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The immunologically active xylan from ultrasound-treated corn cobs: extractability, structure and properties

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Abstract

The extractability of the corn cob xylan, particularly its water-soluble component (ws-X), was studied using extraction methods with and without application of ultrasound in water and aqueous NaOH. The results indicate that ws-X comprise a variety of xylan polymers differing in the proportion of side chains composed of single arabinose and glucuronic acid side chains and 2-O-β-D-xylopyranosyl-α-L-arabinofuranose moieties. The xylan fractions were characterized by yield, composition, structural features, molecular properties and biological response in mitogenic and comitogenic thymocyte tests. Compared to the classically extracted xylans, the ws-X prepared by the assistance of ultrasound had the same primary structure features and similar or slightly higher stimulatory activities in both tests. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Immunologically active; Xylan; Corn cobs

1. Introduction

In the review on hemicelluloses of cereals and grasses (Wilkie, 1979), corn cobs were reported to contain considerable amounts of xylan-rich hemicelluloses A and B differing in composition and structural features. Recent reinvestigations of the corn cob xylan (Ebringerová et al., 1992a) revealed the existence of at least two structurally different components. The major one (wis-X) is a low-substituted arabino-(4-O-methylglucurono) xylan which is water insoluble. The second one (ws-X) is water soluble with a similar xylan backbone, containing in addition to the single arabinosyl and glucuronosyl branches also disaccharide side chains, composed of xylose and arabinose as well as minor amounts of galactose. The last mentioned xylan exhibits useful rheological properties (Ebringerová et al., 1992a) as well as significant immunological activity in vitro comparable with that of the commercial immunodulator Zymosan (Ebringerová et al., 1995).

In view of these facts, attention was paid to the extraction process of the corn cob xylan (Ebringerová et al., 1988) and, currently, also to the application of ultrasound during extraction (Hromádková et al., 1997). Ultrasonic treatment is well established in the processing of plant raw materials, particularly, for extracting low molecular substances (Mason et al., 1996). Recently, ultrasound treatment has been reported to improve the apple pectin technology (Panchev and Kratchanov, 1988; Panchev et al., 1994). However, high intensity ultrasound can break down polymers (Lorimer et al., 1995) which may negatively affect their functional properties. Therefore, we have studied previously (Ebringerová et al., 1997) the effect of ultrasound on the earlier reported biologically active corn cob xylan (Ebringerová et al., 1992a) dissolved in water and aqueous alkaline solutions.

The present study discusses the use of ultrasound in the extraction of the immunologically active corn cob xylan component, its structural and molecular characteristics as well as biological response in mitogenic and comitogenic tests.

2. Experimental

2.1. Materials

Corn cobs were obtained from ZEAINVENT (Trnava, Slovak Republic) in 1995. They were dried on air and ground (particle size 1–2 mm). All chemicals were of analytical grade. Zymosan was obtained from Likospol

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Ltd (Bratislava, Slovak Republic). Experiments and analyses were performed at least in duplicate. Yields are given on a dry weight basis.

2.2. Extraction methods (Fig. 1)

2.2.1. Procedure A

In the first extraction step, ground cobs (10 g) were suspended in 250 ml of 5% NaOH and stirred for 1 h at 60°C. After rapid cooling to room temperature, the insoluble residue I was separated by centrifugation (3000***g***, 15 min). Then, in the second step, it was subsequently treated with 200 ml of 5% NaOH for 1 h at ambient temperature. The remaining insoluble residue II was separated by centrifugation. The extracts from both steps (I, II) were combined and poured into four volumes of ethanol. The precipitate was twice decanted with 80% ethanol, acidified with acetic acid to pH 6, and filtered off. Subsequently, it was suspended in distilled water and subjected to exhaustive dialysis against distilled water in a cellophane bag. The non-dialysable content of the bag was separated by centrifugation into the insoluble and solubilized portions, which were dried by lyophilisation to yield the fractions A/wis-X_{I+II} and A/ws-X_{I+II}.

2.2.2. Procedure B

The procedure differs from procedure A only in the first step, where 5% NaOH containing 3% of H_2O_2 (based on the weight of cobs) was used as the extractant. Fractions B/ws-X_{I+II} and B/wis-X_{I+II} were isolated as described in procedure A.

