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Effect of arabinose substitution on the material properties of arabinoxylan films

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Abstract—This study was undertaken to investigate the effect of arabinose content on film properties. The substrate used was a rye arabinoxylan that had an Ara/Xyl ratio of 0.52 and an average number molecular weight of 305 kDa. Oxalic acid was used to attempt selective removal of the arabinose substituents on the xylan main chain. The debranching of the polymer was coupled with a decrease in molecular weight. The effect of reaction conditions on the decrease in arabinose content and loss of molecular weight was investigated. Optimal conditions were selected using an experimental design. Treatment at lower temperature for longer period of times resulted in debranching with less degradation of the main chain. As the Ara/Xyl ratio was lowered, aggregates began to form in an interval of the Ara/Xyl ratio between 0.31 and 0.23 in a water solution. Precipitation occurred below an Ara/Xyl ratio of 0.1. Thus, removal of arabinose substituents results in a gradual association of unsubstituted chains. There is a linear correlation between arabinose substitution and the moisture content of arabinoxylan at 98% RH. A decrease in arabinose content resulted in the loss of a plasticizing effect, as determined by dynamic mechanical analysis, which is correlated to water binding capacity.

Keywords: Arabinoxylan; Arabinose substitution; Debranching; Moisture content; Material properties

1. Introduction

Arabinoxylan from barley husk has been shown to form films without any addition of plasticizers.[1] It has been shown that xylan films exhibit low oxygen permeability and may be used as oxygen barrier materials in the food packaging industry.[2]

The molecular architecture and degree of substitution of arabinoxylans varies amongst different crops.[3–5] The distribution of arabinose substituents along the backbone is believed to be of great importance since it affects the conformation and the capacity of arabinoxylans to interact with one another.[3] The distribution of the substituents over the main chain is suggested to be non-random and may reflect the functional diversity of xylans in plants.[6–8] Arabinoxylan may become more or less soluble as the number of arabinose side chains increases or decreases.[9] Andrewartha et al.[10] used arabinofuranosidase to debranch wheat flour arabinoxylan, which resulted in a decrease in solubility as the arabinose content decreased. The arabinose residues maintain the molecule in a fully extended conformation. After removal of some of the side branches, the molecule becomes more flexible and hence the viscosity of the solution decreases. Upon additional removal, unsubstituted sections of the xylan backbones may approach each other and form stable inter-chain associations and consequently produce insoluble aggregates.[10] As a result of their rather stiff conformation, arabinoxylans exhibit very high viscosity in aqueous solutions compared to, for example, dextran or arabinan.[3]

Furanosides are hydrolyzed at a greater rate compared to pyranosides.[11–13] Different explanations for this phenomenon have been proposed.[12] Glycosidic linkages at non-reducing ends are hydrolyzed more rapidly than internal glycosidic linkages.[14] Further, terminal linkages are more rapidly hydrolyzed than others.[12] This indicates

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that it is possible to liberate arabinofuranose residues from the xylan backbone of arabinoxylans using acid catalyzed hydrolysis. Whistler et al.[15,16] have shown that the prime action of acid on arabinoxylan in corn hull is the hydrolysis of the arabinofuranose side chains. Zhang et al.[17] even claim that, under gastric acidities (pH 2–3), hydrolysis of arabinoxylans occurs only at side chain linkages.

In this study samples with gradient of arabinose substitution were prepared by controlled acid hydrolysis and their properties have been investigated. The degree of substitution is believed to have a crucial effect on solubility, film formation and material properties.

2. Experimental

2.1. General methods

The substrate used was a high viscosity rye arabinoxylan (LOT: 90501) purchased from Megazyme International Ltd, Ireland. The arabinoxylan was dissolved in water at 1% concentration. Oxalic acid solution was added to a final arabinoxylan concentration of 0.5%. The acid concentration in the reaction tube was varied in an interval between 0.025 and 0.1 M. The temperature in different treatments was varied between 40 and 80 °C. After acid treatment, the arabinoxylan was precipitated with ethanol and dried in a vacuum oven at room temperature overnight. The results of the acid treatment were used to prepare an experimental design plan. The MODDE program (Umetrics Inc., Umeå, Sweden) was used as a tool.

