Arabinose Content of Arabinoxylans Contributes to Flexibility of Acetylated Arabinoxylan Films

A. M. Stepan,[1,2] A. Höije,[1] H. A. Schols,[3] P. de Waard,[4] P. Gatenholm[1,2]

[1]*Department of Chemical and Biological Engineering, Biopolymer Technology, Chalmers University of Technology, SE-412 96 Göteborg, Sweden*
[2]*Wallenberg Wood Science Center, Department of Chemical and Biological Engineering, Biopolymer Technology, Chalmers University of Technology, SE-412 96 Göteborg, Sweden*
[3]*Department of Agrotechnology and Food Sciences, Laboratory of Food Chemistry, Wageningen University, 6703 HD Wageningen, The Netherlands*
[4]*Wageningen NMR Centre, 6700 ET Wageningen, The Netherlands*

Received 27 April 2011; accepted 7 November 2011
DOI 10.1002/app.36458
Published online 22 January 2012 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Arabinoxylans (AX) from rye were partly debranched by chemical hydrolysis methods, and AXs differing in arabinosyl substitution were acetylated using chemical methods. The resulting materials are film forming, and these films underwent molecular structural analysis and were tested for their material properties. The composition and structure of the modified polymers were determined using high performance anion exchange chromatography and two dimensional nuclear magnetic resonance; it was shown that all free hydroxyl groups (of both xylose and arabinose) were acetylated. Further characterizations were done by dynamic mechanical analysis and thermo-gravimetric analysis to evaluate the thermal behavior of the material. The observed glass transition temperatures (T_g) increased with a decrease in arabinosyl substitutions. The thermal degradation temperatures were all close to 380°C. The mechanical properties were characterized with tensile tests of the films. Tensile tests showed that the strain at break, which reflects the flexibility of the material, was significantly higher at higher arabinosyl substitution levels. The elastic Young's modulus was not significantly affected, although a tendency was seen toward a less stiff material at higher arabinosyl substitution. The ultimate strength of the materials was remarkably high in all cases, around 60 MPa, with little difference between them. Considering these properties, a great potential is foreseen in the application of acetylated arabinoxylans as packaging films and as matrix for composites. © 2012 Wiley Periodicals, Inc. J Appl Polym Sci 125: 2348–2355, 2012

Key words: hemicellulose; arabinoxylan; acetylation; degree of substitution; mechanical properties; films

INTRODUCTION

Global warming, carbon dioxide emissions, and a shortage of fossil fuels are the largest environmental problems humanity has faced. Using renewable resources to produce biodegradable plastic materials is one of the obvious solutions to the growing environmental problem of waste disposal and exhaustion of the planet's nonrenewable resources.[1] Hemicelluloses, the second most abundant biopolymers on Earth, and their derivatives have a great potential as bio-based and biodegradable packaging materials, binders, matrix for bio-based composites, extrusion coatings or melt spinning of precursors of carbon fibers. It is also generally highlighted that the structure and functionality of hemicelluloses require further research to provide broader opportunities in material applications.[2,3]

Hemicelluloses comprise a group of biopolymers that are closely associated with cellulose and are usually a mixture of heterogeneous polysaccharides that have a complex composition and structure.[4] The most common monosaccharide units that build up the hemicelluloses are: D-xylose, L-arabinose, D-glucose, D-galactose, D-mannose, D-glucuronic acid (and its 4-O-methyl derivative), and D-galacturonic acid.[3,5,6] Depending on their type and origin, these heteropolymers have an average degree of polymerization around 200.[4,6] Hemicelluloses are often highly branched and have different substitution patterns and frequencies of sugar substituents and/or acetyl or feruloyl groups. The ones containing a high degree of side-chain substitution are more water soluble (and bind less tightly to cellulose), whereas molecules with infrequent side chains are less water soluble (and bind more tightly to cellulose).[6,7] Several studies have been carried out to derivatize free hydroxyl groups of hemicelluloses, which in some cases resulted in preventing chain interactions and in film forming materials. For example, Glasser et al. prepared thermoplastic materials.[8,9] Relevant reviews have been written in the last decade about the isolation, characterization and derivatization of hemicelluloses.[2,10–13]

Correspondence to: P. Gatenholm (paul.gatenholm@chalmers.se).

