Article:

Remón, Javier orcid.org/0000-0003-3315-5933, Li, Tianzong, Chuck, Christopher J. et al. (2 more authors) (2019) Toward Renewable-Based, Food-Applicable Prebiotics from Biomass:A One-Step, Additive-Free, Microwave-Assisted Hydrothermal Process for the Production of High Purity Xylo-oligosaccharides from Beech Wood Hemicellulose. ACS Sustainable Chemistry and Engineering. pp. 16160-16172. ISSN 2168-0485

https://doi.org/10.1021/acssuschemeng.9b03096

Towards renewable-based, food-applicable prebiotics from biomass: A one-step, additive-free, microwave-assisted hydrothermal process for the production of high purity xylo-oligosaccharides from beech wood hemicellulose

Javier Remón[a,c*], *Tianzong Li*[a], *Christopher J. Chuck*[b], *Avtar S. Matharu*[a], *James H. Clark*[a*]

[a]Green Chemistry Centre of Excellence, University of York, Department of Chemistry, Heslington, York, YO10 5DD, UK.

[b]Department of Chemical Engineering, University of Bath, Claverton Down, Bath, BA2 7AY, UK.

[c]Instituto de Carboquímica, CSIC. C/Miguel Luesma Castán 4, 50018 Zaragoza, Spain (Present address).

*Corresponding authors:

james.clark@york.ac.uk (J.H. Clark)

jremon@icb.csic.es (J. Remón)

KEYWORDS: Xylooligosaccharides, renewable prebiotics, biomass, microwaves, hydrolysis.

ABSTRACT: Xylooligosaccharides (XOS) produced from biomass offer a plethora of excellent physicochemical and physiological properties to be used as natural prebiotic nutraceuticals. Herein, this work firstly addresses and optimises a novel one-pot, additive-free, microwave-assisted process to produce high purity XOS from beech wood hemicellulose, studying the influence of the temperature, reaction time, and solid loading. These variables exerted a significant influence, allowing the transformation of hemicellulose into a gas (0-19%), a XOS rich liquid product (9-80%) and a spent solid material (17-90%). The liquid phase consisted of a mixture of XOS with a degree of polymerisation (DP) DP>6 (75-100 C-wt.%) and DP 3-6 (0-10 C-wt.%), together with mono-/di-saccharides (0-1 C-wt.%), carboxylic acids (0-5 C-wt.%), ketones (0-12 C-wt.%) and furans (0-12 C-wt.%). A good compromise between the liquid yield (81%) and XOS purity (96 C-wt.%) was achieved at 172 ºC using a solid loading of 5 wt.% for 47 min. This time could be reduced (33 min) and the solid loading increased (25 wt.%) without substantially altering the XOS (98 C-wt.%) purity, although the liquid yield was reduced. The liquid yield could be increased up to 97% at the expenses of XOS purity (90 C-wt.%) at 177 ºC using a 5 wt.% solid loading for 60 min. For these optima, the microwave production costs shifted between 1.42 and 6.50 €/kg XOS, which is substantially lower than the XOS market price, thus highlighting the high potential of this emerging technology.

INTRODUCTION

The demand to decrease the carbon foot print and combat climate change together with the exponential growth in the demand for energy and chemicals worldwide due to global megatrends, have led researches to explore alternative feedstocks and novel and environmentally friendly technologies to produce these commodities. Nutrition, food security and healthcare become inextricably linked as we strive towards a healthy, sustainable 21st Century. In this regard there has been an increasing interest in the recent past in the manufacturing of renewable value-added products, chemicals and fuels from lignocellulosic biomass accounted for by its renewable origin and the carbon neutral operating conditions [1]. Recently, there has been growing, and significant, interest in the production of xylooligosaccharides (XOS) from hemicellulose-rich feedstocks due to their potential applicability in many markets [2-5].

Hemicellulose, notably xylan, is the second most abundant carbohydrate material after cellulose in lignocellulosic biomass and also is the most important natural precursor for the production of renewable XOS [6]. Chemically, XOS are oligomers with a ramified structure containing several xylose units (normally, from 2 to 20), linked via ß-(1-4)-xylosidic bonds, decorated with several functional groups, such as acetyl groups, uronic acids and arabinose units [3]. These compounds have gained increasing attention in the food and pharmaceutical markets as they have a plethora of excellent physicochemical and physiological properties to be used as natural prebiotic materials [2-5]. Some examples of these features are reducing cholesterol, enhancing bowel function, the absorption of Ca and metabolism of lipid compounds, helping minimise the risk of colon cancer and exerting cytotoxic effects on leukaemia cells. They also have positive effects on type II diabetes mellitus, a non-communicable global concern as highlighted in the United Nations Sustainable Development Goals (SDG 3). XOS are not metabolised by the human digestive system, but enhance that beneficial bacteria grow in the intestine; thus being ideal compounds to be included in drugs and food formulations [3].

Despite these excellent properties, the main challenges facing biomass XOS are related to the development and optimisation of a sustainable and cost-effective technology capable of maximising the yield and purity of the XOS produced [2-5]. Several methods have been explored for the production of XOS [2-5, 7-8]. These methodologies can be classified into two groups: (i) chemical and (ii) enzymatic hydrolyses [3], in which, the former largely consists of an auto-hydrolysis and acid or basic hydrolysis reactions, whilst the latter primarily comprises a biological hydrolysis procedure [2-5, 7-8]. Auto-hydrolysis involves the deacetylation of xylan, leading to the formation of XOS and acetic acid as the main reaction products, together with a variety of un-desired species, including monosaccharaides and furfural. Acidic- and basic-hydrolyses consist of the treatment of lignocellulosic materials with mineral acids such as, diluted H_2SO_4 or strong alkali solutions, such as KOH, NaOH, $Ca(OH)_2$ or NH_4OH. These chemical methodologies result in the production of undesired oxygenates. Therefore, these impurities together with the acidic and/or basic compounds previously used in the hydrolysis reactions hamper the use of the XOS produced for food applications [2-5, 7-8]. Enzymatic hydrolysis does not produce undesirable products, but the reaction time is much longer than that used for auto-hydrolysis and/or acid/basic hydrolysis [3]. Besides, this technology is normally used after having subjected the lignocellulosic biomass to a first pre-hydrolysis step using either an acid or a basic hydrolysis pre-treatment [2-5, 7-8].

These processes are especially attractive and the insights gained are important, but the challenge is achieving a controllable, toxin-free, selective, economical and environmentally-friendly process to produce high purity XOS from biomass. On this matter, the use of microwaves has been recently regarded as a promising heating mechanism for the selective and well-regulated hydrothermal biomass depolymerisation [9-20]. Specifically, microwave heating principle comprises an elevated rate spin of polar molecules, thus resulting in a faster and more pronounced activation of polar species [9-13, 21-23]. Therefore, using microwave assisted heating to run reactions conducted under hydrothermal conditions is regarded as a very favourable strategy to achieve a selective, non-toxic, high purity XOS production from lignocellulosic biomass. It must also be borne in mind that cellulose and hemicellulose own a higher polar character than lignin, and consequently, these two carbohydrates are more reactive when subjected to a microwave-assisted hydrothermal treatment. This different behaviour promotes the preferential reactivity of the biomass carbohydrate matter, without substantially solubilising the lignin content in the biomass [24-25]. Besides, the elevated crystallinity of cellulose as well the great number of hydrogen bonds in its structure hamper the depolymerisation of this carbohydrate [26-27] when subjected to microwave treatment, thus diminishing the reactivity of cellulose in comparison to hemicellulose. Therefore, these intrinsic peculiarities of microwave heating when used under non-catalytic hydrothermal conditions minimise the presence of lignin and cellulose derived species. Moreover, the controllability and selectivity of the process might allow achieving a selective depolymerisation of hemicellulose into soluble oligosaccharides, preventing the further degradation of XOS.

In spite of these favourable features, to the best of our knowledge, the use of microwave-assisted hydrothermal treatment for XOS production from biomass is scarce. A few examples include, Lin *et al.* [4] who studied the production XOS from beech wood xylan, corncob and an industrial hemicellulose effluent catalysed by oxalic, maleic and citric and sulfuric acids. The highest XOS yields (with respect to the xylan content) for the beech wood, corncob and industrial hemicellulose effluent were 39% (126 ºC and 8 min), 27% (120 ºC and 0 min) and 30% (123 ºC and 16 min), respectively. However, these authors focused on the isolation of the xylan and the characterisation of the XOS produced under specific fixed conditions but without an in-depth analysis of the effects of the operating variables on the process. Panthapulakkal *et al.*

[28] and Gulbrandsen *et al.* [29] studied how the temperature influenced the extraction of hemicellulose (xylan) from sugarcane bagasse using microwave-assisted hydrothermal depolymerisation. The highest xylan yield (60%) with respect to the hemicellulose amount of the feedstock was obtained using a reaction time of 10 min, which was much shorter than the reaction time (1.5 h) needed to achieve this yield using conventional heating. Mihiretu *et al.* [8] reported the influence of the temperature (165-200 ºC) and reaction time (8-22 min) on XOS and bio-ethanol production by microwave-assisted hydrothermal depolymerisation of aspen wood and sugar cane bagasse. Maximum extraction yields of 66% and 50% (with respect to the xylan content), were reported for aspen wood and sugarcane bagasse, respectively. However, the hydrolysate contained an important proportion of saccharides, carboxylic acids and furans, thus hindering their use for food applications. As a consequence, more research is still necessary for the optimisation of the process to achieve a product appropriate for human consumption, as commercial XOS purity ranges between 75 and 95% [30].

