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CORNCOB ARABINOXYLAN FOR NEW MATERIALS

Itziar Egüés[a], Agnes M. Stepan[b,d], Arantxa Eceiza[a], Guillermo Toriz[c,d], Paul Gatenholm[b,d], Jalel Labidi[a,*]

[a]Chemical and Environmental Engineering Department, University of the Basque Country, Plaza Europa, 1, 20018, Donostia-San Sebastián, Spain
[b]Department of Chemical and Biological Engineering, Biopolymer Technology, Chalmers University of Technology, SE-412 96 Göteborg, Sweden,

[c]Department of Wood, Cellulose and Paper Research, University of Guadalajara, Guadalajara, Mexico

[d]Wallenberg Wood Science Center, Göteborg, Sweden

itziar.egues@ehu.es
agnes.stepan@chalmers.se
arantxa.eceiza@ehu.es
guillert@chalmers.se
paul.gatenholm@chalmers.se
* jalel.labidi@ehu.es tel.:+34-943017178; fax: +34-943017140

Abstract

Corncob agricultural waste was used as a source of arabinoxylan for preparation of films. Three arabinoxylan samples were prepared: crude extract (CCAX), purified by a washing step, and purified by bleaching CCAX. Films prepared with untreated CCAX were water soluble, yellowish in color and had poor mechanical properties. After the purification processes the Young's modulus increased from ~293 MPa to ~1400-1600 MPa, and strength was improved from ~9 MPa to around 53 MPa, while the strain at break was kept at ~8% both in untreated and purified CCAX. The contact angle was increased from ~21.3° to 67-74° after washing or bleaching CCAX. Acetylation of bleached CCAX showed the highest thermal resistance (325 °C), had low Tg (125 °C) and a high contact angle (80°), and its films were stronger (strength ~67 MPa; Young's modulus ~2241 MPa) and more flexible (~13%). These characteristics make purified CCAX a suitable material to be used as a matrix for film applications.

Keywords: corncob, arabinoxylan, purification, acetylation, films

1. Introduction

Most conventional plastic materials are currently derived from petroleum. They are used everywhere and make life easier, safer and more enjoyable. Worldwide plastic production has increased six times over the past 30 years and is expected to continue growing in the coming years. Part of the fossil fuel based plastics are recycled, others are burned for energy recovery while a significant percentage is rejected as a waste that takes hundreds of years to degrade (Lazarevic, Aoustin, Buclet, & Brandt, 2010).

In recent years, the uncertainty in the supply and price of fossil fuels, as well as environmental pollution, make necessary the investigation of new alternatives to replace them as a source of materials and energy. In this context, utilization of lignocellulosic resources, such as agricultural wastes, is becoming an interesting alternative owing to their renewable origin, the biodegradability of their components and their non-human food application.

Agricultural wastes consist of three main polymeric constituents, namely cellulose, hemicellulose and lignin, which offer high amounts of organic materials that can be employed as biopolymers. Hemicelluloses constitute about 30% of the lignocellulosic total mass, where xylan is one of its major components. The structure of xylan is based on a linear backbone, consisting of β-1,4-linked D-xylopyranose residues, and branches containing acetyl, arabinosyl and glucuronosyl residues (Izydorczyk & Biliaderis, 1995).

The composition of hemicellulose depends on the lignocellulosic source and on the extraction method. Alkaline extraction has been shown to be very effective for the extraction of hemicelluloses from both original or delignified tissues (Bahcegul, Toraman, Ozkan, & Bakir, 2012; García et al., 2013; Van Dongen, Van Eylen, & Kabel, 2011). Alkali treatment removes acetyl and various uronic acid substitutions on hemicelluloses. It also disrupts intermolecular ester and ether bonds in crosslinked xylan with other components such as lignin. Oxidative delignification by sodium chlorite, hydrogen peroxide, ozone, oxygen or air (Peng, Peng, Xu, & Sun, 2012) can also be used to obtain hemicelluloses with less associated lignin.

Hemicellulose utilization is not as extended as others polysaccharides like cellulose or starch. However, some xylan-derived products have already found some commercial applications for production of ethanol, xylitol, and xylooligosaccharides (Deutschmann & Dekker, 2012). Recently, xylans have received a lot of attention as a polymer for materials application, i.e. films. The main xylan polymers-based studies have focused on the formation of films and coatings, foams and gels, and the chemical modifications of xylans to enhance film/foam/gel properties.

The film forming capacity is greatly influenced by the structure of the hemicellulose (Bahcegul et al., 2012; Egüés, Eceiza, & Labidi, 2013). Xylan films have been unsuccessful in materials applications because of their brittleness, low mechanical strength and moisture sensitivity (Zhong, Peng, Yang, Cao, & Sun, 2013). In this sense, special efforts have been made by several authors (Ayoub, Venditti, Pawlak, Sadeghifar, & Salam, 2013; Belmokaddem, Pinel, Huber, Petit-Conil, & Da Silva Perez, 2011; Sun, Sun, Zhao, & Sun, 2004a) to reduce the hygroscopic nature of arabinoxylans by substituting the hydroxyl groups of hemicellulose and increase its hydrophobicity. Surface fluorination of arabinoxylan films was proposed by Gröndahl, Gustafsson, and

Gatenholm (2006) and acetylated arabinoxylan material properties were studied by Stepan, Höije, Schols, de Waard, and Gatenholm (2012). An effective strategy to prepare high-quality xylan film was recently developed by introducing long hydrophobic carbon chains into xylan (Zhong et al., 2013) as well as carboxymethylation (Alekhina, Mikkonen, Alén, Tenkanen, & Sixta, 2013). In all these works, the hemicellulose film formation and material properties were significantly improved.

Several strategies have also been investigated with the aim to address the brittleness of the xylan-based films using different plasticizers (Escalante et al. 2012; Gröndahl, Eriksson, & Gatenholm, 2004; Zhang & Whistler 2004). Goksu, Karamanlioglu, Bakir, Yilmaz, and Yilmazer (2007) observed that the film formation of pure cotton stalk xylan was achieved using 8–14% (w/w) xylan without complete removal of lignin. Höije, Gröndahl, Tømmeraas, and Gatenholm (2005) studied a suitable method for isolating arabinoxylans from barley husks in order to achieve high molecular weight hemicellulose, which is more adequate for the preparation of coatings. The films made by water casting showed similar material properties than obtained by aspen glucuronoxylan films plasticized with 20% of sorbitol (Gröndahl et al., 2004). Hemicellulose based composite materials have also been developed as an attempt to improve their material properties (Peng, Ren, Zhong & Sun, 2011; Saxena, Elder, Kenvin & Ragauskas, 2010). Several articles have been published on the use of xylan and arabinoxylan hemicelluloses for materials (Hansen & Plackett, 2008) and different properties of xylan and mannan based films have also been reviewed by Mikkonen and Tenkanen (2012).

