

Carboxymethylated-, hydroxypropylsulfonated- and quaternized xylan derivative films

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ABSTRACT

Under alkaline/water conditions carboxymethyl, 2-hydroxypropylsulfonate and trimethylammonium-2-hydroxypropyl groups were introduced into xylan in one step with the goal to prepare film specimens. The materials were characterized by NMR, SEC-MALS, TG/DTG/DTA, AFM and mechanical testing. The properties of triple, double and mono-substituted materials were compared. The numerical molar masses of the specimens were from 12.3 to 17.6 kg/mol with M_w/M_n from 1.27 to 1.34. The elastic modulus values are decreasing in order: xylan (X; 7354 MPa) > carboxymethyl xylan (CX; 6090 MPa) > 2-hydroxypropylsulfonate xylan (SX; 6000 MPa) > carboxymethyl/2-hydroxypropylsulfonate xylan (CSX; 4490 MPa) > quaternized xylan (QX; 3600 MPa) > carboxymethyl/quaternary/2-hydroxypropylsulfonate xylan (CQSX; 3380 MPa) > carboxymethyl/quaternary xylan (CQX; 2805 MPa). The onset temperatures of SX (214 °C), CQSX (212 °C), QSX (211 °C) and CQX (207 °C) were higher than for X (205 °C). The roughness values of the film surfaces (3.634 – 18.667 nm) are higher on top than on the bottom of the specimen.

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1. Introduction

Xylan is an important polysaccharide used for composite preparation, film and package applications (Hansen & Plackett, 2008; Köhnke, Lin, Elder, Theliander, & Ragauskas, 2012; Saxena, Elder, Kenvin, & Ragauskas, 2010; Saxena, Elder, & Ragauskas, 2011; Šimkovic, 2013). Carboxymethyl and quaternary xylan (CQX) was recently studied for preparation of an amphoteric polysaccharide derivative of water soluble xylan from bamboo in two steps using microwave (Peng, Ren, Zhong, & Sun, 2012). Carboxymethyl xylan (CX) was also prepared from birch kraft pulp after extraction of xylan and subsequent carboxymethylation (Alekhina, Mikkonen, Alén, Tenkanen, & Sixta, 2014). The reaction has been previously performed, but fully substituted CX was not prepared. By the repeated “slurry” method $DS_C = 1.90$ was reported without considering the molar mass of the produced derivatives (Petzold-Wecke,

Schwikal, Daus, & Heinze, 2014). Carboxymethyl xylan (CX) was also prepared from birch kraft pulp (Alekhina et al., 2014).

Because our beech wood xylan was isolated by NaOH extraction and is only partially soluble in water we used conventional heating instead of microwave to prevent the dramatic molar mass degradation. From our previous studies on the preparation of trimethylammonium-2-hydroxypropyl xylan (QX), 2-hydroxypropylsulfonate (SX) and trimethylammonium-2-hydroxypropyl xylan sulfate (QXS) we have learned that the numerical molar masses (M_n) of the produced material were larger than the water-soluble part of the used xylan (Šimkovic, Gedeon, Uhliariková, Mendichi, & Kirschnerová, 2011a, 2011b; Šimkovic, Tracz, Kelnar, Uhliariková, & Mendichi, 2014). In this work we have used a method in which several ion-exchanging substituents were introduced in one step and the degree of substitution (DS) values were compared with values when single substituent was introduced under analogous conditions. The mechanical properties of the prepared films were studied and compared with data in the literature. The goal was to understand the relation between the chemical structure of the polysaccharide, the type of substituent, molar mass and water solubility together with the effect of

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chemical modification on thermal stability, optical and mechanical properties of the films.

2. Experimental

2.1. Materials

Xylan (X) was extracted from beech sawdust holocellulose with 10% NaOH as previously described for hornbeam wood (Ebringerová, Kramár, & Domanský, 1969). It was characterized as described before (Šimković et al., 2011a, 2011b, 2014). All the chemicals used were of analytical grade and were used without further purification.

CX was prepared by mixing X (1.654 g; 10 mmol) with water (18 ml; 1 mol), containing NaOH (3.2 g, 80 mmol). Subsequently 4.7543 g of ClCH₂COONa (40 mmol; Alfa Aesar) was added and stirred (500 RPM) at 60 °C in a closed container for two hours. The reaction was stopped by dilution, the pH adjusted to 8.0 with HCl, and dialyzed using 10–12 kDa molar mass cut-off dialysis tubing (SERVA # 44126). After preconcentration on a vacuum evaporator (35 °C; 3.3–4 kPa) the solution was freeze-dried [CX, 1.9277 g; C, 34.53; H, 5.34; DS_C = 0.63 (determined with the ¹H-NMR spectroscopy by integration of all xylose anomeric signals and calculated as a ratio of carboxymethylated xylose anomeric signal to all the xylose anomeric signals); yield = (amount of CX obtained/theoretical yield at determined DS) × 100 = 85%].