Thus, this work addresses the toxin/additive-free, selective hydrothermal direct depolymerisation of beech wood hemicellulose into XOS, by thoroughly evaluating the effects of the temperature (150-210 ºC), reaction time (0-60 min) and solid/water ratio (5-25 wt.%). The fact that the toxin-free, microwave-assisted hydrothermal depolymerisation of hemicellulose for food grade XOS production has never been reported before, as well as the process analysis, optimisation and energy and economic assessments performed, demonstrates that this work represents an innovative research for the renewable production of high purity XOS from biomass. This can help to the development of more efficient and greener technologies for the manufacturing of renewable food-applicable additives, comestible nutrients, digestible prebiotics and antioxidant compounds from lignocellulosic biomass.

EXPERIMENTAL

Feedstock characterisation

Xylan obtained from beech wood (cell wall polysaccharide, with 50-600 μm particle size and an average molecular weight of 20000 g/mol, determined by Size Exclusion Chromatography, SEC) was purchased from Serva Electrophoresis GmbH (CAS number 9014-6305), used as received and characterised by means of proximate, ultimate analyses, calorific value and ash content. More detailed information about the procedure followed and apparatus used in these analyses is reported in a previous publication. [18-19, 22] Fibre analysis was conducted following Hu et al. [31] chemical titration method for cellulose and hemicellulose and TAPPI T222 for lignin. Table 1 lists the characterisation results of the hemicellulose used in this work. These results are similar to the characterisation previously reported for beech wood xylan [20, 32].

Table 1. Characterisation of beech wood hemicellulose (xylan)

Proximate analysis (wt.%)		Physico-chemical Properties	
Moisture	8.25±2.44	DP	130
Ash	3.98±1.23	Solubility in water (25 ºC)	insoluble
Volatiles	73.38±3.29	Colour	Brown
Fixed carbon	14.39±2.47	Xylan Purity	90%
Ultimate analysis (wt.%)		Fibre analysis (wt.%)	
C	40.50±0.15	Hemicellulose	90±1.05
H	5.79±0.08	Cellulose	4.75±0.23
O*	53.71±0.15	Lignin	1.27±0.12
HHV (MJ/kg)	15.40±0.12	Ash	3.98±1.23

* Oxygen was calculated by difference.

Experimental system

The experiments were conducted using a CEM-Mars microwave system, equipped with a 70 mL PrepPlus® reactor. For these runs, different solid quantities (beech wood hemicellulose xylan), together with 40 mL of deionised water were placed into the reactor. To achieve reaction conditions from room temperature a ramp time of 5 min was employed. The microwave reactor was operated at dynamic mode, in which power is adjusted to achieve the reaction temperature as quickly and accurately as possible. At full power, the microwave reactor delivers approximately 1800 watts of microwave energy at a frequency of 2450 MHz. After reaction, the microwave own cooling programme was used to reach room conditions. Afterwards, the reactor was opened, the gas phase produced released, and the reaction products, i.e. a liquid/solid mixture (slurry), was recovered in a centrifuge tube. High-speed (25000 rpm), low temperature (10 ºC) centrifugation was employed to achieve an effective solid/liquid separation. Then, the spent solid fraction was dried overnight at 105°C. Finally, both fractions were kept to be characterised in detail.

Experimental design and data analysis

The influence on the process of the temperature (140-210ºC), reaction time (0-60 min) and solid/water ratio (5-25 wt.%) was experimentally analysed using a design of experiments with statistical analysis of the results (DOE+ANOVA). The reaction time was analysed between 0 and 60 min. A 0 min reaction comprises the ramp time (time to reach operation conditions from room temperature) and the cooling time (time to cool down the reactor), this total operating time (including heating and cooling) being fairly similar for all the runs. Hence, all runs comprising a 0 min reaction time can be adequately compared and the reaction time could be satisfactorily examined, as a wide interval (0-60 min) was used to effectively investigate how and to what extend the reaction time affects the process. The experiments were conducted following Box-Wilson design. This corresponds to a 2^k factorial design (k = operating variables and 2^k =

number of experiments (3 and 8, respectively, in this work). Besides, 6 axial experiments were conducted to examine the possible non-linear behaviour of the variables (i.e. main effects and interactions), while 4 centre points experiments (conducted in the middle of the variation interval for each factor) were also conducted to statistically assess the experimental error. This procedure is very convenient to thoroughly study not only linear and quadratic effects of several variables, but all potential interactions between operating conditions as well. Besides, it permits a careful investigation of the process, thus being extremely valuable for the optimisation of the process together with its possible scale-up and commercialisation. The experimental data obtained was analysed by means of an analysis of variance (ANOVA) select the operating variables and interactions with a statistically significant influence in the process. Furthermore, the Pareto principle was used to assess the relative effect of each variable. In this analysis, factors are classified attending their importance, thus the higher Pareto value, the greater the influence. For both statistical analyses, the operating variables were standardised to vary between -1 and 1 (codec variables), which makes the effects of the variables directly comparable regardless their intervals of variation. Interaction plots were developed from the results of the ANOVA analysis (which consider all the experiments conducted according to the factorial design) to explain the effects of the operating variables. Therefore, this procedure takes into account the evolutions attained from the ANOVA analysis of all the experiments performed. Besides, experimental data were added in these figures, when possible. In these interaction figures, only two intervals (upper and lower) are normally represented to carefully explore and explain the interactions found in the responses in a concise manner. Therefore, in some figures only two levels (upper and lower) have been included in the interaction figures; however, for all the responses, the whole interval of variation was sensibly studied and thoroughly discussed.

Response variable and analytical methods

The effects of the operating conditions was analysed on the yields to gas, liquid and solid products (%) together with the detailed chemical composition of the liquid phase produced in carbon basis (C-wt.%). The amount of gas produced was calculated gravimetrically by weight difference following a experimental gravimetric methodology developed previously and reported elsewhere [19]. Under the reaction conditions tested in this work the gas phase largely comprises CO_2 [33-34]. The solid yield was calculated from the dried spent solid recovered after the experiment, while the liquid yield was calculated by mass difference.

Different analytical tools, including Gas Chromatography (GC/MS-FID), High Performance Liquid Chromatography (HPLC) and Total Organic Carbon (TOC), were used to characterise the chemical composition of the hydrolysates (liquid phases). A Vario TOC Cube Analyser was employed for the TOC analyses. An Agilent 1260 apparatus, an Infinity II refraction index detector, and two different columns, were used for the HPLC analyses. DP>6 xylo-oligosaccharides concentration in the hydrolysates was calculated as the difference between the TOC of the solution and the amount of C quantified by chromatography (HPLC and GC). Besides, an ACE C18 column (250 × 4.6 mm, 5 µm particle size) was used for the quantification of ketones and furans. Gas chromatography (GC) analyses were performed in an Agilent 7890 Gas Chromatograph equipped with Flame Ionization (FID) and Mass Spectrometry (MS) detectors. The former detector was used for quantification, while the latter for identification. Carboxylic acids, ketones and furans were quantified by HPLC and GC. More detailed information about the characteristics, experimental conditions used in the analyses, separation columns and chromatography apparatus can be found in our previous publication [20]. Table 2 lists the response variables and the analytical methods used for their calculation.

Table 2. Response variables. Definitions and analytical techniques used in their determination.

Product	Response variable	Analytical method
Solid	$\text{Residual Solid yield (\%)} = \dfrac{\text{mass of solid after reaction (g)}}{\text{initial mass solid (g)}}\ 100$	Gravimetric
Gas	$\text{Gas yield (\%)} = \dfrac{\text{mass gas (g)}}{\text{initial mass solid (g)}}\ 100$	Gravimetric (Reactor mass balance)
Liquid	$\text{Liquid yield (\%)} = \dfrac{\text{mass of liquid products (g)}}{\text{initial mass of solid (g)}}\ 100 = = 100 - \text{Gas yield (\%)} - \mathit{Solid\ yield}\ \text{(\%)}$	Gravimetric (Mass difference)
	$\text{Composition (C} - \text{wt.\%)} = \dfrac{\text{mass of C of each compound}}{\text{total mass of C in solution}}\ 100$	HPLC, GC and TOC

RESULTS AND DISCUSSION

The experimental reaction conditions together with the results produced in the experiments are listed in Table 3. These include the yields to gas, liquid and solid products (%) together with the detailed chemical composition (C-wt.%) of the liquid phase. Table 4 summarises the effects of the operating conditions and interactions obtained from the statistical analysis of the experimental data. Besides, the detailed chemical composition of the liquid phase (mg/mL, wt.% and C-wt.%) is provided as supplementary material in Table S1.

Table 3. Microwave assisted experimental conditions and results

Run	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9-12**	**13**	**14**	**15**	**16**	**17**	**18**
Temperature (ºC)	140	210	140	210	140	210	140	210	175	140	210	175	175	175	175
Time (min)	0	0	60	60	0	0	60	60	30	30	30	0	60	30	30
Solid loading (wt.%)	5	5	5	5	25	25	25	25	15	15	15	15	15	5	25
Overall Product distribution															
Gas yield (%)	2.49	2.99	1.50	15.95	0.10	2.48	0.89	19.33	2.66±1.11	1.50	8.41	0.67	2.67	4.99	2.49
Liquid yield (%)	52.05	56.46	50.48	64.71	14.40	56.90	9.20	45.85	48.82±3.43	14.58	53.58	28.25	79.95	60.76	46.75
Solid yield (%)	45.45	40.55	48.02	19.34	85.50	40.62	89.90	34.82	48.51±2.35	83.92	38.01	71.08	17.38	34.25	50.77
Liquid composition (C-wt.%)															
Oligosaccharides DP>6	100	89.61	98.22	75.28	99.68	87.31	97.65	86.98	88.28±0.40	96.30	87.45	92.65	86.76	88.50	87.61
Oligosaccharides DP3-6	0.00	9.72	0.01	0.00	0.20	7.74	0.00	0.00	9.06±0.45	3.23	0.00	0.00	6.66	9.58	8.22
Saccharides	0.00	0.16	0.06	0.38	0.00	0.25	0.25	0.65	0.19±0.01	0.00	0.47	0.03	0.82	0.18	0.23
Carboxylic Acids	0.00	0.09	0.05	0.84	0.00	0.18	0.09	4.44	0.13±0.03	0.47	1.12	0.09	0.25	0.15	0.14
Ketones	0.00	0.03	0.07	12.32	0.00	1.90	0.00	1.99	1.02±0.54	0.00	4.90	0.89	2.32	0.53	1.39
Furans	0.00	0.39	1.60	11.18	0.12	2.62	2.00	5.94	1.33±0.62	0.00	6.06	6.34	3.19	1.06	2.41

Table 4. Relative influence of the operating conditions on the experimental results.