Smart and Whistler reported film forming hemicellulose acetates already in 1949.[14] Some studies were also carried out on the preparation and thermal properties of arabinoxylan acetates, but the mechanical properties of the films were not evaluated.[15–18] Sun and coworkers reported about extensive studies on different methods of isolation and esterification of hemicelluloses from different sources, in attempts to preserve high molecular weight and improve the thermal stability of the polysaccharides.[19–24] There are further reports on producing thermostable hemicellulose acetates.[25,26] Some research has also been done by Sternemalm and coworkers on the level of arabinosyl substitution in arabinoxylans, but the properties of different derivatives were not investigated.[27,28]

The aim of this study was to investigate the effects of arabinosyl substitution level on material properties of acetylated arabinoxylans (AX) films. Because of application potentials of this material, special attention was paid on the effect of arabinosyl substitution on the mechanical properties of these films. Arabinoxylans from rye were selected as a model of substituted xylans and were debranched to form series of arabinoxylans with a gradient of arabinosyl substitution.

EXPERIMENTAL

Materials

These experiments used rye AX from Megazyme (Ireland) (~ 95% purity, LOT 20601a). The chemicals were purchased from different providers: for acetylation and film casting, acetic acid anhydride from Fluka/Sigma-Aldrich (45830) (Switzerland), pyridine from Riedel-de Haën/Sigma-Aldrich (16037) (Sweden), formamide from Fluka/Sigma-Aldrich (47670) (Sweden), ethanol (95%) from Solveco (1394) (Sweden), diethyl-ether from Fluka (31700) (Switzerland), chloroform from Sigma-Aldrich (372978) (USA) and hydrochloric acid (35%) from VWR International S.A.S. (France). Apart from these, oxalic acid from Fluka/Lab Kemi (75690) (Switzerland) was used to do the debranching of arabinoxylans, and sodiumhydroxide from Riedel-de Haën (30620), sulphuric acid (>95%) from Fisher Scientific (UK), L-(+)- Arabinose (>99%) from Fluka (Germany), D-Xylose(>99%) from Aldrich (WI) and L-(−)-Fucose (>99%) from Sigma-Aldrich (Slovakia) were used in the sugar analysis processes. Potassium sulphate from Riedel-de Haën (31270).

Debranching

Chemical debranching was performed using protocols described elsewhere.[28] The different levels of debranching were done in parallel experiments using 1g of rye AX for each batch. One gram of dry powder AX was added to 100 mL 0.1 M oxalic acid water solution and the reaction took place at 50°C for different periods of time (7 h 20 min, 12 h 40 min, 18 h). The reaction was stopped by adding four times excess of 96% ethanol and refrigerating samples in +4°C. Thereafter, the samples were centrifuged at 2000 × *g* (relative centrifugal speed expressed in units of gravity) for 12 min, and the ethanol was decanted. Samples were washed carefully with ethanol and decanted again, after which they were dried in a freeze drier for 16 h. Characterization and further derivatization were then carried out on the samples.

Sugar analysis

The sample preparation for sugar analysis was carried the following way: 200 g dry AX was placed in a 200 mL beaker and 3 mL of 72% H_2SO_4 was added. Samples were impregnated in a desiccator under vacuum for 15 min. The material was completely covered by the acid and left at 30°C in a water bath for 1 h. Eighty-four milliliter of deionized water was then added. After mixing, it was covered with an aluminum sheet. Hydrolysis was carried out for 1 h in an autoclave at 125°C (samples were removed from the autoclave after 30 more minutes). This hydrolysed material was vacuum filtered (0.22 μm). Samples were diluted to appropriate concentrations, and the internal standard was added.

The sugar composition of control AX and debranched samples were measured using an ICS 3000 Dionex from Dionex Sweden AB equipped with a gradient pump, isocratic post column pump, amperometry detector, column, oven and auto sampler; compartments were provided by Dionex Sweden AB. Operating conditions were the following: all eluents were de-aired and kept under helium pressure (4 psi). Sodium hydroxide solutions were prepared after de-airing deionized water. Sodium hydroxide of 50% solution (UN 1824) was used to prepare the eluents. The system was primed for each solvent. The AEC column was a CarboPac PA 1 analytical 4 × 250 mm column capable of separating monosaccharides was followed by a guard column ProPac PA 1 analytical 4 × 250 mm. Arabinose and xylose were quantified with a calibration curve using fucose as an internal standard.

Acetylation

Esterification of arabinoxylan was carried out using experimental conditions described elsewhere.[29] Dry AX samples (1.00 g) treated with oxalic acid for different times were dispersed in formamide (25 mL). Pyridine (40 mL) was added, followed by acetic acid

anhydride (6.6 mL). After stirring at room temperature for 3 h, another portion of acetic anhydride (6.6 mL) was added, followed by the same amount after another 3 h. After 30 h, the viscous dark solution was poured (under vigorous stirring) into 1.3 L of 2% ice-cold hydrochloric acid. The white fluffy precipitate was filtered on a buchner funnel with filter paper and washed with excess (0.5 L) deionized water, then with 0.5 L methanol and finally with 0.5 L diethyl-ether. The samples were then dried in vacuum at 40°C overnight.

