# Optimization of hemicellulose extraction from sugarcane straw by autohydrolysis

Sílvia Santos Pedrosa[a], Tânia Neto[a], Joana Costa[a]. Manuela Pintado[a], Ana Raquel Madureira[a]

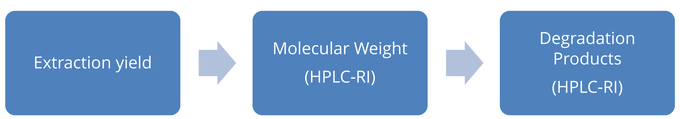
[a] Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Porto, Portuga

## Resume

Sugarcane is an abundant crop and source of several by-products. The fractions obtained from these by-products can be used for several applications for food and animal feed, and cosmetics. This work was focused on the valorisation of sugarcane straw residue as a source of hemicellulose with high content of extractives. Hemicellulose extraction from sugarcane straw was optimized by autohydrolysis in a Parr reactor. Optimization focused on solid/liquid ratio and temperature as two independent variables and function of extraction yields, molecular weight characterization and liquor contamination by degradation products and monosaccharides. After that, extraction conditions were further optimized according to extraction time, based in the same outcomes. Therefore, the best performing extraction conditions were found to be the use of a solid/liquid ratio of 1:18, 165°C temperature and 45 minutes extraction time. Hemicellulose extract was characterized for its chemical composition, molecular weight and thermal profile. Extraction yield was 19.47 ± 0.74 g/100g of straw, with number molecular weight (Mn) of 17.49 kDa and polymerization degree of 116.5.

## Methods and Results

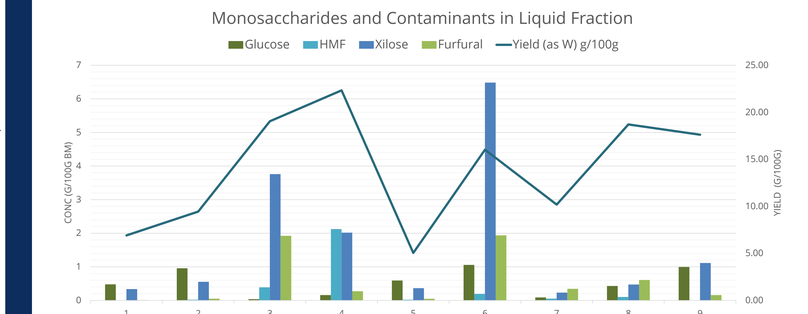
Hemicellulose extraction was performed by autohydrolysis in a Parr Reactor (4551-T-SS-HD-230-VS.50-1000-4848), with water as an extraction solvent. In order to determine the best experimental conditions for the extraction of hemicellulose from sugarcane straw, by autohydrolysis we analysed two independent variables. The two factors analysed were S/L ratio and temperature. Since extraction time, is a temperature dependent variable, it was not studied in this first optimization step.


**Optimization of solid/liquid ratio and temperature autohydrolysis conditions from sugarcane straw**

We investigated the effect of extraction conditions on the molecular weight, presence of monosaccharides and degradation products and extraction yield, as major outputs.

Molecular weight of hemicellulose obtained by autohydrolysis was determined by Size Exclusion Chromatography, using an Agilent 1260 Infinity II LC System coupled to a Refractive Index Detector. A PL aquagel-OH Mixed-M, 4.6 x 250 mm, 8µm and a PL aquagel-OH 7.5 x 300 mm, 20 5 µm columns, at 30 ˚C, coupled to a pre-column, were run at a flow of 0.5 mL/ min with ultrapure water as eluent. Pullulan P-82 standards Shodex (Showa Denko K. K., Tokyo, Japan), ranging from P-5 (5.9 kDa) to P-800 (708 kDa), were used to calibrate the method.