2.2. Analysis

The sugar composition was analyzed with HPAEC (Dionex Corporation, Sunnyvale, CA, USA) equipped with CarboPac™ PA1 (4 × 250 mm) analytical column and electrochemical detector. Fucose was used as internal standard. The complete hydrolysis of polysaccharide samples was done according to Theander and Westerlund.[18]

The molecular weight distributions of the samples were examined using a high performance SEC system (Waters Corporation, Milford, MA, USA). Detectors were RI and MALLS. The eluent used was 0.1 M sodium nitrate with 0.02% sodium azide and flow rate of 0.4 mL/min. The molecular weights of the samples were estimated using pullulan standards.

2.3. Film preparation and analysis

A range of Ara/Xyl ratios between 0.3 and 0.52 were selected for film preparation. The experimental design was used to select conditions for the acid treatment. The selected parameters were 0.1 M acid for a period of 16 h at 46 °C, 16 h at 42 °C and 6 h at 41 °C. The treated arabinoxylans were precipitated with ethanol and freeze dried, then re-dissolved in water. The solutions were poured into Petri dishes and air dried.

For moisture content analysis, arabinoxylan films were put in desiccators containing water. The samples were continuously weighed until equilibrium was reached. Finally, the samples were dried in an oven (105 °C) overnight and weighed again.

The mechanical testing was done on a DMA 7 from Perkin Elmer (Waltham, MA, USA) equipped with a Wetsys humidity generator (Setaram, Caluire, France). The sample width and length were 4 and 12–13 mm, respectively. The dynamic amplitude was set to 5 μm with a static load equal to 120% of the dynamic load. The temperature was held constant at 30 °C and the frequency used was 1 Hz. The humidity scans were performed three times on each sample with an increase of the relative humidity from 0% to 95%. The humidity was raised by increments of 1% RH per minute, followed by 2 min of 1% RH before the next cycle. The storage modulus was recorded during scans.

3. Results and discussion

3.1. Mild acid treatment

The Ara/Xyl ratio in the rye arabinoxylan substrate according to analysis with HPAEC was 0.52. The substrate also contained 2% protein and 3% other sugars according to the manufacturer. The first experiment investigated the change in Ara/Xyl ratio over time during hydrolysis. Figure 1 shows a linear decrease in the

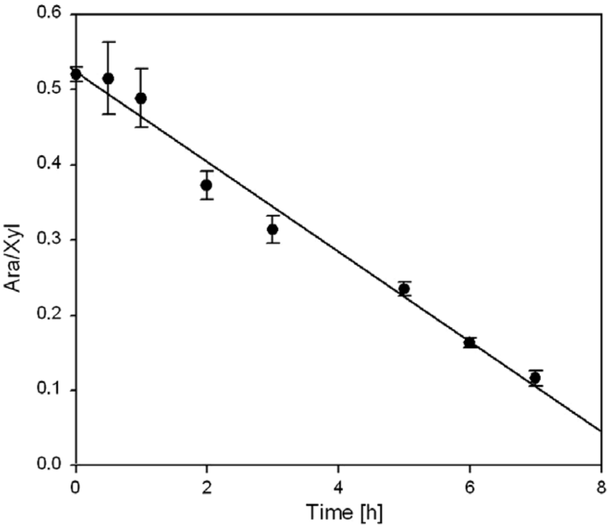

Figure 1. Decrease in arabinose content with treatment time. Samples were done in triplicate. Error bars show the standard deviation. Reaction conditions: 70 °C and 0.05 M of oxalic acid.