Nuclear magnetic resonance analysis

Nuclear magnetic resonance (NMR) measurements were carried out on all four samples (control acetylated AX and three acetylated AXs with different levels of arabinosyl substitution). NMR Samples (2–3 mg) were dissolved in 0.360 mL $CDCl_3$ (99.8 atom%, Sigma Aldrich). NMR spectra were recorded at a probe temperature of 300K on a Bruker Avance-III-600 spectrometer, equipped with a cryo-probe located at Biqualys (Wageningen, The Netherlands). The spectra were calibrated on the solvent signals, chemical shifts are expressed in ppm relative to TMS (tetramethylsilane) at 0.00 ppm. 1D and 2D correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), and heteronuclear multiple quantum coherence (HMQC) spectra were acquired using standard pulse sequences delivered by Bruker. Four hundred experiments of two scans and four scans were recorded for the 1H-COSY and 1H-TOCSY spectra, resulting in measuring times of 0.5 h and 1 h, respectively. The mixing time for the TOCSY spectra was 100 ms. For the [1H, 13C]-HMQC spectra, 512 experiments of four scans were recorded, resulting in a measuring time of 1.2 h.

Casting films

About 1 g of the fluffy white product obtained by AX acetylation was dissolved in chloroform (30 mL). This clear, slightly yellowish solution was used to cast films on glass petri dishes. The films were allowed to dry in a fume hood. They were covered with a glass cover with only a small opening to prevent a too fast chloroform evaporation that would result in an uneven surface or formation of bubbles in the films. The films could be freed from the glass without damage simply by running hot water over the films that had become stuck in the petri dish.

Contact angle measurement

The hydrophobicity of the films was measured by static contact angle with an NRL C.A. Goniometer from Ramé-hart (model 100-00 230) with a lamp from LEP (model 990018). A 5 μL drop of deionized water was placed on the surface of the acetylated AX films, and the contact angle was read after 1 min of contact. Three measurements were done on each sample and average values were calculated.

Water vapor sorption measurement

The acetylated AX films were dried in a vacuum oven for an hour on 105°C. Subsequently, the films were conditioned on 25°C in an air tight container, where water vapor was set at 97% with having a saturated potassium sulphate solution in the container. The equilibrium water content was measured gravimetrically with a balance and calculated as the weight of water in the sample compared to the total weight.

Determining mechanical properties by tensile testing

The mechanical properties of acetylated AX films with a gradient of arabinosyl substitution were evaluated in tensile mode on an Instron 5565A tensile test machine controlled with the BlueHill software from a PC connected to it. Manually controlled pneumatic film clamps were used for the tests. About 50 × 12 × 0.05 mm samples were cut with a paper cutter, and the precise size of each specimen was fed into the software for the calculations. The starting distance between the clamps was 20 mm, and a preload of 0.5 N was applied. The samples were strained at 5 mm/min, and data were collected until the stress drop was greater than 40%. Films were tested in ambient conditions. Four to nine specimens were tested for each sample, depending on the reproducibility of the tests.

Dynamic mechanical analysis

A Perkin-Elmer dynamic mechanical analysis (DMA) 7 operating in tensile mode was used to measure dynamic mechanical properties. The sample size was 20 × 5 × 0.02 mm. The measuring frequency was 1 Hz with an amplitude of 5 μm. Temperature scans were done after an initial drying step (ramp 30–80°C with a rate of 2°C/min, holding at 80°C for 30 min) and conditioning section (isothermal status at 30°C for 15 min after cooling to 30°C with same rate). The temperature scan covered the interval of 30–250°C with a heating rate of 2°C/min. In some cases, data collection was terminated at earlier stages of the experiment due to increasing and sometimes high noise in the signals. Two parallel measurements were made per sample.

Thermo-gravimetric analysis

The thermal stability of the acetylated arabinoxylan was investigated with a Perkin Elmer TGA 7 Thermo-

gravimetric Analyzer and controlled by Start Pyris TGA 7 software from a PC coupled to the device. Samples (2–3 mg) were held at 30°C for 1 min before starting a 10°C/min temperature ramping from 30 to 500°C. The gas flow was 20 mL/min nitrogen.