	R^2	Int.	T	t	C	Tt	TC	tC	T^2	t^2	C^2	TtC	T^2t	T^2C	Tt^2	T^2t^2
Overall Product Distribution																
Gas yield (%)	0.95	2.68	4.27 (33)	n.s.	n.s.	3.75 (26)	n.s.	n.s.	2.88 (26)	n.s.	n.s.	n.s.	3.70 (15)	n.s.	n.s.	n.s.
Liquid yield (%)	0.98	48.92	19.50 (21)	25.85 (7)	-11.14 (17)	n.s.	7.56 (10)	-2.86 (4)	-14.95 (10)	5.08 (6)	4.73 (4)	n.s.	-27.05 (17)	n.s.	-7.28 (5)	n.s.
Solid yield (%)	0.97	47.84	-17.95 (27)	-26.85 (11)	11.40 (17)	-4.25 (6)	-8.30 (11)	n.s.	10.85 (7)	n.s.	-7.60 (6)	n.s.	24.35 (15)	n.s.	n.s.	n.s.
Liquid composition (C-wt.%)																
Oligosaccharides DP>6	0.99	88.58	-4.43 (35)	-2.44 (13)	n.s.	-1.36 (7)	1.29 (6)	1.72 (8)	3.27 (12)	n.s.	n.s.	1.78 (9)	n.s.	1.06 (5)	-2.62 (6)	n.s.
Oligosaccharides DP3-6	0.99	9.01	-1.62 (9)	3.33 (7)	n.s.	-2.16 (13)	n.s.	n.s.	-7.39 (25)	-5.68 (9)	n.s.	n.s.	-5.54 (15)	n.s.	3.77 (10)	6.27 (12)
Saccharides	0.99	0.20	0.24 (20)	0.40 (22)	0.03 (8)	0.04 (4)	0.02 (2)	0.05 (5)	0.04 (3)	0.23 (7)	n.s.	n.s.	-0.28 (14)	0.04 (2)	-0.09 (5)	-0.24 (9)
Carboxylic Acids	0.99	0.14	0.33 (15)	0.08 (13)	n.s.	0.61 (13)	0.46 (10)	0.44 (10)	0.65 (10)	n.s.	n.s.	0.43 (10)	0.56 (6)	0.47 (10)	0.35 (3)	-0.08 (1)
Ketones	0.98	1.15	2.11 (21)	1.39 (14)	n.s.	1.54 (14)	-1.05 (9)	-1.53 (14)	0.97 (6)	n.s.	n.s.	-1.52 (13)	n.s.	-1.07 (9)	n.s.	n.s.
Furans	0.96	1.46	2.25 (22)	-1.58 (14)	n.s.	1.33 (11)	n.s.	-0.90 (8)	1.57 (5)	3.30 (8)	n.s.	-0.97 (8)	3.77 (15)	n.s.	n.s.	-3.35 (9)

n.s. = Non significant term with 95% confidence.

Response = Int + coefficient T $\cdot$ T + coefficient t $\cdot$ t + coefficient C $\cdot$ C + coefficient Tt $\cdot$ T$\cdot$t+ coefficient TC $\cdot$ T$\cdot$ C+ coefficient tC $\cdot$ t C + coefficient T^2 $\cdot$ T^2 + coefficient t^2 $\cdot$ t^2 + coefficient C^2 $\cdot$ C^2 + coefficient TtC $\cdot$ T$\cdot$t$\cdot$C + coefficient T^2t $\cdot$ $T^2\cdot t$ + coefficient T^2C $\cdot$ $T^2\cdot C$ + coefficient Tt^2 $\cdot$ $T\cdot t^2$ + coefficient T^2t^2 $\cdot$ $T^2\cdot t^2$. Standardised codec formula: all operating variables vary between -1 and +1.

Numbers in brackets show the relative influence (%) of each factor on the response variable. These values show the the orthogonal estimated total value (%).

Overall products distribution: gas, liquid and solid yields

The experimental yields of gas, liquid and solid products vary by 0-19%, 9-80% and 17-90%, respectively. The statistical analysis of the experimental data (Table 4) reveals that the temperature (linear and quadratic factors) is the process variable exerting the greatest influence on these yields. In addition, the reaction time and its interaction with the temperature also play a significant influence on these variables, although they have a lower impact. While the solid loading does not significantly influence the gas yield, this variable exerts an important impact on the liquid and solid yields. The influence of the reaction conditions and the most meaningful interactions are represented in Figure 1. In particular, Figure 1 a and b shows the effects on the gas yield (regardless of the initial solid loading, 5-25 wt.%) of the temperature and reaction time, respectively, due to the insignificant influence of the initial solid loading on gas formation. Figure 1 c/f, d/g and e/h plots the effects of the temperature on the liquid/solid yield for a diluted

(5 wt.%) and a concentrated (25 wt.%) initial solid solution using a reaction time of 0, 30 and 60 min, respectively.

Regardless of the feedstock loading (5-25 wt.%), the effects of the temperature on the gas yield depend on the reaction time and vice versa. At low temperature (140-175 ºC), gas formation is negligible for the whole interval of time considered (0-60 min). Conversely, between 175 and 210 ºC, gas production depends on the reaction time with different outcomes taking place; while gas formation does not occur to a substantial extent for a very short reaction time (0 min), increasing the reaction time from 0 to 60 min leads to a linear increase in the gas yield. Augmenting the reaction time at high temperature facilitates a greater development of pyrolysis and decarboxylation reactions [35-37], thus leading to gas formation [38-39].

The effect of the temperature on the liquid and solid yields depends on the reaction time. Regardless of the solid/water ratio used in the experiments, similar evolutions are observed for the liquid and solid yields. For a very short reaction time (0 min; i.e. only 5 min of ramp time), the temperature exerts a weak influence. In particular, a first increase in the temperature from 140 to 175 ºC does not substantially modify the liquid or solid yield and a steady evolution is observed. Conversely, a further increase up to 210 ºC leads to a moderate increase in the liquid yield, this variation being accompanied with a decrease in the solid yield. The positive kinetic temperature influence on the process is responsible for such variations [36, 40], which increases hemicellulose conversion, thus augmenting and reducing the liquid and solid yield, respectively. Hemicellulose depolymerisation starts at a temperature of around 170 ºC when conventional heating is used [41]; however, liquid yields up to 50% can be attained at temperatures as low as 140 ºC when microwave-assisted hydrothermal depolymerisation is used for a reaction time as short as 5 min, which is a clear indicative of the efficiency and selectivity of microwaves during the hydrothermal depolymerisation of hemicellulose [22-23, 25, 42].

