Table 1. Table 1 presents the amount of each sugar which is
207 in monomeric and oligomeric form respect the total amount of the corresponding sugar. In the
208 case of arabinose, practically 50% percent is already in monomeric form after the fractionation
209 process, whereas 95% of the xylose appears as oligosaccharide.
210 The initial total amount of arabinose and xylose (monomers + oligomers) expressed in $g \cdot L^{-1}$ is
211 3.1 and 6.2, respectively. This means that these are the maximum values which both sugars can
212 reach after the hydrolysis process.
213
214

Table 1. Chemical composition of the initial extract.

Compound			
Proteins[a] (mg/L)	1676 ± 113		
Sugars	Monomeric sugars (mg/L)	Oligomeric sugars (mg/L)	% Olig[b]
Glc	195 ± 32	1609± 197	89
Xyl	284± 9	5944± 188	95
Gal	82 ± 26	255± 34	76
Ara	1522± 112	1605± 34	51
Man	70± 9	2± 44	2

[a] Proteins were determined following a standardized Kjeldahl method using a nitrogen to protein conversion factor of 5.7 applicable to wheat bran

[b] %Olig. = percentage of each sugar in oligomeric form: g of each oligomeric sugar/g of the total corresponding sugar x 100

3.2 Catalyst characterization

The properties of the different solid catalysts used in this work are summarized in Table 2. Specific surface area, total pore volume, pore diameter and acidity values were determined. MCM-48 and Al-MCM-48 have specific surface areas of 1298 and 1352 $m^2 \cdot g^1$, and pore volumes of 0.87 and 0.81 $cm^3 \cdot g^1$, respectively. According to these results, no significant changes in surface area and pore volume were observed between these two mesoporous materials. Pore diameter was slightly higher for Al-MCM-48 (2.5 nm) than for MCM-48 (2.2 nm). After $RuCl_3$ or $FeCl_3$ impregnation, a decrease in the specific surface area and pore volume was detected. This fact can be attributed to the partial blocking of the porous network in mesoporous supports. After $RuCl_3$deposition on MCM-48, no changes in the pore size were appreciated. However, pore size slightly increased from 2.5 to 2.7 nm and 2.6 nm after deposition of $RuCl_3$ and $FeCl_3$, respectively, on Al-MCM-48. This indicates slender modifications of pore structure, suggesting a higher pore blocking in Al-MCM-48 supported catalysts than in MCM-48 catalysts. Ruthenium and iron content for $RuCl_3$ and $FeCl_3$-based catalysts was around 4% in both cases, determined by AA.

Acidity is a key parameter of catalysts for hydrolysis reactions. The acidity of the catalysts used in this work presents the following trend: MCM-48 < Al-MCM-48 < $RuCl_3$/MCM-48 < $RuCl_3$/Al-MCM-48 < $FeCl_3$/Al-MCM-48.

238 Table 2. Structural characterization of solid catalysts.

Catalyst	Metal content (%)	S_{BET} ($m^2 \cdot g^{-1}$)	V_{pore}[a] ($cm^3 \cdot g^{-1}$)	D_{pore}[b] (nm)	Acidity (mEq $H^+ \cdot g\ cat.^{-1}$)
MCM-48	-	1298	0.87	2.2	0.293
Al-MCM-48	-	1352	0.81	2.5	0.598
$RuCl_3$/MCM-48	4.1	1032	0.63	2.2	0.738
$RuCl_3$/Al-MCM-48	4.3	1018	0.63	2.7	1.130
$FeCl_3$/Al-MCM-48	4.2	1018	0.59	2.6	1.429

239 [a] Total specific pore volume was evaluated from N_2 uptake at $P/P_0 \geq 0.99$
240 [b] Pore diameter was determined by BJH adsorption average

