Sequential Fractionation of Feruloylated Hemicelluloses and Oligosaccharides from Wheat Bran using Subcritical Water and Xylanolytic Enzymes

Supplementary Material

Andrea C. Ruthes,[a] Antonio Martínez-Abad, [a] Hwei-Ting Tan,[a,b.c] Vincent Bulone[a,b] and Francisco Vilaplana*[a]

[a] *Division of Glycoscience, School of Biotechnology, KTH Royal Institute of Technology, AlbaNova University Centre, SE–106 91 Stockholm, Sweden.*
E-mail: franvila@kth.se

[b] *ARC Centre of Excellence in Plant Cell Walls and School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, Urrbrae, SA 5064 Australia.*

[c] *Centre for Tropical Crops and Biocommodities, Queensland University of Technology, Brisbane, QLD, Australia.*

Table S1. General composition of raw wheat bran (wb) and after destarching (Dwb).

General composition	**Raw wheat bran**	**Destarched wheat bran**
	wb	Dwb
Moisture (%)[a]	**23.3 (0.5)**	**18.5 (1.1)**
Carbohydrate content (mg g^{-1} DW)[b]	595.9 (0.6)	682.4 (0.09)
Rha (%)	-	-
Fuc (%)	-	-
Ara (%)	17.1 (0.2)	23.0 (0.9)
Xyl(%)	29.1 (0.7)	34.8 (1.0)
Man (%)	2.4 (0.7)	2.8 (0.7)
Gal (%)	3.3 (0.1)	2.0 (0.1)
Glc (%)	43.3 (0.1)	34.7 (0.0)
Uronic acids (%)[c]	4.8 (0.1)	2.7 (0.1)
Starch (mg g^{-1} DW)[d]	169.4 (0.6)	28.9 (2.9)
β-D-glucan (mg g^{-1} DW)[e]	57.0 (3.0)	60.8 (1.0)
Protein content (mg g^{-1} DW)[f]	**202.0 (0.1)**	**197.0 (0.5)**
Val (%)	34.2 (1.8)	30.1 (0.2)
Ala (%)	2.9 (0.3)	3.0 (0.1)
Leu (%)	1.7 (0.3)	1.4 (0.1)
Ile (%)	6.3 (0.2)	6.3 (0.0)
Asp (%)	36.6 (0.8)	32.4 (0.6)
Glu (%)	5.8 (0.1)	9.0 (0.1)
Phe (%)	1.9 (0.1)	5.2 (0.0)
Trp (%)	10.6 (0.0)	12.5 (0.0)
Phenolic acid content (mg g^{-1} DW)[g]	**2.2 (0.1)**	**2.4 (0.3)**
p-coumaric acid (%)	3.2 (0.4)	9.7 (1.2)
ferulic acid (%)	96.8 (0.6)	90.3 (1.2)
Klason lignin (mg g^{-1})[h]	**90.0 (3.8)**	**117.8 (1.4)**
Phytic acid (mg g^{-1} DW)[i]	9.6 (0.5)	n.d.
Acetyl content (mg g^{-1} DW)[j]	9.3 (0.1)	8.2 (0.2)

[a] Moisture content was determined gravimetrically after drying in an oven at 110°C for 24 h.
[b] Carbohydrate content was determined by phenol-sulphuric acid method [1].
[c] Uronic acid content was determined by colorimetric method [2].
[d] Starch content was determined using the Total Starch Assay (Megazyme kit).
[e] Mixed-linkage β-D-glucan content was determined enzymatically (Megazyme kit).
[f] Protein content was determined by hydrolysis, derivatization and GC-MS analysis. Amino acids: Val – valine, Ala – alanine, Leu – leucine, Ile – isoleucine, Asp – aspartic acid, Glu – glutamic acid, Phe – phenylalanine, Trp – tryptophan.
[g] Phenolic acid content was determined by saponification, derivatization and GC-MS analysis.[3]
[h] Klason lignin was determined gravimetrically from the residue after acid hydrolysis (H_2SO_4).
[i] Phytic acid was measured enzymatically using the total phosphorous kit
[j] Acetyl content was measured after saponification with NaOH and HPLC-UV analysis

NOTE: The numbers in parenthesis represents the standard deviation of the triplicates.