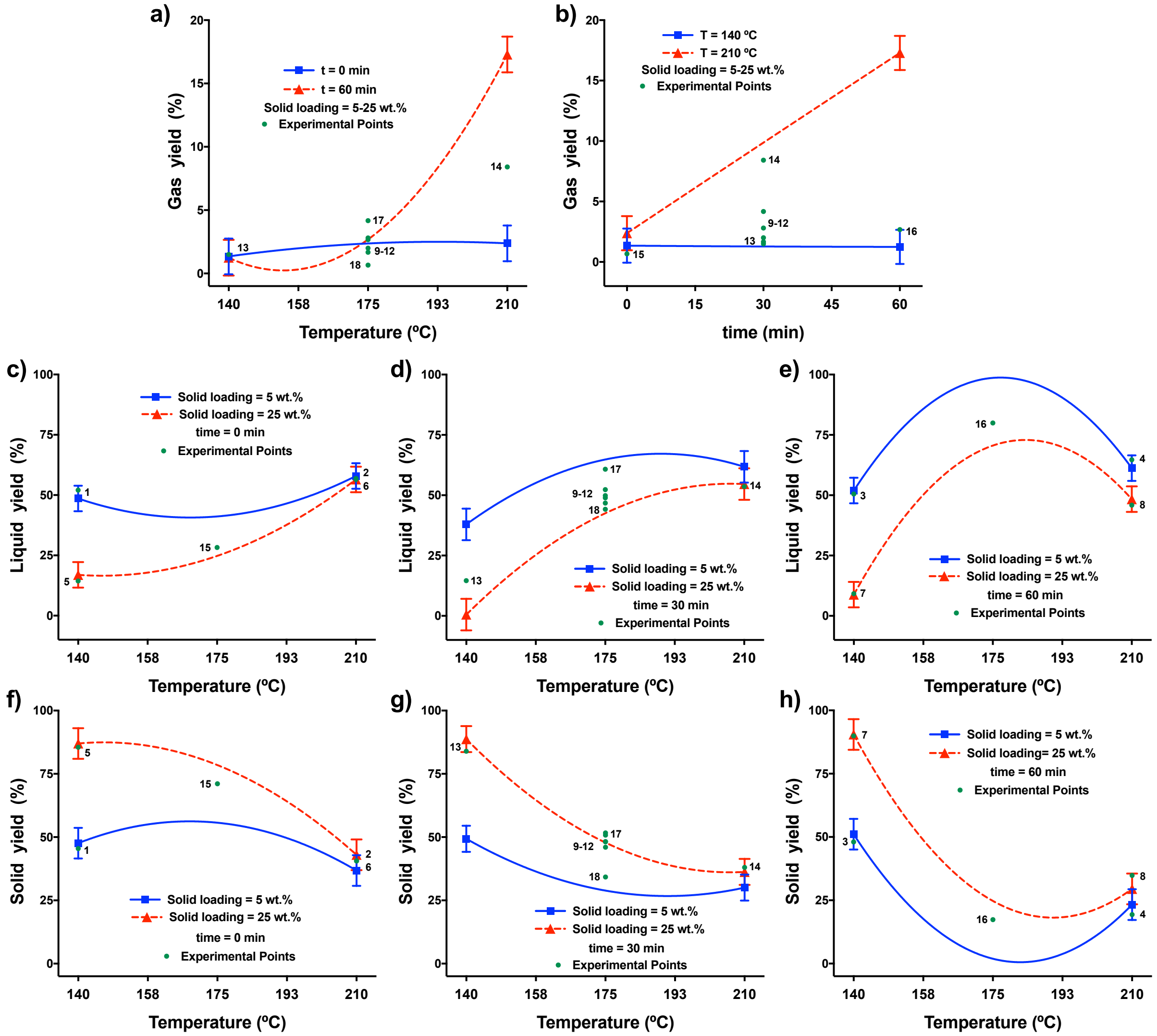


Figure 1. Interactions plots showing the influence of the temperature, reaction time and solid loading on the gas (a and b), liquid (c, d and e) and solid (f, g and h) yields. Bars are LSD intervals with 95% confidence.

Increasing the reaction time modifies the temperature evolution of these variables, especially at high temperature. For a 30 min reaction time, an initial increase in the temperature (140-175 ºC) leads to an increase in the liquid yield together with a decrease in the solid yield due to the positive effect of the temperature [36, 40] as described earlier, while a further increase up to 210 ºC follows the same trend. These variations have their origin from the selective occurrence of some chemical reactions over others. On the one hand, increasing the temperature promotes hemicellulose depolymerisation into water-soluble species, augmenting the yield to liquid and dropping the yield to solid. On the other, this temperature increase also favours gas formation via thermal cracking and deoxygenation of some of the liquid products and part of the original solid using long reaction times [18-20], thus increasing gas formation at the expenses of the liquid and solid yield. These developments increase gas formation, while a trade-off is observed for the liquid and solid yields.

A subsequent increase in the reaction time from 30 to 60 min leads to an increase in the liquid yield and a decrease in the solid yield between 140 and 190 ºC. As a result, for a 60 min reaction increasing the temperature between 140 and 180 ºC leads to a pronounced increase in the liquid yield and decrease in the solid yield. An increase in the reaction time exerts a positive kinetic effect on the process due to the longer exposure of the feedstock to microwaves and therefore, lower temperatures are required in order to reach the same conversion [20]. This temperature-time relationship (interaction) was defined as severity by Prado et al. [41] These authors also reported that increasing the reaction temperature shifted the maximum liquid yield towards a lower residence time due to the lower severity of the process. A further increase in the temperature up to 210 ºC produces a decrease in the liquid yield at the expenses of an increase in the solid yield. The production of solid (largely char and humins) during hemicellulose processing at elevated temperature is responsible for these developments [43-45]. The formation of these macromolecules is originated from the furans produced during the decomposition of hemicellulose by means of a first aldol addition reaction and a subsequent condensation or polymerisation reaction [46-52]. This highlights the importance of achieving an accurate control of the process in order to adequately maximise liquid production, minimising the formation of gas and solid species.

The effect of the solid/water ratio depends on the temperature. Regardless of the reaction time, at low temperature (140-190ºC), an increase in the solid loading decreases the liquid yield and increases the solid yield. This increase in the feedstock loading augments the solid/microwave ratio and decreases the proportion of water (a good microwave absorbent) with respect to biomass in the reactor, thus decreasing the effectiveness of microwave reactions. The effect of the solid loading is more marked at low temperature (140 ºC) and short reaction time (0 min) as the kinetic effect of these variables is weaker than when high temperatures or long reaction times are used. Interestingly, this effect decreases progressively as the temperature increases up to 190 ºC, the temperature at which the effect of the initial solid loading is not significant, as the positive kinetic effect of the reaction temperature might compensate for the lower microwave efficiency taking place when increasing the initial solid/water ratio. As a result, non-statistically significant variations are observed for the liquid or solid yields between 190 and 210 ºC when the solid loading increases from 5 to 25 wt.%. One exception to this takes place for the liquid yield when a long reaction time (60 min) is used due to the large gas formation occurring at high temperatures and long reaction (60 min) times for a concentrated liquid solution (25 wt.%).

Liquid phase chemical composition

The liquid product consists of a mixture of xylooligosaccharides (DP3-6 and DP>6) mono-/di-saccharides (xylose, glucose, fructose, mannose, arabinose, rhamnose and levoglucosan), carboxylic acids (lactic, formic and acetic acids), ketones (levoglucosenone, 1-hydroxy-2-

butanone, 1-hydroxy-2-propanone, 2-hydroxy-gamma-butyrolactone) and furans (5-HMF and furfural). The detailed chemical composition of the hydrolysates is provided as supplementary information. The compounds quantified in the hydroxylates have been previously identified by other authors working on the hydrothermal depolymerisation of hemicellulose [53-54]. The presence of some C6 saccharides in the liquid product might be accounted for by the existence of sugar impurities (largely glucooligosaccharides) in the original feedstock, which can be hydrolysed and released to the solution during the microwave process.

The relative amounts of DP>6 xylooligosaccharides (DP>6-XOS), DP3-6 xylooligosaccharides (DP3-6-XOS), saccharides, carboxylic acids, ketones and furans vary as follows: 75-100 C-wt.%, 0-10 C-wt.%, 0-1 C-wt.%, 0-5 C-wt.%, 0-12 C-wt.% and 0-12 C-wt.%, respectively. Table 4 lists the relative influence of the operating variables on the chemical composition of the liquid product according to the statistical analyses of the experimental data. These analyses show that the temperature (first) and the reaction time (second) are the operating variables with the highest influence on the relative amount of these species in the liquid product. The initial xylan solid loading on its own does not exert a significant influence, however significant interactions are detected for this variable with the temperature and reaction time. In addition, significant interactions between the temperature and reaction time are also significant, which denotes that the effect of the temperature depends on the reaction time and vice versa. Figure 2 a, b and c plots the effects of the temperature on the relative amount of DP>6-XOS for a low (5 wt.%) and a high (25 wt.%) initial solid (xylan) loading using a reaction time of 0, 30, and 60 min, respectively. These effects are shown for the proportions of DP3-6-XOS, saccharides, carboxylic acids, ketones and furans in Figure 2 d-f, g-i, j-l, m-o and p-r, respectively.

The effects of the temperature on the chemical composition of the liquid phase depend on the reaction time and initial solid loading. For a very short reaction time (0 min), the feedstock loading does not substantially influence the composition of the liquid product, and similar evolutions with the temperature are observed regardless of the initial solid/water ratio used in the experiments (5-25 wt.%). Specifically, the liquid phase obtained using a short reaction time is largely made of DP>6-XOS, DP3-6-XOS and furans, the proportions of saccharides, carboxylic acids and ketones in the liquid being lower than 2 C-wt.% in all the cases. At low temperature, the liquid phase primarily is composed of DP>6-XOS due to the progressive depolymerisation of hemicellulose. This implies a first sequential hemicellulose depolymerisation to XOS under microwave heating, with a subsequent depolymerisation to water-soluble XOS without significantly altering the monomeric units comprised in the core xylan original structure [20]. The temperature promotes such transformations due to its positive kinetic influence on depolymerisation and hydrolysis reactions [36, 40]. As a consequence, the proportion of DP>6-XOS in the liquid product decreases with increasing the temperature between 140 and 175 ºC; temperature at which a trade off for these species is observed. The decrease occurring between 140 and 175 ºC is accompanied with an increase in the proportion of furans, which accounts for an increase in the formation of furfural from the acetyl groups bonded to the xylan polymeric core structure [55-58].

A further increase in the temperature from 140 to 210 ºC does not substantially modify the proportion of DP>6-XOS in the liquid phase; however, the relative amount of DP3-6-XOS increases while the proportion of furans decreases. The positive influence of temperature on the hydrolysis and depolymerisation reactions not only increases the liquid yield and the proportion of DP>6-XOS in the liquid, but also it promotes the subsequent transformation of these species into DP3-6-XOS. As a result of this compensation, a trade off is observed for the relative amount of DP>6-XOS in the liquid product, while the relative amount of DP3-6-XOS increases. In addition, the relative amount of furans in the liquid decreases, probably due to light oxygenates transformating into gases under hydrothermal conditions [18-20, 22].