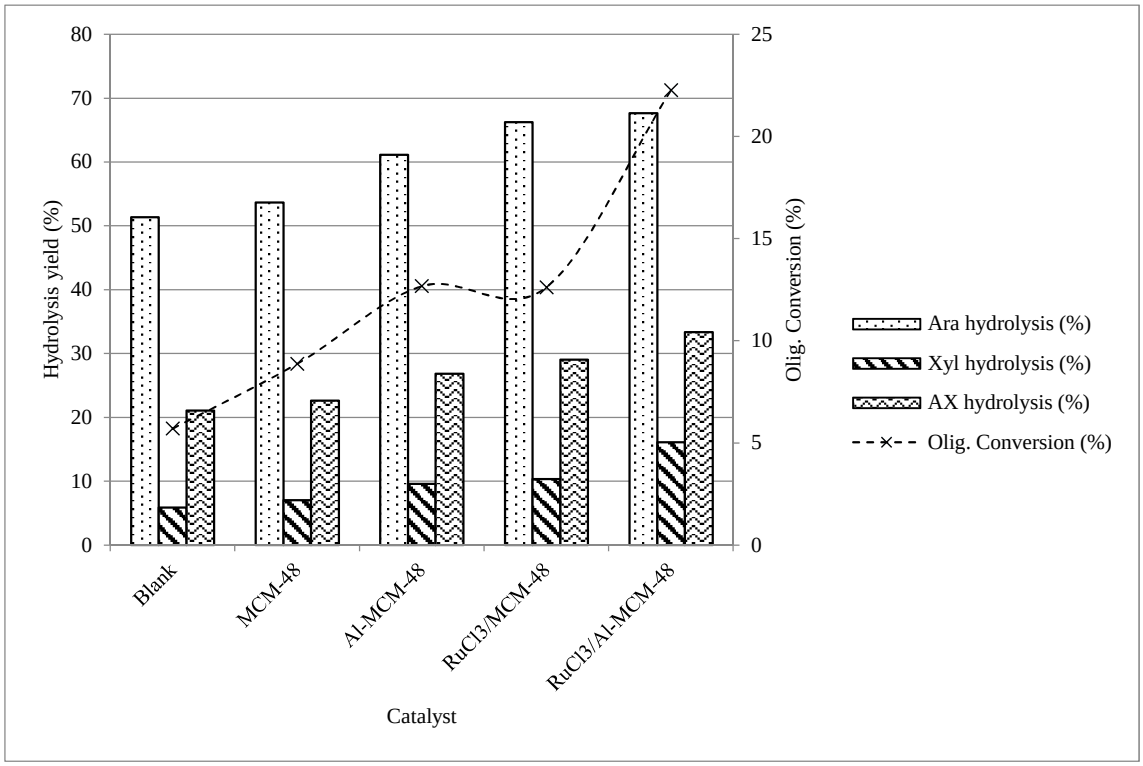
241 3.3 Arabinoxylan hydrolysis experiments

242 The effect of different parameters (type and amount of catalyst, reaction time and different
243 metal cations) was studied in the hydrolysis of an arabinoxylan enriched extract obtained from
244 destarched wheat bran. The results are discussed in terms of hydrolysis yield of arabinose and
245 xylose oligomers. The formation of furfural, as the main degradation product derived from
246 dehydration of arabinose and xylose, was also taken into account.

247 3.3.1 Effect of different mesoporous silica materials and $RuCl_3$ based-catalysts

248 A first screening of different mesoporous silica supports and the corresponding $RuCl_3$ based-
249 catalysts was carried out. MCM-48, Al-MCM-48, $RuCl_3$/MCM-48 and $RuCl_3$/Al-MCM-48 were
250 tested in the arabinoxylan hydrolysis (Fig. 2). The experimental conditions were 180 ºC, 15
251 minutes and 50 bar N_2. The ratio of catalyst to carbon content in hemicelluloses of the initial
252 extract was 0.6 $g \cdot g^{-1}$, which is the same ratio used in the previous fractionation from wheat bran.
253 The trend observed in total AX hydrolysis yield is as follows: Blank ≈ MCM-48 < Al-MCM-48
254 < $RuCl_3$/MCM-48 < $RuCl_3$/Al-MCM-48. This is in agreement with the acidity values of
255 catalysts: higher acidities result in higher hydrolysis yields. In the absence of catalyst (blank
256 reaction), the amount of monomeric arabinose and xylose turns out to be the same as in the
257 initial AX solution (Table 1). At 180 ºC, the pK_w of water is low, which means that the amount
258 of protons derived from water is relatively high (Bandura and Lvov, 2006). However, as it is
259 shown in Fig. 2, the water protonation is not enough to cause the breakdown of arabinoxylan
260 molecules into monomers under these conditions. MCM-48 is the least acidic catalyst and its
261 surface acidity is due to silanol groups (Si – OH), which correspond to weak acid sites (Xue et.,

2004). The weak nature of these sites does not practically improve AX hydrolysis, although a slight increase in arabinose oligomers hydrolysis is observed. Al-MCM-48 exhibits a higher catalytic activity in this hydrolysis process. Specially, Al-MCM-48 improves the conversion of arabinose oligomers into monomers. During Al-MCM-48 synthesis, a silicon atom (Si^{+4}) is replaced by an aluminum atom (Al^{+3}) in a tetrahedral network. Thus, a cation, usually a proton, is required to balance the aluminum tetrahedron. This compensation proton derives in a Brønsted acid site, which increases the total acidity of the Al-MCM-48 (Kao et al., 2008). In Al-MCM-48, Brønsted acidity is higher than Lewis acidity due to the large amount of tetracoordinated aluminum on the silica surface (Krithiga et al., 2005). $RuCl_3$ supported catalysts ($RuCl_3$/MCM-48 and $RuCl_3$/Al-MCM-48) demonstrated to be more active in AXs hydrolysis than the corresponding mesoporous silica materials (MCM-48 and Al-MCM-48, respectively). Higher activity of $RuCl_3$ catalysts in hydrolysis reactions is related to their global acidity. $RuCl_3$-based catalysts show higher acidity than the corresponding mesoporous silica support, which is due to the high acidity of $RuCl_3$ (Guisnet et al., 1993). In addition to this, several authors have already reported that cations with moderate Lewis acidity, such as Ru^{+3}, are found to be active in hydrolysis of cellobiose or cellulose (Jing et al., 2016; Shimizu et al., 2009). In all the cases, furfural formation was negligible in comparison to the amount of arabinose and xylose.