2.2.3. Procedure C

The sample (2 g) was suspended in 30 ml of distilled water, sonicated at 50 ± 3°C for 20 min. The extract I was dialysed and lyophilized (C/ws-X_I), and the insoluble residue I was treated with 5% NaOH, as described above, yielding fractions C/ws-X_{II} and C/wis-X_{II}.

2.2.4. Procedure D

In this case, the sonication was performed in 1% NaOH at 60 ± 3°C for 30 min and the extract I was precipitated into four volumes of ethanol as in procedure A to yield fraction D/ws-X_I. Residue I was further processed according to procedure A yielding fractions D/ws-X_{II} and D/wis-X_{II}.

2.2.5. Procedure E

The experiment was carried out according to procedure D, but sonication was performed in 5% NaOH at 60 ± 3°C for 10 min. From the first step, xylan from fraction E/ws-X_I was separated as described above. The water-insoluble portions from both extracts (I, II) were combined and recovered as fraction E/wis-X_{I+II}.

2.3. Ultrasonic treatment

Irradiation was carried out using the Ultragen system PERSON (Nitra, Slovak Republic, 20 kHz) at 100 W sonic power and sonication time intervals of 10 min. The dispersion was sonicated the given time in a glass beaker of standard geometry in order to preserve the same ultrasonic intensity of 8 W cm^{-2} in all experiments.

2.4. General analyses

The methods of hydrolysis, paper chromatography of neutral and acidic sugars, and GC analysis of neutral sugars as alditol trifluoracetates were described previously (Hromádková and Ebringerová, 1995). The uronic acid content was determined by potentiometric titration (Kohn et al., 1986).

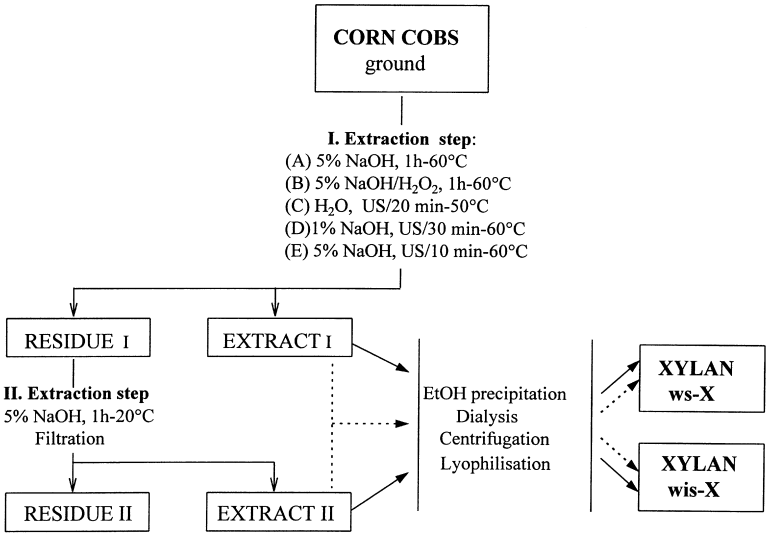

Fig. 1. Scheme for the isolation of the xylan component of corn cobs.

2.5. Estimation of molecular weight distribution

Molecular weight distributions were estimated by HPGPC on Separon HEMA-BIO 100 and 1000 columns, calibrated from relative molecular weight 10×10^3 to 8×10^5 by the aid of pullulan standards P10–P800, as previously described in detail (Odonmažig et al., 1994).

2.6. NMR spectroscopy

NMR spectra were recorded at 40°C on a FT-NMR AVANCE DPX-300 Bruker spectromer (^{1}H at 300.13 MHz and ^{13}C at 75.46 MHz) equipped with a selective excitation unit and gradient enhanced spectroscopy kit (GRASP) for generation of Z-gradients up to 50 Gauss/cm in a 5 mm inverse probe.

The ^{1}H detected two-dimensional heterocorrelated (HSQC) experiment (Schleucher et al., 1994) was conducted with composite GARP sequence decoupling; 128 scans pre t_1; 256 experiments zero-filled to 1 K points; processed with shifted sine-bell window (SSB = 3) in both dimensions. The two-dimensional COSY experiment was performed according to Davis et al. (1991) using gradient pulses with double quantum filter.