For preparation of carboxymethyl/trimethylammonium-2-hydroxypropyl xylan (CQX) 1.654 g (10 mmol) of X was mixed with water (16.21 ml; 1 mol in total) containing 6.4 g NaOH (160 mmol). Subsequently 4.7543 g of ClCH₂COONa (40 mmol) and 8.94 ml of glycidyltrimethylammonium chloride (GTMAC, 60% solution, Aldrich; 40 mmol) were added and stirred as above for two hours. The solution was adjusted to pH 10.0, dialyzed and worked up as above [CQX; 2.1384 g; C, 42.49; H, 7.03; N, 2.32; DS_Q = (nitrogen content/nitrogen content of fully substituted derivative) × DS of fully substituted derivative [(2 × amount of xylose units not substituted with 4-O-methyl-D-glucuronate unit) + (3 × amount of xylose units substituted with 4-O-methyl-D-glucuronate unit)] = (2 × 0.86) + (3 × 0.14) = 2.14] = 0.35; DS_C = DS_{NMR} – DS_Q = 0.60 – 0.35 = 0.25; yield = 90%].

The carboxymethyl/2-hydroxypropylsulfonate xylan (CSX) was prepared by mixing X (1.654 g; 10 mmol) with water (18 ml; 1 mol) containing NaOH (6.4 g; 160 mmol) and subsequent addition of 8.5836 g of 3-chloro-2-hydroxy-1-propanesulfonic acid, sodium salt (CHPS, 40 mmol; Aldrich) and 4.7543 g of ClCH₂COONa (40 mmol). The sample was stirred (500 RPM) at 60 °C for two hours and worked up as above [CSX, 1.9140 g; S, 1.12; C, 36.97; H, 5.34; DS_S = (sulphur content/sulphur content of fully substituted derivative) × DS of fully substituted derivative = 0.08; DS_C = DS_{NMR} – DS_S = 0.55 – 0.08 = 0.47; yield = 52%].

The carboxymethyl/2-hydroxypropylsulfonate/trimethylammonium-2-hydroxypropyl xylan (CSQX) was prepared by mixing X (1.654 g; 10 mmol) with water (16.21 ml; 1 mol in total) containing NaOH (6.4 g; 160 mmol) and subsequent addition of 4.7543 g of ClCH₂COONa (40 mmol), 8.5836 g of CHPS (40 mmol) and 8.94 ml of GTMAC (40 mmol). The reaction was run for two hours at 60 °C and worked up as above [CSQX; 1.6769 g; N, 2.25; C, 41.01; H, 6.95; S, 0.72; DS_Q = 0.32; DS_S = 0.05; DS_{NMR} = 0.66; DS_C = DS_{NMR} – DS_Q – DS_{SA} = 0.66 – 0.32 – 0.05 = 0.29; yield = 37%].

For comparison an analogous experiment was performed at the ratio of X/NaOH/CHPS/H₂O = 10/80/40/1000 with a 2 h treatment and worked up as above for the preparation of SX (1.7994 g; C, 36.71; H, 6.11; S, 2.33; DS_S = 0.37; DS_{NMR} = 0.32; yield = 80%). Similarly, QSX (1.5643 g; N, 1.62; C, 41.05; H, 6.68; S, 1.85; DS_Q = 0.23; DS_S = 0.13; DS_{NMR} = 0.39; yield = 53%) was obtained by the reaction

of X/NaOH/GTMAC/CHPS/H₂O = 10/160/40/40/1000 for 2 h, with the treatment and work up as above. QX was prepared at X/NaOH/GTMAC/H₂O = 10/80/40/1000, a two hour treatment and worked up as above (1.1325 g; N, 2.08; C, 40.20; H, 6.72; DS_Q = 0.25; DS_{NMR} = 0.46; yield = 47%). The SX and QSX were also prepared previously (Šimković et al., 2011a), but the NMR data of these derivatives were not completely assigned and are also listed in Table 1.

2.2. Analytical methods

Solution NMR measurements were performed as described previously (Šimković et al., 2011a). The elemental composition of specimens was performed using a FLASH 2000 Organic elemental analyzer (Thermo Fisher Scientific; furnace temperature: 950 °C; PTFE column, 6 o.d./5 mm i.d. × 2 m; 65 °C; helium as carrier and reference gas at 140 and 100 ml/min; oxygen flow: 250 ml/min; 720 s run time; 12 s sampling delay; 5 s injection end).

SEC-MALS characterization was performed using 0.1 M acetate buffer at pH 4.5 as SEC mobile phase (two TSK gel columns; G4000 and G3000; Tosoh Bioscience; at 35 °C; flow rate: 0.8 mL/min; dn/dc = 0.146 g/ml; sample concentration: 3 mg/ml).