Corncob is one of the largest sources of available biomass in the corn-processing industry. Therefore, it has been widely used by many researches mainly for production of xylooligosaccharides, xylose, xylitol and ethanol (Aachary & Prapulla, 2008). The use of xylan from corncob as films has been restricted as a co-component in edible films with gluten (Kayserilioglu, Bakir, Yilmaz, & Akkas, 2003), with chitosan (Li, Shi, Wang, & Du, 2011) and in composites with montmorillonite (Ünlü, Günister, & Atıcı, 2009) and sepiolite (Sárossy, et al. 2012). The present study focuses on the extraction of corncob arabinoxylan (CCAX) by delignification and alkaline extraction, to be used as a single compound in the first step of materials production. Ultrafiltration technique was also used to enhance high molecular weight molecule isolation. Moreover, washing and bleaching treatments were proposed for arabinoxylan purification and film quality improvement, and for removing the xylan´s brownish color, which can limit the spectrum of its applications.

All the materials (initial CCAX, purified and acetylated CCAXs) were thoroughly characterized by IR-ATR, TGA, DSC, SEC, HPLC and H-NMR, and their material properties, i.e. the thermal stability, water contact angle and mechanical properties, were evaluated.

2. Materials and Methods

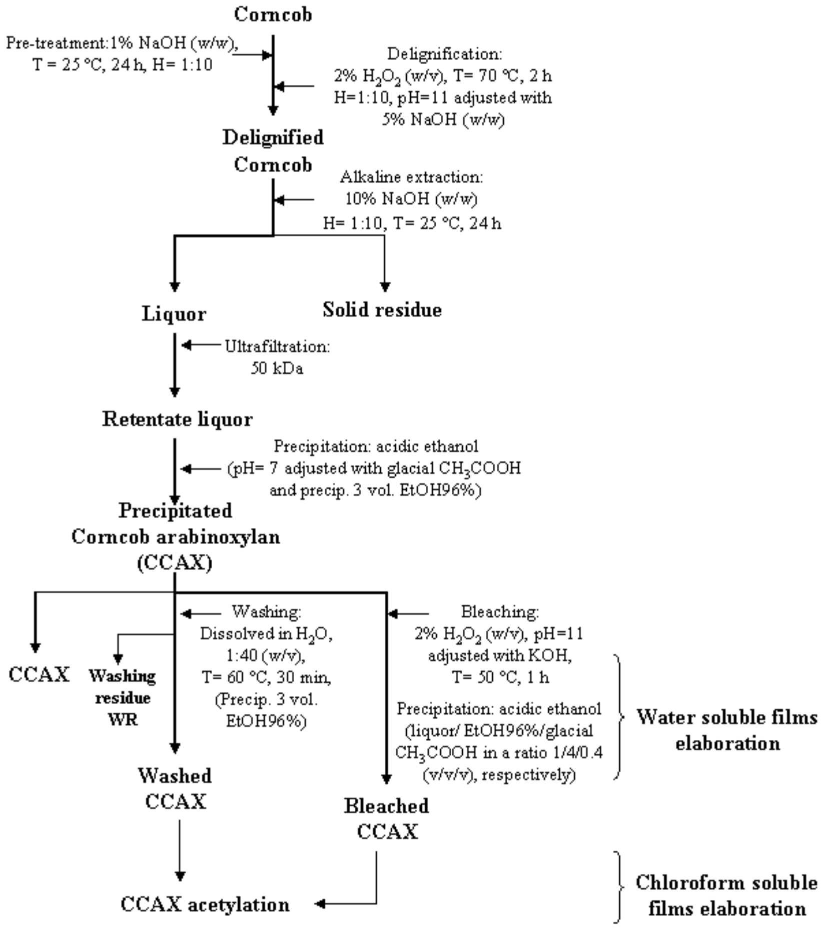
2.1 Arabinoxylan extraction

Corncob was collected from different places in northern Spain. It was ground in a hammer mill and sieved to obtain 4 to 6 mm-sized fractions. The hemicellulose extraction process is summarized in Fig. 1. The raw material was pre-treated with 1% NaOH (w/w) with a solid:liquid ratio of H= 1:10 (w/v), for 24 h at room temperature. After that, the swollen raw material was delignified at H= 1:10 (w/v) using hydrogen peroxide 2% (w/v) at pH 11 (adjusted with NaOH) and 70 ºC for 2 h. The delignified raw material was washed with distilled water and oven dried at 50 ºC. The material was then subjected to alkaline extraction using 10% NaOH (w/w) at 25 ºC for 24 h for hemicellulose extraction.

2.1.1 Ultrafiltration

The alkaline liquor obtained was ultrafiltrated using a 50 kDa ceramic membrane (Membralox XLAB 5, supplied by PALL Corporation, equipped with a 3 L double jacket feed tank, volumetric recirculation pump, membrane module and manometers) with the aim to concentrate the liquor and retain high molecular weight components, which are more suitable for material applications. The ultrafiltration temperature was controlled using a constant cooling water flux around the tank to avoid hydrolysate degradation by temperature. The pumping pressure was adjusted to its maximum level (4 bar) to improve the permeate flow rate. The ceramic membrane used (multichannel type with an external diameter of 10 mm and a length of 250 mm) was supplied by TAMI Industries. The CCAX molecular weight was previously determined to choose a suitable cut-off of the ceramic membrane. After ultrafiltration, CCAX was precipitated from retentate liquor by adjusting the pH to 7 with glacial acetic acid, and then using 3 volumes of ethanol. CCAX was then filtered and washed with excess ethanol. This CCAX was freeze-dried for further characterization, purification and preparation of films. The CCAX extraction yield was about 27.42% with respect to the initial dry corncob material.

Figure 1. Scheme of corncob arabinoxylan extraction, purification treatments, acetylation and film elaboration.



2.2 Arabinoxylan purification

The obtained CCAX powder had a yellowish appearance, and was subjected to either additional washing or bleaching processes as shown in Fig. 1. Washing was carried out by dissolving CCAX in deionized water at H= 1:40 (w/v), under stirring at 60 ºC for 30 minutes. Afterwards, washed CCAX (denoted as W) was precipitated by adding three volumes of ethanol and was then filtered and washed with excess ethanol. The washing liquor (which consisted of water and ethanol for precipitation and rinsing) was subjected to rotary evaporation in order to obtain the water soluble impurities (called washing residue, WR), which were used for further characterization. To remove possible residual lignin, which could provide films their yellowish appearance, CCAX was also subjected to a bleaching step. In this case, 1 g of CCAX was dissolved in 50 mL of deionized water, and 12 mg of diethylene triamine pentaacetic acid (DTPA) was then added as chelating agent. After 24 h under stirring, 2 mL of hydrogen peroxide (33% p/v, 110 vol.) was added, followed by sodium silicate (23 mg); the pH of the solution was adjusted to 11 using a few drops of 0.1 M KOH and bleaching was performed at 50 ºC for 1 h. Bleached CCAX (denoted as B) was recovered using acidic ethanol (hemicellulose liquor/ethanol/glacial acetic acid in a ratio of 1/4/0.4), and was filtered and washed with excess ethanol. Both washed and bleached CCAX were dried under vacuum at 40 ºC for further characterization, water soluble film preparation and acetylation.

The yields obtained after washing treatment and after bleaching treatment resulted on 70% and 66% (w/w) sample recovery respectively, which corresponded with 19.2% and 18.2% (w/w) of initial dry corncob.