2.7. Immunological activity tests

Samples were subjected to an assay for their mitogenic and comitogenic activities as described in a previous paper (Ebringerová et al., 1995) using Zymosan as positive control. The stimulation index was measured by the incorporation of ^{3}H-thymidine in rat thymocytes cultivated in absence (SI_{mit}) and presence (SI_{comit}) of phytohaemagglutinin (PHA). The direct mitogenic effect was expressed as: (SI_{mit}) = mean cpm for test compound/mean cpm for control. The comitogenic effect was expressed as: (SI_{commit}) = mean cpm for PHA + test compound/mean cpm for PHA. The mean cpm ± SD for control cultures without any addition was 912 ± 323 and for cultures incubated with PHA it was 1165 ± 622.

3. Results and discussion

3.1. Extraction of the corn cob xylan component

Prior to developing the ultrasound-mediated extraction procedures, we have determined (Ebringerová et al., 1997) the conditions (sonication time, power and temperature) in which the structural properties and biological activity of the water-soluble corn cob xylan (Ebringerová et al., 1992a) showed no significant changes after sonication in water, 1% NaOH, and 5% NaOH. The previously (Hromádková et al., 1997) developed two-step extraction procedures, without (classical) and with application of ultrasound in the first step, and constant conditions in the second (washing) step, were carried out according to the scheme in Fig. 1. The extraction temperature was held at 50–60°C in order to avoid severe alkaline degradation of the xylan macromolecule.

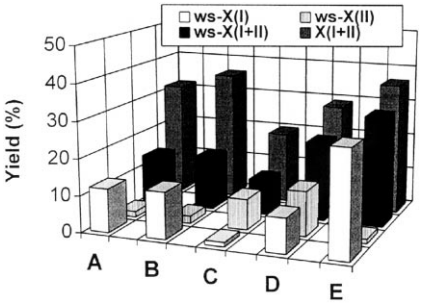

Fig. 2. Yield of ws-X fractions isolated from corn cobs by classical (A, B) and ultrasound mediated extractions (C–E) in the I and II extraction steps and the total extracted xylan X_{I+II}.

The extractability of the xylan components under the conditions used is shown in Fig. 2, expressed in terms of the yield of ws-X fractions obtained in both extraction steps (I, II) and total extractable xylan (X_{I+II}) comprising both ws-X and wis-X components. In comparison with both classical procedures, significant changes were observed after application of ultrasound in the total yield of the xylan and its water-soluble component, in dependence on the used extractant. Both classical procedures, using 5% NaOH without (A) and with addition of H_2O_2 (B), gave about the same yields (13.5 and 14.7%, respectively) of ws-X, released mainly (~80%) in the first extraction step. The ws-X components represented about 40% of the total extracted xylan. After a short application of ultrasound in 5% NaOH (E), the yield of ws-X_1 increased dramatically (28.8%) and exceeded twice the yield of the classically extracted xylan. However, the total yield of the extracted xylan resembled that obtained by the classical procedure A. The ultrasonic effect was less pronounced in 1% NaOH (D) where the yield of X_{I+II} was lower. Nevertheless, the yield of ws-X_{I+II} was still about 50% higher in this case, when compared with the classical procedures. The observed beneficial sonication effect on the extractability of the ws-X component can be explained by both the mechanical disruption of the cell walls (Mason et al., 1996) and breaking of inter- and intramolecular xylan linkages, enhanced in the presence of hot alkali (Ebringerová et al., 1997). As a result, the accessibility, solubility and diffusion of the dissolved molecules from the cell walls increased. This was also confirmed by the low sonication effect in water (procedure C).

3.2. Composition and molecular properties of xylan fractions

Table 1 summarizes the sugar composition of the xylan preparations. The ws-X fractions resembled in composition the previously reported (Donnelly et al., 1973)

Table 1
Neutral sugar composition of the xylan fractions derived from classical and ultrasound-mediated extraction methods