280

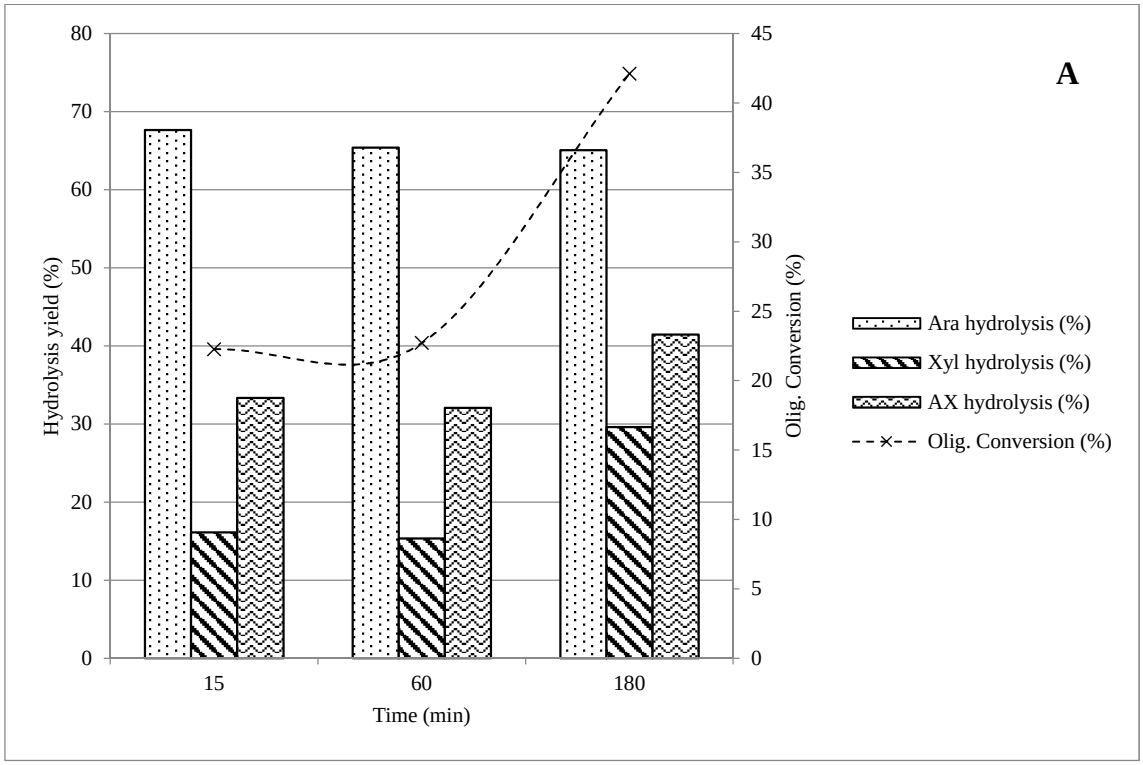
281 Fig. 2. Comparison of different catalysts in arabinoxylan hydrolysis yield and oligomers
282 conversion. Reaction conditions: 180 ºC, 50 bar N_2, 15 min, 0.6 g catalyst·g C^{-1} in initial
283 hemicelluloses.