2.3 Arabinoxylan acetylation

Washed and bleached CCAXs were subjected to acetylation for thermoplastic materials production according to Stepan et al. (2012) and Zinbo and Timell (1965). In brief, 1 g of each purified CCAX was dispersed in 25 mL of formamide under vigorous stirring at room temperature until a homogeneous phase was obtained. Then, pyridine (40 mL) was added. Every three hours, 6.6 mL of acetic acid anhydride was added up to a total of three additions. Thirty hours later, the viscous dark solutions were poured into 1.3 L of 2% ice-cold hydrochloric acid (w/w). Washed-acetylated CCAX (called WA) and bleached-acetylated CCAX (called BA) were filtered and washed with excess deionized water, followed by methanol and diethyl-ether. This washing step allowed the removal of unreacted acetic acid and pyridine from the samples. The acetylated CCAXs were dried in vacuum at 40 ºC for further characterization and film elaboration. The degree of substitution (DS) of the acetylated sample was determined by ^{1}H-NMR analysis. The obtained recovered yield (based on recovered mass) was about 80% for washed acetylated sample and 130% for bleached acetylated sample. The high recovery yield of BA may reflect the successful conversion of OH groups to $COOCH_3$, without apparent arabinoxylan depolymerization.

2.4 Characterization of different arabinoxylans

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) was performed using a Nicolet Nexus 670 FT-IR spectrometer (Madison, WI, USA) equipped with a single horizontal Golden Gate ATR cell (Specac, Kent, United Kingdom). Spectra were recorded using a spectral width ranging from 600 to 4000 cm^{-1}, with a resolution of 4 cm^{1} and an accumulation of 20 scans.

The thermal degradation of the samples was studied with a TGA/SDTA 851 analyzer from Mettler Toledo. Samples of 5 mg were heated from 25 to 600 ºC at a rate of 10ºC/min under constant nitrogen flow. The glass transition temperature (Tg) of all samples was determined using a differential scanning calorimeter with a Mettler Toledo DSC 822 equipped with a Sample Robot TSO 801 RO. About 5 mg of the sample sealed in perforated aluminium pans was used for each experiment. To avoid water interferences related to humidity, all samples were first heated to 110 ºC, cooled to 25 ºC and then reheated to 400 ºC under nitrogen gas at a flow rate of 10 mL/min.

For monomeric sugar quantification, CCAX samples were subjected to a post-hydrolysis process using sulphuric acid at a concentration of 4% (w/w) for 60 min. Quantification of monomeric sugars was carried out in a high performance liquid chromatography unit (HPLC) Jasco LC Net II/ADC equipped with a refractive index detector and a photodiode array detector. A Phenomenex Rezex ROA HPLC column with 0.005 N H_2SO_4 prepared with HPLC grade degassed water was used as the mobile phase (0.35 mL/min flow, 40 ºC and injection volume 40 μL). High purity standards of

D (+) glucose, D (+) xylose, D (-) arabinose, (supplied by Sigma Aldrich) were used for the calibration curves.

Klason lignin of the samples was determined following the method proposed by Theander and Westerlund (1986). In brief, 3 mL of 72% of H_2SO_4 was added to 200 mg sample and was maintained for 30 min in a water bath (30 ºC, 1 h). Then, 84 mL of deionized water was added, and sample solution was hydrolyzed at 121 ºC for 1 h in autoclave. After that, the solid residue was filtrated, oven dried and weighted as Klason lignin.

The weight-average (Mw) and number-average (Mn) molecular weights and polydispersity index (PDI= Mw/Mn) of the samples were determined by size exclusion chromatography (SEC) in a HPSEC–MALS–RI–UV aqueous system Waters 2690 (Waters Corporation, Milford, MA, USA). The system had an online degasser; auto sampler; column oven and three serial connected columns (Shodex OHpak SB-803, 804 and 806 M HQ) controlled at 50 ºC. The detectors were multi-angle laser light scattering (MALS; DSP equipped with a He–Ne laser at 632.8 nm, Wyatt Technology Corp., Santa Barbara, CA, USA); refractive index (RI) controlled at 35 ºC (Optilab DSP, Wyatt Technology Corp.) and a UV–vis detector set to record at 280 nm (Shimadzu SPD-10A, Shimadzu Corp., Kyoto, Japan). The eluent used was sodium nitrate (0.1 M $NaNO_3$) containing 0.02% sodium azide (NaN_3). The flow rate was 0.4 mL/min. All samples were dissolved in 0.1 M $NaNO_3$ solution at a concentration of 2% (w/w) and filtered (GHP syringe filter, 0.45 µm, PALL). The dn/dc used was 0.146 mL/g (Dervilly et al., 2002). The range of angles available for determination was 30-150º. The weight- and number-average molecular weights were determined using ASTRA3 software.

The degree of acetylation was calculated by ^{1}H-NMR spectrometry using a Bruker 500 MHz spectrometer at a frequency of 250 MHz with an acquisition time of 0.011 s, at room temperature. The spectrum was recorded over 32 scans, and DMSO-d_6 was used as the solvent for acetylated CCAX.

2.5 Film elaboration and material properties

Water soluble films were prepared by solvent casting using 0.5 g of sample in 20 mL of deionized water. In the case of acetylated CCAX, chloroform was used. The homogeneous solutions were placed into petri dishes with a diameter of 6 cm. Water soluble films were dried at 25 ºC and 50% RH in a climate-controlled chamber, whereas chloroform soluble films were dried at ambient conditions.

The hydrophobic/hydrophilic character of the films was evaluated by the water contact angle. A static contact angle with an NRL C.A and Goniometer from Ramé-hart (model 100-00 230) with a lamp from LEP (model 990018) was used. A 5 µL drop of deionized water was placed on the surface of each film. The contact angle was read immediately and after 45 s contact. The average value of five measurements was calculated.

Tensile tests were conducted in a DMA Q800 (TA Instruments) with an attached RH control unit to allow for controlled relative humidity during the tests. Samples were cut from the films using parallel razor blades with an inter-distance of 5.73 mm. The film thickness was measured using a micrometer (Mitutoyo). The samples were mounted in the testing chamber with an approximate distance between the clamps of 1.4 cm. A conditioning step of isothermal status at 25 ºC, 50% RH, for 30 min was performed before testing the samples. A preload of 0.01 N was used, and the stress was ramped with 0.25 N/min from 0 to 18000 N. Three replicates were tested for each film. In the case of acetylated CCAX films, MTS (Insight 10) equipment provided with pneumatic clamps (Advantage Pneumatic Grips) and with a loading cell of 250 N was used. The stress/strain measurements were performed using a video extensometer with a digital video camera (EX Sigma, 105mm 1:2.8 DG Macro) connected to a PC. The starting distance between the clamps was 25 mm, and the films were strained at 3 mm/min at ambient conditions. The results were averaged from a minimum of five specimens.