Method	Xylan fraction	Neutral sugar composition (mol%)					GlcA[a] (%)	Ara/Xyl (mol ratios)	Xyl/GlcA (mol ratios)
		Ara	Xyl	Man	Glc	Gal			
Without ultrasound									
A	ws-X_{I+II}	21.1	68.2	0	5.5	5.2	7.5	0.31	11.8
	wis-X_{I+II}	3.4	91.1	tr	2.0	3.5	3.9	0.04	32.7
B	ws-X_{I+II}	14.5	81.5	0	1.1	2.9	6.5	0.18	15.5
	wis-X_{I+II}	5.8	88.9	tr	3.5	1.8	nd	0.06	nd
With ultrasound									
C	ws-X_I	31.7	55.8	0	6.2	6.3	nd	0.57	nd
	ws-X_{II}	22.2	73.7	0	0	4.1	8.1	0.30	11.8
	wis-X_{II}	8.8	91.2	0	0	0	nd	0.10	nd
D	ws-X_I	23.9	62.5	2.6	4.0	6.0	8.9	0.38	9.0
	ws-X_{II}	15.6	78.5	0	2.4	3.5	4.5	0.20	23.5
	wis-X_{II}	8.5	87.9	1.6	1.6	0.4	nd	0.10	nd
E	ws-X_I	18.3	73.1	1.4	4.8	2.4	9.9	0.25	9.4
	wis-X_{I+II}	6.9	74.1	4.4	14.7	0	3.1	0.09	32.5

[a]Determined as 4-O-methylglucoronic acid; tr, traces; nd, not determined.

hemicellulose B component of different genetic populations of corn cobs, but showed a larger range of the arabinose to xylose ratios: 0.18–0.57, against the reported 0.14–0.21. This is due to the fractional extraction of the ws-X polymers by the different extractants used in our study. The wis-X fractions had very low arabinose to xylose ratios (0.04–0.10) and correspond to the hemicellulose A (Donnelly et al., 1973). All xylan preparations contained variable amounts of glucuronic acid (mainly its 4-O-methyl derivative), glucose and galactose. The arabinose to xylose ratio of ws-X_1 from procedure E ranged between values obtained by the classical procedures. Higher ratios were found in the ws-X fractions isolated by ultrasonication in 1% NaOH (procedure D) and water, particularly in the first step.

The results indicate a great diversity of the ws-X component and various locations of ws-X and wis-X polymers in the cell walls, similarly to that reported for the rye bran (Hromádková and Ebringerová, 1992) and beechwood xylan components (Ebringerová et al., 1992b). Probably, due to the above mentioned sonication effects, the lower substituted xylans from the less accessible regions of the cell walls were also degraded and solubilized. In accordance, the yields of wis-X fractions, which are the least extractable ones (Fig. 2, difference between the yields of X_{I+II} and ws-X_{I+II}), showed a decreasing tendency in the order C > D > E, and were substantially lower than those from procedures A and B.

The molecular properties of the ws-X fractions were characterized by HPGPC (Table 2). All xylan fractions showed a bimodal distribution with a minor (2–9%) high molecular component (peak I, M_w~400–900 kDa), similar to that previously reported for the water-soluble corn cob xylan prepared in a technical scale (Ebringerová et al., 1992a). The results in Table 2 support the previous suggestions on the fractional extraction of ws-X enhanced by the sonication effects. The lowest relative molecular mass (M_w) of the main molecular component (peak II) and highest degree of polydispersity (M_w/M_n) were determined in ws-X from procedures C and D.

3.3. Structural characterization

The $^{13}C-NMR$ spectra of the water soluble xylan preparations obtained by the ultrasound-mediated extraction showed no significance in comparison to that of the ws-X from classically extracted corn cobs (Ebringerová et al.,

Table 2
Molecular mass distribution[a] of ws-X fractions derived from classical (A, B) and ultrasound-mediated (C–E) extractions

Xylan fraction	Peak I			Peak II		
	$M_w \times 10^{-3}$	M_w/M_n	Area (%)[b]	$M_w \times 10^{-3}$	M_w/M_n	Area (%)[b]
A/ws-X_{I+II}	577	1.02	7	94	1.36	93
B/ws-X_{I+II}	466	1.03	8	71	1.81	92
C/ws-X_{II}	586	1.01	2	37	4.01	98
D/ws-X_I	419	1.03	6	54	4.30	94
E/ws-X_I	882	1.01	9	61	2.03	91

[a]Measured on Separon HEMA B10–100 and 1000 columns, calibrated with pullulan standards; [b]calculated from the peak areas of the RI-detected chromatograms.