Table S2. Detailed yields, monosaccharide composition and phenolic acid content of the extracts (E) and residues (R) after alkaline [NaOH and $Ca(OH)_2$] and subcritical water extraction (SWE)

Composition	Alkaline Extraction				Subcritical Water Extraction (SWE)											
					100°C		120°C		140°C		160°C					
	NaOH		$Ca(OH)_2$		pH 5.0		pH 5.0		pH 5.0		pH 5.0		pH 7.0		pH 9.0	
	NE	NR	CE	CR	HE	HR	HE	HR	HE	HR	HE	HR	HE	HR	HE	HR
Yield (% DW)[a]	**31.3**	**14.8**	**12.4**	**37.0**	**13.3**	**47.2**	**9.2**	**42.5**	**15.6**	**26.6**	**20.9**	**39.0**	**22.3**	**39.3**	**15.1**	**38.0**
Carbohydrate content (mg g^{-1})[b]	**871.9**	**750.0**	**882.2**	**837.9**	**679.7**	**474.9**	**794**	**892.7**	**780**	**779.3**	**870.2**	**761.8**	**919.8**	**694.1**	**897.8**	**785.0**
AX (%)[c]	72.8	43.8	80.6	57.0	24.1	59.0	51.8	58.5	55.9	59.4	73.1	57.2	74.3	60.1	77.7	57.9
Glc (%)[d]	22.3	51.9	14.1	39.7	73.0	37.5	41.4	36.5	37.8	35.5	23.4	38.1	21.8	34.6	18.3	38.9
A/X[e]	0.90	0.63	1.03	0.59	0.50	0.65	0.62	0.66	0.60	0.73	0.48	0.77	0.51	0.54	0.68	0.86
Ara (%)	34.4	16.9	40.9	21.2	8.0	23.2	19.9	23.3	20.9	25.0	23.8	24.9	25.2	21.1	31.5	26.9
Xyl (%)	38.4	26.9	39.8	35.8	16.1	35.8	31.9	35.2	35.0	34.4	49.3	32.3	49.1	39.0	46.2	31.1
Man (%)	0.5	2.9	0.7	1.4	0.8	1.4	1.3	1.3	1.3	2.0	0.4	1.4	0.6	2.6	0.5	1.4
Gal (%)	4.4	1.4	4.6	1.9	2.0	2.1	5.5	3.7	5.0	3.1	3.1	3.3	3.3	2.7	3.5	1.8
Glc (%)	22.3	51.9	14.1	39.7	73.0	37.5	41.4	36.5	37.8	35.5	23.4	38.1	21.8	34.6	18.3	38.9
Uronic acid (%)[f]	3.6 (0.2)	n.d.[g]	3.3 (0.1)	n.d.	1.8 (0.1)	n.d.	1.9 (0.1)	n.d.	1.8 (0.1)	n.d.	1.9 (0.1)	n.d.	1.9 (0.1)	n.d.	1.9 (0.1)	n.d.
Protein content (mg g^{-1})[h]	**125.9 (7.7)**	**n.d.**	**149.8 (13.8)**	**n.d.**	**100.0 (10.0)**	**n.d.**	**172.9 (2.9)**	**n.d.**	**80.1 (0.8)**	**n.d.**	**64.8 (0.4)**	**n.d.**	**70.9 (0.87)**	**n.d.**	**60.2 (0.2)**	**n.d.**
Phenolic acid content (mg g^{-1})[i]	**0.2 (0.0)**	**0.3 (0.0)**	**0.2 (0.0)**	**0.3 (0.0)**	**1.3 (0.2)**	**1.5 (0.2)**	**2.2 (0.4)**	**3.8 (0.4)**	**2.2 (0.1)**	**4.1 (0.3)**	**2.0 (0.3)**	**3.7 (0.4)**	**3.8 (0.2)**	**3.0 (0.3)**	**0.8 (0.1)**	**0.5 (0.1)**
Ferulic acid (%)	98.7 (0.6)	94.5 (1.8)	98.4 (1.8)	100.0 (0.0)	97.6 (0.5)	97.5 (0.3)	97.6 (1.0)	86.1 (0.9)	98.6 (1.7)	86.5 (0.9)	97.9 (1.6)	95.8 (0.5)	98.3 (3.2)	93.7 (0.6)	98.9 (0.8)	97.7 (0.3)
$M_w \times 10^5$ (g mol^{-1})	**4.6**	**-**	**4.7**	**-**	**n.d.**	**-**	**3.2**	**-**	**2.1**	**-**	**1.9**	**-**	**2.7**	**-**	**3.0**	**-**