| Run # | S/L ratio | Temp (°C) | Heating rate (°C/min) | Cooling rate (°C/min) | Yield (%) | Sugars (g/100g BM) | Degradation prod. (g/100g BM) |
|---|---|---|---|---|---|---|---|
| 1 | 1/8 | 150 | 3,56 | 1,46 | 6,91 | 1,355 | 0,119 |
| 2 | 1/16 | 150 | 3,18 | 3,27 | 9,44 | 2,372 | 0,237 |
| 3 | 1/8 | 180 | 2,99 | 1,96 | 19,04 | 4,518 | 3,384 |
| 4 | 1/16 | 180 | 3,40 | 3,80 | 22,33 | 3,094 | 3,241 |
| 5 | 1/12 | 143 | 2,44 | 1,95 | 5,05 | 1,534 | 0,175 |
| 6 | 1/12 | 186 | 3,53 | 2,77 | 16,02 | 9,146 | 3,842 |
| 7 | 1/6 | 165 | 3,26 | 1,66 | 10,18 | 0,842 | 0,622 |
| 8 | 1/18 | 165 | 3,35 | 3,09 | 18,71 | 1,926 | 1,223 |
| 9 | 1/12 | 165 | 3,13 | 3,52 | 17,62 | 3,580 | 0,708 |
| 10 | 1/12 | 165 | 3,33 | 3,30 | 16,95 | | |
| 11 | 1/12 | 165 | 3,22 | 3.41 | 16,24 | | |

Figure 1: Description of experimental conditions and results of hemicellulose extraction from sugarcane straw using S/L ratio and Temperature as variables.

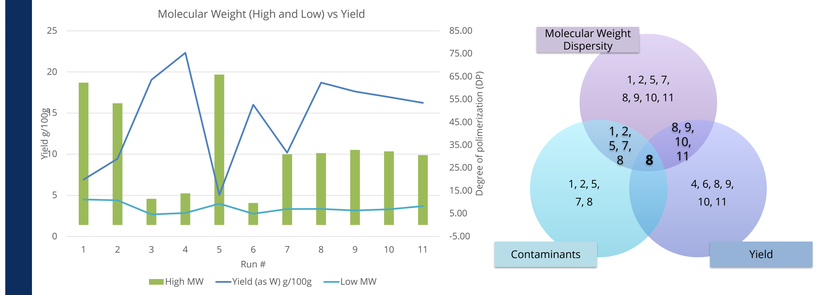



Figure 2: Resume of characterization of hemicellulose liquor obtained in the different experimental conditions.

According to extraction yield, presence of contaminants (monosaccharides and degradation products) and wide molecular weight sample dispersity, the experimental condition chosen was run #8 – 1:18 solid/liquid ratio, 165ºc temperature.

**Optimization of hemicellulose extraction time from sugarcane straw**

After selecting the best condition in the optimization of soli/liquid ratio and temperature experimental conditions, we investigated the effect of extraction time.
In a similar approach we chose molecular weight, presence of monosaccharides and degradation products and extraction yield, as major outputs.
The condition that provided higher molecular weight dispersity are runs 8.1, 8.2, 8,4 and 8.6. Regarding contaminants the run 8.5 produced the most monosaccharide hydrolysis and degradation products. Regarding extraction yield, the condition that provided best performance is run 8.6.

Table 3: Description of experimental conditions and results of hemicellulose autohydrolysis extraction conditions using time as variable.

| Run # | S/L ratio | Temp (°C) | Time (min) | Heating rate (°C/min) | Cooling rate (°C/min) | Yield liquid fraction (%) | Sugars (g/100g BM) | Contaminant (g/100g BM) |
|---|---|---|---|---|---|---|---|---|
| 8.1 | 1/18 | 165 | 20 | 3.21 | 7.16 | 15.85 | 3.315 | 0.241 |
| 8.2 | 1/18 | 165 | 20 | 2.90 | 5.77 | 16.44 | 3.029 | 0.247 |
| 8.3 | 1/18 | 165 | 30 | 2.89 | 5.90 | 20.31 | 3.289 | 0.347 |
| 8.4 | 1/18 | 165 | 30 | 3.72 | 4.98 | 18.22 | 3.605 | 0.274 |
| 8.5 | 1/18 | 165 | 60 | 3.01 | 6.25 | 23.28 | 4.584 | 0.991 |
| 8.6 | 1/18 | 165 | 45 | 2.82 | 5.58 | 21.66 | 3.976 | 0.733 |