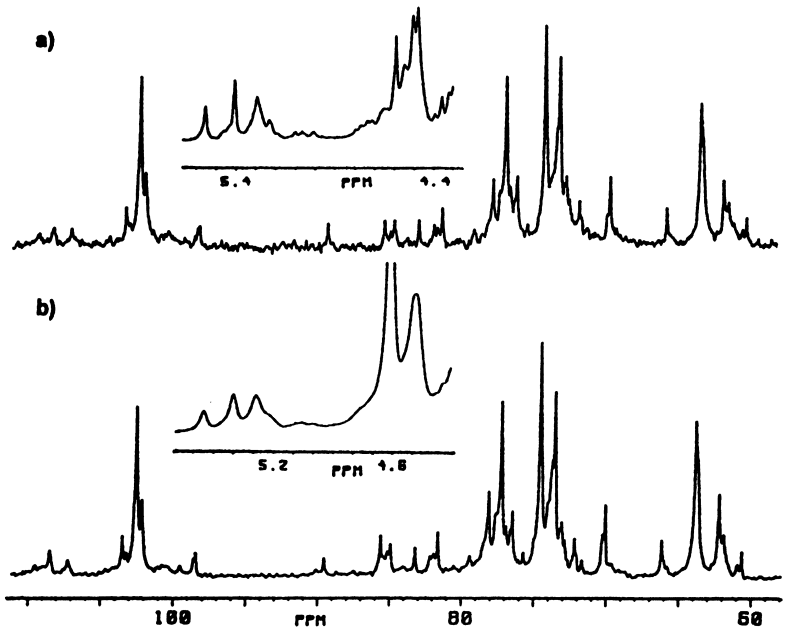

Fig. 3. $^{13}C-$NMR spectra (in D_{2O}) of xylan fractions (a) B/ws-X_{I+II}, and (b) E/ws-X_I. Inserted are the partial $^{1}H-$NMR spectra of the fractions.

1992a). This is demonstrated by the $^{1}H-$ and $^{13}C-$NMR spectra (Fig. 3) of the xylan representants of both techniques (B/ws-X_{I+II} and E/ws-X_I). Therefore, the last mentioned xylan will be discussed in more detail. Most of the major resonances were assigned in the previous paper (Ebringerová et al., 1992a) by reference to data in literature. They were confirmed in this paper after inspection of the two-dimensional $^{1}H–^{1}H-$NMR COSY (Fig. 4) and $^{1}H–^{13}C-NMR$ COSY spectra (Fig. 5).

There were at least nine groups of anomeric resonances (with integrated intensities in brackets) in the $^{13}C-$NMR spectrum of E/ws-X_1 at 109.5/109 (4.1%), 108.5 (8.2), 107.3 (5.4), 103.5 (8.0), 103.2 (4.9), 102.6/102.2 (59.6), 100.6/101.4 (2.4), 99.4 (2.4), and 98.6 (5.0) ppm. In the case of B/ws-X_{I+II}, the corresponding intensities (%) were: 3.8, 4.3, 4.1, 8.1, 4.0, 68.8, 1.9, 0.8 and 4.2. Some groups contained more than one resonance. The signals of the first two groups were assigned to C-1 of terminal α-arabinofuranosyl residues linked to O-2 and O-3 (disubstitution) and O-3 (monosubstitution) of the β-xylopyranosyl residues, respectively (Ebringerová et al., 1990). This was confirmed by the presence of the corresponding protons at 5.26, 5.32 and 5.42 derived from the correlated $^{1}H–^{13}C-$NMR COSY spectrum. In accordance, the triade of C-2 resonances at 82.2, 82.0 and 81.7 ppm, and C-4 signals at 85.0, 85.2 and 85.6 ppm were present. Such groupings of arabinose signals are typical of arabinoxylans isolated from cereal grains (Ebringerová et al., 1990; Bengtsson and Aman, 1990; Hoffmann et al., 1992; Vinkx et al., 1995; Izydorczyk and Biliaderis, 1995).