Increasing the reaction time not only does modify the chemical composition of the liquid phase, but also the effects the temperature and initial solid loading exert on the composition of this fraction. The variations in the proportion of DP>6-XOS/DP3-6-XOS/saccharides/carboxylic acids/ketones/furans when increasing the reaction time can be analysed by comparing Figure 2 a/d/g/j/m/p (0 min) with 2 b/e/h/k/n/q (30 min) and/or 2 c/f/i/l/o/r (60 min). The effect of the reaction time depends on the temperature and initial solid loading. Between 140 and 175 ºC, the reaction time significantly influences the proportions of DP>6-XOS, DP3-6-XOS, saccharides and furans; the relative amounts of carboxylic acids and ketones being negligible and unaffected. Regardless of the initial solid/water ratio, an initial increase in the reaction time from 0 to 30 min leads to decreases in the relative amounts of DP>6-XOS and furans. These decreases are accompanied with a substantial and moderate increase in the proportion of DP3-6-XOS and saccharides, respectively. The positive reaction time kinetic influence on the process is responsible for such variations as the exposure to microwaves of the feedstock together with the reaction intermediates/products increases. Therefore, the hydrolysis and depolymerisation reactions take place to a greater extent, thus promoting the depolymerisation of DP>6-XOS into DP3-6-XOS and saccharides as well as the subsequent transformation of furans into gaseous compounds via decarboxylation and thermal decomposition reactions [35, 37, 59].

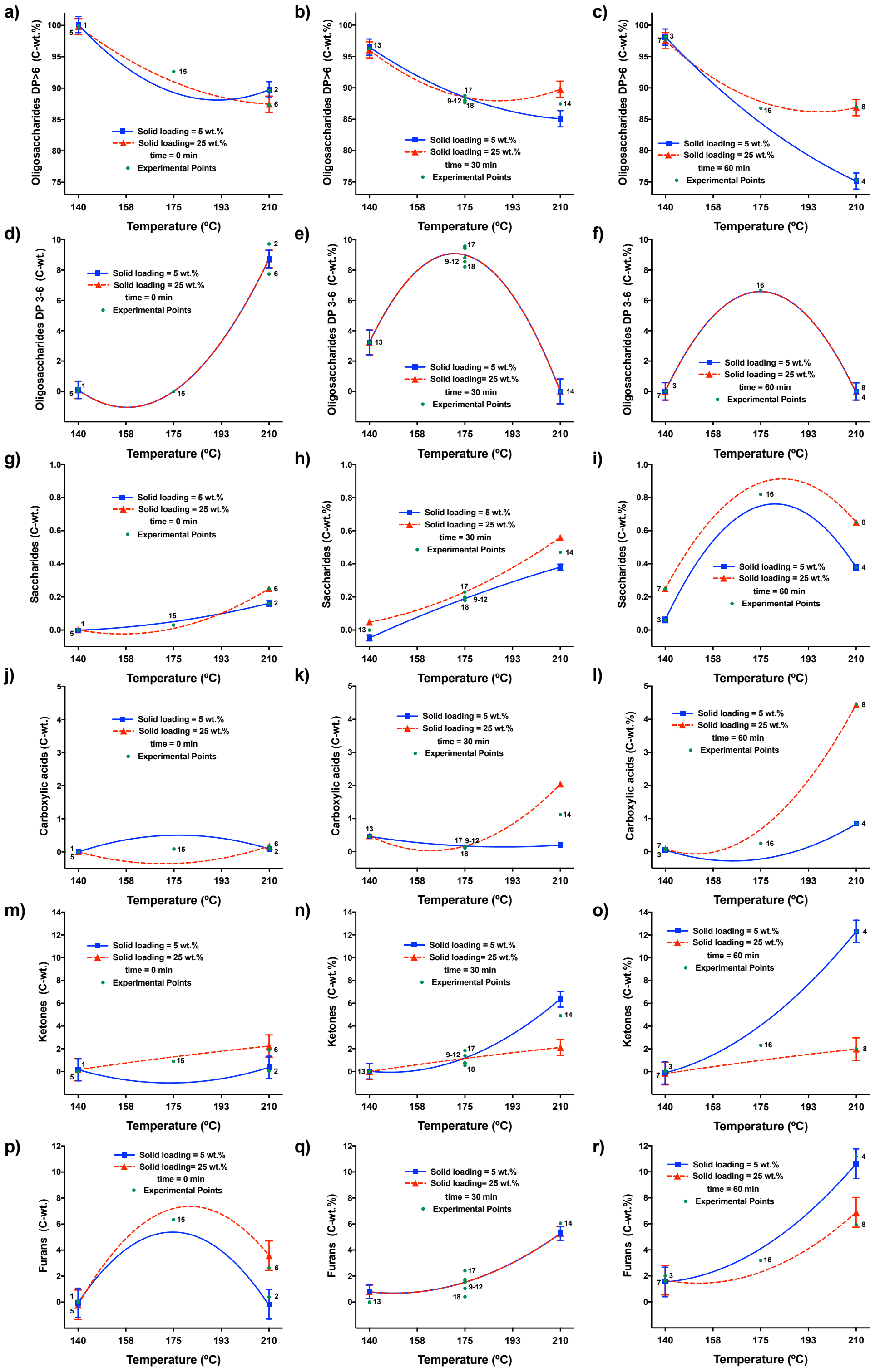


Figure 2. Interactions plots showing the influence of the temperature, reaction time and solid loading on the chemical composition of the liquid phase: proportions of oligosaccharides DP>6 (a,b,c), oligosaccharides DP3-6 (d,e,f), saccharides (g, h, i), carboxylic acids (j, k, l), ketones (m, n, o) and furans (p, q, r). Bars are LSD intervals with 95% confidence.

At high temperature (175-210 ºC), the same increase in the reaction time does not substantially modify the relative amount of DP>6-XOS in the liquid due to the compensation between DP>6-XOS production and depolymerisation as described earlier. Conversely, significant variations are observed for the relative amounts of DP3-6-XOS, saccharides, carboxylic acids and furans. In particular, the relative amounts of DP3-6-XOS and furans decrease; these variations being accompanied with increases in the proportions of carboxylic acids or ketones due to the progressive transformation of the former saccharides into the latter small oxygenates. While for a low initial feedstock loading (5 wt.%), the relative amounts of DP3-6-XOS and furans decrease at the expenses of an increase in the proportion of ketones, for a high solid loading (25 wt.%) the proportion of carboxylic acid in the liquid increases. These variations modify the effects of the temperature on the composition of the liquid phase for an intermediate (30 min) reaction time. At low temperature, the effect of the solid loading is negligible. Regardless of the solid/water ratio, an increase in the temperature between 140 and 175 ºC decreases in the proportion of DP>6-XOS and increases the relative amounts of DP3-6-XOS and saccharides due positive effect of the temperature on depolymerisation reactions thus promoting the transformation of DP>6-XOS into lower molecular weight XOS and saccharides [36, 40]. Within this temperature interval, the proportions of carboxylic acids, ketones and furans, are negligible.

A further increase in the temperature up to 210 ºC does not substantially modify the proportion of DP>6-XOS, especially for a high initial solid loading. However, the relative amount of DP3-6-XOS decreases and the proportions of saccharides and furans increase regardless of the initial solid amount. Elevated temperatures and long reaction times promote hydrolysis reactions, thus increasing the liquid yield and the proportion of DP>6-XOS in the liquid. In addition, this positive kinetic effect also favours the subsequent transformation of DP>6-XOS into DP3-6-

XOS, saccharides and small oxygenates. This seems to indicate that under microwave heating, the first hemicellulose depolymerisation steps into XOS and soluble DP>6-XOS are the limiting rate stages, while the subsequent fragmentation reactions into saccharides and light oxygenates are much faster. Hence, it is very important to achieve an accurate process control to reach an excellent compromise between DP>6-XOS production and degradation. Conversely, different evolutions take place for the proportions of carboxylic acids and ketones. In particular, these variations are accompanied with an increase in the proportions of ketones and carboxylic acids for a low (5 wt.%) and a high initial solid loading (25 wt.%), respectively.

A subsequent increase in the reaction time from 30 to 60 min leads to different variations in the liquid chemical composition depending on the temperature and initial solid/water ratio. At low temperature (140-175 ºC), the effect of the feedstock loading is relatively weak. The relative amount of DP>6-XOS is not greatly modified, while the proportion of DP3-6-XOS and saccharides decreases and increases, respectively. This suggests the transformation of the former into the latter due to the positive kinetic effect of the reaction time on depolymerisation reactions [36, 40]. In addition, the relative amounts of carboxylic acids, ketones and furans do not increase with time, and their proportion in the liquid is negligible, which might indicate that secondary reactions are substantially inhibited when processing the material at low temperature [20]. Conversely, at elevated temperature (175-210 ºC), greater variations in the liquid composition take place with increasing reaction time. Besides, the initial solid loading exerts a substantial influence, with different developments occurring. On the one hand, for a low initial solid loading (5 wt.%), increasing the reaction time from 30 to 60 min leads to a substantial reduction in the proportion of DP>6-XOS along with a moderate increase in the proportion of saccharides, without significantly modifying the relative amount of DP3-6-XOS. These variations produce a sharp increase in the proportions of ketones and furans due to the greater extension of secondary decomposition reactions at high temperatures and long reaction times [20]. On the other hand, when a concentrated suspension (25 wt.%) is used, the relative proportion of DP>6-XOS remains relatively steady with increasing the reaction time from 30 to 60 min, as a lower organic/water ratio hinders the extension of hydrolysis reactions. In addition, the proportion of carboxylic acids increases, while the relative amounts of ketones and furans are not modified with this increase in the reaction time.