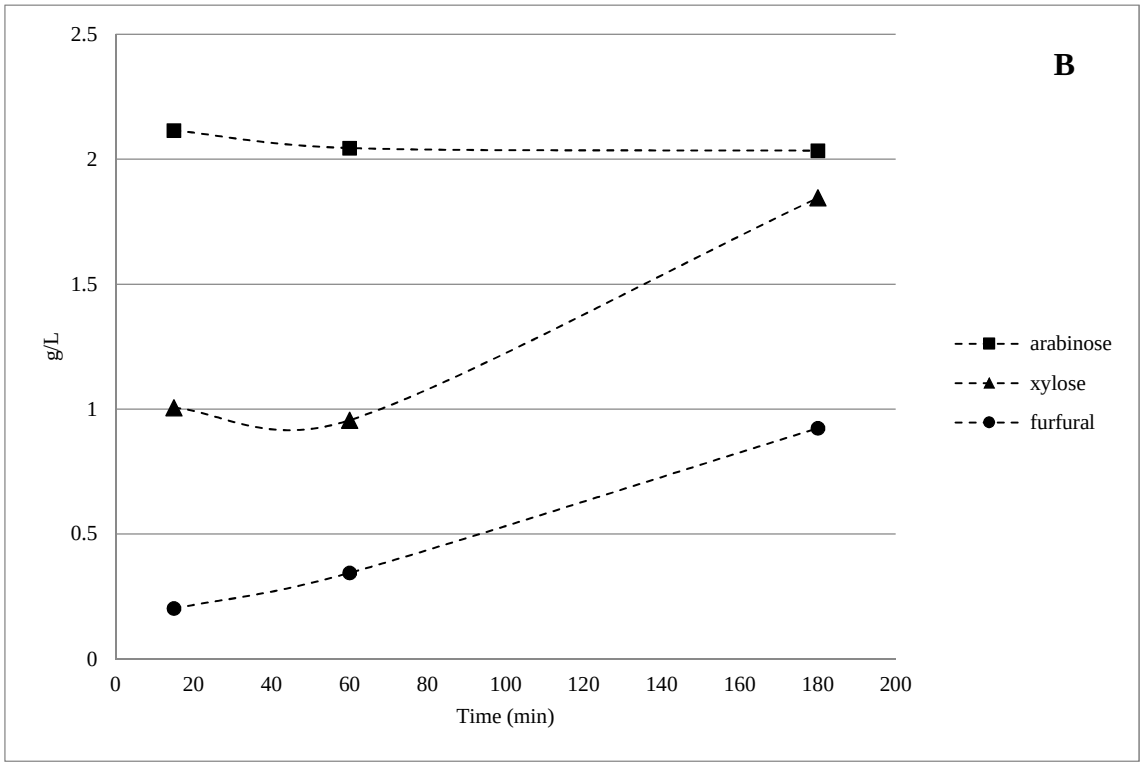
284 Although it has been shown that a more acidic catalyst leads to a higher conversion of AXs into
285 monomeric sugars, hydrolysis yields and oligomer conversion under these experimental
286 conditions were still very low. $RuCl_3$/Al-MCM-48, as the most active catalyst, was chosen for a
287 further study to improve hydrolysis yield.

288 3.3.2 Effect of catalyst amount and reaction time

289 $RuCl_3$ supported on mesoporous Al-MCM-48 was used to study the influence of reaction time
290 and catalyst loading on hydrolysis yield. First, a low amount of catalyst (0.6 g·g C^{-1}) was tested
291 at 180 ºC, 50 bar N_2 and different reaction times (15-180 min) (Fig. 3). A maximum of 68% in
292 the hydrolysis yield of arabinose oligomers is achieved at 15 minutes of reaction, as a
293 consequence of their degradation into furfural for longer times. No difference is observed in
294 terms of arabino-oligosaccharides hydrolysis yield for times beyond 15 minutes. However, with
295 this low amount of catalyst, the maximum hydrolysis yield of xylose oligomers is obtained after

180 minutes of reaction, where the concentration of furfural becomes also important due to xylose degradation, as it is shown in Fig. 3B. This means that monomeric xylose is slowly formed and partially degraded into furfural after 3 hours. Part of xylose obtained over time has had probably enough time to be converted into furfural. At short times, xylose detected is low because xylose oligomers have not been hydrolyzed. At longer times, this amount is higher but hydrolysis yield of xylose oligomers is still low (≈ 30%) due to the slow xylose formation from xylo-oligosaccharides and fast degradation into furfural. The large quantity of furfural also evidences the slow hydrolysis rate of xylose oligomers into xylose compared to the high degradation rate of xylose into furfural.