[a] Yields were determined gravimetrically based on the dry weight (DW) of the samples; [b] Carbohydrate content was determined by phenol-sulphuric acid method [1]; [c] Arabinoxylan (AX) content was calculated based on the total of arabinose (Ara) and xylose (Xyl) composition. This value includes the residual Ara potentially present in arabinogalactan; [d] Glucose (Glc) content can be potentially assigned to mixed-linkage β-D-glucans in the extracts (E) after TFA hydrolysis and to cellulose in the residues (R) after H_2SO_4 hydrolysis; [e] A/X is the ratio between Ara and Xyl; [f] Uronic acid content was determined by colorimetric method [2]; [g] n.d. – Not determined; [h] Protein content was determined by Bradford colorimetric method [4]; [i] Phenolic acid content was determined by saponification, derivatization and GC-MS analysis.[3] Ferulic acid content is presented as % of the total phenolic acid content. NOTE: The numbers in parenthesis represents the standard deviation of the triplicates.

Table S3. Glycosidic linkage composition of the high molar mass (H) fractions from alkaline and subcritical water extraction.

	Linkage composition (%mol)[a]					
	NE-H	**HE-120-H**	**HE-140-H**	**HE-160-5-H**	**HE-160-7-H**	**HE-160-9-H**
Total Ara (%)[b]	39.9 (2.7)	29.8 (2.7)	25.7 (1.1)	24.7 (0.8)	25.3 (0.8)	22.8 (0.7)
Araf-(1→	30.4 (1.4)	23.7 (1.5)	20.3 (0.5)	18.9 (0.4)	19.9 (0.6)	18.7 (0.5)
→2)-Araf-(1→	2.1 (0.1)	1.5 (0.1)	1.5 (0.1)	1.4 (0.1)	1.7 (0.0)	1.4 (0.0)
→3)-Araf-(1→	4.9 (0.6)	1.4 (0.6)	1.4 (0.1)	1.2 (0.0)	1.9 (0.1)	1.7 (0.1)
→5)-Araf-(1→	1.6 (0.5)	2.4 (0.3)	1.8 (0.3)	2.7 (0.2)	1.4 (0.1)	1.0 (0.1)
→2,3,5)-Araf-(1→	-	0.8 (0.1)	0.7 (0.1)	0.5 (0.1)	0.5 (0.0)	-
Total Xyl (%)[b]	54.2 (3.6)	48.1 (3.2)	49 (1.9)	49.1 (0.5)	54.3 (2.3)	63.1 (2.2)
Xylp-(1→	5.4 (0.4)	2.4 (0.5)	0.3 (0.0)	1.8 (0.1)	2.5 (0.1)	2.5 (0.2)
→4)-Xylp-(1→	25.9 (0.3)	28.4 (1.1)	31.8 (1.2)	30.1 (0.1)	31.4 (1.4)	39.7 (0.8)
→2,4)-Xylp-(1→	3.7 (0.8)	2.6 (0.4)	3.1 (0.1)	3.2 (0.0)	4.6 (0.8)	4.1 (0.1)
→3,4)-Xylp-(1→	7.7 (1.0)	5.5 (0.5)	5.0 (0.3)	6.5 (0.1)	7.4 (0.7)	7.8 (0.3)
→2,3,4)-Xylp-(1→	11.5 (1.1)	9.2 (0.7)	8.7 (0.5)	7.6 (0.1)	8.4 (0.6)	9.0 (0.9)
Total Glc (%)[b]	1.7 (0.3)	15.6 (1.8)	22.0 (2.4)	21.0 (0.4)	17.4 (1.9)	12.1 (0.4)
Glcp-(1→	0.2 (0.0)	1.3 (0.3)	3.0 (0.5)	2.4 (0.1)	2.0 (0.5)	1.2 (0.0)
→3)-Glcp-(1→	0.2 (0.0)	2.3 (0.3)	7.0 (0.8)	5.8 (0.0)	5.0 (0.5)	3.1 (0.1)
→4)-Glcp-(1→	1.3 (0.2)	10.2 (0.3)	12.0 (1.1)	12.8 (0.3)	10.4 (0.8)	7.8 (0.2)
Total Gal (%)[b]	3.7 (0.9)	6.3 (1.0)	3.2 (0.7)	4.9 (0.2)	2.5 (0.4)	2.0 (0.1)
Galp-(1→	1.2 (0.2)	1.7 (0.3)	0.8 (0.1)	0.9 (0.0)	0.9 (0.2)	0.8 (0.1)
→3)-Galp-(1→	0.5 (0.2)	0.8 (0.1)	0.7 (0.2)	0.8 (0.1)	0.4 (0.1)	0.3 (0.0)
→4)-Galp-(1→	1.1 (0.2)	0.3 (0.1)	0.7 (0.1)	1.3 (0.1)	0.6 (0.1)	0.6 (0.0)
→3,6)-Galp-(1→	0.9 (0.3)	3.6 (0.5)	1.0 (0.3)	1.8 (0.0)	0.7 (0.1)	0.3 (0.0)
Total AX (%)[c]	84.9	72.2	74.1	72.3	80.6	83.2
A/X[d]	0.74	0.62	0.52	0.50	0.47	0.36