These variations modify the effect of the temperature and solid/water ratio when a long reaction time (60 min) is used. Specifically, increasing the temperature leads to a substantial decrease in the relative amount of DP>6-XOS. This decrease is more pronounced than that observed for shorter reaction times and, it depends on the initial solid loading when a temperature higher than 175 ºC is used. In addition, the maximum value reached for the relative amount of DP3-6-XOS decreases with increasing the reaction time, since longer reaction times favour the depolymerisation of these species towards the formation of saccharides. As a result, the proportion of saccharides displays a pronounced increase between 140 and 175 ºC and a subsequent decrease between 175 and 190 ºC. This is accounted for by the positive effect of the reaction time on the process, which promotes saccharides formation at low temperature, but also helps their decomposition if high temperatures are used [41]. In addition, despite using a long reaction time, the proportions of carboxylic acids, ketones and furans are very low (< 3 C-wt.%) at low temperature (140 and 175 ºC). However, increasing the temperature when a long reaction time is used promotes the formation of these species, which is in agreement with the decomposition occurring for the relative amount of DP>6-XOS, DP3-6-XOS and saccharides. When a low initial solid amount is employed, the decrease observed for these compounds take place along with increases in the relative amounts of furan and ketones, while for a high solid loading the relative amount of carboxylic acids increases; these differences being accounted for by the different organics/water and organics/microwave power ratios used, as described earlier.

The effect of the solid/water ratio depends on the temperature and reaction time. In general, the longer the reaction time, the greater is the influence of the solid loading on the composition of the liquid phase. For a very short reaction time (0 min), the composition of the liquid phase does not greatly depend on the solid loading. Conversely, increasing the reaction time significantly modifies the effect of solid/water ratio on the chemical composition of the liquid phase. Specifically, when an intermediate (30 min) reaction time is used, the effect of the solid loading depends on the reaction temperature. While at temperatures lower than 175 ºC the initial solid loading does not exert a significant impact on the chemical composition, between 175 and 210 ºC the solid/water ratio has a significant influence. An increase in the initial solid loading from 5 to 25 wt.% leads to increases in the proportions of DP>6-XOS, saccharides and carboxylic acids, which results in a significant decrease in the relative amount of ketones. Moreover, the effect of the solid loading becomes more important when the reaction time increases. As a result, when a reaction time of 60 min is used, not only the effect of the solid loading is significant at lower temperatures (than for a 30 min reaction time), but also more pronounced variations are observed. Specifically, substantial increases in the proportions of DP>6-XOS, saccharides and carboxylic acids take place along with substantial depletions in the relative amounts of ketones and furans between 160 and 210 ºC. These developments are presumably the result of the lesser spread of hydrolysis and depolymerisation reactions due to the lower amount of water in the reaction medium. This helps stop the process at earlier reaction stages, thus hampering the extension of secondary reactions.

Theoretical optimisation and energy and cost assessments

Three different optimisation scenarios were sought using the empirical correlations developed from the statistical analysis of the results. The first and second optimisations are directed towards the production of a liquid product containing a high concentration of XOS, thus maximising the liquid yield and concentrations of DP>6-XOS and DP3-6-XOS as well as minimising the presence of other species in the liquid product. The first scenario does not consider any restrictions for the temperature, reaction time or solid loading, while for the second optimisation the temperature and time are minimised and the solid loading maximised for energy saving purposes. The third optimisation comprises the maximisation of the liquid yield without any restriction in the chemical composition of the liquid product. For all the optimisations, the gas and solid yields are also minimised to ensure the selective transformation of hemicellulose to liquid products. Besides, a relative importance (from 1 to 5) has been assigned to each constraint to come up with operating conditions meeting all the restrictions (Table 5).

Taking these restrictions into consideration, the optimum temperature range to selectively convert hemicellulose into liquid soluble species is between 172 and 177 ºC. A good compromise (Opt. 1) between the liquid yield (81%) and XOS relative proportion (96 C-wt.%) takes place at 172 ºC using a solid loading of 5 wt.% for 47 min. Opt. 2 reveals that the reaction time could be reduced and the solid/water ratio increased. In particular, a liquid product containing a high concentration (97 C-wt.%) of XOS could be produced at 172 ºC, using a 25 wt.% initial solid loading for 33 min. However, the liquid yield is reduced down to 51%. Opt. 3 reveals that the highest liquid yield occurs when a 5 wt.% solution is treated at 177 ºC for 60 min. Under these conditions, a relatively high XOS proportion (90 C-wt.%) XOS liquid product is produced in a very high yield (99%).

When XOS are produced by water or stream treatment under conventional heating, a variety of other compounds (impurities), such as monosaccharides, carboxylic acids (acetic acid), furans (furfural) and ketones and lignin and proteins derived compounds appear in the reaction medium. To produce food-grade XOS the liquid produced must be purified to remove such impurities, leading to a commercial product with a XOS purity ranging from 75-95%. Therefore, the yields and XOS purities achieved under optimum conditions in this work are much higher than those previously reported during the production of XOS from biomass [8, 28-29, 60-61]. The purity of this product might be increased with readily available technologies, including, solvent extraction and precipitation, adsorption, chromatographic separation, and membrane technology (ultrafiltration and nanofiltration).

Table 5. Optimisation: Restrictions and theoretical and experimental optimum values.

Optimisation	**1**			**2**			**3**		
	Objective	**Theoretical**	**Exp.**	**Objective**	**Theoretical**	**Exp.**	**Objective**	**Theoretical**	**Exp.**
Temperature (ºC)	none	172		Minimise (3)	172		none	177	
Time (min)	none	47		Minimise (3)	33		none	60	
Solid loading (wt.%)	none	5		Maximise (3)	25		none	5	
Overall Product distribution									
Gas yield (%)	Minimise (3)	2 ±1.37	2.16	Minimise (3)	2.39±1.37	3.57	Minimise (5)	3±1.37	2.20
Liquid yield (%)	Maximise (3)	81±3.97	81.22	Maximise (3)	43.14±3.97	44.50	Maximise (5)	98±3.97	96.71
Solid yield (%)	Minimise (3)	15±4.89	15.19	Minimise (3)	50.63±4.89	51.93	Minimise (5)	1±4.89	1.09
Liquid composition (C-wt.%)									
Oligosaccharides DP>6	Maximise (5)	87.±0.78	87.03	Maximise (5)	89±0.78	89.17	none	84±0.78	83.73
Oligosaccharides DP3-6	Maximise (5)	9±0.62	9.19	Maximise (5)	10±0.62	9.50	none	7±0.62	6.64
Saccharides	Minimise (5)	0.4±0.01	0.42	Minimise (5)	0.3±0.01	0.33	none	1±0.01	0.76
Carboxylic Acids	Minimise (5)	0.1±0.03	0.1	Minimise (5)	0.14±0.03	0.20	none	0.1±0.03	0.10
Ketones	Minimise (5)	2.3±0.62	2.35	Minimise (5)	1±0.62	0.60	none	5±0.62	4.46
Furans	Minimise (5)	1.8±0.88	1.81	Minimise (5)	1±0.88	0.20	none	5±0.88	4.38

An energetic assessment was conducted to assess the energy input and economic aspects of the microwave process. The Energy consumption of the hydrothermal microwave reactions was measured using an Energenie (ENE007) a power meter plugged between the reactor plug and the electric wall socked. To estimate the production cost, only the microwave reaction step, which is considered the cost-limiting factor, was considered. Future work to assess the possibility of using this microwave-assisted hydrothermal process at large scale should consider other factors such as the costs of the feedstock, pre-processing and purification treatments. However, it is expected that the high selectivity of microwave-assisted hydrothermal treatments towards the depolymerisation of the hemicellulose fraction in lignocellulosic biomass, together with the excellent process control of microwave heating, allowing the minimisation of impurities in the XOS product, will help decrease the cost of the possible pre- and post-processing steps.

Under optimum conditions, the total electric energy required for running the microwave reaction (including both electric energy to microwaves and microwaves to thermal energy) for Opts. 1, 2 and 3 is 0.044 kWh (0.16 MJ), 0.038 kWh (0.14 MJ) and 0.08 kWh (0.29 MJ), respectively. The theoretical energy to heat the feedstock (hemicellulose/water solution) up to the operating temperature required (172-177 ºC) is around 0.025 MJ, thus giving an energy efficiency for the microwave process between 10 and 15%. However, it must be borne in mind that heat losses might occur, as the microwave reactor used at this development stage (lab scale) might not be completely adiabatic. As a result, extra consumption is necessary to provide the microwave energy to keep up the temperature for the whole reaction time used in each experiment to compensate for heat losses, thus decreasing the energy efficiency in comparison to the theoretical value. In addition, lab scale microwaves run at 2.45 GHz, and consequently have a low electrical efficiency, while industrial large-scale microwaves are capable of working at much lower frequencies (from 896 to 922 MHz), which allows attaining electrical efficiencies greater than 85% [32, 62], thus helping increase the overall energy efficiency.

Assuming an energy cost of around 0.16 €/kwh and taking into account the amounts of XOS produced in Opts. 1-3, the total cost of the microwave reaction for the production of XOS from hemicellulose is 4.40 €/kg XOS, 1.42 €/kg XOS and 6.50 €/kg XOS, respectively. The market price for XOS ranges between 30 and 90 €/kg, depending on the purity. In this regard, it is important to note that this controllable microwave XOS production process is still at bench-scale and its competitiveness (<7 €/kg XOS) is thought to increase even more according to the scale-up principle economic aspects, which clearly highlights the potential of this emerging technology. In addition, the process is conducted only employing water as the reaction medium, without any acid/basic catalysts, thus helping the production of a product suitable for human consumption. Therefore, these promising results might represent a step-change towards an economically competitive and environmentally friendly production of renewable based, food-applicable prebiotics from biomass.