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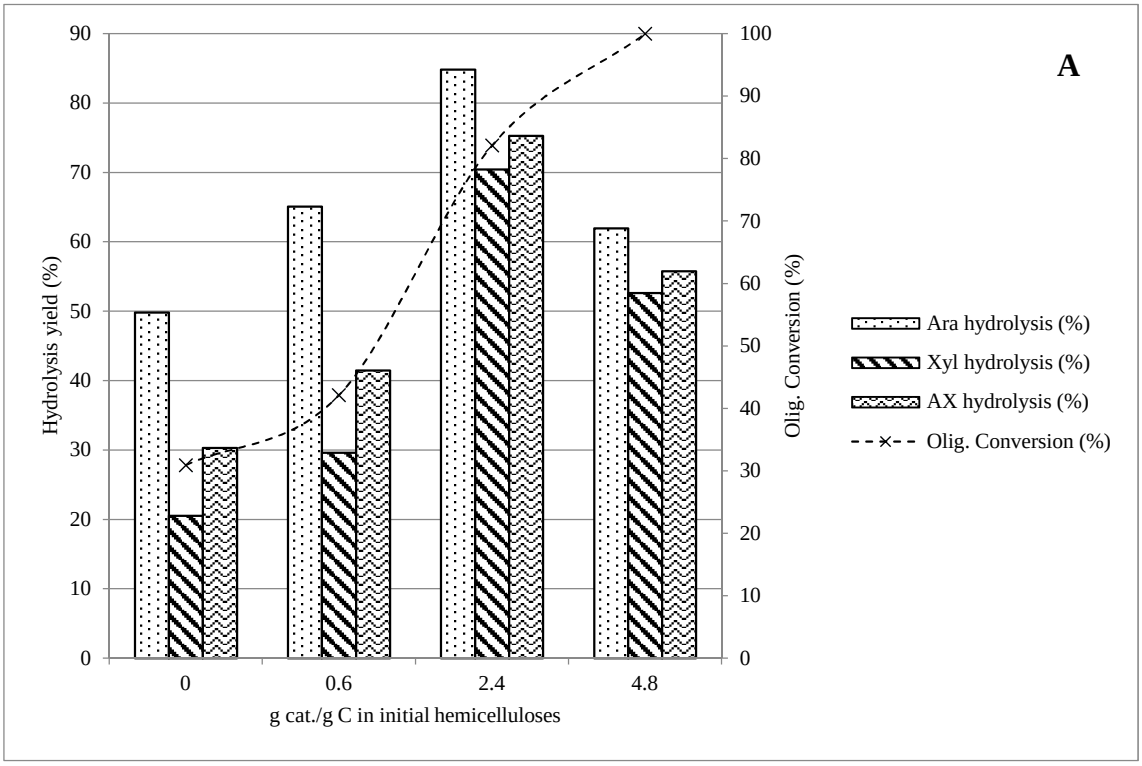
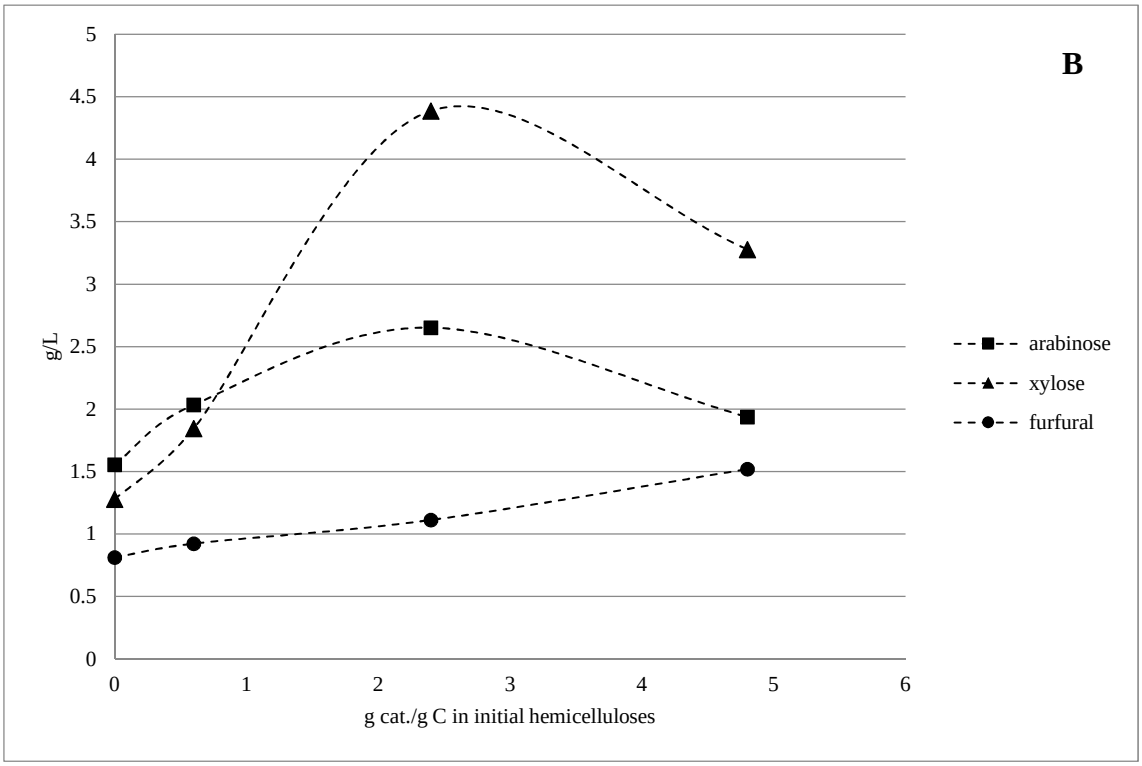
307 Fig. 3. Effect of time in arabinoxylan hydrolysis with low amount of catalyst. Reaction
308 conditions:180 ºC, 50 bar N_2, catalyst: RuCl3/Al-MCM-48, catalyst loading: 0.6 g catalyst·g C^{-1}
309 in initial hemicelluloses. A) Hydrolysis yield and oligomers conversion, B) Composition of the
310 liquid after hydrolysis (g·L^{-1}).