[a] The linkage composition (%mol) was obtained after analysis of the partially-methylated alditol acetates (PMAA) by GC-MS and corrected to the monosaccharide composition.

[b] The total monosaccharide composition (%mol) was obtained after TFA hydrolysis and further analysis of the alditol acetates by GC-MS.

[c] The total arabinoxylan (AX) content is calculated from the linkage composition of the Xyl and Ara*f* residues.

[d] The arabinose-to-xylose (A/X) ratio is calculated from the monosaccharide composition.

NOTE: The numbers in parenthesis represents the standard deviation of the triplicates.

Table S4. Glycosidic linkage composition of the low molar mass (L) fractions from alkaline and subcritical water extraction.

	Linkage composition (%mol)[a]					
	NE-L	**HE-120-L**	**HE-140-L**	**HE-160-5-L**	**HE-160-7-L**	**HE-160-9-L**
Total Ara (%)[b]	29.3 (2.9)	17.9 (1.4)	26.2 (2.2)	21.6 (0.5)	31.1 (3.1)	26.1 (0.4)
Araf-(1→	22.1 (1.4)	8.4 (0.4)	16.6 (0.5)	16.2 (0.3)	19.2 (1.6)	19.9 (0.3)
→2)-Araf-(1→	2.0 (0.3)	n.d.	1.4 (0.5)	1.3 (0.1)	0.9 (0.3)	1.7 (0.0)
→3)-Araf-(1→	3.1 (0.6)	0.5 (0.1)	1.5 (0.2)	1.3 (0.0)	0.9 (0.3)	2.3 (0.0)
→5)-Araf-(1→	2.0 (0.3)	6.2 (0.7)	3.8 (0.1)	2.8 (0.1)	6.2 (0.4)	1.6 (0.0)
→2,3,5)-Araf-(1→	n.d.	2.1 (0.1)	2.6 (0.8)	0.2 (0.0)	3.8 (0.4)	0.6 (0.0)
Total Xyl (%)[b]	31.3 (2.9)	35.0 (1.3)	41.2 (2.5)	48.8 (0.9)	48.6 (1.6)	49.7 (1.3)
Xylp-(1→	3.5 (0.3)	6.3 (0.4)	1.2 (0.2)	2.0 (0.0)	1.1 (0.5)	3.6 (0.3)
→4)-Xylp-(1→	13.4 (1.1)	19.2 (0.3)	22.6 (0.8)	27.6 (0.5)	30.4 (0.9)	25.5 (0.5)
→2,4)-Xylp-(1→	3.1 (0.6)	1.6 (0.1)	2.5 (0.6)	3.1 (0.2)	3.5 (0.2)	3.5 (0.5)
→3,4)-Xylp-(1→	6.0 (0.3)	2.7 (0.1)	5.7 (0.9)	6.7 (0.1)	5.8 (0.7)	7.6 (0.2)
→2,3,4)-Xylp-(1→	5.3 (0.6)	5.3 (0.4)	9.3 (0.0)	9.5 (0.1)	7.8 (0.7)	9.5 (0.3)
Total Glc (%)[b]	29.4 (2.3)	41.8 (2.4)	28.9 (1.9)	25.3 (1.4)	20.4 (2.0)	20.2 (0.6)
Glcp-(1→	12.5 (0.5)	6.2 (0.5)	5.3 (0.6)	2.7 (0.1)	2.2 (0.7)	1.4 (0.2)
→3)-Glcp-(1→	2.4 (0.9)	4.3 (0.1)	5.2 (0.4)	6.1 (0.6)	5.1 (0.9)	5.9 (0.0)
→4)-Glcp-(1→	8.3 (0.7)	23.8 (1.5)	17.7 (0.7)	15.8 (0.7)	13.0 (0.4)	12.2 (0.1)