RESULTS AND DISCUSSION

Debranching of arabinoxylan

A gradient of chemically debranched rye AX was produced to investigate the relation between arabinosyl substitution and material properties. The highest arabinosyl substituted sample was a non debranched (NDB) sample with an arabinose to xylose ratio (A/X) of 0.5, and the lowest arabinosyl substitution was A/X = 0.27 for the sample debranched for the longest time (DB3). This means that different amounts of arabinose side groups were removed from the xylose backbone of the AX, while at the same time trying to maintain a high molecular weight and avoid the main chain degradation. A detailed study in Ref. 28 showed that it is possible to be able to control acidic debranching of arabinoxylans without a loss of molecular weight. The experiments in this study were done to achieve a designed gradient of arabinose to xylose ratios (A/X) starting from the A/X = 0.52 in rye AX and covering 0.4, 0.3, and 0.2 A/X ratios. The high performance anion exchange chromatography confirmed success in performing a gradient of debranching of AX with A/X ratios from 0.52 to 0.41, and then 0.33 and, finally, to 0.27 (samples NDB, DB1, DB2, and DB3 respectively; (Table I)]. The debranched samples were the raw material for the acetylation.

NMR Results

The assignment of the sugar signals in the proton spectra (Fig. 1) is based on 2D COSY and TOCSY experiments (data not shown). Chemical shifts of protons attached to non acetylated carbons are in accordance with Vliegenthart et al.[30] Acetylation results in downfield shifts for both proton and carbon signals. The HSQC spectrum (Fig. 2) connects the proton and carbon chemical shifts. The carbon chemical shifts of acetylated carbons are in accordance with those of acetylated xylose and arabinose.[31]

TABLE I
Sugar Composition of the Samples Treated for Different Periods of Time for Debranching

Sample name	Treatment time	A/X ratio
NDB	0 min	0.52
DB1	7 h 20 min	0.41
DB2	12 h 40min	0.33
DB3	17 h 59 min	0.27

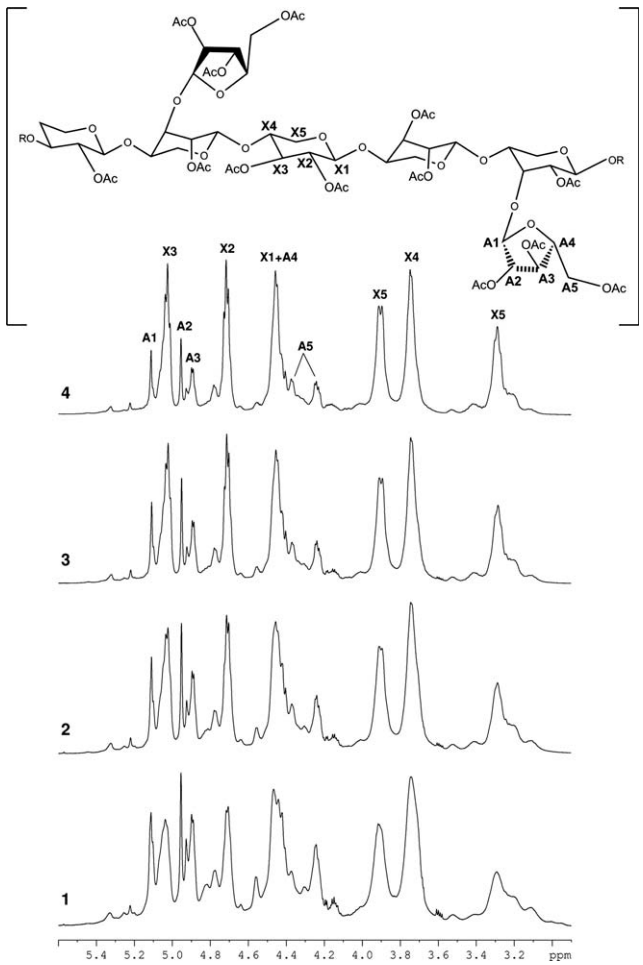

Figure 1 Pictorial representation of structure of acetylated AX and 1D proton spectra of samples: sample 1: AcAX; sample 2: AcAXDB1; sample 3: AcAXDB2; sample 4: AcAXDB3. Assignments are indicated, X = xylose; A = arabinose. The acetyl region on the right is scaled down 10 times to keep high resolution of xylose-arabinose region.

1-4-linked xylose residues are dominant in the sample with the lowest arabinosyl substitution (sample 4) as seen in the 1D spectrum (Fig. 1). From the position of the H-2 and H-3 signals in the 1H spectrum resonating at higher ppm values than the H-1 signal, it is obvious that all carbons 2 and 3 of xylose are acetylated. The positions of the H-1, H-4, and H-5 signals indicate that these carbons are not acetylated. Carbons 1 and 4 are involved in the 1-4 linkage of the xylose backbone and C-5 is used for ring closure, confirming the pyranose ring structure of xylose. In the 1D spectrum signals for smaller levels of arabinose can be recognized. The positions in the proton spectrum indicate that the C-2, C-3, and C-5 carbons of arabinose are acetylated, whereas arabinose C-4 is used for furanose ring closure and arabinose C-1 is involved in the linkage with a xylose residue in the xylan backbone. Many possibilities in substitution patterns exist for the arabinose-substituted xylose