Ara/Xyl ratio as the reaction proceeds. The Ara/Xyl ratio decreased from 0.52 to 0.12 during the 7 h reaction. This behaviour is in accordance with previous observations that furanosides are hydrolyzed faster than pyranosides.[12]

To investigate the extent of backbone chain hydrolysis, the treated arabinoxylan was analyzed using SEC to determine how the treatment affected the molecular weight. Figure 2 shows the molecular weight distributions of samples treated for 10 min, 1 h, 2 h and 3 h compared to the original material. As the reaction progresses, a broadening of the molecular weight distribution appears, with a tail of low molecular weight species. This is consistent with the hydrolysis not being a random process.[12] More products of a low degree of polymerization and fewer intermediate sized products are formed than would be expected from a completely random process.

The samples treated for 5 and 6 h showed the presence of agglomerates. Nieduszynski and Marchessault.[19] have showed insolubility as a result of the ability of unsubstituted xylan chains to interact. According to Dea el al.[20] association of unbranched chains occurs preferentially compared to branched regions. Less densely substituted xylans can be separated from aqueous solution as crystalline aggregates.

The next variable that was investigated was the treatment temperature. A linear dependence between the Ara/Xyl ratio and treatment temperatures is seen in Figure 3. At 80 °C, the reaction yields an Ara/Xyl ratio of 0.05. At this temperature, precipitation of the arabinoxylan was clearly observed.

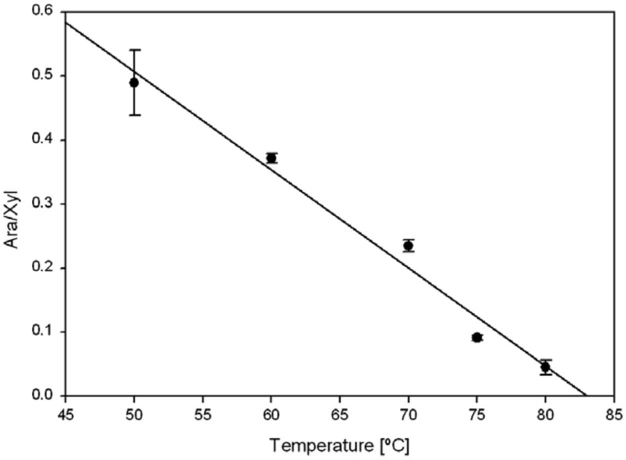

Figure 3. Decrease in arabinose content at different temperatures. Samples were done in triplicate. Error bars show standard deviation. Reaction conditions: 5 h and 0.05 M of oxalic acid.

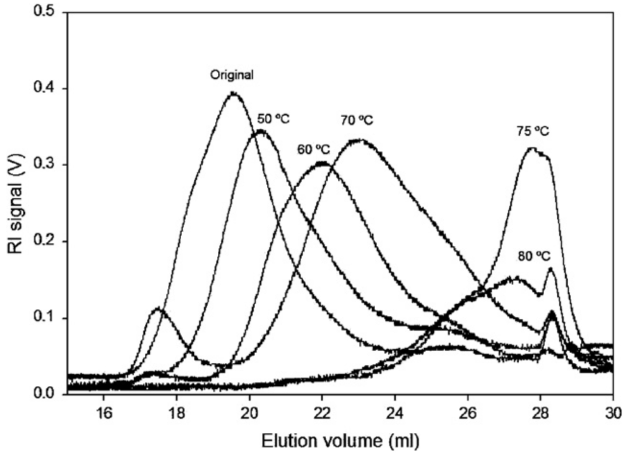

Figure 4. Molecular weight distributions of samples with mild acid treatment at different temperatures.

Figure 4 shows the molecular weight distribution of the samples prepared at different temperatures. Again, aggregate formation is seen at a reaction temperature of 70 °C. This corresponds to an Ara/Xyl ratio of 0.23. No aggregate formation is seen in Figure 2 at an Ara/Xyl ratio of 0.31. Thus it seems that aggregate formation occurs at a ratio of between 0.31 and 0.23 and precipitation takes place at Ara/Xyl ratios below 0.1. Treatment at 75 and 80 °C resulted in extensive degradation of the main chain, as seen in Figure 4. For this reason, no determination of the average number molecular weight of theses samples was made.