→4,6)-Glcp-(1→	6.3 (0.3)	7.6 (0.3)	0.7 (0.1)	0.8 (0.1)	n.d.[f]	0.7 (0.3)
Total Gal (%)[b]	9.0 (0.5)	5.3 (0.6)	3.6 (0.6)	4.3 (0.2)	n.d.	4.1 (0.3)
Galp-(1→	5.4 (0.1)	3.8 (0.1)	1.4 (0.1)	1.0 (0.0)	n.d.	1.1 (0.0)
→3)-Galp-(1→	1.2 (0.1)	0.6 (0.3)	0.5 (0.2)	0.6 (0.0)	n.d.	0.5 (0.0)
→4)-Galp-(1→	0.5 (0.1)	0.7 (0.1)	0.3 (0.1)	0.9 (0.1)	n.d.	1.3 (0.2)
→3,6)-Galp-(1→	1.9 (0.2)	<0.1	0.5 (0.1)	1.5 (0.1)	n.d.	0.6 (0.0)
Total AX (%)[c]	49.5	43.7	68.3	66.4	68.7	71.3
A/X[d]	0.94	0.51	0.64	0.44	0.64	0.53

[a] The linkage composition (%mol) was obtained after analysis of the partially-methylated alditol acetates (PMAA) by GC-MS and corrected to the monosaccharide composition.

[b] The total monosaccharide composition (%mol) was obtained after TFA hydrolysis and further analysis of the alditol acetates by GC-MS.

[c] The total arabinoxylan (AX) content is calculated from the linkage composition of the Xyl*p* and Ara*f* residues.

[d] The arabinose-to-xylose (A/X) ratio is calculated from the monosaccharide composition.

[f] n.d. – Not detected.

NOTE: The numbers in parenthesis represents the standard deviation of the triplicates.

Table S5. Peak annotation and assignment of the oligosaccharide mass profiles obtained by ESI-MS.

Oligosaccharide family	*m/z*	Proposed assignation*
Arabinoxylan oligosaccharides (AXOs)	305	$[P_2+Na]^+$
	437	$[P_3+Na]^+$
	569	$[P_4+Na]^+$
	701	$[P_5+Na]^+$
	833	$[P_6+Na]^+$
	965	$[P_7+Na]^+$
	1097	$[P_8+Na]^+$
	1229	$[P_9+Na]^+$
	1361	$[P_{10}+Na]^+$
Feruloylated Arabino-xylo-oligosaccharides (FAXOs)	613	$[P_3F+Na]^+$
	745	$[P_4F+Na]^+$
	877	$[P_5F+Na]^+$
	1009	$[P_6F+Na]^+$
	1141	$[P_7F+Na]^+$
Hexo-oligosaccharides (galactan oligosaccharides, cello-oligosaccharides and mixed-linkage β-D-glucan oligosaccharides)	527	$[H_3+Na]^+$
	689	$[H_4+Na]^+$
	851	$[H_5+Na]^+$
	1013	$[H_6+Na]^+$
	1175	$[H_7+Na]^+$
	1337	$[H_8+Na]^+$
	1499	$[H_9+Na]^+$

*P – pentose; F – ferulic acid; H - hexose. The subscript number is related to the degree of polymerization (DP).