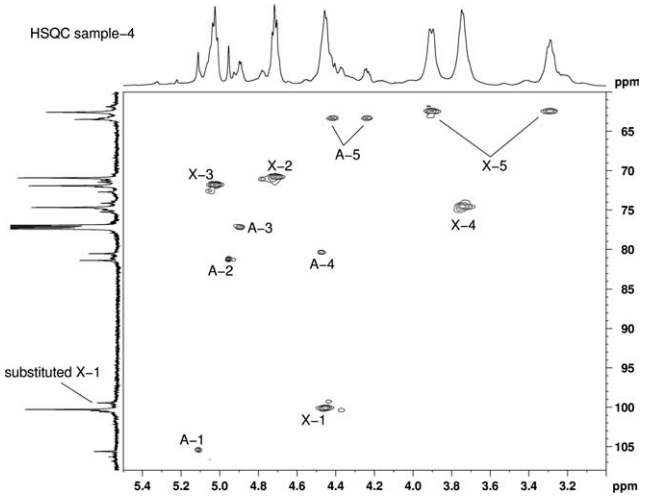

Figure 2 HSQC spectrum of sample 4 (AcAXDB3) together with the 1D proton (horizontal) and 1D carbon (vertical) spectra. Assignments are indicated, X = xylose; A = arabinose.

residues,[30] all resulting in different chemical shifts for the substituted xyloses and their neighboring residues. It was not possible to assign all these different signals. As an example, in the carbon spectrum, a signal close to the C-1 of a xylose not substituted by arabinose is indicated probably to be assigned to a xylose substituted with arabinose. Small cross peaks in the HSQC spectrum with carbon signals close to this C-1 indicate that proton signals connected to these carbons are overlapping with xylose H-1 and arabinose H-4 and H-5 signals.

Decreasing amounts of arabinose are clearly visible in a comparison of the integrals of xylose and arabinose proton signals in the spectra of samples 1 to 4 in Figure 1. The ratio between arabinose and xylose was calculated by dividing the integral of arabinose H-1 at 5.11 ppm by the integral of the xylose H-5 at 3.91 ppm, it was estimated to be A/X = 0.42, 0.28, 0.24, and 0.21 for samples 1, 2, 3, and 4, respectively. The integral of the total sugar protons was calibrated at 1.000 and the integrals of the acetyl signals were estimated to be 1.012, 1.023, 1.042, and 0.995 for samples 1, 2, 3, and 4, respectively. Comparing the integral of all sugar protons with the integral of the CH_3 protons of the acetyl group, the ratio was exactly 1:1 for each sample. This indicates that all the free positions of both the xylose and arabinose residues were acetylated in all four samples.

Acetylated arabinoxylan films

After evaluating different acetylation methods and starting materials (data not published), chemical acetylation was selected for further studies based on a method published by,[29] which was also used in the studies of Grondahl.[18] The products of the acetylation after drying were white fluffy materials, which were easily dissolved in chloroform when they had completely dried. The chloroform solution was then cast into films as described in the experimental section. The clear, transparent films had a yellowish color in thicker films. The static contact angle of deionized water (after 1 min) on the films' surface was 66, 63, 61, and 61° as the level of arabinosyl substitution decreased. Nonacetylated films soak water rapidly (contact angle, 0°). The mean of the contact angle measurements might indicate a trend, but there were no significant differences between the samples because of the overlap of the standard deviation of the measurements. The message of these results is the decreased hydrophilicity of the acetylated samples compared with non acetylated AX films.

Water vapor sorption

The water vapor sorption of the acetylated AX films indicated that the acetylated samples are interacting with water to a low extent, since at 97% relative humidity the water content of the samples was 8, 6, 6, and 7% for the samples AcAX, DB1, DB2, and DB3, respectively, which is comparable with previous studies done by Grondahl et al.[17,18]

Mechanical properties

The acetylated AX films cast from chloroform were tested for mechanical properties on an Instron 5565A tensile test machine. Tensile stress and strain were followed along with the Young's modulus (Fig. 3). The debranched samples showed higher deviations in the strain at break or stress at break between the different specimens. In the tensile test, the more debranched samples showed no cross-section area change, no necking was observed and the film broke at smaller extension rates than the other samples. This is supported by the Young's modulus of the different samples shown in Figure 3(a). It is clearly expressed that the more debranched the AX was, the higher modulus the acetylated sample had, resulting in a stiffer material. The tensile strain at break varied between 7 and 22% and showed a clear trend, with the lowest strain at break for the acetylated AXs that were debranched to a higher degree, see Figure 3(b). The necking of the sample and the cross-section area change caused by stretching could clearly be seen and measured in the non debranched sample (NDB), which had a 22% strain at break. DB1 and DB2 samples had strains at break around 13% where already no necking was observable to the naked eye, and DB3 broke at a relatively early stage of the test without necking phenomena, showing 7% strain at break. The maximum tensile stress, which is practically the