The signal at 107.3 ppm was tentatively assigned to C-1 of the 2-linked α-arabinofuranosyl residue of a disaccharide side chain, terminated by a β-xylopyranosyl residue, in the previous paper on corn cob xylan (Ebringerová et al., 1992a). These results were supported by methylation analysis. The presence of 2-O-β-D-xylopyranosyl-α-L-arabinofuranose side chains in corn cob heteroxylans was determined by the methylation analysis of oligosaccharidese isolated from xylan hydrolysates (Wilkie, 1979; Kusakabe et al., 1983). Recently, Saulnier et al. (1995) have isolated from corn bran, and structurally characterized, the mentioned dissacharide feruloylated at position O-5 of the arabinose residue. In our study, the C–H shift correlation experiment revealed the position of the anomeric resonance of the 2-linked arabinose unit at 5.58 ppm which has a cross-peak in the COSY spectrum (Fig. 4) with a signal at 4.28 ppm. It must arise from H-2 which correlated with the signal at 89.6 ppm, assigned to C-2 of the 2-linked α-arabinofuranosyl unit (Fig. 5). Saulnier et al. (1995) reported the value of 89.9 ppm for the corresponding feruloylated disaccharide. The additional peak in the region of the C-4 resonances at 85.4 ppm, correlated with H-4 at 4.17 ppm, can be ascribed to C-4 of the 2-linked arabinose unit. In both B/ws-X_{I+II} and E/ws-X_I, the 2-linked arabinose made up about one third of the arabinose component, whereas the proportion of arabinose residues involved in disubstitution was higher in B/ws-X_{I+II}.

The couple of chemical shifts at ~107 and 89 ppm as well as an additional downfield shifted proton signal (~5.5), which are diagnostic for the presence of 2-O-β-D-xylopyranosyl-α-L-arabinofuranose side chains, can be seen also in the spectra of the heteroxylans from corn bran (Hromádková and Ebringerová, 1995) and some arabinoxylans from rye (Nilsson et al., 1996) and wheat (Hoffmann et al., 1991; Annison et al., 1992). Although some proton signals in wheat arabinoxylan were discussed in relation to

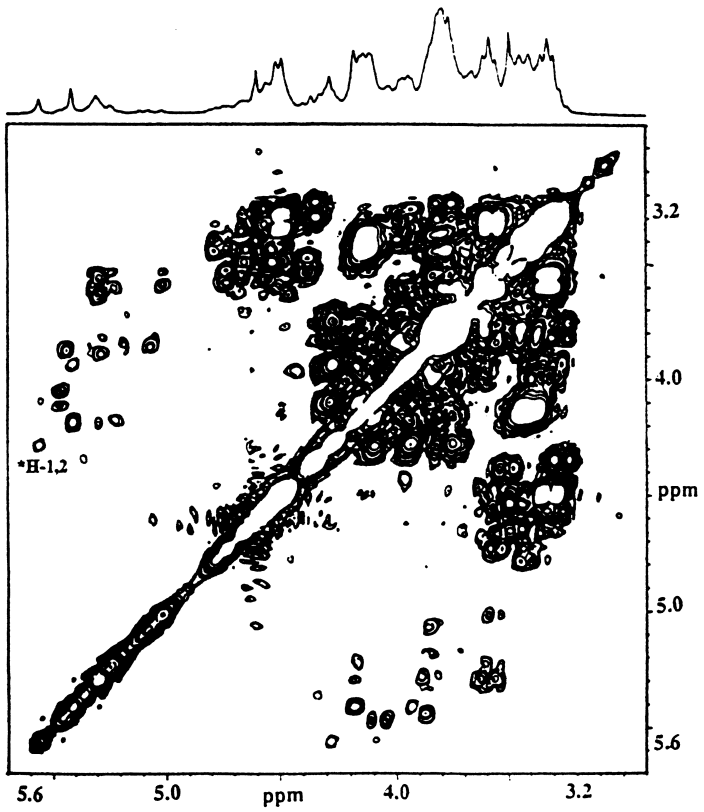

Fig. 4. Homonuclear $^{1}H—^{1}H$ – NMR COSY spectrum of E/ws-X_1; *cross peak of the H-1 and H-2 protons of the 2-linked α-arabinofuranosyl residue of the disaccharide side chain.