Table S6. Detailed yields, monosaccharide composition and phenolic acid content of SWE residue after enzymatic treatment [Feruloyl sterase (FE) and Xylanase (X)] followed by a second round of SWE

SWE + Enzymatic treatment + SWE 160°C, pH 7.0160°C, pH 7.0								
	Control		**FE**		**X**		**FE+X**	
	ZE	**ZR**	**ZE**	**ZR**	**ZE**	**ZR**	**ZE**	**ZR**
Yield (% DW)[a]	6.9	79.9	5.8	70.1	8.0	75.5	17.9	66.2
Carbohydrate content (mg g^{-1})[b]	308.2	331.2	839.9	456.5	894.7	473.4	871.6	408.4
AX (%)[c]	70.4	58.2	72.6	59.2	73.1	58.9	62.6	59.1
Glc (%)[d]	22.6	37.9	20.0	36.8	18.0	37.1	30.0	36.8
A/X[e]	0.88	0.77	0.97	0.79	1.20	0.84	0.97	0.78
Rha (%)	1.5	-	1.9	-	2.0	-	1.8	-
Ara (%)	32.9	25.3	35.8	26.0	39.8	26.9	30.8	26.0
Xyl (%)	37.5	32.9	36.8	33.1	33.3	31.9	31.9	33.1
Man (%)	0.8	2.3	0.9	2.2	1.9	2.1	1.4	2.2
Gal (%)	4.7	1.8	4.5	1.9	5.0	1.9	4.1	1.9
Glc (%)	22.6	37.9	20.0	36.8	18.0	37.1	30.0	36.8
Phenolic acid content (mg g^{-1})[f]	-	1.0 (0.003)	1.8 (0.00)	-	-	1.1 (0.07)	3.8 (0.03)	-
Ferulic acid (%)[g]	-	100 (0.10)	100 (0.05)	-	-	100 (0.03)	100 (0.06)	-

[a] Yields were determined gravimetrically based on the dry weight (DW) of the samples;
[b] Carbohydrate content was determined by phenol-sulphuric acid method [1];
[c] Arabinoxylan (AX) content was calculated based on the total of arabinose (Ara) and xylose (Xyl) composition. This value includes the residual Ara potentially present in arabinogalactan;
[d] Glucose (Glc) content can be potentially assigned to mixed-linkage β-D-glucans in the extracts (E) after TFA hydrolysis and to cellulose in the residues (R) after H_2SO_4 hydrolysis;
[e] A/X is the ratio between Ara and Xyl;
[f] Phenolic acid content was determined by saponification, derivatization and GC-MS analysis.[3]
[g] Ferulic acid content is presented as % of the total phenolic acid content.
NOTE: The numbers in parenthesis represents the standard deviation of the triplicates.

Table S7. Scavenging activity of different concentrations of wb alkaline and subcritical water extracts against DPPH• and ABTS•+

