Differences in extractability under subcritical water reveal interconnected hemicellulose and lignin recalcitrance in birch hardwoods

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Supplementary Material

Table S1. Complete monosaccharide composition of raw material, extracts and residue (quantification)

Composition (mg g^{-1})	Birch chips[a]	B1[b]	B2[b]	B3[b]	B4[b]	B5[b]	B6[b]	Residue[a]
Ara	2.9	27.4	9.8	1.7	1.3	1.4	0.6	0.2
Rha	3.7	14.8	16.3	8.8	6.8	3.3	0.8	0.4
Gal	8.1	30.7	26.1	15.3	13.9	16.7	13.4	1.4
Glc	420.9	234.3	45.3	7.8	4.3	4.1	4.1	616.2
Xyl	264.7	148.4	643.0	739.1	723.6	626.6	342.4	116.5
Man	18.8	180.4	39.0	5.9	3.0	2.1	2.5	15.0
GalA	17.7	69.5	26.8	16.9	15.4	10.8	5.3	2.3
GlcA	0.3	4.0	4.1	3.0	2.8	3.4	1.9	0.3
MeGlcA	29.1	5.7	52.6	75.2	90.8	78.7	39.3	0.3
Total	766.5	715.1	849.0	851.4	840.1	728.0	402.4	752.6

[a]Carbohydrate content obtained by two step sulfuric hydrolysis
[b]Carbohydrate content obtained by methanolysis

Table S2.Oligosaccharide assignment of the mass profiles obtained by ESI-MS.

Proposed assignation	m/z (M+Na$^+$)	m/z (M+2Na$^+$-H)	Proposed assignation	m/z (M+Na$^+$)	m/z (M+2Na$^+$-H)
X_2	305	327	$X_5U^{4m}Ac_3$	1017	1039
X_2Ac_1	347	369	$X_6U^{4m}Ac_1$	1065	1087
X_2Ac_2	389	411	X_7Ac_3	1091	1113
X_3	437	459	X_8	1097	1119
X_3Ac_1	479	501	$X_6U^{4m}Ac_2$	1107	1129
X_2U^{4m}	495	517	X_7Ac_4	1133	1155
X_3Ac_2	521	543	X_8Ac_1	1139	1161
$X_2U^{4m}Ac_1$	537	559	$X_6U^{4m}Ac_3$	1149	1171
X_4	569	591	X_7U^{4m}	1155	1177
$X_2U^{4m}Ac_2$	579	601	X_7Ac_5	1175	1197
X_4Ac_1	611	633	X_8Ac_2	1181	1203
X_3U^{4m}	627	649	$X_6U^{4m}Ac_4$	1191	1213
X_4Ac_2	653	675	$X_7U^{4m}Ac_1$	1197	1219
$X_3U^{4m}Ac_1$	669	691	X_8Ac_3	1223	1245
X_4Ac_3	695	717	X_9	1229	1251
X_5	701	723	$X_7U^{4m}Ac_2$	1239	1261
$X_3U^{4m}Ac_2$	711	733	X_8Ac_4	1265	1287
X_5Ac_1	743	765	X_9Ac_1	1271	1293
X_4U^{4m}	759	781	$X_7U^{4m}Ac_3$	1281	1303
X_5Ac_2	785	807	X_8U^{4m}	1287	1309
$X_4U^{4m}Ac_1$	801	823	X_8Ac_5	1307	1329
X_5Ac_3	827	849	X_9Ac_2	1313	1335
X_6	833	855	$X_7U^{4m}Ac_4$	1323	1345
$X_4U^{4m}Ac_2$	843	865	$X_8U^{4m}Ac_1$	1329	1351
X_5Ac_4	869	891	X_9Ac_3	1355	1377
X_6Ac_1	875	897	X_{10}	1361	1383
$X_4U^{4m}Ac_3$	885	907	$X_8U^{4m}Ac_2$	1371	1393
X_5U^{4m}	891	913	X_9Ac_4	1397	1419
X_6Ac_2	917	939	$X_8U^{4m}Ac_3$	1413	1435
$X_5U^{4m}Ac_1$	933	955	X_9U^{4m}	1419	1441
X_6Ac_3	959	981	X_9Ac_5	1439	1461
X_7	965	987	$X_8U^{4m}Ac_4$	1455	1477
$X_5U^{4m}Ac_2$	975	997	$X_9U^{4m}Ac_1$	1461	1483
X_6Ac_4	1001	1023	$X_8U^{4m}Ac_5$	1497	1519
X_7Ac_1	1007	1029	$X_9U^{4m}Ac_2$	1503	1525
$X_5U^{4m}Ac_3$	1017	1039	$X_9U^{4m}Ac_3$	1545	1567

NOTE: The assignation of the XOs to the m/z has been performed in-house using ChemDraw.