311 To overcome this degradation is necessary to speed up the xylo-oligosaccharides hydrolysis step
312 but inhibiting further degradation. Three different amounts of catalyst (0.6, 2.4 and 4.8 g·g C^{-1})
313 were tested at 180 ºC and 3 hours under 50 bar of N_2 (Fig. 4). The hydrolysis yield of xylose
314 oligomers reaches 70% after 3 hours with a catalyst loading of 2.4 g·g C^{-1}. Under these
315 conditions, the hydrolysis yield of arabino-oligosaccharides is already 85%. When catalyst
316 loading is increased from 0.6 to 2.4 g·g C^{-1}, monomeric xylose and arabinose in the liquid after
317 hydrolysis rise in a greater proportion than furfural. This means that arabinose and xylose
318 formation is faster than the consecutive degradation into furfural. This was also observed and
319 well explained by Sahu et al. (2012). They studied the effect of substrate/catalyst ratio on
320 hemicelluloses hydrolysis using HUSY zeolite as catalyst. Interactions between the substrate
321 (hemicelluloses) and available active sites in catalyst decrease as increasing substrate/catalyst

ratio (decreasing catalyst amount); in addition to this, once xylose is formed, it may undergo non-catalytic degradation reactions. In that work, a xylose+arabinose yield of 35% was reported when substrate/catalyst ratio was 130. However, when this ratio was 10, the yield of arabinose+xylose was 56%. They concluded that the reaction rate of hemicelluloses into xylose was higher than xylose to furfural when the amount of catalyst was increased. In the present work, when catalyst loading was further increased (from 2.4 to 4.8 g·g C^{-1}), the oligomer conversion was total. However, after 3 hours of hydrolysis, a dramatic decrease in xylose and arabinose is observed and black sediment appears. This black sediment corresponds probably to humins derived from furfural: arabinose and xylose are degraded to furfural and this later to humins; that explains a drop in arabinose and xylose but not a sharp increase in furfural as it is shown in Fig. 4B.