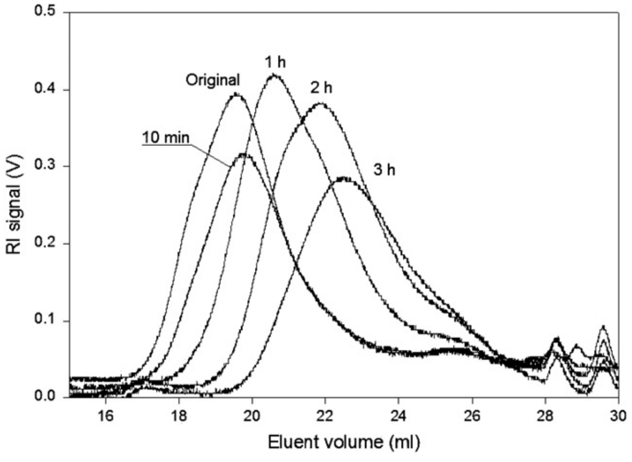

Figure 2. Molecular weight distributions of samples treated with mild acid for 0–3 h.

The samples treated at 50 °C for a period of 5 h had a molecular weight of 169 kDa and an Ara/Xyl ratio of 0.49. The original material had an M_n of 305 kDa and an Ara/Xyl ratio of 0.52. In the previously described experiment, the samples treated at 70 °C for 1 h had an identical Ara/Xyl ratio but a considerably lower molecular weight (123 kDa). This fact indicates that reducing the treatment temperature from 70 to 50 °C and using longer treatment times give similar debranching but cause less degradation of the main chain.

To investigate this effect further and examine how other variables and interaction effects, such as acid

Table 1. Full factorial experimental plan at three levels

Exp. no.	Exp. name	Run order	Time [h]	Temperature [°C]	Acid concn [M]	Ara/Xyl [%]	Mol. weight [Da]
1	N1	1	6	40	0.025	0.520	193,000
2	N2	8	16	40	0.025	0.483	197,400
3	N3	7	6	60	0.025	0.363	85,900
4	N4	9	16	60	0.025	0.240	27,000
5	N5	6	6	40	0.100	0.445	165,200
6	N6	10	16	40	0.100	0.409	129,400
7	N7	2	6	60	0.100	0.196	18,300
8	N8	5	16	60	0.100	0.072	Precipitated
9	N9	3	13	50	0.050	0.342	86,400
10	N10	4	13	50	0.050	0.312	99,500
11	N11	11	13	50	0.050	0.338	106,100

concentration, affect the reaction, an experimental design plan was developed. The full factorial design is given in Table 1; this is a set of experimental runs where each factor is investigated at both levels of all other factors. The levels chosen for reaction time, temperature and acid concentration were 6–16 h, 40–60 °C and 0.025–0.1 M, respectively.

One value is missing in the molecular weight column owing to too extensive degradation of the polymer. Two separate models were calculated, one with the Ara/Xyl ratio and one with the molecular weight as the response. These are referred to as models one and two, respectively. The temperature was found to have the strongest effect on the Ara/Xyl ratio in the selected interval. Apart from the main factors, only interaction terms involving temperature as a factor had a significant effect.

Figure 5a shows a contour plot of model 1, depicting how the Ara/Xyl ratio varies in the temperature and treatment time interval in which the model is valid. The acid concentration was set to a constant value of 0.1 M. The plot shows a non-linear relationship between treatment time and temperature, due to interaction factors involving temperature in the model. Only main effects were significant factors affecting the average number molecular weight. A contour plot for model two is seen in Figure 5b. Since the model included no interaction effects between the factors, only linear relationships between the different factors were distinguished.

When the contour plots for the two models (Fig. 5a and b) are compared it can be seen that for high temperatures (above 53 °C), reduction of the treatment temperature and subsequent prolongation of the treatment time would generate an arabinoxylan with a constant Ara/Xyl ratio but with a higher molecular weight. Reducing the treatment temperature and prolonging treatment times thus seems to lead to a decreased rate of main chain degradation with a preserved rate of debranching. This is the same effect that was noted above, and is here confirmed. However, at treatment temperatures below 50 °C, the lines describing constant Ara/Xyl ratio and molecular weight become parallel. It can be concluded that below this temperature the described effect diminishes. It can also be noted that the effect described by BeMiller,[12] that an increased acid concentration would promote hydrolysis of terminal linkages, cannot be seen in this study.