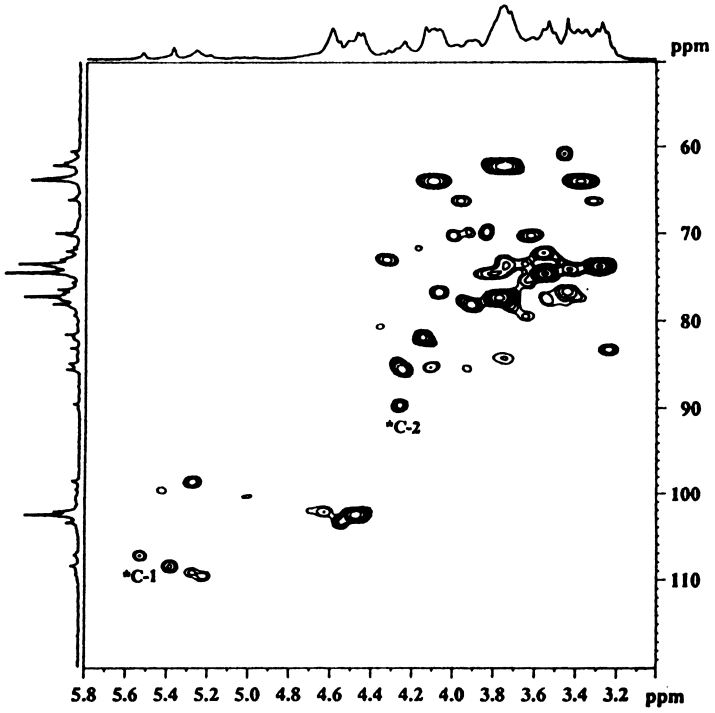

Fig. 5. Heteronuclear$^{1}H—^{13}C$ – NMR COSY spectrum of E/ws-X_1; *C-1 and *C-2 resonances of the 2-linked α-arabinofuranosyl residue of the disaccharide side chain.

the presence of substituted arabinose units by Vinkx et al. (1995), the above mentioned diagnostic carbon signals could not be observed in the reported ^{13}C – NMR spectrum.

The strong signals at 102.6/102.2 ppm, correlating with the proton resonances at 4.50–4.70 ppm, were attributed to the anomeric resonances of unsubstituted and substituted xylopyranosyl units of the xylan backbone, and that at 103.5/103.2 ppm, correlating with proton resonances at 4.55 ppm, had been assigned to non-reducing terminal xylosyl units (Ebringerová et al., 1992a). The resonances of their C-5/C-4 signals were found at 66.2 and 70.3 ppm, respectively (Bock and Thogersen, 1984). However, the proportion of C-1 resonance of the 2-linked arabinose units corresponded more to that of the resonance at 103.2 than at both 103.2 and 103.5 ppm. The last mentioned resonance might arose from otherwise linked terminal xylopyranosyl units and/or β-linked glucose (Bock and Thogersen, 1984; Bock et al., 1984). By methylation analysis of the corn cob xylan (Ebringerová et al., 1992a), 4-linked glucopyranosyl units were determined in considerable quantity. The C-4 and C-6 signals of such units could be found at 79.8 and 60.8 ppm, respectively, although their intensities are lower when compared to that of the C-1 resonance at 103.5 ppm.

The proportions of the arabinose to xylose ratios of B/ws-X_{I+II} (0.27) and E/ws-X_I (0.17), calculated from the intensities of the respective resonances with exclusion of the signal at 103.5 ppm, corresponded well with the values of 0.25 and 0.18, respectively, determined by the sugar analysis. In xylans from procedure E, glucose was determined in high amounts, particularly in the wis-$X_{I,II}$ fraction (14.7%). Also mannose, not found in the classically extracted xylans, appeared in the xylans from procedures D and E, particularly in the wis-$X_{I,II}$ fractions. This can be explained by the release of degraded cell wall glucan and glucomannan as a consequence of the sonication effects.

The weak signals at 100.6–101.4 ppm showed only one weak cross peak with the anomeric proton resonance at 5.04 pm. In this region, α-galactopyranosyl units used to resonate (Bock and Thogersen, 1984). Galactose was reported (Wilkie, 1979) to terminate oligosaccharide side chains isolated from annual plant heteroxylans.