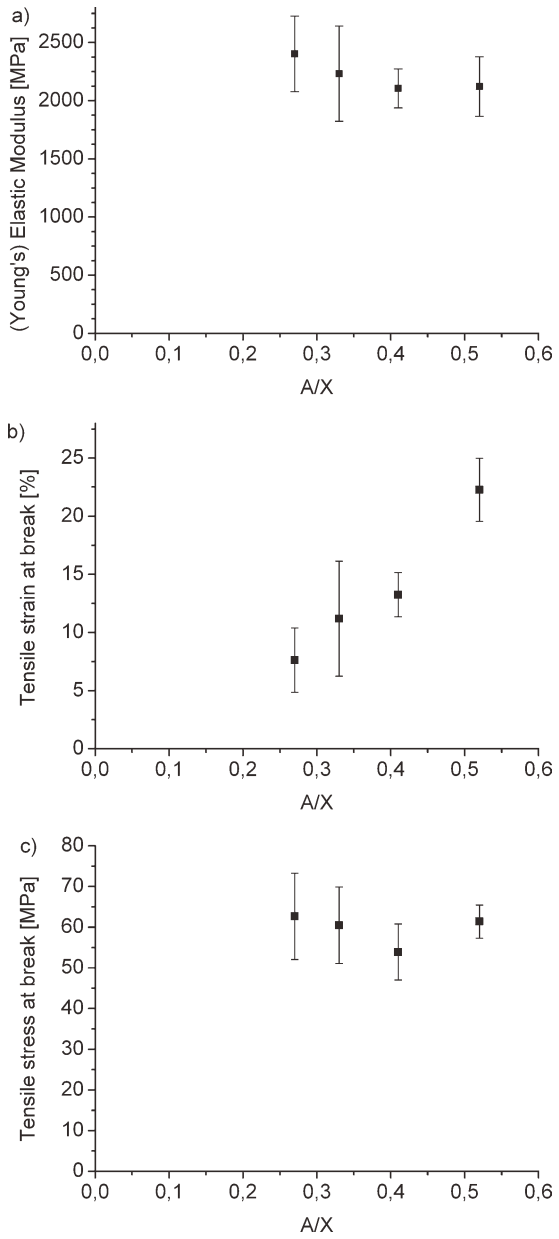

Figure 3 Effect of arabinosyl substitution on Young's modulus (a), strain at break (b) and tensile strength (c) of acetylated AX films.

same as the stress at break for these samples, was around 60 MPa and is a relatively good strength for such materials, see Figure 3(c). These results show that flexible acetylated films can be prepared at higher arabinose content without the need of an addition of an external plasticizer. Figure 4 shows that the higher the arabinosyl substitution is on the acetylated AX, the more ductile the material is.

Thermo-mechanical properties of the films (DMA, TGA)

DMA and TGA were both run on the acetylated films in order to determine the response of the material to different temperatures. The DMA showed clear transition points for each sample between 150 and 200°C. As there is a gradient scale of arabinosyl substitution between the samples, there is also a difference in their behavior at elevated temperatures. Figure 5 shows the change in Young's elastic modulus of the acetylated AXs as the temperature increases. It is clear that the no-debranched acetylated AX with A/X = 0.52 has the lowest temperature range for glass transition region, at 130–170°C. The DB1 sample has the second highest ratio, of A/X = 0.41, and the second lowest transition region, between about 150 and 190°C. The DB2 and DB3 samples, with A/X ratios of 0.33 and 0.27, respectively, have approximately the same glass transition temperature range, around 170–220°C. This is not very surprising, because these A/X ratios are closer to each other. Nevertheless, the DB3, with a lower A/X ratio, does show a slightly higher temperature range for glass transition. Consequently, the different T_g values are in an order corresponding to the gradient substitutions. The highest arabinose

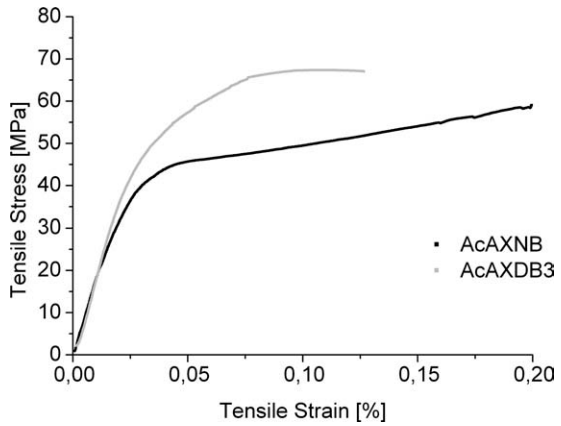

Figure 4 Effect of arabinosyl substitution on tensile behavior of acetylated AX films.