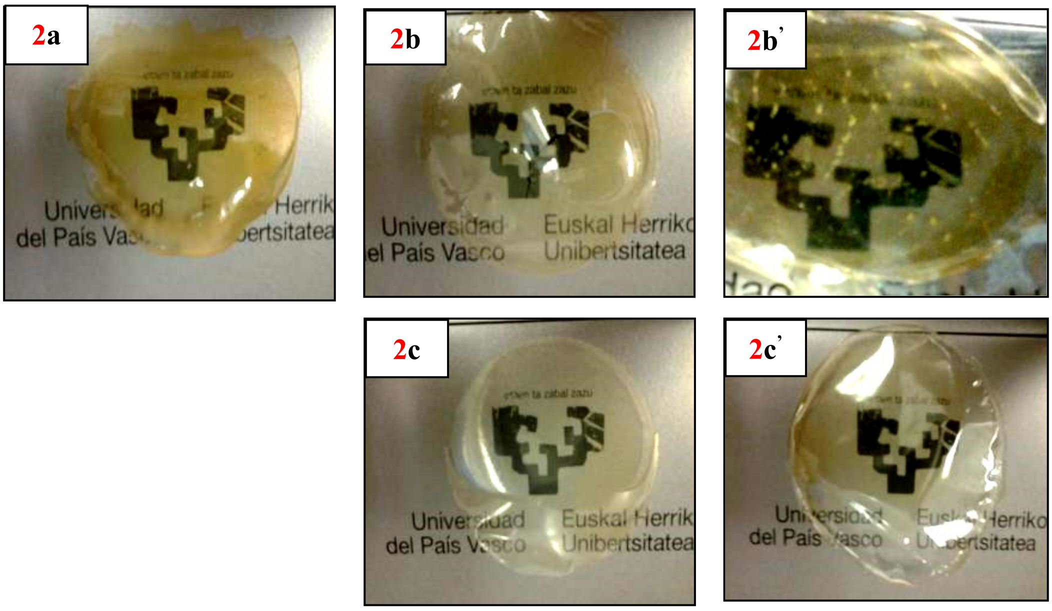
A DMA Q800 (TA Instruments) connected to a humidity controller was used for dynamic mechanical analysis under different humidity conditions. Samples for analysis were cut from the films using parallel razor blades with an inter distance of 5.73 mm. The film thickness was measured using a micrometer (Mitutoyo). The samples were mounted in the testing chamber with an approximate distance between the clamps of 1.5 cm. The samples were loaded at a 1 Hz frequency, 125% force track, 0.1 N preload force and an amplitude of 10 μm. The temperature was fixed at 25 °C, and the humidity was ramped with 1% RH per 10 min starting at 10% RH running up to 90% RH.

3. Results and discussion

3.1 Elaboration of films

The films obtained using CCAX, as well as the purified and acetylated ones, are shown in Figure 2. All films look transparent, are easy to handle and are without apparent fractures, with the exception of the film made using untreated CCAX (Fig. 2a), which contained some opaque zones and was more brittle. As can be observed, the yellow appearance of untreated CCAX film was slightly reduced after the washing step (Fig. 2b), whereas considerable color removal was obtained by bleaching (Fig. 2c). On the other hand, the film made with washed-acetylated CCAX (Fig. 2b’) showed some aggregations, owing to partial insolubility of the sample in acetylation medium and then in chloroform. The partial insolubility showed by WA could be owed to its higher lignin content (vide infra, Table 1) and/or by supramolecular aggregation of hemicellulose, which could leave the arabinoxylan less accessible to acetylation reagents and further reaction. However, the bleached-acetylated CCAX was completely soluble in acetylation medium and also in chloroform and presented a homogeneous and less colored plastic film (Fig.2c’). The thickness of the films was about 0.05 ± 0.02 mm.

Figure 2. Water soluble films made of: untreated CCAX (2a); washed CCAX (W), (2b); bleached CCAX (B), (2c). Chloroform soluble films made of purified and acetylated samples: washed-acetylated CCAX (WA), (2b’); bleached-acetylated CCAX (BA), (2c’). All films were cast from solution at 2.5 wt% xylan concentration.



3.2 FT-IR spectra

Fourier transform infrared spectra of different samples are shown in Fig. 3. The bands found at 3420 and 2920-2850 cm^{-1} indicate the OH stretching and CH bond deformation of CH_2-CH_3 groups (Fig.3a). Especially in the untreated CCAX, two highlighted peaks at 1560 and at 1405 cm^{-1} can be observed that correspond to CH and OH bending, respectively (Sun & Tomkinson, 2002). These peaks are also observed in the washing residue. Further peaks found in the washing residue at 1012, 920 and 648 cm^{-1} indicate the presence of CH_3COONa salts, whereas the peak observed at 808 cm^{-1} could indicate C-H of the aromatic ring of residual lignin (highlighted also in untreated

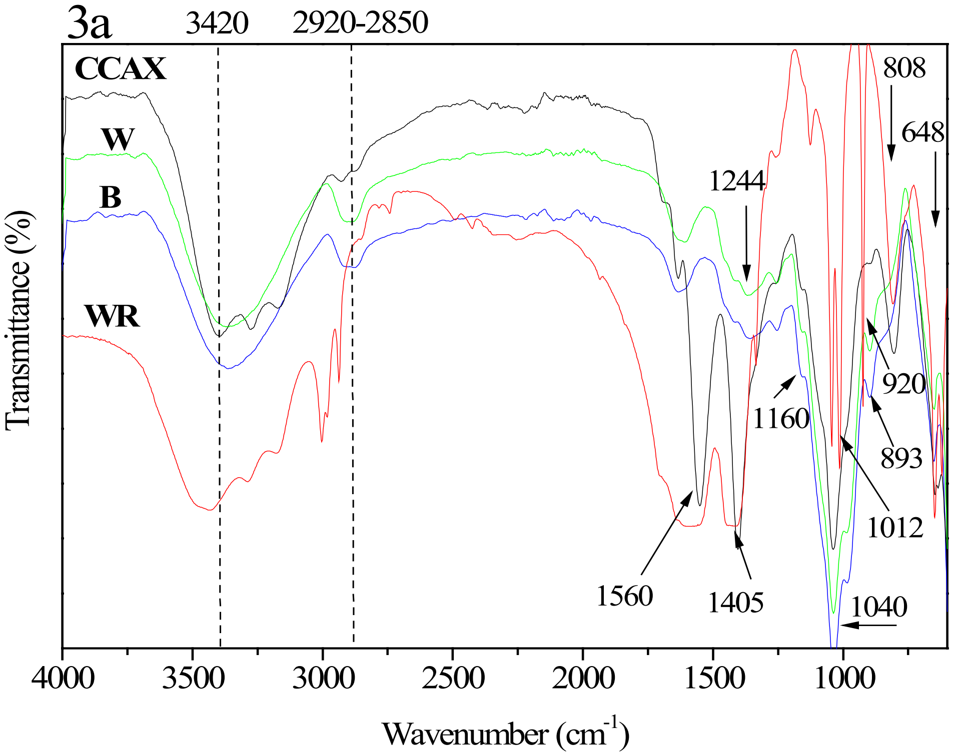
CCAX). Both, salts and residual lignin, could precipitate together with CCAX from the retentate liquor, being the origin of the poor material's properties of films prepared from CCAX (see Table 3). In fact, after CCAX purification these peaks disappeared, either after washing or bleaching treatment.

In washed (W) and bleached sample (B), -C–O- stretching band at 1244 cm^{-1} could be detected. On the other hand, the presence of xylan at 1040 cm^{-1} and arabinose residues at 1160 cm^{-1} was observed, whereas the domain of β-glycosidic bonds between sugars is shown at 893 cm^{-1}. These facts indicate that CCAX was constituted by a xylan backbone with arabinose residues, in addition to salt and residual lignin impurities, which were removed after the purification processes.