CONCLUSIONS

This work firstly addresses the microwave-assisted hydrothermal depolymerisation of beech wood hemicellulose for the production of high purity, food-applicable xylooligosaccharides with high potential to be used as natural and renewable prebiotics. A good compromise between liquid yield (81%) and XOS purity (96 C-wt.%) was achieved at 172 ºC using a solid loading of 5 wt.% for 47 min. In addition, at this temperature (172 ºC), the reaction time could be reduced (33 min) and the solid loading increased (25 wt.%), without substantially altering the purity of the liquid XOS product (98 C-wt.%) at the expenses of liquid production (51%). Besides, these liquid yields could be substantially increased using a reaction temperature of 177 ºC and a 5 wt.% feedstock loading for 60 min. This allowed the solubilisation of 97% of hemicellulose into a liquid product, but decreased the XOS purity down to 90 C-wt.%. At optimum conditions, the total electric energy required for running the microwave reaction varied between 0.038 kWh (0.14 MJ) and 0.08 kWh (0.29 MJ). These different energy costs and the products yield achieved, varied the XOS production costs between 1.42 and 6.50 €/kg XOS, thus clearly highlighting the potential of this emerging technology.

ACKNOWLEDGEMENTS

The authors acknowledge the Industrial Biotechnology Catalyst (Grants Innovate UK, BBSRC, EPSRC and EP/N013522/1) and EPSRC research grant number EP/K014773/1 for financial support. In addition, Javier Remón Núñez would like to express his gratitude to the Spanish Ministry of Science, Innovation and Universities for the Juan de la Cierva fellowship (FJCI-2016-30847) awarded.

Supporting Information

Operating conditions, overall yields (gas, liquid and solid yields) and detailed chemical composition (mg/mL, wt.% and C-wt.%) of the liquid product (hydrolysate).

REFERENCES

1. Ayhan, D., Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. *Energy Conversion and Management* **2008,** *49* (8), 2106-2116.
2. Bian, J.; Peng, P.; Peng, F.; Xiao, X.; Xu, F.; Sun, R. C., Microwave-assisted acid hydrolysis to produce xylooligosaccharides from sugarcane bagasse hemicelluloses. *Food chemistry* **2014,** *156*, 7-13.
3. Carvalho, A. F. A.; Neto, P. d. O.; da Silva, D. F.; Pastore, G. M., Xylo-oligosaccharides from lignocellulosic materials: Chemical structure, health benefits and production by chemical and enzymatic hydrolysis. *Food Research International* **2013,** *51* (1), 75-85.
4. Lin, Q.; Li, H.; Ren, J.; Deng, A.; Li, W.; Liu, C.; Sun, R., Production of xylooligosaccharides by microwave-induced, organic acid-catalyzed hydrolysis of different

xylan-type hemicelluloses: Optimization by response surface methodology. *Carbohydrate polymers* **2017,** *157*, 214-225.

5. Miguez, B.; Gomez, B.; Parajo, J. C.; Alonso, J. L., Potential of Fructooligosaccharides and Xylooligosaccharides as Substrates To Counteract the Undesirable Effects of Several Antibiotics on Elder Fecal Microbiota: A First in Vitro Approach. *J Agric Food Chem* **2018,** *66* (36), 9426-9437.

6. Scheller, H. V.; Ulvskov, P., Hemicelluloses. *Annu Rev Plant Biol* **2010,** *61*, 263-289.

7. Amorim, C.; Silverio, S. C.; Rodrigues, L. R., One-step process for producing prebiotic arabino-xylooligosaccharides from brewer's spent grain employing Trichoderma species. *Food chemistry* **2019,** *270*, 86-94.

8. Mihiretu, G. T.; Brodin, M.; Chimphango, A. F.; Oyaas, K.; Hoff, B. H.; Gorgens, J. F., Single-step microwave-assisted hot water extraction of hemicelluloses from selected lignocellulosic materials - A biorefinery approach. *Bioresource technology* **2017,** *241*, 669-680.

9. Budarin, V. L.; Clark, J. H.; Lanigan, B. A.; Shuttleworth, P.; Macquarrie, D. J., Microwave assisted decomposition of cellulose: A new thermochemical route for biomass exploitation. *Bioresource technology* **2010,** *101* (10), 3776-3779.

10. Budarin, V. L.; Shuttleworth, P. S.; Dodson, J. R.; Hunt, A. J.; Lanigan, B.; Marriott, R.; Milkowski, K. J.; Wilson, A. J.; Breeden, S. W.; Fan, J.; Sin, E. H. K.; Clark, J. H., Use of green chemical technologies in an integrated biorefinery. *Energy Environ. Sci.* **2011,** *4* (2), 471-479.

11. de Melo, E. M.; Clark, J. H.; Matharu, A. S., The Hy-MASS concept: hydrothermal microwave assisted selective scissoring of cellulose for in situ production of (meso)porous nanocellulose fibrils and crystals. *Green Chem.* **2017,** *19* (14), 3408-3417.

12. Fan, J.; De bruyn, M.; Budarin, V. L.; Gronnow, M. J.; Shuttleworth, P. S.; Breeden, S.; Macquarrie, D. J.; Clark, J. H., Direct microwave-assisted hydrothermal depolymerization of cellulose. *J Am Chem Soc* **2013,** *135* (32), 11728-11731.

13. Fan, J.; De bruyn, M.; Zhu, Z.; Budarin, V.; Gronnow, M.; Gomez, L. D.; Macquarrie, D.; Clark, J., Microwave-enhanced formation of glucose from cellulosic waste. *Chemical Engineering and Processing: Process Intensification* **2013,** *71*, 37-42.

14. Li, F.; Liu, L.; An, Y.; He, W.; Themelis, N. J.; Li, G., Hydrothermal liquefaction of three kinds of starches into reducing sugars. *Journal of Cleaner Production* **2016,** *112*, 1049-1054.

15. Mahmood, N.; Yuan, Z.; Schmidt, J.; Xu, C., Depolymerization of lignins and their applications for the preparation of polyols and rigid polyurethane foams: A review. *Renewable and Sustainable Energy Reviews* **2016,** *60*, 317-329.

16. Rosso, D.; Fan, J.; Montoneri, E.; Negre, M.; Clark, J.; Mainero, D., Conventional and microwave assisted hydrolysis of urban biowastes to added value lignin-like products. *Green Chem.* **2015,** *17* (6), 3424-3435.

17. Singh, R.; Krishna, B. B.; Mishra, G.; Kumar, J.; Bhaskar, T., Strategies for selection of thermo-chemical processes for the valorisation of biomass. *Renewable Energy* **2016,** *98*, 226-237.

18. Lorente, A.; Remón, J.; Budarin, V. L.; Sánchez-Verdú, P.; Moreno, A.; Clark, J. H., Analysis and optimisation of a novel "bio-brewery" approach: Production of bio-fuels and bio-chemicals by microwave-assisted, hydrothermal liquefaction of brewers' spent grains. *Energy Conversion and Management* **2019,** *185*, 410-430.

19. Remón, J.; Randall, J.; Budarin, V. L.; Clark, J. H., Production of bio-fuels and chemicals by microwave-assisted, catalytic, hydrothermal liquefaction (MAC-HTL) of a mixture of pine and spruce biomass. *Green Chemistry* **2019,** *21* (2), 284-299.

20. Remón, J.; Santomauro, F.; Chuck, C. J.; Matharu, A. S.; Clark, J. H., Production of fermentable species by microwave-assisted hydrothermal treatment of biomass carbohydrates: reactivity and fermentability assessments. *Green Chemistry* **2018,** 20, 4507-4520.

21. De bruyn, M.; Fan, J.; Budarin, V. L.; Macquarrie, D. J.; Gomez, L. D.; Simister, R.; Farmer, T. J.; Raverty, W. D.; McQueen-Mason, S. J.; Clark, J. H., A new perspective in bio-refining: levoglucosenone and cleaner lignin from waste biorefinery hydrolysis lignin by selective conversion of residual saccharides. *Energy Environ. Sci.* **2016,** *9* (8), 2571-2574.

22. Li, T.; Remón, J.; Jiang, Z.; Budarin, V. L.; Clark, J. H., Towards the development of a novel “bamboo-refinery” concept: Selective bamboo fractionation by means of a microwave-assisted, acid-catalysed, organosolv process. *Energy Conversion and Management* **2018,** *155*, 147-160.

23. Li, T.; Remón, J.; Shuttleworth, P. S.; Jiang, Z.; Fan, J.; Clark, J. H.; Budarin, V. L., Controllable production of liquid and solid biofuels by doping-free, microwave-assisted, pressurised pyrolysis of hemicellulose. *Energy Conversion and Management* **2017,** *144*, 104-113.

24. Remón, J.; Matharu, A. S.; Clark, J. H., Simultaneous production of lignin and polysaccharide rich aqueous solutions by microwave-assisted hydrothermal treatment of rapeseed meal. *Energy Conversion and Management* **2018,** *165*, 634-648.

25. Zhou, L.; Budarin, V.; Fan, J.; Sloan, R.; Macquarrie, D., Efficient Method of Lignin Isolation Using Microwave-Assisted Acidolysis and Characterization of the Residual Lignin. *ACS Sustainable Chemistry & Engineering* **2017,** *5* (5), 3768-3774.

26. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapple, M.; Ladisch, M., Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour Technol* **2005,** *96* (6), 673-686.

27. Jiang, Z.; Yi, J.; Li, J.; He, T.; Hu, C., Promoting effect of sodium chloride on the solubilization and depolymerization of cellulose from raw biomass materials in water. *ChemSusChem* **2015,** *8* (11), 1901-1907.

28. Panthapulakkal, S.; Pakharenko, V.; Sain, M., Microwave Assisted Short-Time Alkaline Extraction of Birch Xylan. *Journal of Polymers and the Environment* **2013,** *21* (4), 917-929.

29. Gulbrandsen, T. A.; Johnsen, I. A.; Opedal, M. T.; Toven, K.; Oyaas, K.; Pranovich, A.; Mikkola, J.-P.; Hoff, B. H., Extracting hemicelluloses from softwood and bagasse as oligosaccharides using pure water and microwave heating. *Cellulose Chemistry and Technology* **2015,** *49* (2), 117-126.