Table S3. Assignment of the main ^{13}C-^{1}H 2D HSQC correlation signals in DMSO-d6 obtained from the lignin populations in the Birch (B1-B6) extracts

Lignin cross-Signal	δ_C (ppm)	δ_H (ppm)	Description
1	72.0	4.84	C_α/H_α in $\beta O4$
1	85.9	4.09	C_β/H_β in $\beta O4$ in Syringyl units
1	83.3	4.28	C_β/H_β in $\beta O4$ in Guaiacyl units
2	86.9	5.40	C_α/H_α in phenylcoumaran
2	53.2	3.42	C_β/H_β in phenylcoumaran
3	85.1	4.64	C_α/H_α in resinol structure
3	53.5	3.04	C_β/H_β in resinol structure
3	71.1	4.16	C_γ/H_γ in resinol structure
3	71.1	3.78	C_γ/H_γ in resinol structure
4	81.0	5.06	C_α/H_α in Spirodienone
4	59.8	2.73	C_β/H_β in Spirodienone
5	106.0-106.5	7.20-7.30	C_2/H_2, C_6/H_6 in a oxidized side Syringyl units
6	55.6	3.71	C/H in Methoxy Group
$S_{2,6}$	103.9	6.67	C_2/H_2 and C_6/H_6 in Syringyl units
G_2	110.8	7.93	C_2/H_2 in Guaiacyl units
G_5	114.9	6.67-6.90	C_5/H_5 in Guaiacyl units
G_6	118.8	6.74	C_6/H_6 in Guaiacyl units

NOTE: The inter-monolignol linkages were assigned according to Balakshin and Rencoret.[i]

Table S4. Assignment of the main ^{13}C-^{1}H 2D HSQC correlation signals in DMSO-d6 obtained from the lignin-carbohydrate complexes (LCC) in the Birch (B1-B6) extracts

| LCC cross-Signal	δ_C (ppm)	δ_H (ppm)	Description
PG	99-104	4.8-5.3	Phenyl Glycoside
BE$_1$(α)	80.1-82.5	4.4-4.7	Benzyl ether to C6/C5
BE$_2$(α)	81.7-82.6	5.04-5.26	Benzyl ether to C2/C3 in α
γ-ester	60.5-63.0	4.1-4.3	γ-ester

NOTE: Lignin Carbohydrate bonds were assigned accordingly to previous work based mostly on model compound[ii]

Table S5. Assignment of the main ^{13}C-^{1}H 2D HSQC correlation signals in DMSO-d6 obtained from the carbohydrate structural units in the Birch (B1-B6) extracts