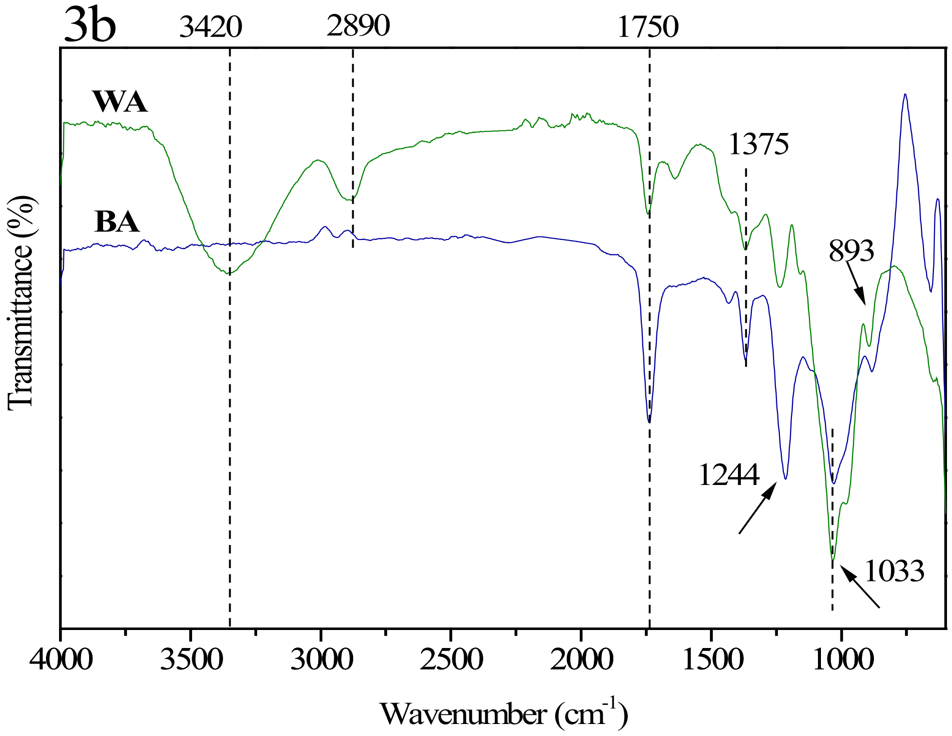
In the case of the bleached-acetylated CCAX (denoted BA) shown in Fig. 3b, evidence of acetylation was observed by the presence of three important ester bands at 1750 cm^{-1}, C=O ester, at 1375 cm^{-1}, $–C–CH_3$, and at 1244 cm^{-1} , –C–O– stretching band (Sun et al., 2004a). The lack of peaks at 1840-1760 cm^{-1} suggests that the product is free of the unreacted acetic anhydride (Sun et al., 2004a). In the case of washed-acetylated CCAX (denoted WA), an unreacted hydroxyl group (–O–H) could be observed at 3420 cm^{-1}, indicating an incomplete acetylation, which could explain its partial insolubility in chloroform (Fig. 2b'). The low acetylation efficiency (DS less than 0.10) might be owed to higher lignin content that promotes aggregation and formation of hemicelluloses supramolecular structures that hinder the acetylation reaction (Westbye, Köhnke, Glasser, & Gatenholm, 2007). The poor quality and low solubility in chloroform of the WA film, in addition to its yellowish appearance, made it unsuitable for further film characterization.

Figure 3. Fourier transform infrared spectroscopy (FT-IR) spectra. Fig. 3a: untreated corncob arabinoxylan (CCAX), purified CCAX (W= washed, B= bleached) and washing residue (WR). Fig. 3b: spectra of acetylated CCAX: (WA= washed-acetylated, BA= bleached-acetylated).

3a



3b



3.3 Thermal analysis

The thermal behavior of samples is shown in Fig. 4a and b. A sudden drop in the TGA curve of untreated CCAX can be seen during the early stage of heating, derived from water loss. CCAX was then stable up to 220 ºC (Fig. 4a) whereas the maximum weight loss rate was observed at 280 ºC (Fig.4b). After the washing step, the thermal stability of the W sample improved up to 230 ºC (Fig.4a) and the maximum weight loss occurred at 300 ºC (Fig.4b). However, after bleaching of CCAX (B), the CCAX thermal stability decreased slightly to 210 ºC, probably due to the presence of acetic acid from the precipitation stage (see Section 2.2) and/or less lignin content (see Table 1). On the other hand, a significant thermal stability improvement was achieved after acetylation (BA), (T= 325 ºC), with maximum weight loss at 375 ºC, indicating a higher thermal resistant behavior, desired for thermoplastic processing conditions. This higher thermal stability reflected the decrease in the number of hydroxyl groups, which are oxidized during heating (Fundador, Enomoto-Rogers, Takemura, & Iwata, 2012). Finally, a small peak at 450 ºC (not detected in the other samples, Fig. 4b) could indicate the presence of residual lignin in CCAX.

The results obtained by differential scanning calorimetry (DSC) are shown in Fig. 5. As can be observed, DSC thermographs also revealed a clear upward shift of the degradation temperature (T_d) of the bleached acetylated sample (BA). CCAX, W and B samples gave a broader endothermic peak, attributed to the disintegration of intramolecular hydrogen bonds and degradation, whereas a more open structure could be observed in the BA sample, owed to lower hydrogen bonding due to acetylation (Ren, Sun, Liu, Lin, & He, 2007; Sun, Sun, Tomkinson, & Baird, 2003). The transition temperature (Tg) of xylan is reported to be in the range of 167-180 ºC (Irvine, 1984). In this study a Tg of 175 ºC was observed in CCAX. Furthermore, a melting temperature of salt impurities was observed in CCAX (T_m = 325 ºC), which disappeared after the washing or bleaching treatment. On the other hand, no clear Tg value was found for W sample, however, a glass transition temperature at 130 ºC was detected for sample B. The lower Tg of B as compared to CCAX is probably due to the presence of acetic acid, derived from the precipitation step (see section 2.2), as it has been found that acetic acid reduce Tg in other polysaccharides such as starch (Shogren, 2000). After bleaching and acetylation, the Tg of BA was lowered to 125 ºC, which resulted from less hydrogen bonding after acetylation.

Figure 4. Thermogravimetric analysis. Fig 4a-b: TGA and DTG of untreated CCAX, purified CCAX (W= washed, B= bleached), and bleached-acetylated CCAX (BA).