AFM imaging was performed using a Digital Instruments Dimension 3100 Series Scanning Probe Microscope (SPM) equipped with a NanoScope IIIa SPM Controller. The top and the bottom surfaces of the films were imaged at a scan size of 5 μm in tapping mode using an etched silicon probe (TESP) at a scan rate of 1.969 Hz. Five images were obtained for each side of individual samples, which were then processed prior to analysis. Raw images were flattened so as to remove the any vertical offset between the scan lines arising out of possible vertical scanner drift, image bow and skips. These were then subject the 'XY PlaneFit' command to remove any tilt or bow from the images before the roughness and section analyses were performed. Analysis of the captured images involved calculating the root mean square (RMS) roughness values of both sides and performing a Section analysis by cutting cross-sectional lines between opposite corners of the images. The RMS roughness value is obtained as the standard deviation of the Z (height) values within a given area, using the following equation:

$$\text{RMS} = \left(\frac{\sum_{i=1}^N (Z_i - Z_{\text{ave}})^2}{N} \right)^{1/2}$$

where Z_{ave} refers to the average Z value within the given area, Z_i is the current Z value, and N is the number of points within a given area or section analysis profile. The roughness values are reported as averages for the five images taken. Section analysis provides with the depth, height, width and the angular measurements across an indicated section. Horizontal and vertical distance, the mean roughness and the maximum difference in height on the surface relative to the mean plane (R_{mzx}) are reported as obtained between the chosen cursor points, ~2.12 μm apart. All other methods were described previously (Šimković et al., 2014).

2.3. Film preparation

All the films were prepared by the casting method using plastic Petri dishes (7 cm in diameter) and one gram of lyophilized sample dissolved in 50 ml of water and stirred overnight at 1250 RPM.

Table 1
NMR data of samples (ppm).

Sample/unit	H ₁ /C ₁	H ₂ /C ₂	H ₃ /C ₃	H ₄ /C ₄	H _{5ax} , H _{5eq} /C ₅	H ₆ /C ₆	CH ₃
CX/MeGlcA	5.29/98.36	3.57/72.15	3.76/73.12	3.22/83.10	4.33, –/72.94	–/177.18	3.46/60.74
CX/Xyl ^a	4.63/102.25	3.41/77.09	3.63/73.53	3.81/76.72	3.41, 4.11/63.57	–	–
CX/Xyl ^b	4.48/102.45	3.29/73.58	3.56/74.42	3.79/77.15	3.38, 4.11/63.66	–	–
CX/Xyl ^c	4.58/102.39	3.16/82.72	3.66/73.61	3.81/77.64	3.37, 4.08/63.52	–	–
CX/Xyl ^d	4.52/102.36	3.38/72.88	3.48/83.80	3.92/76.57	3.40,	4.10/n.a.	–
CQX/MeGlcA	5.26/98.40	3.58/72.19	3.77/73.00	3.26/83.15	4.30, –/73.22	–/177.24	3.47/60.41
CQX/Xyl ^a	4.63/101.98	3.46/77.13	3.65/n.a.	3.80/76.98	3.40, 4.10/63.62	–	–
CQX/Xyl ^b	4.48/102.48	3.31/73.59	3.57/74.59	3.78/77.32	3.39, 4.11/63.74	–	–
CQX/Xyl ^c	4.57/102.34	3.18/82.63	3.66/73.64	3.81/77.76	3.37, 4.09/63.50	–	–
CSX/MeGlcA	5.27/98.48	3.59/72.07	3.77/73.06	3.27/82.91	4.34, –/72.91	–/177.23	3.48/60.41
CSX/Xyl ^a	4.63/101.16	3.45/77.51	3.66/73.63	3.81/77.00	3.41, 4.11/63.92	–	–
CSX/Xyl ^b	4.48/102.43	3.31/73.57	3.58/74.56	3.79/77.30	3.41, 4.12/63.65	–	–
CSX/Xyl ^c	4.57/102.33	3.18/82.74	3.67/73.63	3.82/77.74	3.39, 4.09/63.5	–	–
CSX/Xyl ^d	4.51/n.a.	3.42/72.98	3.48/83.82	3.91/76.84	3.40, 4.10/n.a.	–	–
CSQX/MeGlcA	5.26/98.40	3.58/72.31	3.76/73.12	3.27/83.15	4.30, –/73.24	–/177.30	3.47/60.47
CSQX/Xyl ^a	4.63/101.97	3.47/77.54	3.65/n.a.	3.80/77.00	3.40, 4.11/63.55	–	–
CSQX/Xyl ^b	4.48/102.51	3.31/73.60	3.57/74.60	3.79/77.32	3.40, 4.12/63.7	–	–
CSQX/Xyl ^c	4.57/102.27	3.18/82.61	3.67/73.64	3.81/77.54	3.38, 4.10/63.60	–	–
CSQX/Xyl ^d	4.50/n.a.	3.40/72.83	3.47/83.44	3.89/76.40	3.40, 4.10/63.55	–	–
SX/MeGlcA	5.26/98.44	3.58/71.98	3.76/72.99	3.26/83.09	4.29, –/73.15	–/177.37	3.47/60.46
SX/Xyl ^a	4.63/102.20	3.47/77.60	3.65/73.85	3.80/77.03	3.40, 4.10/63.71	–	–
SX/Xyl ^b	4.48/102.54	3.31/73.62	3.56/74.58	3.79/77.31	3.39, 4.11/63.80	–	–
SX/Xyl ^c	4.56/102.32	3.21/82.51	3.65/73.85	3.80/77.26	3.38, 4.09/63.85	–	–
QSX/MeGlcA	5.28/98.30	3.58/72.18	3.75/73.12	3.21/83.36	4.32, –/72.96	–/177.54	3.46/60.81
QSX/Xyl ^a	4.63/102.16	3.43/76.74	3.62/73.97	3.80/76.88	3.39, 4.10/63.68	–	–
QSX/Xyl ^b	4.48/102.50	3.29/73.56	3.56/74.44	3.79/77.20	3.38, 4.10/63.68	–	–
QSX/Xyl ^c	4.57/102.24	3.18/82.37	3.63/73.97	3.80/77.29	3.37, 4.10/63.68	–	–

n.a. – not assigned; OCH₃ – methylester signal on 4-O-methyl-D-glucuronic acid unit.