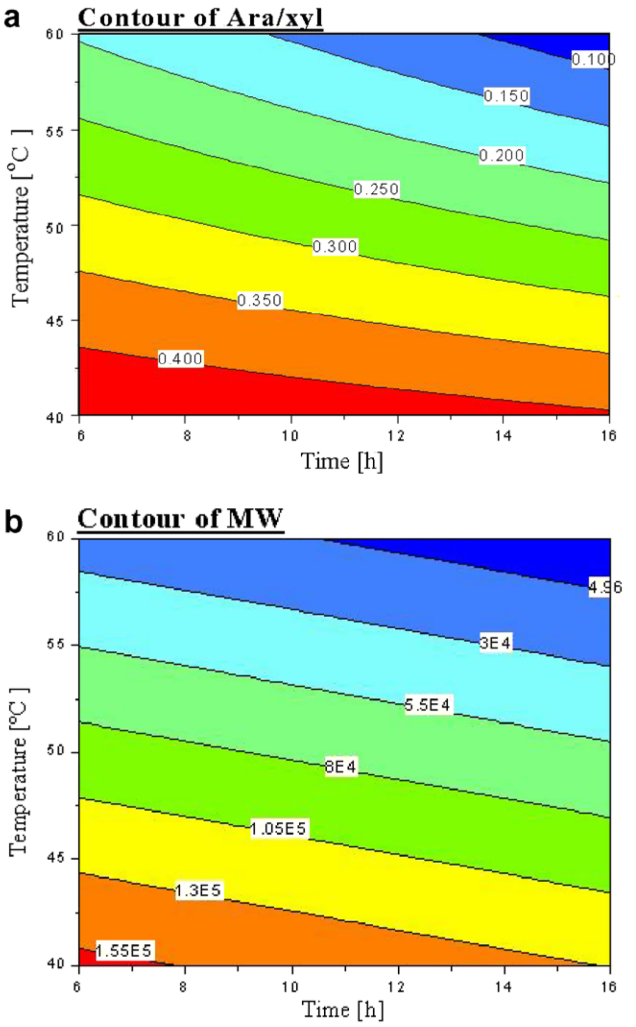

Figure 5. Contour plot of the factors of treatment temperature versus time for the (a) Ara/Xyl ratio and (b) molecular weight as the response. The acid concentration was held constant at 0.1 M.

Table 2. Moisture content and molecular weight at different Ara/Xyl ratio

Ara/Xyl	Moisture content (%)	M_n (kDa)
0.36	57	130
0.41	59	167
0.44	63	238
0.52	72	305

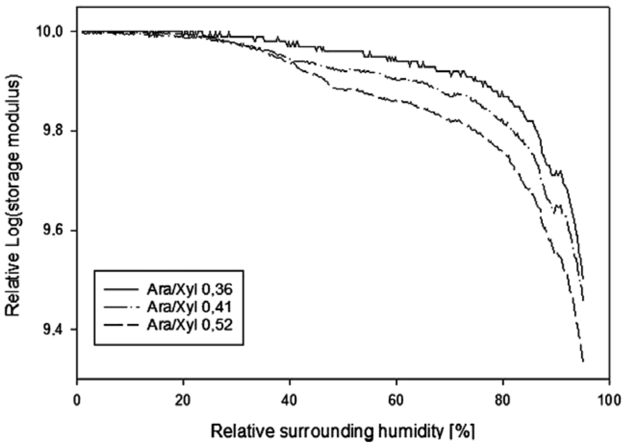

Figure 6. Measurement of storage modulus at different relative surrounding humidities for three samples of arabinoxylan with different Ara/Xyl ratios.