Carbohydrate cross-Signal	δ_C (ppm)	δ_H (ppm)	Description
Ara	107.8	4.75	C_1/H_1 in β-L-arabinopyranoside
C_6	60.0	3.56	C_6/H_6 in β-D-gluco-/mannopyranoside
X	101.5	4.36	C_1/H_1 in β-D-xylopyranoside
X_{2Ac}	73.7	4.45	C_2/H_2 in 2-O-acetyl-β-D-xylopyranoside
$X_{(2Ac)}$	100.0	4.44	C_1/H_1 in 2-O-acetyl-β-D-xylopyranoside
X_{3Ac}	75.0	4.78	C_3/H_3 in 3-O-acetyl-β-D-xylopyranoside
X_{23Ac}	71.4	4.49	C_2/H_2 in 2,3-O-acetyl-β-D-xylopyranoside
X_4	75.3	3.45	C_4/H_4 in β-D-xylopyranoside
X_5	62.9	3.13/3.78	C_5/H_5 in β-D-xylopyranoside
X(nr)	103.4	4.14	C_1/H_1 in β-D-xylopyranoside of xylans non reducing end
$X_{t,\alpha}(r)$	92.3	4.84	C_1/H_1 in α-D-xylopyranoside of xylans reducing end
$X_{t,\beta}(r)$	97.3	4.20	C_1/H_1 in β-D-xylopyranoside of xylans reducing end
M	100.3	4.51	C_1/H_1 in β-D-mannopyranoside
M_{2Ac}	70.8	5.21	C_2/H_2 in 2-O-acetyl β-D-mannopyranoside
$M_{t,\alpha}(r)$	93.5	4.87	C_1/H_1 in α-D-mannopyranoside of mannans reducing end
Gal	105.3	4.23	C_1/H_1 in α-D-galactopyranoside
MeGlcA	96.8	5.11	C_1/H_1 in 4-O-methyl-α-D-glucuronic Acid
MeGlcA	81.4	3.07	C_4/H_4 in 4-O-methyl-α-D-glucuronic Acid

NOTE: The annotation of the carbohydrate structures was performed according to the work by Telemann and Ralph[iii].