^a Xyl unit substituted with MeGlcA unit.

^b Non-substituted Xyl unit.

^c Xyl unit substituted at C-2.

^d Xyl unit substituted at C-3.

3. Results and discussion

3.1. Carboxymethylation and multiple derivatization

According to the results from ¹H-NMR spectroscopy of CX in D₂O solution when the molar ratio of reactants was at X/NaOH/ClCH₂COONa/H₂O = 10/80/40/1000, the DS_C = 0.63 was determined by integration of the anomeric signals. Based on HSQC, COSY, TOCSY and HMBC experiments CX was assigned with the predominant substituent at C-2 of xylopyranose unit (Table 1). By using the integration of anomeric signals except the determined DS_C also 4-O-methyl-D-glucuronic acid content was determined to be 20% of xylose substituted also at C-2 position. According to the HMBC experiment the signals of the methylene group from the carboxymethyl substituents are at 4.07, 4.20/82.72 ppm (H/C) for substituent at C-2. Analogous signals for C-3 are at 4.13, 4.27/83.80 ppm. This indicates that carboxymethyl-groups were linked at both the C-2 and C-3 positions. Other signals related to these substitutions were also assigned as indicated in Table 1.

When quaternized under analogous conditions the DS_Q of QX calculated from elemental analysis was 0.25 as compared to DS_{NMR} = 0.46. The uronic acid content determined also by NMR was 22% which corresponds to DS_U = 0.48. The anomeric NMR signals of unsubstituted, quaternized and xylose substituted with uronic acid were slightly overlapping which resulted in some inaccuracies of the results as seen from the difference of the DS values. For analogous conditions when modified with ClCH₂CHOHCH₂SO₃Na the calculated DS_S of SX was 0.17 in comparison to DS_{NMR} = 0.32 and DS_U = 0.32. So when modified with single alkylating agent according to ¹H NMR the DS of introduced groups decreased in order DS_C > DS_Q > DS_S, while the calculated values according to elemental analysis were smaller than those obtained by NMR. When we

calculate the total charge of the samples taking into account also the negative charge of 4-O-methyl-D-glucuronic acid units than the total charge of all the specimen is negative and decrease in order CX (DS = –1.03) > SX (DS = –0.69) > X (DS = –0.33) > QX (DS = –0.23). We suppose that the negative groups are bonded with sodium cations.

When X was simultaneously carboxymethylated and quaternized at the molar ratio of X/NaOH/ClCH₂COONa/GTMAC/H₂O = 10/160/40/40/1000, a double substituted CQX-derivative was prepared. According to NMR the chemical shift of anomeric signals of both xylose substituted units were identical at 4.57/102.35 ppm (H/C). So the total DS of both substituents (0.60) could be determined and calculated by integration of all anomeric ¹H-NMR signals. Together with DS_Q (0.35) calculated from elemental analysis also DS_C (0.25) could be calculated by subtraction of DS_Q from the value determined by NMR (DS_{NMR}). The 4-O-methyl-D-glucuronic acid content of CQX was 12%. According to two-dimensional NMR experiments it could be also stated that both substituents are linked to C-2 and C-3 of xylopyranose units (Table 1). This is contrary to results on consecutive carboxymethylation and quaternization of arabinoxylan from *Dendrocalamus membranaceus* Munro species (Peng et al., 2012), where no adequate analytical support proof was given for substituent at C-3 xylopyranose unit. The DS_C and DS_Q values we obtained in one step are comparable to values they obtained when the reaction was run in two steps.

For carboxymethylated/2-hydroxypropylsulfonate xylan (CSX) at ratio X/NaOH/ClCH₂COONa/HPS/H₂O = 10/160/40/40/1000 also a double substituted CSX-derivative with 18% of 4-O-methyl-D-glucuronic acid content was assigned (Table 1). Some of the methylene groups from 2-hydroxypropylsulfonate groups could not be exactly assigned due to overlapping with the methylene

Table 2
SEC-MALS characterization of samples at pH 4.5.

