Screening of sugars and phenolics released during pretreatment of miscanthus, maize and sugar cane bagasse for potential added value products from c4 crops

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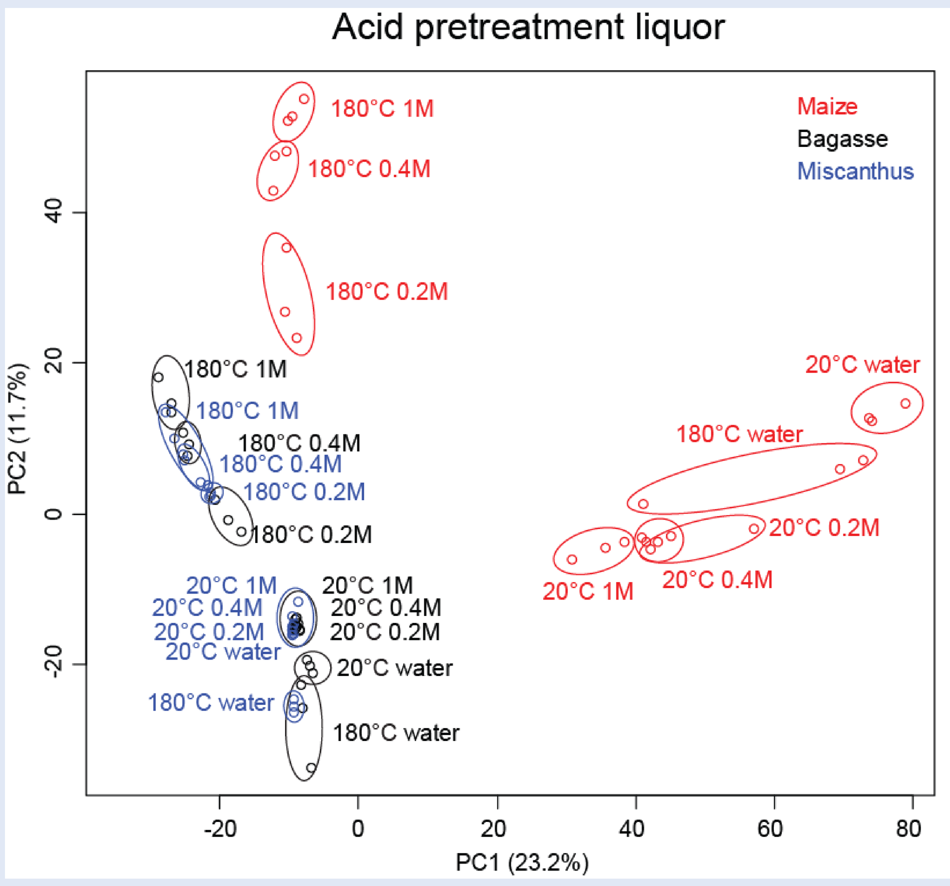
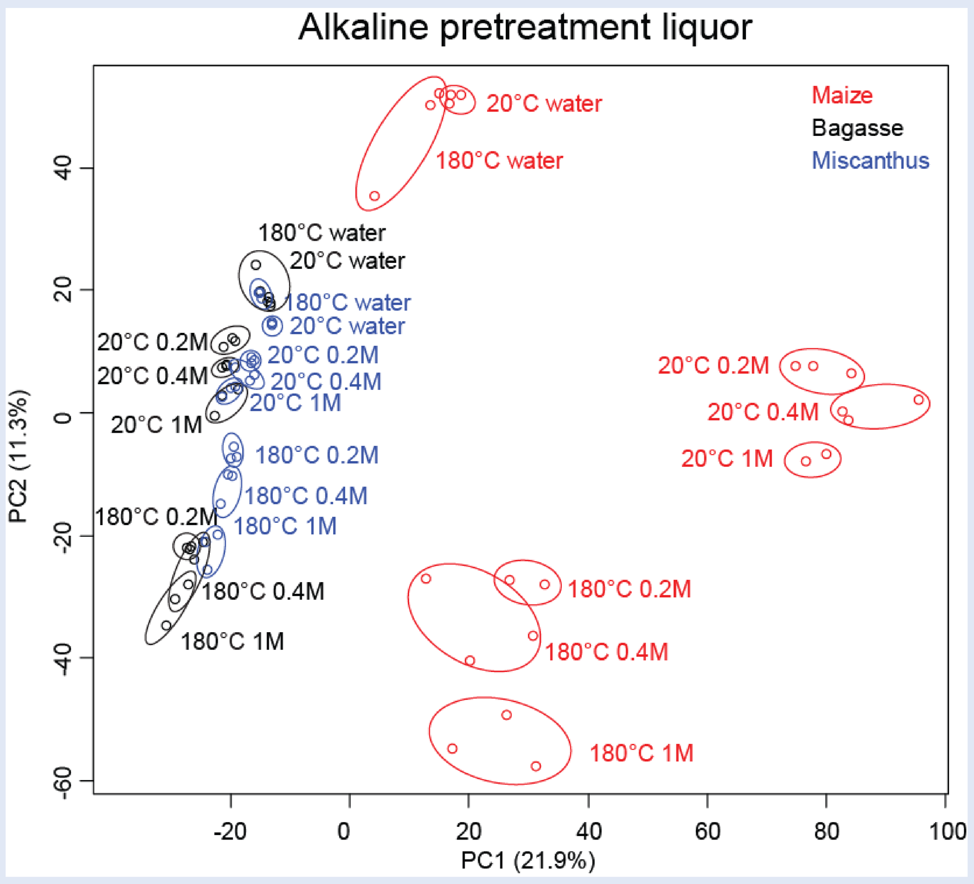
INTRODUCTION