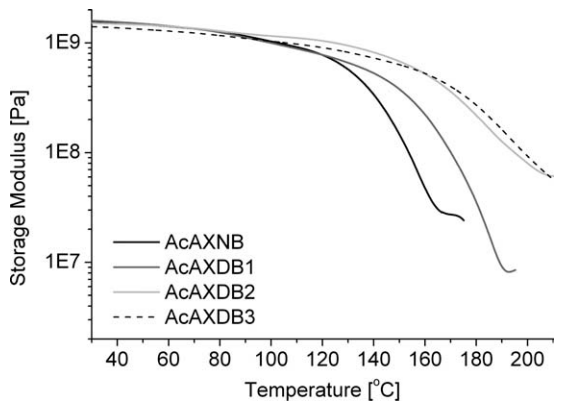

Figure 5 Temperature scan of acetylated arabinoxylan samples at different levels of arabinosyl substitution (smoothed average curves).

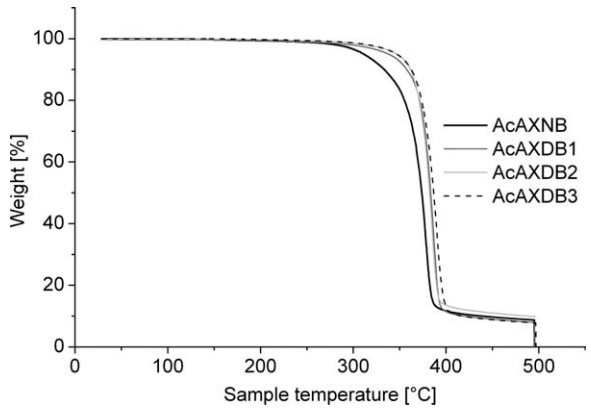

Figure 6 Thermo-gravimetric analysis of the acetylated arabinoxylan samples at different levels of arabinosyl substitution.

substituted sample had the lowest transition temperature, and the less arabinosyl substitution a sample had, the higher was the transition temperature. TGA confirmed thermal decomposition around 380°C (Fig. 6) supporting the theory of glass transition regions around 150–200°C. The specific transition temperatures were determined visually in Figure 5. The highest arabinose substituted sample for decomposing temperatures was the only one that was more separated from the other three samples with a decomposition temperature some degrees lower (Fig. 6). The other values for the decomposition temperatures were close to each other, and no clear trend correlations could be seen among them.

CONCLUSIONS

The aim of this work was to show the effect of arabinosyl substitution of acetylated AXs on material properties. The series of AXs with a gradient of arabinosyl substitution were prepared and fully acetylated. The chemical composition of AX and acetylated samples was determined by means of sugar analysis and 2D NMR. The films were cast, and their material properties were evaluated. A clear conclusion is that the strain at break, which reflects the flexibility of the acetylated material, is higher at higher arabinosyl substitution levels. The elastic Young's modulus is not affected to any great extent, but a tendency can be seen toward a less stiff material at a higher arabinosyl substitution. The ultimate strength of the materials also does not differ very much, and all of the materials are remarkably strong (up to 60 MPa). Thermal transition regions follow a trend with respect to the level of arabinosyl substitution of the sample, where the lowest T_g is found for the highest substitution levels and the highest T_g for the lowest. The existence of these glass transition temperatures enables thermal processing of these materials around temperatures of 150–200°C. Thermo gravimetric analysis confirmed that the arabinose content contributes slightly to the thermal stability of the acetylated arabinoxylans. Optical and barrier properties are currently being investigated in our laboratories. We envision the potential application of acetylated arabinoxylan to be packaging film and matrix in composites.

Elina Mabasa Bergström from Inventia is acknowledged for help in the DMA measurements. Anders Mårtensson from Chalmers University of Technology is acknowledged for his help in making TGA measurements. The Knut and Alice Wallenberg Foundation is gratefully acknowledged for donating a base for a Swedish research center, the Wallenberg Wood Science Center, thus financing this work.