Sample	M_p [kg/mol]	M_n [kg/mol]	M_w [kg/mol]	M_w/M_n	Recovered mass [%] ^a
CX	21.3	12.3	16.0	1.29	77.6
QX	25.1	17.6	22.4	1.27	50.8
SX	21.1	15.4	20.8	1.20	68.6
CQX	23.4	15.1	20.3	1.34	83.0
CSX	21.5	15.7	18.8	1.20	83.1
QSX	21.0	13.8	18.1	1.30	85.2
CSQX	22.9	15.2	19.6	1.30	85.2

^a "Recovered Mass" is the mass of sample (g) eluting (i.e. recovered) from the SEC columns, expressed as % of the sample injected mass (sample concentration times injection volume). The Recovered Mass is calculated from the area of the DRI concentration detector after accurate calibration.

groups of both substituents. The DS_{NMR} was 0.55 and the calculated $DS_C = DS_{NMR} - DS_S = 0.55 - 0.08 = 0.47$. For preparation of QSX when using the analogical conditions as above (see Experimental section) the obtained DS_Q (0.23) was bigger than DS_S (0.13). The calculated $DS_U = 0.31$ contributed to the total negative charge of the specimen expressed as $DS = -0.21$. So the charges of double substituted samples decrease in order $CSX (-0.91) > QSX (-0.21) > CQX (-0.14)$.

When all three alkylating agents were introduced in one step at ratio $X/NaOH/ClCH_2COONa/GTMAC/CHPS/H_2O = 10/160/40/40/40/1000$, $DS_C = DS_{NMR} - DS_Q - DS_S = 0.66 - 0.32 - 0.05 = 0.29$ was calculated. So the DS values for individual types of substituents for the prepared derivative are decreasing in order: $DS_Q > DS_C > DS_S$. This means that the reactivity of the three alkylating agents when present in the reaction mixture in an excess of NaOH is decreasing in that order. The 4-O-methyl-D-glucuronic acid content of the mixed derivative was 15% and the cross-peaks for carboxymethyl-group substituents determined by HMBC experiments were identical with those for CX. The remaining signals could not be exactly assigned due to overlapping. Also the assignments of mono-substituted SX and QSX (Table 1) gave very close chemical shifts as those of carboxymethyl-derivatives. The yields of obtained derivatives were decreasing in order: $CQX (90\%) > CX (85\%) > SX (80\%) > QSX (53\%) > CSX (52\%) > QX (47\%) > CSQX (37\%)$. It indicates that the simultaneous presence of carboxymethylated and quaternizing agents improves the yield in comparison to sole carboxymethylation. The yield of CSX when two negatively charged agents were used resulted in much lower value. The combination of two negatively and one positively charged agent further decreased the yield. The total charge of all studied samples decreased in order: $CX (-1.03) > CSX (-0.91) > SX (-0.69) > X (-0.33) > CQX (-0.32) > QX (-0.23) > QSX (-0.21) > CQX (-0.14)$. So in all samples the negatively charged ion-exchanging groups were in surplus.

3.2. SEC-MALS analysis

Results in Table 2 indicate the effect of chemical modification on solubility of the films as well as on the determined molar masses. As described previously, when the film prepared from X was characterized by SEC-MALS, its numerical average molar mass (M_n) was the smallest from all studied samples with the biggest polydispersity (M_w/M_n) and the smallest amount of recovered mass (Šimković et al., 2014). As seen from Table 2 there is a sharp peak before the main peak in the SEC-profile of X. By the chemical modification the solubility of samples increased, which is represented by increased values of recovered mass. As seen from the results in Table 2 the obtained SEC-MALS data are very similar for all studied samples which could be expected due to identical reaction conditions. The highest mass value at the peak (M_p) was obtained for QX which had the lowest value of recovered mass. The second lowest mass recovered was observed on SX. Generally all three monosubstituted samples had lower mass recovered values than the multiple

substituted ones. For QSX sample some aggregation was observed due to additional peak observed on light scattering signal. This might be explained by the interaction of oppositely charged groups. The polydispersity values of all the samples are much smaller than values obtained on carboxymethylated/quaternized-xylan when microwave was used (Peng et al., 2012). This might be affected by the source of xylan. On the other hand the M_w obtained by the authors when carboxymethylated in the first step was bigger than ours, but the values determined after quaternization in the second step are comparable with ours.

3.3. Thermal stability

According to the previous TG/DTG/DTA studies the thermal degradation of X in air environment starts at 205 °C (Šimković et al., 2014). For CX film the onset temperature (OT) is at 204 °C (Table 3) with first DTG peak at 247 °C at the rate of 1.94 mg/min. Additionally there are two more DTG peaks at 350 and 720 °C and six DTA peaks observed for this film. The DTG peak at 350 °C is probably related to residual cellulose represented by the unrecovered portion determined by SEC-MALS as stated previously (Šimković et al., 2014). Although SX sample was studied before on different TG/DTG/DTA instrument, we are listing it also here due to different conditions to compare with other samples under the identical instrumental and experimental conditions (Šimković et al., 2011a). The onset temperature (214 °C) is the highest from all studied samples. The first DTG peak is at 262 °C with first DTA peak at 271 °C. Additionally there are four other DTG and DTA peaks and thermal degradation is finished at 625 °C. The QX sample prepared under identical conditions as CX and SX has OT at 201 °C. There were three DTA and two DTG maxima observed, while first DTA maxima for all three mono-substituted samples are very close to each other. The residues of QX from 300 up to 1000 °C are smaller than for two other mono-substituted specimens.