The last two signals at 99.4 and 98.6 ppm, correlated with proton resonances at δ5.48 and 5.32, respectively, were assigned to C-1 and H-1 of the α-glucuronic acid and its 4-O-methyl ether which are known as single side chains of non-endospermic heteroxylans (Wilkie, 1979; Kusakabe et al., 1983). The resonances of C-4 and the methoxyl group of the methylated glucuronic acid were seen at 83.2, and 60.6 ppm, respectively. The doublet at 177.4 ppm is assigned to C-6 of the uronic acid units. The xylose to uronic acid ratio of E/ws-X_I (8.7), calculated from the NMR data, corresponded well with the value of 9.4, estimated from the uronic acid content (Table 1). In the case of B/ws-X_{I+II}, the corresponding xylose to uronic acid ratios were 14.6 and 15.5, respectively.

The NMR spectra of the ws-X_I fractions isolated by ultrasound in water and 1% NaOH (not shown) had a distinct resonance at δ56.2 (H-1~3.55 ppm) as well as several very weak and badly resolved resonances in the downfield region (116–165 ppm). They might arise from ferulic acid, known to be linked to the arabinosyl side chains of heteroxylans isolated from monocyotyledons (Saulnier et al., 1995). Such residues could have resisted during the weak alkaline conditions of procedures C and D. The presence of phenolic acids in D/ws-X was determined also by FT-IR and FT-Raman spectroscopies (Kačuráková et al., 1997).

3.4. Biological activity of the ws-xylans

In Fig. 6a and b, the immunostimulatory activity in the mitogenic and comitogenic thymocyte tests, respectively, of the ws-X fractions, isolated from the classical and ultrasound-mediated extractions, are shown. Evidently, the application of ultrasound under the used irradiation conditions had no detrimental effect on the biological response of the isolated ws-xylan component. The ws-X fractions obtained by short application of ultrasound showed immunostimulatory activities in the comitogenic test which are comparable with those of the classically extracted xylans. A higher biological response in the mitogenic test was shown only by D/ws-X_{II}. In all cases, the immunological activity of the ws-X preparations in both tests was higher in

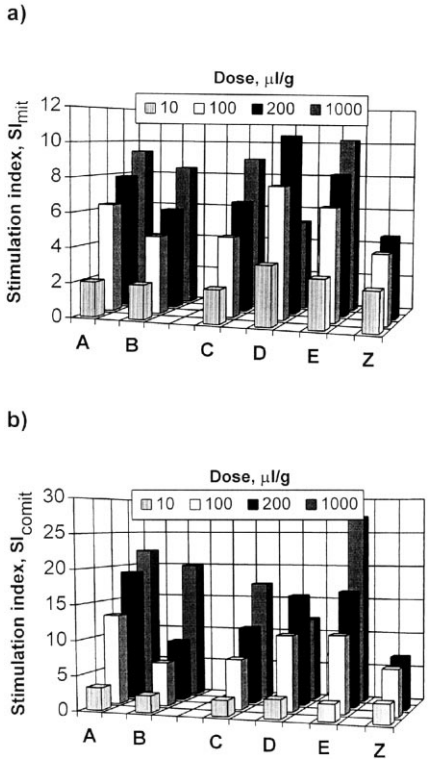

Fig. 6. Stimulatory activities of xylans prepared by the classical, A (ws-X_{I+II}) and B (ws-X_{I+II}), and ultrasound-mediated procedures, C (ws-X_{II}), D (ws-X_I) and E (ws-X_I), in (a) mitogenic test, (b) comitogenic test; Z: the positive control Zymosan.

comparison to that of the commercial immunomodulator Zymosan from yeast.

4. Conclusion

The ws-X component of corn cobs comprises a variety of xylan polymers differing in the proportions of arabinose and xylose, and in the types and proportion of side chains. The couple of chemical shifts at ~107, 89, and ~5.5 ppm can be used to recognize the presence of 2-O-β-D-xylopyranosyl-α-L-arabinofuranose side chains in heteroxylans. Except for the proportions of the present side chains, there were no substantial differences in the structural features of the ws-X preparations isolated by the classical or ultrasound-mediated extraction procedures. Nevertheless, differences may occur in the fine structure of the xylan chains, i.e. in the distribution of the branches along the xylan chains. During the comparative studies of the production of the immunologically active ws-X component of corn cobs, the short ultrasound treatment (up to 30 min) was found to intensify the extraction process of the ws-xylan and its biological response. This is of major importance from the economical point of view and makes the ultrasound-mediated extraction process very advantageous.

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