The viability of cellulosic fuels hangs on the reduction of the costs involved in the conversion processes. An approach to reach a realistic cost for conversion of biomass into large volumes/low cost products is **by identifying added value products** that can be obtained during the production of biomass derived fuels. This biorefinery concept involves the benefits of reducing the cost of the overall process**, replace petroleum derived materials and chemicals, and reduce waste streams**. Pretreatments have been considered for a long time as the key to increase the efficiency of pretreatments in terms of increased fuel production after fermentation. An interesting new approach to evaluate pretreatmens is the preservation of the valuable chemicals in the pretreatment liquors in order to add value to the process from products obtained by fractionation of the biomass. In the present work we characterise the sugars and lignin derivatives present in the **pretreatment liquor of maize, miscanthus and sugar cane bagasse under a range of acid and alkaline pretreatments**.

EFFECT OF PRETREATMENTS





To reveal the general similarities and differences between the pretreatment conditions, all samples were integrated and aligned after UHPLC-MS analysis and PCA was performed. The composition of the pretreatment liquor with acid 20 °C is not very different from water pretreatment. However, the pretreatment liquor with acid 180 °C clearly separated from the 20 °C, indicating that the **acid needs higher temperatures to be effective**. In terms of biomass, the PC1- and PC2-based PCA plots showed a **high similarity between miscanthus and bagasse**, while maize samples were clearly different.

MONOSACCHARIDES

The monosaccharide composition of these liquors was analysed using high-performance anion-exchange chromatography (HPAEC). In most conditions used, **glucose** and **xylose** were the most abundant monosaccharides, these are followed by **arabinose** (up to 39%), **galactose** (up to 10%) and **mannose** (up to 6%). Alkaline conditions release a complex mixture of monosaccharides with a large representation of C5 sugars. Acid conditions, on the other hand, produce liquors with higher proportion of glucose, particularly at low temperatures.

CHARACTERISED COMPOUNDS

bagasse

	water 20 °C	water 180 °C	0.2 M NaOH 20 °C	0.2 M NaOH 180 °C	0.4 M NaOH 20 °C	0.4 M NaOH 180 °C	1 M NaOH 20 °C	1 M NaOH 180 °C
monomeric acids								
1 benzoic acid	295	7217	67652	114765	76175	109028	75669	104939
2 *p*-coumaric acid	6668	20777	70675	169709	119810	222879	245659	369714
3 ferulic acid	72	581	19252	37541	42517	43488	63507	64413
dimers with glycerol aliphatic chain								
4 G(8-O-4)Gglycerol 1	10	38	80	580	92	1381	100	2656
5 G(8-O-4)Gglycerol 2	29	98	167	1668	217	3175	269	5510
6 G(8-O-4)Sglycerol 1	47	103	799	2419	1043	5720	1154	14754
7 G(8-O-4)Sglycerol 2	48	110	594	2444	709	6467	801	14947
8 S(8-O-4)Sglycerol 1	9	33	190	935	319	3017	315	9469
9 S(8-O-4)Sglycerol 2	4	30	60	804	91	2524	137	7727
ferulic acid containing dimers								
10 G(8-O-4)ferulic acid 1	188	208	10941	13445	13834	12398	15361	15378
11 G(8-O-4)ferulic acid 2	94	126	7733	10033	10931	12316	13641	9476
12 S(8-O-4)ferulic acid 1	6	45	861	8737	1554	8774	1970	7134
13 S(8-O-4)ferulic acid 2	11	59	1111	7532	1781	8296	2096	9286
14 G(8-5)ferulic acid	3	29	1743	4570	2132	4003	2968	3177
15 S(8-5)ferulic acid	6	79	898	12988	1395	13044	3171	13415
16 ferulic acid(8-5)G	44	821	1325	120892	1472	86698	1337	60799
17 ferulic acid(8-5)ferulic acid 1	2	15	818	3538	1144	4388	2489	5130
18 ferulic acid(8-5)ferulic acid 2	9	238	3410	22644	8283	29181	14562	42472

miscanthus

	water 20 °C	water 180 °C	0.2 M NaOH 20 °C	0.2 M NaOH 180 °C	0.4 M NaOH 20 °C	0.4 M NaOH 180 °C	1 M NaOH 20 °C	1 M NaOH 180 °C
monomeric acids								
1 benzoic acid	39	5529	33318	71394	45682	72479	48139	70987
2 *p*-coumaric acid	6	9446	60403	86250	98954	121878	209581	173383
3 ferulic acid	33	335	21671	21058	31556	28290	44645	43874
dimers with glycerol aliphatic chain								
4 G(8-O-4)Gglycerol 1	17	92	153	522	131	1589	173	4167
5 G(8-O-4)Gglycerol 2	41	297	386	2174	435	4528	542	11432
6 G(8-O-4)Sglycerol 1	92	169	705	974	935	3514	1211	10172
7 G(8-O-4)Sglycerol 2	54	144	453	2515	593	6909	831	19912
8 S(8-O-4)Sglycerol 1	39	108	196	755	242	2137	338	8485
9 S(8-O-4)Sglycerol 2	18	74	33	1237	58	2805	149	12244
ferulic acid containing dimers								
10 G(8-O-4)ferulic acid 1	361	159	19369	8215	21451	9190	25050	9032
11 G(8-O-4)ferulic acid 2	207	111	19436	9619	24514	11429	30389	5478
12 S(8-O-4)ferulic acid 1	181	146	1322	6940	1560	6684	2294	6222
13 S(8-O-4)ferulic acid 2	175	159	1106	9062	1457	7589	2086	8065
14 G(8-5)ferulic acid	80	49	13837	13749	12409	12752	11903	12403
15 S(8-5)ferulic acid	118	93	2011	26398	2521	21996	3080	25700
16 ferulic acid(8-5)G	19	37	740	67863	783	38336	630	28023
17 ferulic acid(8-5)ferulic acid 1	2	2	904	2734	1798	3420	2855	4033
18 ferulic acid(8-5)ferulic acid 2	18	45	5686	11220	8007	18032	13989	26838

maize

	water 20 °C	water 180 °C	0.2 M NaOH 20 °C	0.2 M NaOH 180 °C	0.4 M NaOH 20 °C	0.4 M NaOH 180 °C	1 M NaOH 20 °C	1 M NaOH 180 °C
monomeric acids								
1 benzoic acid	232	2486	6676	18368	12043	20825	13100	17315
2 *p*-coumaric acid	1865	6200	20541	39664	41984	55743	69022	78481
3 ferulic acid	3037	1939	23819	26599	36647	34566	48658	46241
dimers with glycerol aliphatic chain								
4 G(8-O-4)Gglycerol 1	5	14	22	382	35	829	34	1638
5 G(8-O-4)Gglycerol 2	5	44	50	816	66	1604	52	3052
6 G(8-O-4)Sglycerol 1	117	108	493	918	571	1938	636	5647
7 G(8-O-4)Sglycerol 2	100	81	375	1511	409	2881	463	6618
8 S(8-O-4)Sglycerol 1	59	84	160	490	209	1022	273	3805
9 S(8-O-4)Sglycerol 2	58	101	188	1253	114	1723	224	4181
ferulic acid containing dimers								
10 G(8-O-4)ferulic acid 1	136	137	6541	6559	7587	6461	9024	9494
11 G(8-O-4)ferulic acid 2	151	110	7038	6243	9587	6003	10568	5153
12 S(8-O-4)ferulic acid 1	87	42	961	6000	1342	4737	1545	5192
13 S(8-O-4)ferulic acid 2	61	76	993	4829	1041	4522	1268	4935
14 G(8-5)ferulic acid	2	37	137	388	128	3356	123	1139
15 S(8-5)ferulic acid	16	5	6897	11540	9550	8099	6893	7437
16 ferulic acid(8-5)G	21	78	2051	54000	2352	28328	874	19045
17 ferulic acid(8-5)ferulic acid 1	3	8	1556	6050	3087	6984	5483	8355
18 ferulic acid(8-5)ferulic acid 2	20	392	7488	17099	12006	22215	17651	30718

Eighteen **phenolic compounds** were structurally characterized based on their MSMS fragmentation and their respective ion traces. Benzoic **1**, *p*-coumaric **2** and ferulic acid **3** are among the highest accumulating compounds (based on MS traces).

Conclusions:

- Miscanthus and Bagasse pretreatment product profile are most similar to each other, while Maize presents a different profile of chemicals released.
- Xylose predominates liquor monosaccharides of Miscanthus and bagasse, while glucose predominates in maize.
- The alkaline preatreatment liquors were substantially more enriched in phenolic compounds as compared to acid. Grasses contain high levels of benzoate, *p*-coumarate and ferulate esters and these compounds are released in alkaline conditions.

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