The double substituted film QSX has the highest OT from all double-substituted samples (211 °C; Table 3). With three DTA and four DTG peaks the process of thermal degradation is the simplest from all three double substituted samples. The first DTG is also at higher temperature than for CQX and CSX samples. The residues are slightly bigger up to 400 °C than for QX, while at higher temperature they are smaller. The CQX film has OT at 207 °C and first DTA and DTG peaks are also at lower temperature than for QSX. There are four DTA exotherms and DTG peaks observed for this sample, indicating the four step degradation process with last DTA and DTG peak at 500 °C. The residues at 300 and 400 °C are smaller than for QSX film. When X was carboxymethylated and hydroxypropylsulfonated in one step, the most complicated DTG and DTA profiles were observed for CSX film in comparison to other samples (Table 3). The onset temperature was the smallest (187 °C). The first and biggest DTG peak was observed at 243 °C with the degradation rate of 1.49 mg/min. The first DTA peak was at 273 °C, which was probably related to carboxymethyl-group degradation because an analogous peak was observed at 273 °C for CX. There are five

Table 3
TG/DTG/DTA data of studied films.

Sample	OT ^a [°C]	DTA peaks [°C]		DTG peaks Δ [K/mg]			Residue [%] at [°C]			
				[°C]	[mg/min]	200	300	400	500	1000
CX	204	273	0.0651	247	1.94	91	49	38	34	7
		304	0.0605	350						
		363	0.0972	720						
		694	0.1176							
		769	0.1482							
		842	0.0326							
SX	214	271	0.0268	262	0.61	85	46	33	21	5
		371	0.0559	338	0.52					
		401	0.0499	400	0.03					
		510	0.1858	485	0.09					
		584	584	0.04						
QX	201	270	0.045	264	6.63	88	37	27	10	2
		422	0.1263	499	0.77					
		505	0.1357							
QSX	211	282	0.0364	271	4.92	88	44	30	4	1
		438	0.1232	312	0.66					
		489	0.1742	478	1.51					
				509	0.44					
CQX	207	267	0.0344	254	4.46	87	39	26	5	2
		342	0.0878	340	0.42					
		463	0.2144	441	0.76					
		500	0.2022	500	0.46					
CSX	187	273	0.0278	243	1.49	89	50	40	37	13
		314	0.0498	259	1.45					
		370	0.0571	349	0.23					
				692	0.90					
		708	0.1432	708	0.84					
		762	0.1940	782	0.04					
		800	0.0565							
CQSX	212	274	0.0234	261	4.42	87	41	27	10	2
		436	0.1311	432	0.55					
		522	0.1983	479	0.72					
				540	0.36					

^a Onset temperature.

additional DTG peaks at 259, 349, 692, 708 and 782 °C. Also the DTA curve has five additional peaks at 314, 370, 708, 762, and 800 °C. The residue at 1000 °C is the biggest from all studied samples (13%).

The OT of CQSX is at 212 °C, which is the second highest value for all studied samples. The first DTG maximum is at 261 °C with the third highest degradation rate (4.42 mg/min) after QSX (4.92 mg/min) and CQX (4.46 mg/min). There are three additional DTG peaks at 432, 479, and 540 °C, while there were three DTA peaks observed at 274, 436 and 522 °C. The residue at 1000 °C is the same as for CQX and QX (2%). So according to the measured onset temperatures of the studied samples the thermal stability decreases in order: SX (214 °C) > CQSX (212 °C) > QSX (211 °C) > CQX (207 °C) > X (205 °C) > CX (204 °C) > QX (201 °C) > CSX (187 °C). It seems that all the samples could survive the heating over 100 °C required for sterile conditions.

3.4. Mechanical properties

From the results in Table 4 it is obvious that tensile strength and stiffness of all modified samples were reduced in comparison with X, analogously as for quaternized and sulfated amphoteric xylan films (Šimkovic et al., 2014). It indicates that modifications lead to change in supramolecular structure of xylan and interactions between its chains. The thickness of the films is in the range of 0.03–0.30 mm. The modulus values are decreasing in order: X > SX > CX > CSX > QSX > QX > CQSX > CQX, while the tensile strength values are decreasing as follows: X > CX > CQSX > QX > CSX > QSX > CQX > SX. The elongation values

are decreasing along the line: QX > X > CQSX > CQX > CX > CSX = QSX > SX. When compared with the previous work in the present work QX sample with lower DS was prepared, which resulted in higher modulus and tensile strength values. The double substituted samples QSX and CSX are having similar modulus and tensile strength values as sulfated/quaternized xylan. On the other side the quaternized/sulfated analog behaved in similar way as SX by having low tensile strength value. We think this results could be assigned to interaction of the introduced substituents. On QSX molar mass aggregation was observed. The lower modulus for CQX might be due to less aggregation as the oppositely charged spacers are of different length than equally long one attached on QSX. Interestingly the CSX sample with the highest DS of anionic groups from double substituted derivatives have the highest modulus while there was no molar mass aggregation observed for other samples than QSX. The triple substituted CQSX sample resulted in higher modulus than CQX and bigger tensile strength than all three double substituted derivatives. This indicates that the effect of multiple modification on structural parameters and interactions is probably more complex in comparison with single substituted sample. It was also observed by others that chemical modification of xylan negatively affects the mechanical properties (Alekhina et al., 2014). The mechanical properties of films are comparable with nanocrystalline cellulose enforced films made from ramie, hardwood, sugar cane bagasse, cotton, luffa cylindrical or wheat straw (Bras, Viet, Bruzzese, & Dufresne, 2011). Also nanocomposite films based on xylan-rich hemicelluloses and cellulose nanofibers have similar mechanical properties (Peng, Ren, Zhang, Sun, 2011). The modulus

Table 4
Mechanical properties of studied xylan films.