Sample		DPPH• [a]					ABTS•+ [b]				
		Concentration (μg mL^{-1})									
		10	30	50	100	EC_{50}	10	30	50	100	EC_{50}
Control	Ascorbic acid	67.1 ± 0.61	76.6 ± 0.20	81.1 ± 0.06	81.6 ± 0.03	3.9 ±0.39	84.8 ± 0.17	95.4 ± 0.06	95.5 ± 0.04	95.7 ± 0.07	2.9 ± 0.24
	Ferulic acid	70.2 ± 0.09	74.7 ± 0.00	82.6 ± 0.50	84.0 ± 0.09	4.4 ± 0.39	66.2 ± 0.67	68.6 ± 0.09	77.8 ± 0.74	85.0 ± 0.64	6.1 ± 0.13
	WE-AX	-5.2 ± 0.95	-4.7 ± 1.77	-7.2 ± 0.00	-6.2 ± 1.45	1174.7 ± 5.35	0.9 ± 0.27	2.8 ± 0.10	4.6 ± 0.25	5.3 ± 0.25	1147.7 ±0.63
Low molar mass (L)	NE-L	-6.8 ± 0.23	-3.4 ± 0.10	-0.1 ± 0.01	2.1 ± 0.25	285.6 ± 1.15	-	-	-	4.3 ± 0.25	
	HE-120-5-L	11.3 ± 0.12	22.0 ± 0.00	28.5 ± 0.79	36.5 ± 0.47	135.6 ± 1.12	-	-	-	8.1 ±0.10	-
	HE-140-5-L	16.7 ± 0.38	16.7 ± 0.06	20.1 ± 1.52	26.5 ± 1.46	377.6 ± 2.24	-	-	-	8.1 ± 0.10	-
	HE-160-5-L	5.8 ± 1.20	9.7 ± 0.09	14.3 ± 0.93	20.2 ± 0.73	246.7 ± 2.58	-	-	-	8.1 ± 0.10	-
	HE-160-7-L	15.5 ± 0.26	16.7 ± 0.12	19.7 ± 0.38	36.1 ± 0.35	78.79 ± 1.12	-	-	-	9.1 ± 0.10	-
	HE-160-9-L	5.8 ± 0.18	7.8 ± 0.16	9.6 ± 0.18	14.4 ± 0.10	631.4 ± 3.36	-	-	-	2.6 ± 0.20	-
High molar mass (H)	NE-H	-7.9 ± 1.43	-2.8 ± 0.41	0.3 ± 0.47	1.8 ± 0.40	294.9 ± 1.34	2.2 ± 0.05	5.3 ± 0.25	7.0 ± 0.15	9.2 ± 0.21	679.8 ± 0.38
	HE-120-5-H	22.6 ± 0.73	27.9 ± 0.50	35.4 ± 0.15	50.7 ± 0.25	75.0 ± 0.75	-	-	-	7.6 ± 0.10	-
	HE-140-5H	23.2 ± 1.46	27.6 ± 0.99	35.3 ± 0.29	50.5 ± 0.50	79.59 ± 0.84	-	-	-	9.4 ± 0.10	-
	HE-160-5-H	19.8 ± 0.54	24.3 ± 0.22	41.9 ± 0.80	49.9 ± 0.01	86.47 ± 0.84	-	-	-	10.2 ± 0.20	-
	HE-160-7-H	37.9 ± 0.26	40.5 ± 0.18	41.9 ± 0.26	55.1 ± 0.06	53.6 ± 1.34	10.7 ± 0.05	13.5 ± 0.10	17.2 ± 0.20	23.8 ± 0.10	286.1 ± 0.19
	HE-160-9-H	0.00 ± 0.26	12.0 ± 0.15	19.7 ± 0.38	20.6 ± 0.35	136.0 ± 0.96	-	-	-	3.7 ± 0.10	-

* Data are expressed as means ± standard deviations of triplicate measurements.

[a] DPPH• scavenging activity was measured according to Brand-Willians et al., [5].

[b] DPPH•+ scavenging activity was measured according to Re et al., [6].

Figure S1. Mass pofiling of the oligomeric fractions by ESI-MS. (a) Low molar mass fraction after membrane filtration (HE-160-7-L); (b) Enzymatic treatment of the residue: control (C) and feruloyl esterase (FE). NOTE: P: pentose, H: hexose.