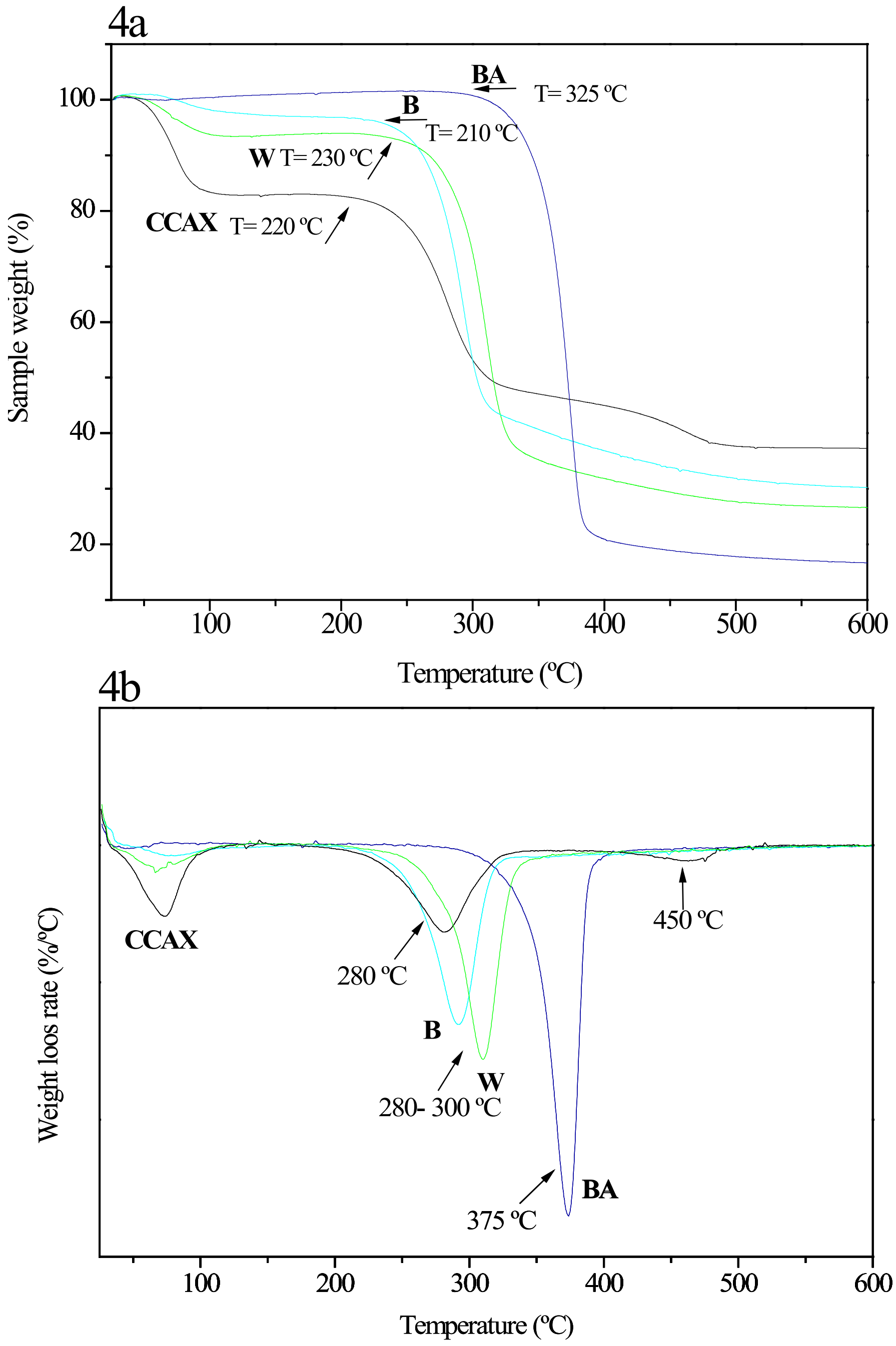
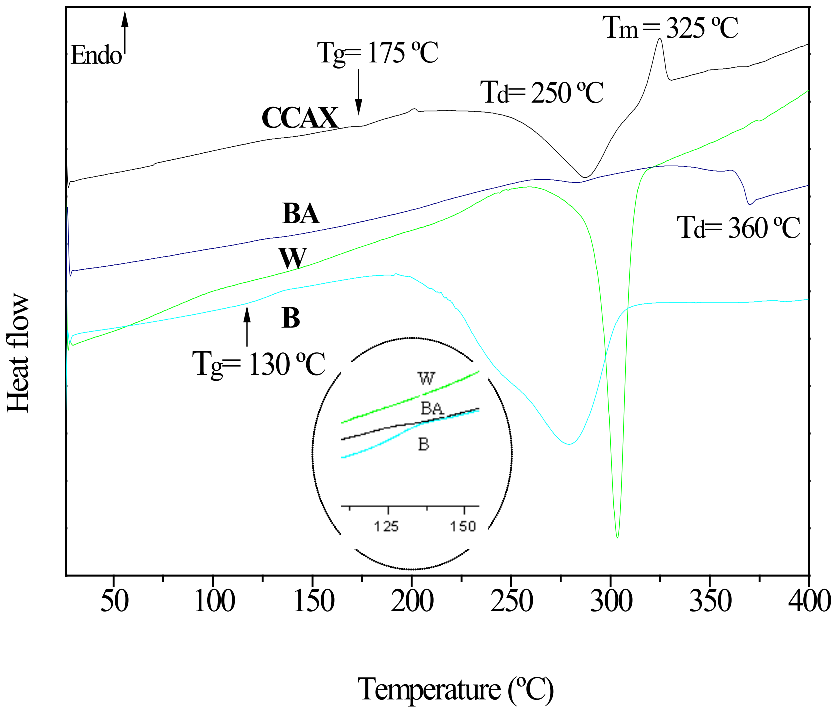


Figure 5. Differential scanning calorimetry (DSC) of untreated corncob arabinoxylan (CCAX), purified CCAX (W= washed, B= bleached) and bleached-acetylated CCAX (BA)



3.4 High performance liquid chromatography (HPLC) and Klason lignin

As pointed out in Table 1, the sugar content and composition of corncob arabinoxylan remained constant after the washing and the bleaching treatment (~ 86-87% xylose, 10-11% arabinose and 1-3% glucose). Also, the yields of detected sugar were improved with the purification processes. On the other hand, the residue obtained from the washing step had low sugar content. The results confirmed that the extracted corncob hemicellulose in this study was constituted mainly of xylose units, followed by small quantities of arabinose and glucose. On the other hand, high contents of Klason lignin were obtained in CCAX and WR, about 6.0% and 6.20%, respectively. These results are in agreement with IR, where lignin presence was more evident in CCAX and WR. Furthermore, the peak observed in CCAX in DTG at 450 ºC, was also in accordance with this result. On the other hand, a slightly lower percentage Klason lignin was obtained after bleaching process, about 4.60%. It should be born in mind that Klason lignin includes besides lignin other molecules that are not soluble in the extraction medium, such as supramolecular aggregates, cellulose etc. Furthermore, the partial insolubility showed by sample W (subjected to a washing step) in the acetylation process could be owed to the higher lignin content and/or supramolecular aggregate formation of hemicellulose. Consequently, bleaching process improved the acetylation procedure by decreasing the Klason lignin content and by opening the hemicellulose structure.

Table 1. Sugar analysis and Klason lignin content of different samples: corncob arabinoxylan (CCAX), washed CCAX (indicated as W), bleached CCAX (indicated as B) and washing residue (WR).

		Sugar analysis (%)				
Sample	Glucose[a]	Xylose[a]	Arabinose[a]	Ara/Xyl ratio	Yield (%)[b]	Klason lignin(%)[c]
CCAX	1.60	87.6	10.8	0.12	66.30	6.00
W	2.16	86.6	11.2	0.13	92.60	5.50
B	1.97	86.6	11.5	0.13	85.10	4.60
WR	9.5	19.04	71.4	3.75	2.10	6.20

[a] Percentage with respect to the detected total sugars (w/w).