Sample/DS ^a	<i>E</i> (MPa)	Thickness (mm)	σ_b (MPa)	ε_b (%)
X/0.33	7355 ± 380	0.05	108.0 ± 19	3.9 ± 0.9
CX/1.03	6090 ± 660	0.07	93.0 ± 9	2.7 ± 0.7
SX/0.69	6000 ± 190	0.03	44.4 ± 31	0.8 ± 0.6
QX/0.23	3600 ± 315	0.15	70.1 ± 3.6	4.5 ± 0.9
CQX/0.14	2805 ± 405	0.15–0.25	50.8 ± 4.1	3.3 ± 0.8
CSX/0.91	4490 ± 810	0.11	66.1 ± 7.3	2.4 ± 0.8
QSX/0.21	3810 ± 500	0.13	59.3 ± 3.6	2.4 ± 0.2
CQSX/0.32	3380 ± 340	0.11–0.3	73.2 ± 4.9	3.4 ± 0.6

^aThe prevailed anionic DS.

values are bigger than obtained on CX from birch extracts (Alekhina et al., 2014) or arabinoxylans from wheat grain (Ying et al., 2013). Also the tensile strength determined on oat spelt xylan film or composites films containing up to 10% of cellulose whisker was smaller than determined on our specimens (Saxena, Elder, Pan, & Ragauskas, 2009).

3.5. AFM analysis

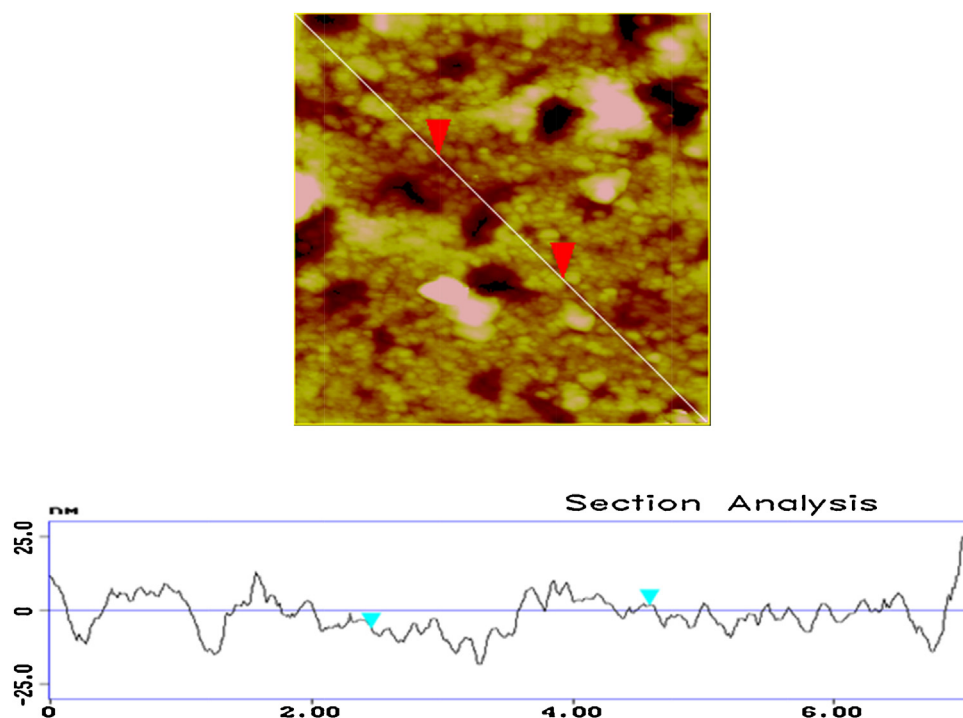
The determined average roughness values of all samples are listed in Table 5. They are from 3.634 to 18.667 nm and are larger for the top side than for the bottom side of the surface. This could be explained by the effect of the environment above the Petri dish during the drying process. Due to the smallest recovered amount of sample according to SEC-MALS analysis the roughness value of X is affected by the presence of water-insoluble particles. The images of X, QX and SX were already presented before (Šimković et al., 2014). The top surface of CX height image (Fig. 1) looks different than the previous three with lot of irregularities. The roughness of the monosubstituted samples seems to be decreasing in the same order as the DS values of the introduced groups in the specimens. For the double substituted specimen surfaces the biggest value was observed on the top side of CSX specimen containing two negatively

Table 5
Roughness values [nm].