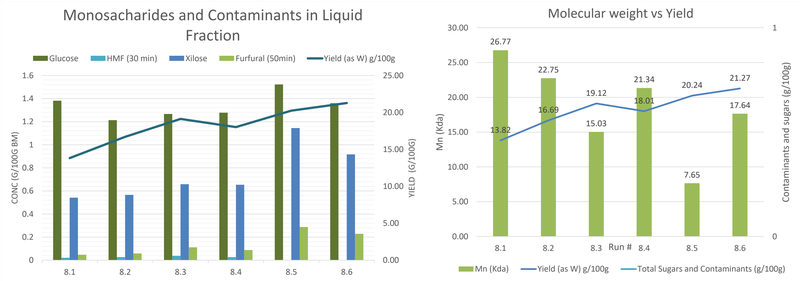
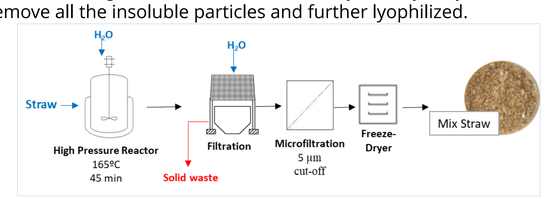



Figure 3: Resume characterization of hemicellulose liquor obtained by optimization of extraction time.

According to extraction yield, presence of contaminants (monosaccharides and degradation products) and wide molecular weight sample dispersity, the experimental condition chosen was run #8.6 – 1:18 solid/liquid ratio, 165ºC temperature and 45 minutes extraction time.

**Characterization of hemicellulose liquor from sugarcane straw**

.Hemicellulose from sugarcane straw is obtained by autohydrolysis and further filtered (5µm mesh) to remove all the insoluble particles and further lyophilized.




Straw and bagasse mix fractions were characterized as their sugars content by the National renewable energy laboratory (NREL) procedure and molecular by HPLC-RI.

Table 1: Physicochemical characterization of straw hemicellulose liquor.

| | Chemical characterization | | | | | |
|---|---|---|---|---|---|---|
| | Glucose (g/100g) | Xilose (g/100g) | Arabinose (g/100g) | Sol. Lignin (g/100g) | Acetic Acid | Humidity (g/100g) |
| Straw hemicellulose | 12.46 ±0.25 | 40.33 ±1.35 | 10.71±0.22 | 8.35±0.18 | 5.34±0.17 | 15.64±0.10 |
| | Molecular Weight Characterization | | | | | |
| MW (kDa) | | MP (kDa) | | Mn (kDa) | | PD |
| 38.44 | | 2.57 | | 17.49 | | 116.6 |

## Conclusions

We were able to successfully obtain a hemicellulose extract from sugarcane straw by-product by autohydrolysis. We optimized the extraction conditions using water as a extraction solvent and using S/L ratio, temperature and time as variables. Extraction conditions performance were analysed as a function of extraction yield, lack of contaminants and wide molecular weight dispersity. Overall, best performing extraction conditions were, – 1:18 solid/liquid ratio, 165ºC temperature and 45 minutes extraction time. Obtained hemicellulose extract is mainly composed by xylan and arabinan and average number molecular weight (Mn) of 17.49 kDa and polymerization degree of 116.5.

Acknowledgements
This work was co-financed by Compete 2020, Portugal 2020 and the European Union, through the European Regional Development Fund through Alchemy project - Capturing high value from industrial fermentation bio products (POCI−01−0247−FEDER−027578), and by Fundação para a Ciência e a Tecnologia (FCT) through project UIDB/50016/2020. The authors would also like to thank Amyris Inc. (Emeryville, CA) the scientific support and collaboration.

Cofinanciado por: