

## Supplementary Material

### **Sequential extraction of hemicelluloses by subcritical water improves saccharification of hybrid aspen wood grown in greenhouse and field conditions**

Pramod Sivan<sup>‡a</sup>, Emilia Heinonen<sup>‡a,b</sup>, Madhavi Latha Gandla<sup>c</sup>, Amparo Jiménez-Quero<sup>a</sup>,  
Hüsamettin Deniz Özeren<sup>a</sup>, Leif J. Jönsson<sup>c</sup>, Ewa J Mellerowicz<sup>d</sup> and Francisco Vilaplana<sup>a,b\*</sup>

<sup>a</sup> Division of Glycoscience, Department of Chemistry, KTH Royal Institute of Technology, AlbaNova University Centre, 106 91 Stockholm, Sweden

<sup>b</sup> Wallenberg Wood Science Centre, KTH Royal Institute of Technology, 100 44 Stockholm, Sweden

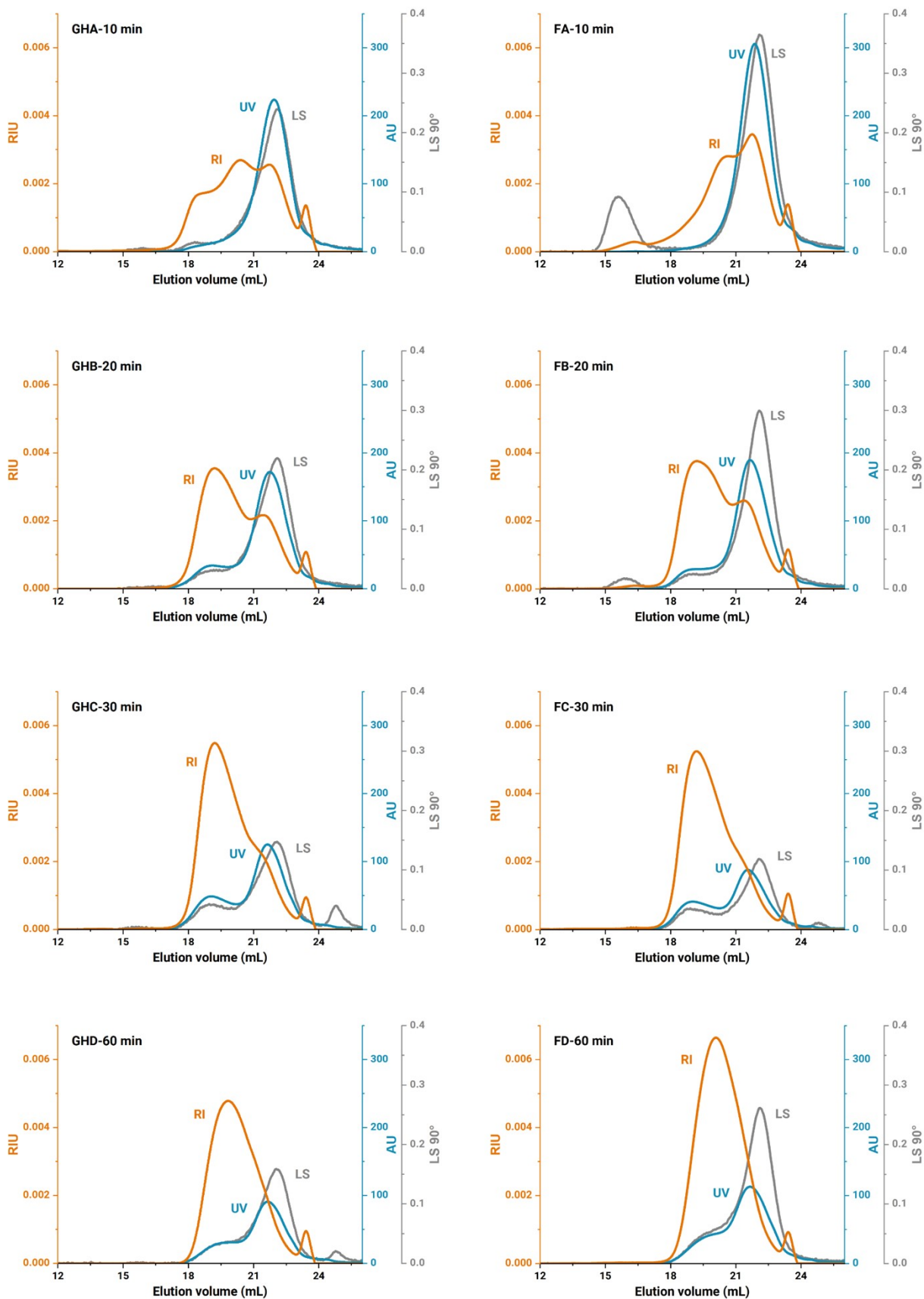
<sup>c</sup> Department of Chemistry, Umeå University, 901 87 Umeå, Sweden

<sup>d</sup> Umeå Plant Science Centre, Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, 901 83 Umeå, Sweden

<sup>‡</sup>These authors contributed equally to this work.

\*Corresponding author: Francisco Vilaplana ([franvila@kth.se](mailto:franvila@kth.se))

**Figure S1.** Chromatograms from the multiple-detector SEC: profiles from refractive index (orange), UV (blue) and MALLS detectors (grey). GHA = greenhouse, FA = Field



**Table S1.** Monosaccharide composition of raw material, extracts and residue from greenhouse grown aspen

<b>Composition (mg per gm)</b>	<b>Starting material</b>	<b>A1</b>	<b>A2</b>	<b>A3</b>	<b>A4</b>	<b>Residue</b>
Ara	2,9	31,4	10,7	4,3	2,2	0,4
Rha	4,6	42,3	26,6	18,6	13,2	1,1
Gal	10,2	107,5	31,1	18,6	15,5	2,7
Glc	441,1	79,6	24,2	7,3	3,3	564,0
Xyl	228,2	175,9	408,9	568,0	600,1	164,9
Man	28,3	118,3	45,8	16,4	5,8	21,7
GalA	4,4	159,5	64,2	27,7	20,2	1,8
GlcA	1,4	15,6	8,2	7,5	6,9	0,8
MeGlcA	n.d	56,0	75,4	112,5	127,3	n.d
<b>Total</b>	<b>721,2</b>	<b>786,1</b>	<b>695,0</b>	<b>781,0</b>	<b>794,4</b>	<b>757,4</b>

**Table S2.** Monosaccharide composition of raw material, extracts and residue from field grown aspen

<b>Composition (mg per gm)</b>	<b>Starting material</b>	<b>A1</b>	<b>A2</b>	<b>A3</b>	<b>A4</b>	<b>Residue</b>
Ara	4,3	47,6	15,6	5,1	3,9	0,6
Rha	4,4	33,7	20,0	15,5	12,8	0,9
Gal	6,7	38,6	17,3	12,7	16,7	3,0
Glc	461,5	168,2	32,2	7,6	8,0	579,0
Xyl	247,7	183,0	484,4	622,5	692,0	168,2
Man	22,1	133,6	36,5	11,4	7,3	16,8
GalA	4,3	136,1	50,5	25,9	21,7	1,6
GlcA	0,8	6,2	4,3	4,4	4,7	0,5
MeGlcA	n.d	57,3	95,1	132,8	167,3	n.d
<b>Total</b>	<b>751,8</b>	<b>804,3</b>	<b>755,9</b>	<b>838,0</b>	<b>934,4</b>	<b>770,6</b>

**Table S3.** P- values of monosaccharide composition of wood from green house (G) and field (F) grown aspen

	G	F	p-Value
Pectin (%)	3.27±0.02	2.73±0.02	<0.0001*
Mannan (%)	3.94±0.40	2.95±0.40	0.0001*
Xylan (%)	31.00±0.30	32.40±0.30	0.0511

**Table S4.** P- values of MeGlcA to Xyl ratio of sequential subcritical extracts from green house (G) and field (F) grown aspen

	G	F
10-20 min	0.0036*	0.0040*
20-30 min	0.1811	0.0515
30-60 min	0.0079*	0.0394*

**Table S5.** P- values of lignin monomer and S/G ratio of sequential subcritical extracts from greenhouse (G) and field (F) grown aspen

Greenhouse (G)	S	G	H	S/G
10-20 min	0.0004*	0.3364	0.0026*	<0.0001*
20-30 min	0.1744	0.0217*	0.0017*	0.0006*
30-60 min	0.0040*	0.0006*	<0.0001*	<0.0001*
Field (F)	S	G	H	S/G
10-20 min	0.0074*	<0.0001*	<0.0001*	<0.0001*
20-30 min	<0.0001*	<0.0001*	<0.0001*	<0.0001*
30-60 min	<0.0002*	<0.0001*	<0.0001*	<0.0001*

**Table S6.** P- values of cellulose microfibril width of wood (SM) and SWE residue (R) from green house (G) and field (F) grown aspen

	SM	R	p-Value	
			SM vs R	G vs F
G	14.0±0.48	12.00±0.08	0.0237*	<0.0001*
F	17.0±0.24	15.07±0.60	<0.0001*	



**Table S7.** P- values of BET surface area of wood (SM) and SWE residue (R) from green house (G) and field (F) grown aspen