Sample	Top surface/section roughness	Bottom surface/section roughness
X	14.784/6.122 or 15.794	4.651/3.473 or 2.382
CX	15.624/6.902 or 8.341	5.275/3.308 or 5.979
SX	8.153/4.109 or 3.371	4.687/3.444 or 4.967
QX	10.114/6.151 or 4.250	8.653/7.697 or 9.896
CQX	11.337/7.142 or 6.815	6.319/2.883 or 3.742
CSX	18.667/51.099 or 20.710	3.634/4.459 or 2.690
QSX	6.400/6.359 or 5.267	5.310/5.078 or 4.634
CQSX	6.475/2.158 or 3.143	5.134/3.764 or 4.164

Key: Section roughness values of individual profiles in the section analysis of two cuts set from corners of the image and crossing in the middle of the image; the first value is from profile set from left top corner of the image.

charged substituents, while the smallest value was determined on the bottom side of the same specimen. The smallest difference of roughness values between the top and the bottom side of the film was observed on QSX sample. The CQX sample had the biggest roughness value on the bottom side and the second biggest on the top side of film from all three double substituted samples. The triple substituted CQSX film top surface (Fig. 2) produced also similar

**Fig. 1.** AFM high image and section analysis of top side of CX film.

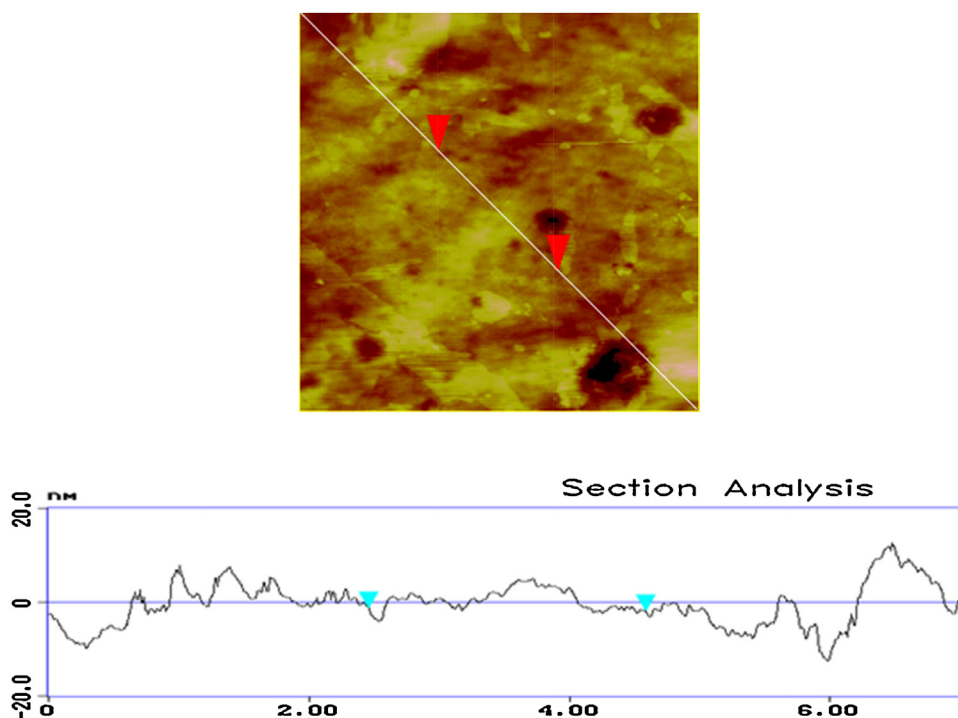


Fig. 2. AFM high image and section analysis of top side of CQX film.

roughness values on both sides as QSX sample. In both cases the DS_Q substituent was the predominant although the anionic character of ion-exchanging groups in all specimens prevailed when all ionic groups were taken in account. The section analyses were made on the images in such way that two sections were made on each image from corner to opposite corner. The highest roughness value on the individual profiles was observed again for CSX top side (Table 5). It seems that there is some effect of the negative charged groups on the resulting increase of roughness values due to some repulsion forces. This was observed also on carboxymethylated glucomannan polysaccharide from *Auricularia auricular* (Yang et al., 2011).

4. Conclusions

The CQX, CSX, QSX and CQSX films were prepared in one step by using up to three alkylating agents. When singly-substituted under identical conditions the DS decreases in the order: $DS_C > DS_Q > DS_S$, while when all three alkylating agents are introduced in one step the obtained DS's were decreased differently ($DS_Q > DS_C > DS_S$). For doubly substituted derivatives the yield decreased in order CQX (90%) > QSX (53%) > CSX (52%). This could be explained by the difference of the reaction mechanism of negatively charged alkylchlorides and positively charged epoxy-agent. The mechanical properties of films were comparable with previously published data mentioned above. It is obvious that the level of mechanical parameters of all modified samples is still relatively good and balanced, suitable for wide range of applications (comparable with many "commodity" polymers). The roughness values of all studied films were smaller on the bottom than on top of the films probably due to environment action during the drying process. The anionic substituents prevailed in all samples, but were probably inhomogeneously distributed through-out the specimen.

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