[b] Yield: sugars detection respect to initial sample (w/w).

[c] Klason lignin: percentage respect to the initial sample (w/w), average of two experiments (less than ± 0.3 experimental error).

3.5 Size Exclusion Chromatography (SEC)

The weight-average (M_w) and number-average (M_n) molecular weights and polydispersity index (PDI) of the samples are shown in Table 2. As observed, the permeate liquor was constituted of low molecular weight components Mw= 3943 g/mol. The molecular weight fraction of CCAX was about Mw= 21170 g/mol, and showed a high polydispersity. After the purification treatments the detected molecular weights were similar for both washed and bleached treatments, i.e., Mw= 25370 for W and Mw= 26200 for B. Although W and B samples have higher molecular weights than CCAX, the elution profiles were similar (not shown). These results showed that the molecular weight of sample B was not affected by bleaching process. On the other hand, the polydispersity index decreased both in W and B comparing to CCAX, probably due to the purification and precipitation steps. The molecular weight of the acetylated sample could not be analyzed due to its incompatibility with the mobile phase. However, taking into account the high recovery yield in BA (Section 2.3), it would be expected that the sample has the same or higher molecular weight.

Table 2. Molecular weight of precipitated components from different ultrafiltration fractions: corncob arabinoxylan (CCAX) and purified ones: washed CCAX (indicated as W) and bleached CCAX (indicated as B).

Sample	M_w[c]	M_n[d]	PDI[e]
Permeate fraction (< 50 kDa)[a]	3943	2303	1.71
Retentate fraction, CCAX (>50 kDa)[b]	21170	8852	2.39
W	25370	18570	1.37
B	26200	20030	1.30

[a] Molecular weight of the precipitated components from permeate liquor.
[b] Molecular weight of the precipitated components from retentate liquor, called as CCAX.
[c] M_w: weight-average molecular weight (g/mol).
[d] M_n: number-average molecular weight (g/mol).
[e] PDI: Polydispersity index (Mw/Mn).

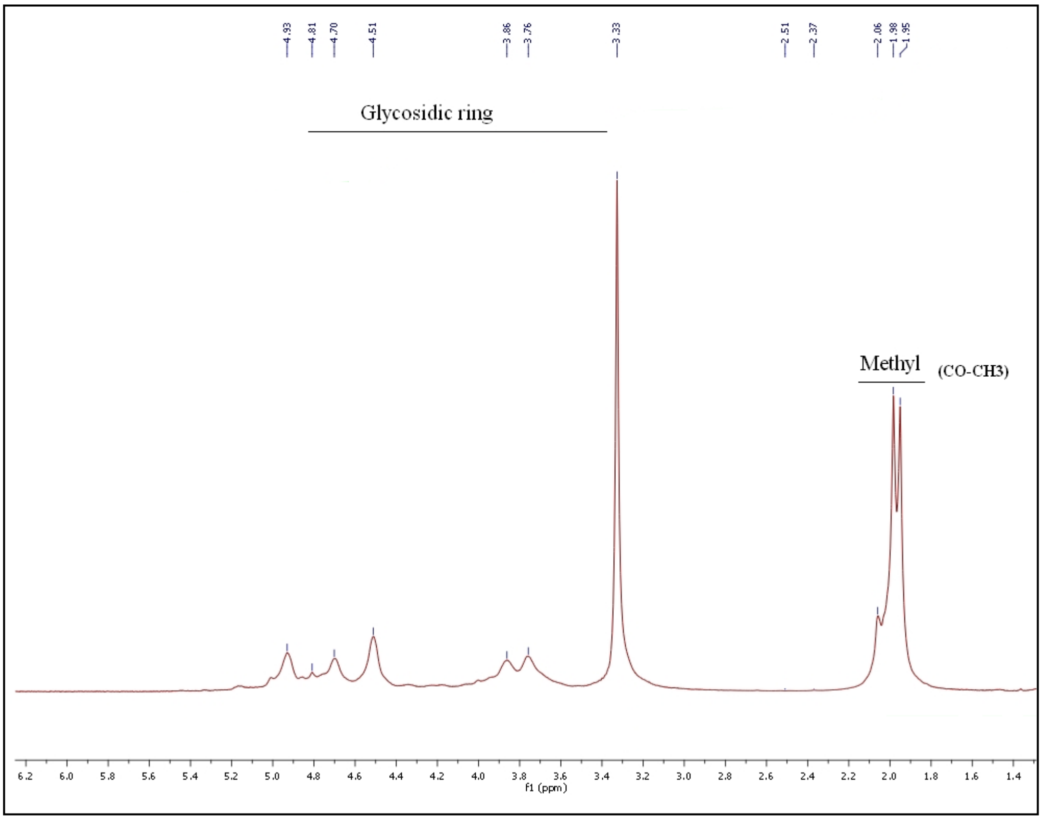
3.6 Nuclear magnetic resonance (H NMR)

The degree of substitution (DS) of the acetylation reaction was determined by ^{1}H NMR following the method used by Belmokaddem et al. (2011). The area of methyl protons of ester chains at 1.9-2.0 ppm (I_{methyl}) and the area of anomeric proton of xylan at 4.5 ppm ($I_{anomericXylan}$) were compared as shown in Eq. (1):

$$DS = {I_{methyl}} \Big/ {3 \times I_{anomericXylan}} \qquad (1)$$

In this study, the value of DS for bleached-acetylated CCAX was DS= 1.90. Taking into account that the average number of available OH groups per sugar unit is DS= 2, the degree obtained can be considered an acceptable value. As can be seen in Fig. 6, the methyl protons of the acetyl groups gave a strong peak at 1.9-2 ppm. Furthermore, the spectrum showed the typical signal of anhydroxylose protons between 3.0 and 4.2 ppm and its anomeric proton at 4.5 ppm. α-D-arabinofuranosyl anomeric proton signal can be observed as well at 5.0 ppm (Sun, Sun, Sun, & Su, 2004).

Figure 6. ^{1}H NMR spectrum of bleached-acetylated CCAX (dissolved in DMSO-d_6).



3.7 Mechanical properties and contact angle

As summarized in Table 3, the purification processes dramatically improved CCAX tensile properties. Specifically, the washing step and bleaching treatment allowed to obtain higher tensile stress (from 9 to 53 MPa) and higher Young's modulus (from 293 to 1400-1600 MPa), whereas the elongation at break was similar. The elongation of the film made of untreated CCAX film (8.10%) could have been owed by its hygroscopic nature in which water acts as a plasticizer. Lignin removal did not affect the elongation results of films made of W and B samples (about 7-8%), where water could act again as main plasticizer. However, the removal of sodium acetate improved significantly the mechanical properties on these films, whereas the possible residue of acetic acid found in sample B did not affect the mechanical properties. Corn hull arabinoxylan films were produced by Zhang and Whistler (2004) with similar material properties (stress at break= 53.8 MPa, strain= 6.2% and Young modulus= 1316 MPa). Also, strong water soluble films of delignified arabinoxylans from barley husks were made by Höije, Gröndahl, Tømmeraas and Gatenholm (2005) (stress at break= 50 MPa, strain= 2.5% and Young's modulus= 2900 MPa).