3.2. Material properties of films

Films were prepared from debranched arabinoxylan. The films formed were all translucent. No difference in appearance, structure or brittleness between films with different Ara/Xyl ratios could be observed. Table 2 shows how the moisture content at equilibrium varied for arabinoxylan films of different Ara/Xyl ratios (98% surrounding relative humidity, mean value of two samples). There is a linear correlation between arabinose substitution and moisture content of arabinoxylan at 98% RH. The moisture content varies between 57% and 72%, which indicates that arabinoxylans are very hygroscopic. This agrees with the observations of Höije et al.,[1] who found that the water content of arabinoxylans isolated from barley husk can be as high as 82% at 100% RH.

The results of the dynamic mechanical testing with a moisture scan are shown in Figure 6. The material is plasticized by increased moisture content, which can be seen as a drop in the storage modulus. Two transitions can be seen in the graph, one secondary transition occurring between 40% and 50% surrounding relative humidity, and the main transition (T_g) at 70% surrounding relative humidity. The secondary transition can be attributed to the effect of the arabinose substituents. An increase in the arabinose content of the samples shows a clear plasticizing effect on the material. Arabinoxylans with higher arabinose content have lower *E*-modulus at RH above 40%. This is plausibly caused by the increase in hydration, shown above, as the arabinose content is raised.

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References

1. Höije, A.; Gröndahl, M.; Tømmeras, K.; Gatenholm, P. *Carbohydr. Pol.* **2005**, *61*, 266–275.
2. Gröndahl, M.; Eriksson, L.; Gatenholm, P. *Biomacromolecules* **2004**, *5*, 1528–1535.
3. Izydorczyk, M. S.; Biliaderis, C. G. *Carbohydr. Pol.* **1995**, *28*, 33–48.
4. Chanliaud, E.; Saulnier, L.; Thibault, J. F. *J. Cereal Sci.* **1995**, *21*, 195–203.
5. Höije, A.; Sandström, C.; Roubroeks, J. P.; Andersson, R.; Gohil, S.; Gatenholm, P. *Carbohydr. Res.* **2006**, *341*, 2959–2966.
6. Gruppen, H.; Kormelink, F. J. M.; Voragen, A. G. J. *J. Cereal Sci.* **1993**, *18*, 111–128.
7. Izydorczyk, M. S.; Biliaderis, C. G. *Carbohydr. Pol.* **1994**, *24*, 61–71.
8. Dervilly-Pinel, G.; Tran, V.; Saulnier, L. *Carbohydr. Pol.* **2004**, *55*, 171–177.
9. Perlin, A. S. *Cereal Chem.* **1951**, *28*, 382–393.
10. Andrewartha, K. A.; Phillips, D. R.; Stone, B. A. *Carbohydr. Res.* **1979**, *77*, 191–204.
11. Sjöström, E. *Wood Chemistry: Fundamentals and Applications*; Academic Press, 1993.
12. BeMiller, J. N. *Adv. Carbohydr. Chem. Biochem.* **1967**, *22*, 25–108.
13. Kennedy, J. F. *Carbohydrate Chemistry*; Oxford University Press, 1988.
14. Hollo, J.; Laszlo, E.; Szejtli, K.; Zala, G. *Starch* **1964**, *16*, 211–214.
15. Whistler, R. L.; BeMiller, J. N. *J. Am. Chem. Soc.* **1955**, *78*, 1163–1165.
16. Whistler, R. L.; Corbett, W. M. *J. Am. Chem. Soc.* **1955**, *77*, 6328–6330.
17. Zhang, P.; Zhang, Q.; Whistler, R. L. *Cereal Chem.* **2002**, *80*, 252–254.
18. Theander, O.; Westerlund, E. A. *J. Agric. Food Chem.* **1986**, *34*, 330–336.
19. Nieduszynski, I. A.; Marchessault, R. H. *Biopolymers* **1972**, *11*, 1335–1344.
20. Dea, I. C. M.; Rees, D. A.; Beveridge, R. J.; Richards, G. N. *Carbohydr. Res.* **1973**, *29*, 363–372.