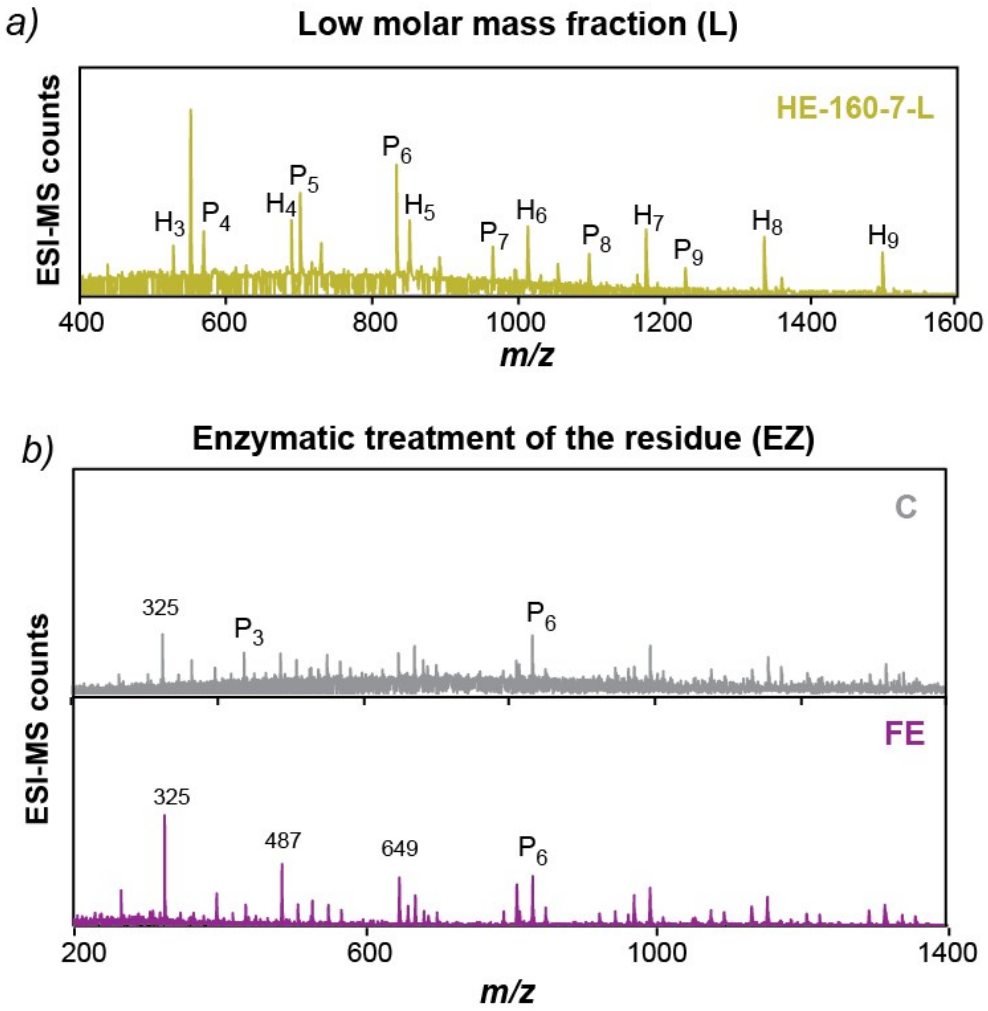
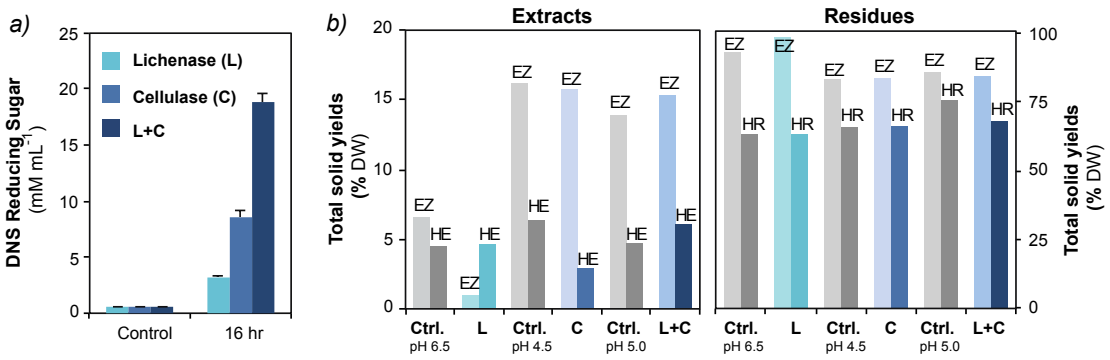


Figure S2. Valorization of the crosslinked residue by treatment with auxiliary enzymes (lichenase and/or cellulase) followed by subcritical water extraction. (a) Activity of the enzymatic treatments as measured by reducing sugar analysis: L: Lichenase; C: cellulase; L+C: combination of lichenase and cellulase; (b) Total solid yields of the extracts and the residue after the enzymatic treatment (EZ) and the subsequent subcritical water extraction (HE: extract; HR: residue).



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