30. Aachary, A. A.; Prapulla, S. G., Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications. *Comprehensive Reviews in Food Science and Food Safety* **2011,** *10* (1), 2-16.

31. Hu, L.; Luo, Y.; Cai, B.; Li, J.; Tong, D.; Hu, C., The degradation of the lignin in Phyllostachys heterocycla cv. pubescens in an ethanol solvothermal system. *Green Chemistry* **2014,** *16* (6), 3107-3116.

32. Beneroso, D.; Monti, T.; Kostas, E. T.; Robinson, J., Microwave pyrolysis of biomass for bio-oil production: Scalable processing concepts. *Chemical Engineering Journal* **2017,** *316*, 481-498.

33. Thiruvenkadam, S.; Izhar, S.; Yoshida, H.; Danquah, M. K.; Harun, R., Process application of Subcritical Water Extraction (SWE) for algal bio-products and biofuels production. *Applied Energy* **2015,** *154*, 815-828.
34. Toor, S. S.; Rosendahl, L.; Rudolf, A., Hydrothermal liquefaction of biomass: A review of subcritical water technologies. *Energy* **2011,** *36* (5), 2328-2342.
35. Dimitriadis, A.; Bezergianni, S., Hydrothermal liquefaction of various biomass and waste feedstocks for biocrude production: A state of the art review. *Renewable and Sustainable Energy Reviews* **2017,** *68*, 113-125.
36. Kumar, M.; Olajire Oyedun, A.; Kumar, A., A review on the current status of various hydrothermal technologies on biomass feedstock. *Renewable and Sustainable Energy Reviews* **2018,** *81*, 1742-1770.
37. Xu, C.; Lancaster, J., Conversion of secondary pulp/paper sludge powder to liquid oil products for energy recovery by direct liquefaction in hot-compressed water. *Water Res* **2008,** *42* (6-7), 1571-1582.
38. Egües, I.; Alriols, M. G.; Herseczki, Z.; Marton, G.; Labidi, J., Hemicelluloses obtaining from rapeseed cake residue generated in the biodiesel production process. *Journal of Industrial and Engineering Chemistry* **2010,** *16* (2), 293-298.
39. Pińkowska, H.; Wolak, P.; Oliveros, E., Hydrothermolysis of rapeseed cake in subcritical water. Effect of reaction temperature and holding time on product composition. *Biomass and Bioenergy* **2014,** *64*, 50-61.
40. Muppaneni, T.; Reddy, H. K.; Selvaratnam, T.; Dandamudi, K. P. R.; Dungan, B.; Nirmalakhandan, N.; Schaub, T.; Omar Holguin, F.; Voorhies, W.; Lammers, P.; Deng, S., Hydrothermal liquefaction of Cyanidioschyzon merolae and the influence of catalysts on products. *Bioresource technology* **2017,** *223*, 91-97.
41. Prado, J. M.; Lachos-Perez, D.; Forster-Carneiro, T.; Rostagno, M. A., Sub- and supercritical water hydrolysis of agricultural and food industry residues for the production of fermentable sugars: A review. *Food and Bioproducts Processing* **2016,** *98*, 95-123.
42. Briens, C.; Piskorz, J.; Berruti, F., Biomass valorization for fuel and chemicals production - A review. *International Journal of Chemical Reactor Engineering* **2008,** *6*, 1-51.
43. Remón, J.; García, L.; Arauzo, J., Cheese whey management by catalytic steam reforming and aqueous phase reforming. *Fuel Processing Technology* **2016,** *154*, 66-81.
44. Remón, J.; Laseca, M.; García, L.; Arauzo, J., Hydrogen production from cheese whey by catalytic steam reforming: Preliminary study using lactose as a model compound. *Energy Conversion and Management* **2016,** *114*, 122-141.
45. Remón, J.; Ruiz, J.; Oliva, M.; García, L.; Arauzo, J., Cheese whey valorisation: Production of valuable gaseous and liquid chemicals from lactose by aqueous phase reforming. *Energy Conversion and Management* **2016,** *124*, 453-469.
46. Chheda, J. N.; Dumesic, J. A., An overview of dehydration, aldol-condensation and hydrogenation processes for production of liquid alkanes from biomass-derived carbohydrates. *Catalysis Today* **2007,** *123* (1-4), 59-70.
47. Huber, G. W.; Dumesic, J. A., An overview of aqueous-phase catalytic processes for production of hydrogen and alkanes in a biorefinery. *Catalysis Today* **2006,** *111* (1-2), 119-132.
48. Kirilin, A. V.; Tokarev, A. V.; Kustov, L. M.; Salmi, T.; Mikkola, J. P.; Murzin, D. Y., Aqueous phase reforming of xylitol and sorbitol: Comparison and influence of substrate structure. *Applied Catalysis A: General* **2012,** *435-436*, 172-180.

49. Rackemann, D. W.; Bartley, J. P.; Doherty, W. O. S., Methanesulfonic acid-catalyzed conversion of glucose and xylose mixtures to levulinic acid and furfural. *Industrial Crops and Products* **2014,** *52*, 46-57.

50. Taylor, M. J.; Durndell, L. J.; Isaacs, M. A.; Parlett, C. M. A.; Wilson, K.; Lee, A. F.; Kyriakou, G., Highly selective hydrogenation of furfural over supported Pt nanoparticles under mild conditions. *Applied Catalysis B: Environmental* **2016,** *180*, 580-585.

51. Tuteja, J.; Nishimura, S.; Ebitani, K., One-Pot Synthesis of Furans from Various Saccharides Using a Combination of Solid Acid and Base Catalysts. *Bulletin of the Chemical Society of Japan* **2012,** *85* (3), 275-281.

52. Yan, K.; Wu, G.; Lafleur, T.; Jarvis, C., Production, properties and catalytic hydrogenation of furfural to fuel additives and value-added chemicals. *Renewable and Sustainable Energy Reviews* **2014,** *38*, 663-676.

53. Möller, M.; Schröder, U., Hydrothermal production of furfural from xylose and xylan as model compounds for hemicelluloses. *RSC Advances* **2013,** *3* (44), 22253-22260.

54. Negahdar, L.; Delidovich, I.; Palkovits, R., Aqueous-phase hydrolysis of cellulose and hemicelluloses over molecular acidic catalysts: Insights into the kinetics and reaction mechanism. *Applied Catalysis B: Environmental* **2016,** *184*, 285-298.

55. Pintiaux, T.; Viet, D.; Vandenbossche, V.; Rigal, L.; Rouilly, A., High Pressure Compression-Molding of alpha-Cellulose and Effects of Operating Conditions. *Materials* **2013,** *6* (6), 2240-2261.

56. Mamman, A. S.; Lee, J.-M.; Kim, Y.-C.; Hwang, I. T.; Park, N.-J.; Hwang, Y. K.; Chang, J.-S.; Hwang, J.-S., Furfural: Hemicellulose/xylosederived biochemical. *Biofuels, Bioproducts and Biorefining* **2008,** *2* (5), 438-454.

57. Sheldon, R. A., Green and sustainable manufacture of chemicals from biomass: state of the art. *Green Chem.* **2014,** *16* (3), 950-963.

58. Zhang, Q.; Xu, Y.; Li, Y.; Wang, T.; Zhang, Q.; Ma, L.; He, M.; Li, K., Investigation on the esterification by using supercritical ethanol for bio-oil upgrading. *Applied Energy* **2015,** *160*, 633-640.

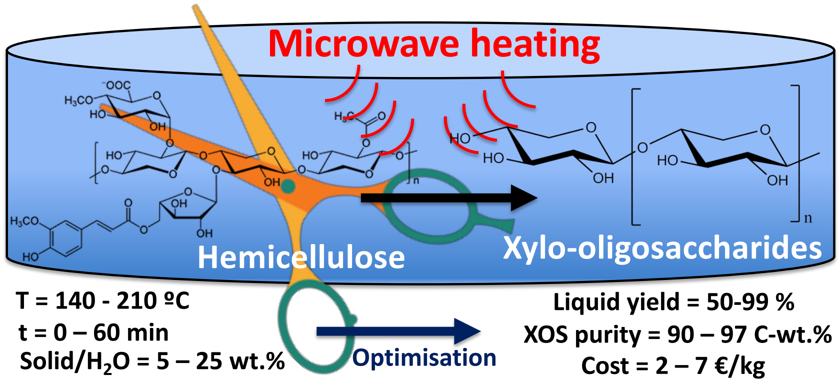
59. Gollakota, A. R. K.; Kishore, N.; Gu, S., A review on hydrothermal liquefaction of biomass. *Renewable and Sustainable Energy Reviews* **2018,** *81*, 1378-1392.

60. Jacobs, A.; Palm, M.; Zacchi, G.; Dahlman, O., Isolation and characterization of water-soluble hemicelluloses from flax shive. *Carbohydrate research* **2003,** *338* (18), 1869-1876.

61. Jacobson, K.; Maheria, K. C.; Kumar Dalai, A., Bio-oil valorization: A review. *Renewable and Sustainable Energy Reviews* **2013,** *23*, 91-106.

62. Kostas, E. T.; Beneroso, D.; Robinson, J. P., The application of microwave heating in bioenergy: A review on the microwave pre-treatment and upgrading technologies for biomass. *Renewable and Sustainable Energy Reviews* **2017,** *77*, 12-27.

For Table of Contents Use Only.



Novel microwave-assisted hydrothermal process assessed and optimised for the production of food-applicable prebiotic nutraceuticals from beech wood hemicellulose.