Figure S1. Comparison of sugar composition of the extracts by methanolysis and sulfuric hydrolysis
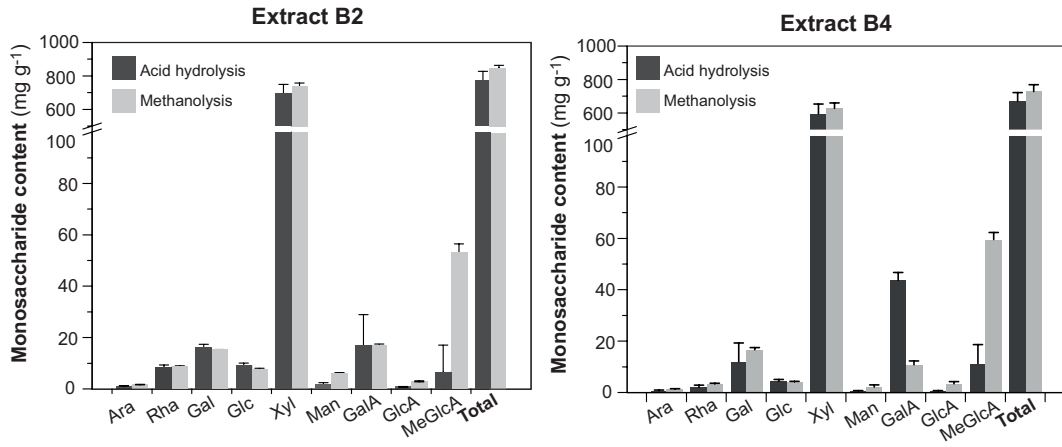
Figure S2. Refractive index (DRI) and ultraviolet (UV) detection traces from size-exclusion chromatography (SEC) of the birch extracts. The carbohydrates are low UV absorbing and are mostly detected by the DRI signal, whereas lignin are strong UV absorbers and can be detected by both DRI and UV. The evolution of the UV and DRI traces offers indirect evidence of the hemicellulose and lignin contributions to the molar mass distributions, respectively. For the initial extracts B1-B4, a "decoupling" from DRI and UV traces can be observed. This indicates that the hemicellulose populations with larger molar mass are only limitedly connected to lignin, and only the smaller eluting compounds assigned to lignin contribute to the UV signal. However, for the recalcitrant extracts B5-B6 both DRI and UV signals coincide, which may indicate that the hemicellulose and lignin populations are largely interconnected.
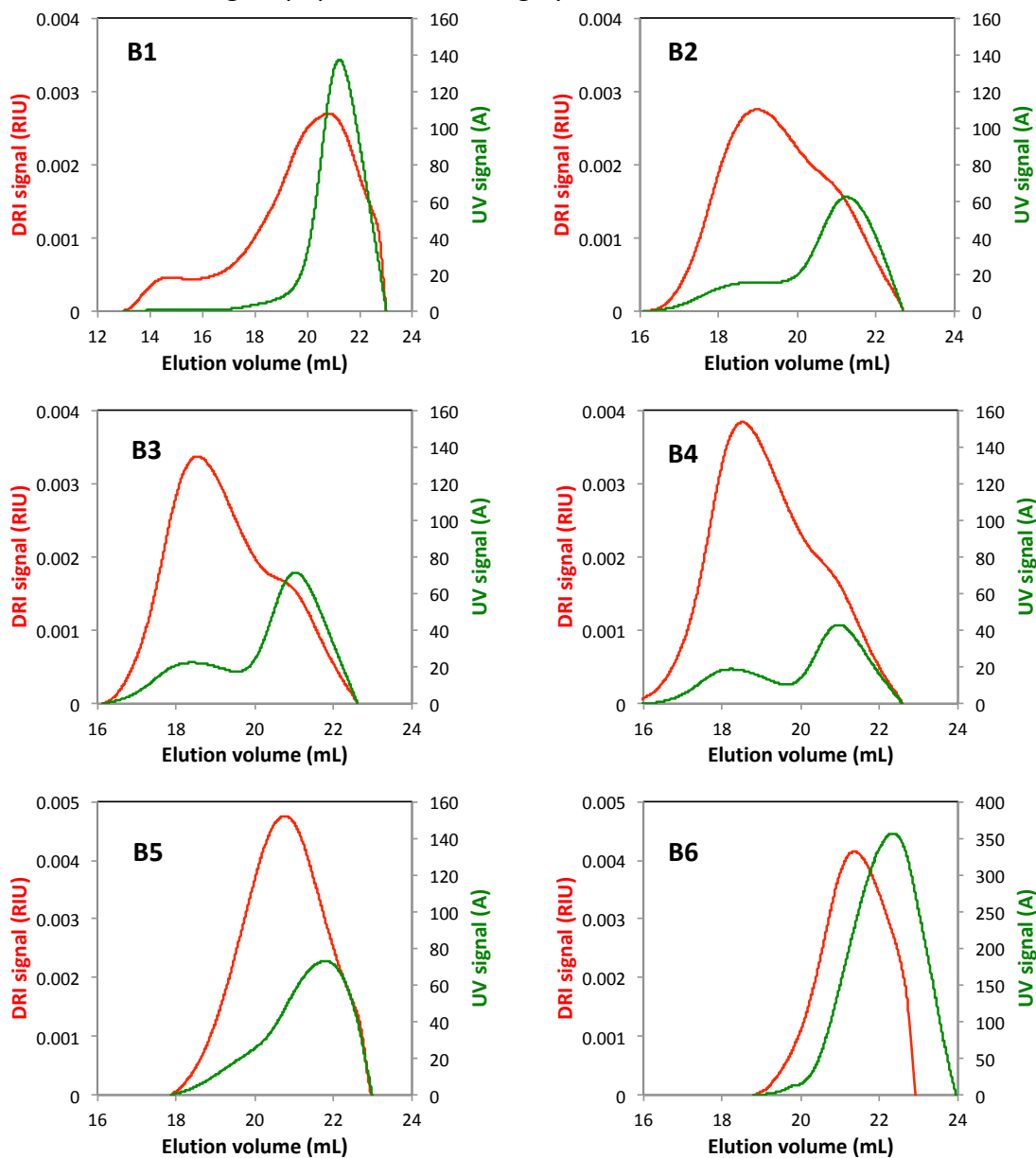