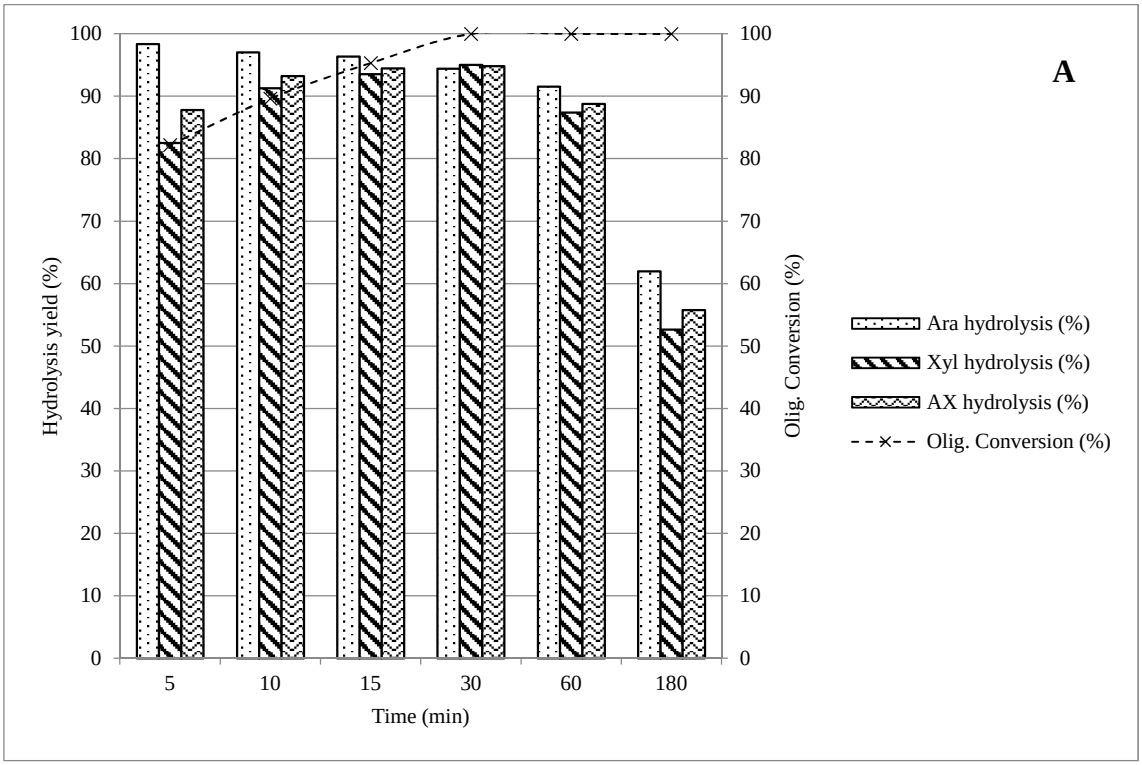


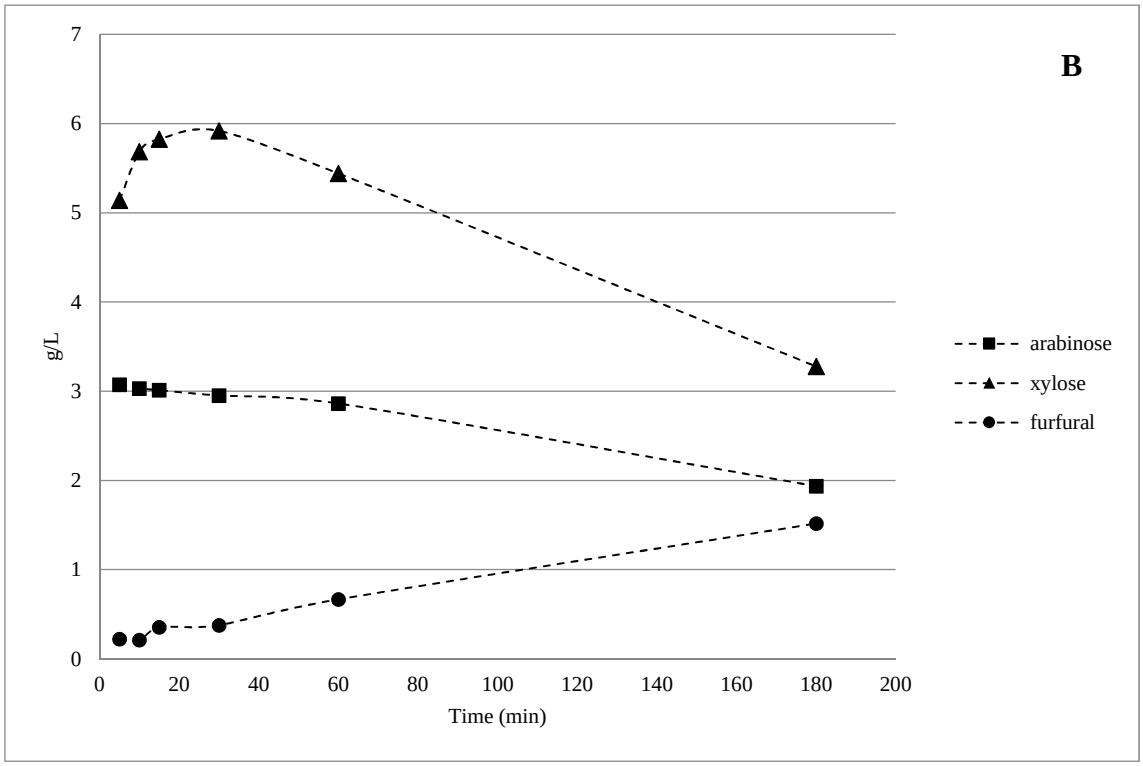
334

335 Fig. 4. Effect of the amount of catalyst in arabinoxylan hydrolysis. Reaction conditions: 180 ºC,
336 3 h, 50 bar N_2, catalyst: $RuCl_3$/Al-MCM-48. A) Hydrolysis yield of arabinoxylans and
337 oligomers conversion, B) Composition of the liquid after hydrolysis ($g \cdot L^{-1}$).

338 In order to shorten reaction times and subsequent degradation, the highest catalyst loading (4.8
339 $g \cdot g\ C^{-1}$) was tested at shorter times (< 180 min) (Fig. 5). The hydrolysis yield of arabinose
340 oligomers is almost complete after 5 minutes (98%). Longer times make arabinose degrade
341 slowly into furfural. The hydrolysis of xylose oligomers gets the maximum yield after 15-30
342 minutes (94-95%), and then xylose starts to be degraded. Total conversion of oligomers is
343 reached after 15 minutes. The amount of furfural obtained rises after 30 minutes, due to
344 arabinose and xylose degradation. The hydrolysis of arabinose oligomers is always higher than
345 that of xylose. Arabinose side chains are linked by α-glycosidic bonds, whereas xylose units
346 from the backbone are connected by means of β-glycosidic bonds. α-glycosidic bonds are more
347 easily hydrolysable than β-glycosidic linkages, what explains the faster release of arabinose
348 molecules than xylose. This is well reported by Negahdar et al. (2016).

In conclusion, a high arabinoxylan hydrolysis yield was achieved at 180 ºC, 15 minutes and 50 bar N_2 with a catalyst loading of 4.8 g·g C^{-1}. Arabinose and xylose oligomers hydrolysis yields were 96 and 94%, respectively. Under these conditions, the process is optimized since xylose and arabinose reach practically the maximum amount possible. Moreover, the amount of furfural was negligible in comparison to arabinose and xylose.