In this study, the acetylation process allowed the highest values of tensile strength, 67 MPa, 13.4% of elongation at break and 2241 MPa of Young's modulus. The elongation was improved, due to internal plasticization of acetyl groups. At the same time, the strength and modulus were enhanced, owed to the elimination of OH groups and reduced water sensitivity. Acetylation of bleached xylan resulted in a material with better mechanical properties.

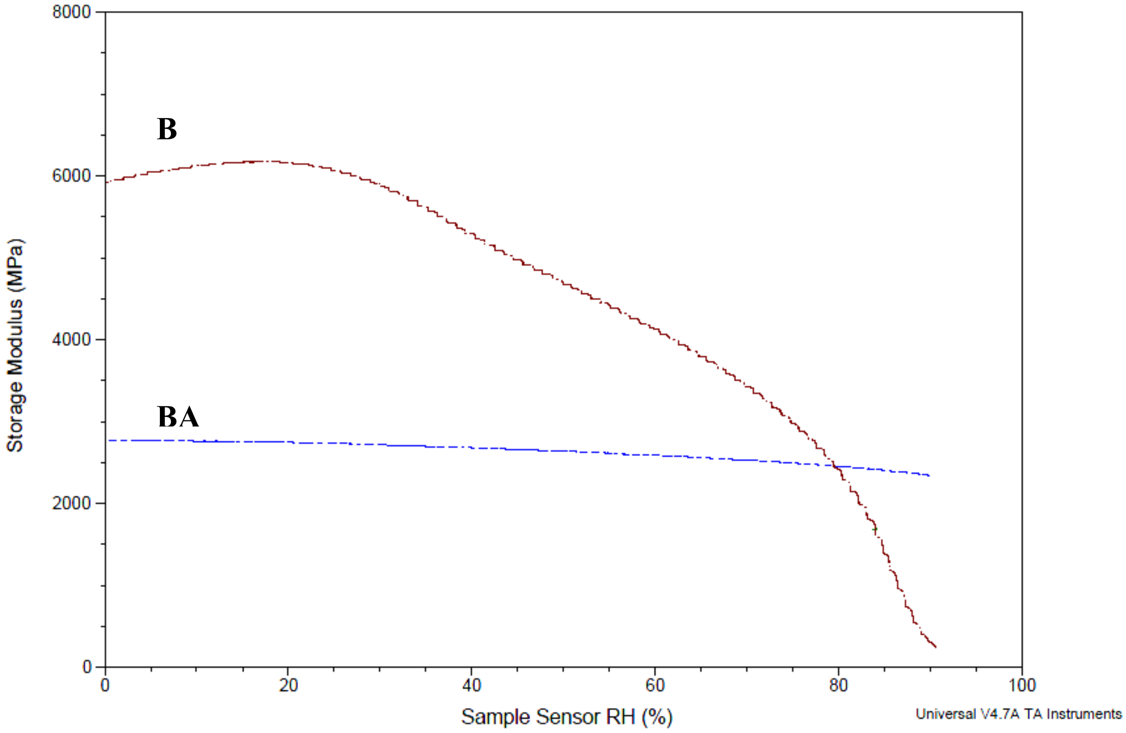
Table 3. Mechanical properties and water contact angle of water soluble films made of: corncob arabinoxylan (CCAX), washed CCAX (W), bleached CCAX (B); and chloroform based film prepared with bleached-acetylated CCAX (BA).

Sample	Stress (MPa)	Strain (%)	Young's modulus (MPa)	Contact angle (°)	
				t= 0 s	t= 45 s
CCAX	9.02 ± 0.74	8.10 ± 2.50	293 ± 90	59.0 ± 5.3	21.3 ± 1.7
W	53.54 ± 12.8	7.08 ± 1.31	1662 ± 268	78.8 ± 3.0	67.9 ± 5.0
B	53.56 ± 4.66	8.17 ± 0.93	1432 ± 437	77.6 ± 4.9	74.3 ± 3.9
BA	67.30 ± 12.2	13.4 ± 1.55	2241 ± 353	82.1 ± 5.9	80.5 ± 5.8

A sudden drop of the contact angle was observed in the film made of CCAX at 45 s, from 59° to 21.3°. This was probably due to its hygroscopic nature and the presence of sodium acetate salt in CCAX. The contact angle of the film made of CCAX increased considerably from ~21.3° to 67-74° with purification treatments. The higher contact angle showed by films made of W and B initially as well as at 45 sec, showed the effectiveness of the purification processes on the removal of salts. However, the contact angle of W and B samples decreased considerably after one minute, showing the hygroscopic nature of arabinoxylan. On the other hand, the contact angle of BA film showed about 82-80°. A contact angle of 45° was reported for unmodified switchgrass arabinoxylan by Ayoub et al. (2013) where the contact angle was improved to 83° by hemicelluloses acetylation (D.S= 1.25). Although in this study initially bleached-acetylated film showed a similar contact angle as that of the purified ones (W and B), this sample clearly showed more water resistant behavior over time.

Humidity scans in DMA show the influence of humidity on material storage modulus. As shown in Fig. 7, the storage modulus of films made with bleached CCAX was highly dependent on RH. The storage modulus started to decrease at 25% RH and was reduced 50% of the initial modulus value at 80% RH. The material becomes rapidly less stiff. Dammströn, Salmén and Gathenholm (2005) observed a decrease in modulus at about 85% RH for pure aspen glucuronoxylan. Escalante et al. (2012) also observed that the arabinoglucuronoxylan film softened at relative humidity of 80% RH. This result showed the hygroscopic nature of the film made of bleached arabinoxylan, where the humidity acts as plasticizer and reduces the stiffness of the material. However, the storage modulus became independent of RH after acetylation. This is in agreement with previous research showing a decrease in water diffusion with acetylation (Ayoub et al., 2013). The increase of RH softening the material by addition of MFC or nanocellulose reinforcement was also reported (Mikkonen et al., 2011; Stevanic et al., 2011). The strategy used in this study, allowed obtaining acetylated CCAX that is completely independent of surrounding humidity. This hydrophobic character could be related to the acetyl groups attached on the hemicellulose by acetylation process.

Figure 7. Mechanical properties of bleached (B) and bleached-acetylated film (BA) against humidity.



4. Conclusions

In this contribution, the extraction, purification and acetylation of corncob arabinoxylan was developed until a suitable matrix for film production was obtained. Washing and bleaching treatments of corncob arabinoxylan allowed improving considerably the material properties of films made with non-purified corncob arabinoxylan. The poor properties of films made with non-purified corncob arabinoxylan were caused mainly by the presence of sodium acetate salts. The salt impurities were eliminated both by washing or bleaching treatments. Moreover, bleaching reduced the Klason lignin content providing less colored film and a suitable hemicellulose for further acetylation process. In this case, the acetylation improved the arabinoxylan mechanical properties even more. By combining bleaching and acetylation of the CCAX it was also possible to prepare a hemicellulose film, which has mechanical properties independent from the surrounding RH. This dramatically broadens possible fields of application, a fact that has been sought for hemicellulose films since long. Bleached-acetylated CCAX represents a suitable material for further material applications.

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