Figure S3. Sequencing of the X_4Ac_nmU isomer series: A. Single ion chromatogram for the extract B3 by LC-ESI-MS. B. Single ion chromatogram for the extract B5 by LC-ESI-MS. The isomer X_4mU (*m/z* 880) shows a single peak *(1)*. The isomer X_4AcmU (*m/z* 922) shows one single peak (2), which is significantly abundant in extract B5. The isomer X_4Ac_2mU (*m/z* 964) show two predominant peaks *(3)* and *(4)* which are predominantly abundant in extract B3. The isomer X_4Ac_3mU (*m/z* 1006) show one predominant peak, which is only detectable in extract B3. C. Fragmentation spectra by LC-ESI-MS/MS of the peaks (1-4), where the specific placement of the acetyl groups on the Xyl*p* units can be assigned. Unfortunately, the regioselective position of the acetyl group on the *O*-2 or *O*-3 of the Xyl*p* units cannot be assigned. The isomer X_4Ac_3mU (*m/z* 1006) could not be sequenced successfully.
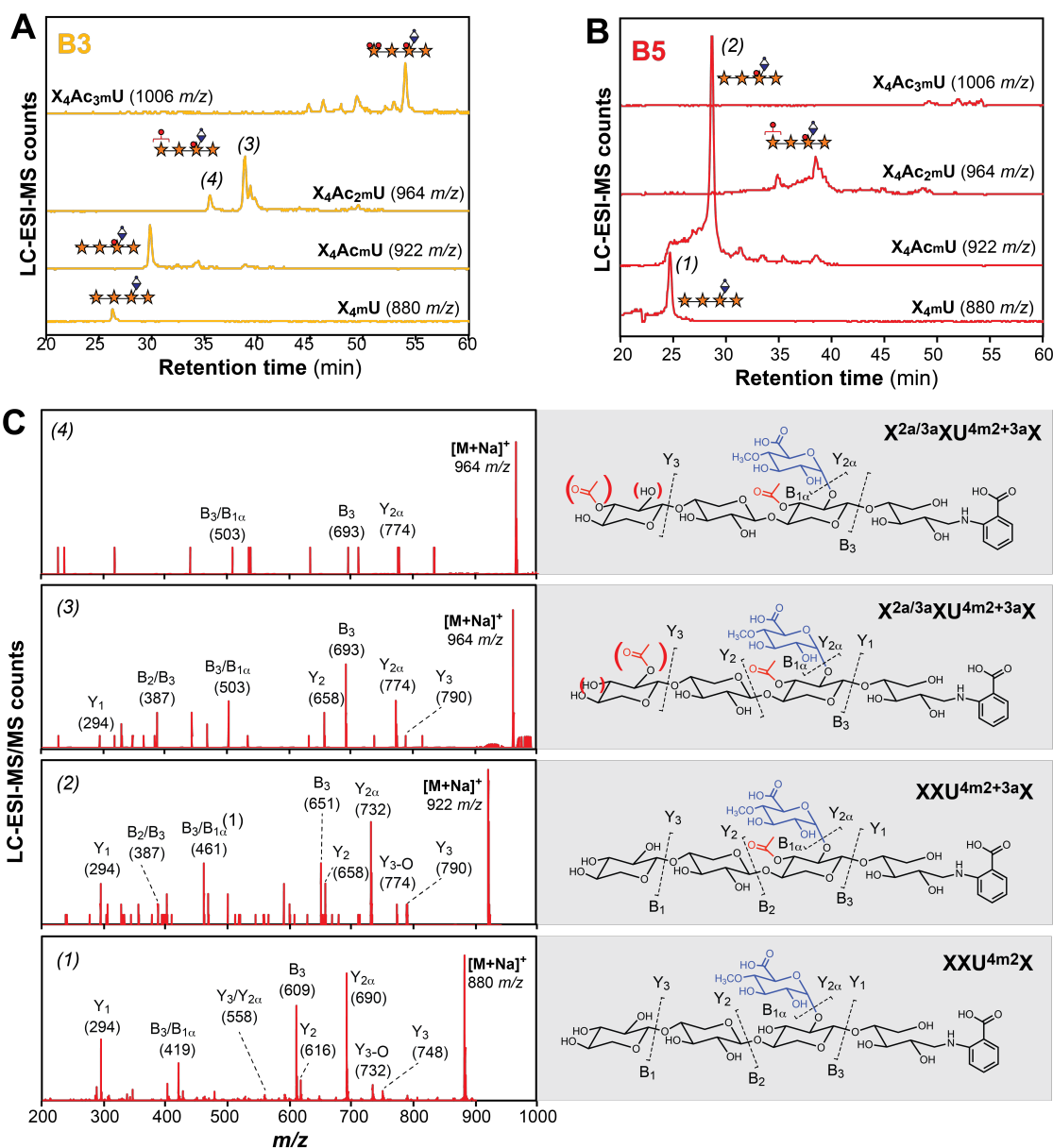
NOTE: The assignation of the fragmentations has been performed in-house using ChemDraw.

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