	SM	R	p-Value
G	2.20±0.08	3.07±0.08	0.0004*
F	1.40±0.06	1.98±0.60	0.0017*

**Table S8.** P- values of relative crystallinity index of wood (SM) and SWE residue (R) from green house (G) and field (F) grown aspen

	SM	R	p-Value
G	42.80±0.11	48.60±0.11	<0.0001*
F	43.70±0.05	50.20±0.60	<0.0001**

**Table S9:** Composition sugars released during saccharification from wood (SM) and residue of sub-critical extraction process from filed grown aspen

	NT	SW	PL(AP)	AP	PL(SW/AP)	SW/AP
Arabinose	0.001±0.000	0.000±0.000	0.003±0.000	0.000±0.000	0.001±0.000	0.000±0.000
Galactose	0.001±0.000	0.001±0.000	0.005±0.002	0.000±0.000	0.003±0.000	0.000±0.000
Glucose	0.052±0.002	0.312±0.023	0.057±0.014	0.403±0.020	0.069±0.018	0.002±0.024
Xylose	0.010±0.000	0.091±0.007	0.100±0.028	0.005±0.002	0.102±0.011	0.000±0.002
Mannose	0.003±0.000	0.005±0.000	0.012±0.005	0.004±0.000	0.011±0.001	0.000±0.000
Total	0.068±0.002	0.409±0.030	0.178±0.050	0.412±0.022	0.186±0.030	0.603±0.027

**Table S10.** Composition sugars released during saccharification from wood (SM) and residue of sub-critical extraction process from greenhouse grown aspen

	NT	SW	PL(AP)	AP	PL(SW/AP)	SW/AP
Arabinose	0.001±0.000	0.000±0.000	0.003±0.000	0.000±0.000	0.001±0.000	0.000±0.000
Galactose	0.001±0.000	0.001±0.000	0.008±0.002	0.000±0.000	0.003±0.000	0.000±0.000
Glucose	0.080±0.001	0.291±0.010	0.069±0.008	0.409±0.036	0.056±0.011	0.552±0.019
Xylose	0.014±0.000	0.086±0.004	0.137±0.000	0.005±0.001	0.104±0.008	0.011±0.002
Mannose	0.004±0.000	0.006±0.000	0.018±0.005	0.003±0.000	0.014±0.001	0.004±0.000
Total	0.101±0.001	0.384±0.014	0.235±0.015	0.417±0.036	0.178±0.020	0.567±0.022

**Table S11.** P- values of glucose production rate from saccharification of wood (SM) and SWE residue (R) from green house (G) and field (F) grown aspen

	SM	R	p-Value
G-NT	0.77±0.07	1.14±0.13	<0.0001*
G-AP	2.36±0.11	2.60±0.50	0.0054*
F-NT	0.31±0.04	0.92±0.09	<0.0001*
F-AP	2.35±0.40	2.35±0.12	0.6556

\*NT= Non-treated; AP=Acid pretreated

**Table S12.** P-values from the comparison of glucose production rate (GPR) , yields of glucose (Glc), xylose (Xyl) and total sugar from saccharification of field grown aspen wood after sub-critical extraction (SW) process with those of different treatments. NT= Non-treated, AP= Acid-Pretreated.

Treatment	GPR	Glc	Xyl	Total sugar
NT	<,0001	<,0001	<,0001	<,0001
AP	<,0001	<,0001	0.1979	<,0001
SW/AP	<,0001	<,0001	0,0016	<,0001

**Table S13.** P-values from the comparison of glucose production rate (GPR), yields of glucose (Glc), xylose (Xyl) and total sugar from saccharification of green house grown aspen wood after sub-critical extraction (SWE) process with those of different treatments. NT= Non-treated, AP= Acid-Pretreated,

Treatment	GPR	Glc	Xyl	Total sugar
NT	<,0001	<,0001	<,0001	<,0001
AP	<,0001	<,0001	<,0001	<,0001
SW/AP	<,0001	<,0001	<,0001	<,0001

**Table S14.** Evolution of the pH of the extracts and the blank (buffer passed through cells without sample under same experimental conditions). G1, G2, G3 and G4 (technical replicates of greenhouse grown aspen samples); F1, F2, F3 and F4 (technical replicates of field grown aspen samples).

	Blank	10 min	20 min	30 min	60 min
G1	5,01	4,90	4,87	4,85	4,82
G2	5,01	4,91	4,88	4,87	4,83
G3	5,01	4,92	4,88	4,87	4,82
G4	5,00	4,91	4,87	4,85	4,80
F1	5,01	4,86	4,87	4,85	4,81
F2	5,03	4,84	4,88	4,85	4,82
F3	5,00	4,81	4,85	4,82	4,79
F4	5,01	4,81	4,83	4,80	4,79