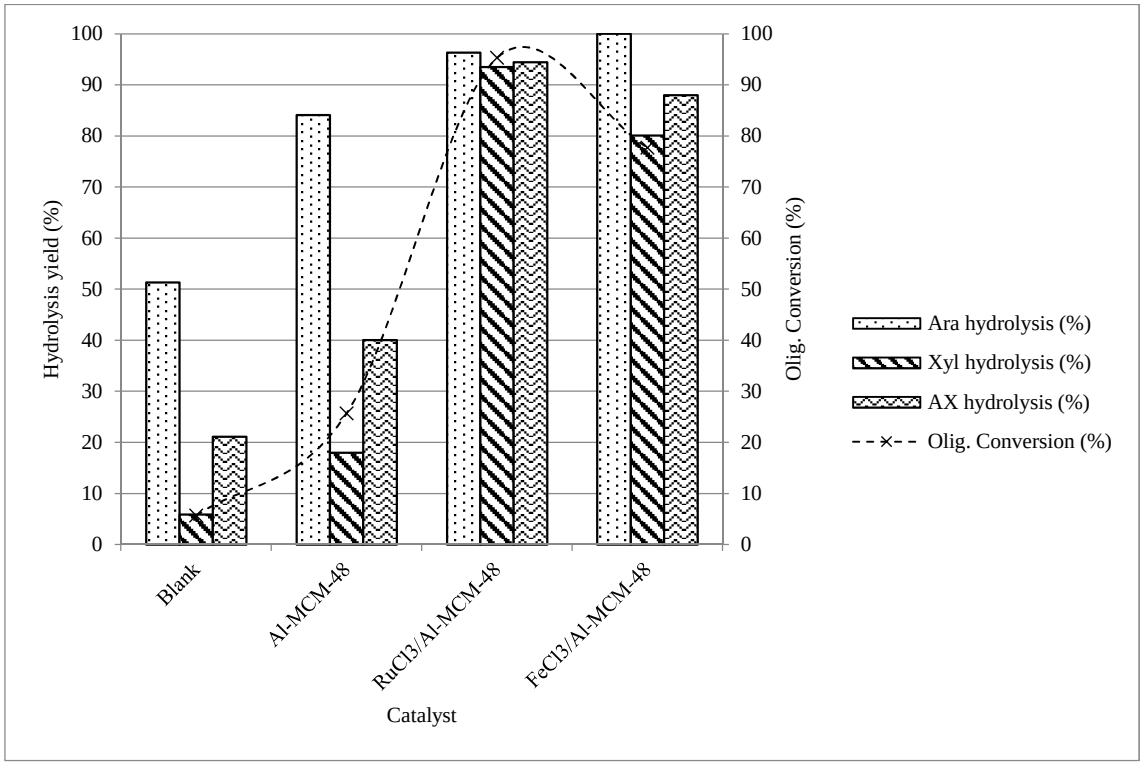
355

356 Fig. 5. Effect of time with high amount of catalyst in arabinoxylan hydrolysis. Reaction
357 conditions: 180 ºC, 50 bar N_2, catalyst: $RuCl_3$/Al-MCM-48, catalyst loading: 4.8 g catalyst·g C^{-1}
358 in initial hemicelluloses. A) Hydrolysis yield of arabinoxylans into arabinose and xylose and
359 oligomers conversion, B) Composition of the liquid after hydrolysis (g·L^{-1}).

360 3.3.3 Effect of cation (Ru^{+3}, Fe^{+3})

361 The effect of different cations (Ru^{+3}, Fe^{+3}) was studied using $RuCl_3$ and $FeCl_3$ supported on Al-
362 MCM-48 as catalysts. Experiments were performed at 180 ºC, 15 minutes and 50 bar N_2 using
363 4.8 g·g C^{-1}, which was optimized in previous sections. For comparison, a reaction with bare Al-
364 MCM-48 was also carried out to point out the effect of the metal precursors. Results are shown
365 in Fig. 6. It can be clearly seen that the incorporation of $RuCl_3$ or $FeCl_3$enhances arabinoxylan
366 hydrolysis. The hydrolysis of arabinose oligomers is almost complete with both supported
367 catalysts. However, a higher hydrolysis yield of xylose oligomers is obtained with $RuCl_3$-
368 catalyst, despite the greater acidity of $FeCl_3$/Al-MCM-48 (Table 2). Fe^{+3} and Ru^{+3} have been
369 demonstrated to be active in hydrolysis of cellobiose and cellulose (Jing et al., 2016; Shimizu et
370 al., 2009). Nevertheless, higher reaction rates were observed for catalysts with moderate Lewis

371 acidity, such as Ru^{+3}, than for those with high Lewis acidity, such as Fe^{+3}. This could explain
372 the better catalytic activity of $RuCl_3$ catalysts in comparison with $FeCl_3$ in arabinoxylan
373 hydrolysis (Shimizu et al., 2009).



374

375 Fig. 6. Effect of cation (Ru^{+3}, Fe^{+3}) in arabinoxylan hydrolysis and oligomers conversion.
376 Reaction conditions: 180 ºC, 15 min, 50 bar N_2, catalyst loading: 4.8 g catalyst·g C^{-1} in initial
377 hemicelluloses.

378 4. Conclusions

379 The use of heterogeneous catalysts has been demonstrated to be a good option for hydrolysis of
380 real arabinoxylans derived from wheat bran. The hydrolysis yield is improved by increasing the
381 acidity of the heterogeneous catalyst (MCM-48 < Al-MCM-48 < $RuCl_3$/MCM-48 < $RuCl_3$/Al-
382 MCM-48). Cations with moderate Lewis acidity (Ru^{+3}) present a higher activity in hydrolysis
383 processes than those with high Lewis acidity (Fe^{+3}). In this work, a high hydrolysis yield of
384 arabinoxylans into the corresponding monomers (94 and 96% for xylose and arabinose,
385 respectively) is achieved at 180 ºC after 15 minutes using an amount of $RuCl_3$/Al-MCM-48
386 equal to 4.8 g·g^{-1}.

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