REFERENCES

1. Ragauskas, A. J.; Williams, C. K.; Davison, B. H.; Britovsek, G.; Cairney, J.; Eckert, C. A.; Frederick, W. J.; Hallett, J. P.; Leak, D. J.; Liotta, C. L.; Mielenz, J. R.; Murphy, R.; Templer, R.; Tschaplinski, T. Science 2006, 311, 484.
2. Heinze, T.; Koschella, A.; Ebringerova, A. ACS Symp Ser 2004, 864, 312.
3. Timell, T. E. Wood Sci Technol 1967, 1, 45.
4. Tombs, M.; Harding, S. An Introduction to Polysaccharide Biotechnology; Taylor & Francis Ltd., London, 1997.
5. Spiridon, I.; Popa, V. I. Amer Chem Soc (ACS) Conf Proc 2008, 35, 289.
6. Sun, R.; Sun, X. F.; Tomkinson, J. ACS Symp Ser 864, 2 2004.
7. Kabel, M.; Borne, H. V. d.; Vincken, J.-P.; Voragen, A. G. J.; Schols, H. A. Carbohydr Polym 2007, 69, 94.
8. Glasser, W. G.; Jain, R. K.; Sjostedt, M. A.: Int. Pat. No.: US005430142 A, 25pp 1995.
9. Jain, R. K.; Sjostedt, M.; Glasser, W. G. Cellulose 2000, 7, 319.
10. Ebringerova, A.; Heinze, T. Macromol Rapid Commun 2000, 21, 542.
11. Hansen, N. M. L.; Plackett, D. Biomacromolecules 2008, 9, 1493.
12. Lindblad, M. S.; Albertsson, A.-C. Amer Chem Soc (ACS) Conf Proc 2005, 30, 491.
13. Spiridon, I.; Popa, V. I. Amer Chem Soc (ACS) Conf Proc 2005, 35, 475.
14. Smart, C. L.; Whistler, R. L. Science 1949, 110, 713.
15. Buchanan, C. M.; Buchanan, N. L.; Debenham, J. S.; Gatenholm, P.; Jacobsson, M.; Shelton, M. C.; Watterson, T. L.; Wood, M. D. Carbohydr Polym 2003, 52, 345.
16. Buchanan, C. M.; Buchanan, N. L.; Debenham, J. S.; Gatenholm, P.; Jacobsson, M.; Shelton, M. C.; Watterson, T. L.; Wood, M. D. ACS Symp Ser 2004, 864, 326.
17. Grondahl, M.; Gatenholm, P. Am Chem Soc (ACS) Conf Proc 2005, 19, 509.
18. Grondahl, M.; Teleman, A.; Gatenholm, P. Carbohydr Polym 2003, 52, 359.
19. Fang, J. M.; Sun, R.; Fowler, P.; Tomkinson, J.; Hill, C. A. S. J Appl Polym Sci 1999, 74, 2301.
20. Fang, J. M.; Sun, R. C.; Tomkinson, J.; Fowler, P., Carbohydr Polym 1999, 41, 379.
21. Ren, J. L.; Sun, R. C.; Liu, C. F.; Cao, Z. N.; Luo, W. Carbohydr Polym 2007, 70, 406.
22. Sun, R.; Fang, J. M.; Tomkinson, J.; Hill, C. A. S. J Wood Chem Technol 1999, 19, 287.

23. Sun, R.; Fang, J. M.; Tomkinson, J.; Jones, G. L. Ind Crops Prod 1999, 10, 209.
24. Sun, R. C.; Fang, J. M.; Tomkinson, J.; Geng, Z. C.; Liu, J. C. Carbohydr Polym 2000, 44, 29.
25. Skalkova, P.; Srokova, I.; Erbingerova, A.; Csomorova, K.; Janigova, I. Cell Chem Technol 2006, 40, 525.
26. Xu, C.-L.; Leppaenen, A.-S.; Eklund, P.; Holmlund, P.; Sjoeholm, R.; Sundberg, K.; Willfoer, S. Carbohydr Res 2010, 345, 810.
27. Hoije, A.; Sternemalm, E.; Heikkinen, S.; Tenkanen, M.; Gatenholm, P. Biomacromolecules 2008, 9, 2042.
28. Sternemalm, E.; Hoije, A.; Gatenholm, P. Carbohydr Res 2008, 343, 753.
29. Zinbo, M.; Timell, T. E. Sven Papperstidn 1965, 68, 647.
30. Vliegenthart, J. F. G.; Hoffmann, R. A.; Kamerling, J. P. Xylan Xylanases. Visser, J.; Beldman, G.; Kuster van Someren, M.A.; Voragen, A.G. J., Eds.; 1992; p 17.
31. Bock, K.; Pedersen, C. Adv Carbohydr Chem Biochem 1983, 41, 27.