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Abstract: This work addresses a novel and green process for the co-production of lignin and oligosaccharides from rapeseed meal, examining the effects of the temperature (150-210 °C), reaction time (0-60 min) and catalyst amount (1-4 mol/L, CH<sub>3</sub>COOH) on the process. The yields to gas, liquid and solid varied by 0-18%, 22-64% and 34-74%, respectively. The solid consisted of high purity lignin (26-88 wt.%) together with unreacted cellulose (0-28 wt.%), hemicellulose (0-28 wt.%) and proteins (11-28 wt.%). Increasing the temperature and/or reaction time produced an increase in the liquid yield and a decrease in the solid yield due to the solubilisation of the cellulosic and hemicellulosic contents of the feedstock. Acetic acid exerted a positive catalytic effect, promoting the solubilisation of cellulose and hemicellulose and preventing humins formation. The relative amounts (wt.%) of C, H, O and N in the solid fraction shifted between 46-63, 5.8-6.4, 28-42 and 2-6, respectively. Py-GC/MS analysis revealed that the solid decomposed into phenols (1-19%), sugars (0-15%), N-compounds (0-31%), carboxylic acids (37-75%), hydrocarbons (4-20%) and furans (1-8%). The liquid phase comprised oligo- and mono/di-saccharides (33-51 C-wt.%, 0-3 C-wt.% and 0-6 C-wt.%) and carboxylic acids (40-62 C-wt.%). The progressive solubilisation of cellulose and hemicellulose produced an increase in the proportion of C together with a decrease in the amounts of H and O in the solid product, which also accounted for the increase and decrease observed in the proportions of phenols and sugars, respectively. An optimum was found at 186 °C using an acid concentration of 1 mol/L and a total reaction time of 2 min. These conditions maximise the solubilisation of cellulose and hemicellulose without altering the lignin content of the solid; thus allowing the selective and simultaneous production of high purity (85 wt.%) lignin together with a rich oligosaccharide (51 C-wt.%) solution. The acid can be recovered from the sugar mixture, which not only improves the efficiency of the process but also allows the production of a pure saccharide (92 C-wt.%) product.



Dear Professor Keat Teong Lee,

Please find enclosed the revised manuscript entitled: “**Simultaneous production of lignin and polysaccharide rich aqueous solutions by microwave-assisted hydrothermal treatment of rapeseed meal**” by J. Remón, A.S. Matharu and J.H. Clark.

We have also enclosed a separate letter with the detailed response to the reviewers. We have revised the work taking into consideration the suggestions of the reviewers, which we consider clearly contribute to the improvement of the work.

We believe that the concerns of the reviewers have been adequately answered. Therefore, we hope that the work may now be published in Energy Conversion and Management.

Yours sincerely,

Prof. James H. Clark

Dr. Javier Remón

**Highlights (85 characters including spaces)**

- Novel and environmentally friendly methodology for rapeseed meal valorisation
- Simultaneous production of lignin and saccharides from rapeseed meal
- Selective cellulose and hemicellulose dissolution to produce saccharide-free lignin
- Optimum conditions for rapeseed meal valorisation: 186 °C, 3 min and 1 mol/L CH<sub>3</sub>COOH

1 **Simultaneous production of lignin and polysaccharide rich aqueous solutions by microwave-**  
2 **assisted hydrothermal treatment of rapeseed meal**

3  
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9

10 **Abstract**

11 This work addresses a novel and green process for the co-production of lignin and oligosaccharides  
12 from rapeseed meal, examining the effects of the temperature (150-210 °C), reaction time (0-60 min)  
13 and catalyst amount (1-4 mol/L, CH<sub>3</sub>COOH) on the process. The yields to gas, liquid and solid varied  
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15 together with unreacted cellulose (0-28 wt.%), hemicellulose (0-28 wt.%) and proteins (11-28 wt.%).  
16 Increasing the temperature and/or reaction time produced an increase in the liquid yield and a decrease  
17 in the solid yield due to the solubilisation of the cellulosic and hemicellulosic contents of the  
18 feedstock. Acetic acid exerted a positive catalytic effect, promoting the solubilisation of cellulose and  
19 hemicellulose and preventing humins formation. The relative amounts (wt.%) of C, H, O and N in the  
20 solid fraction shifted between 46-63, 5.8-6.4, 28-42 and 2-6, respectively. Py-GC/MS analysis  
21 revealed that the solid decomposed into phenols (1-19%), sugars (0-15%), N-compounds (0-31%),  
22 carboxylic acids (37-75%), hydrocarbons (4-20%) and furans (1-8%). The liquid phase comprised  
23 oligo- and mono/di-saccharides (33-51 C-wt.%, 0-3 C-wt.% and 0-6 C-wt.%) and carboxylic acids  
24 (40-62 C-wt.%). The progressive solubilisation of cellulose and hemicellulose produced an increase in  
25 the proportion of C together with a decrease in the amounts of H and O in the solid product, which  
26 also accounted for the increase and decrease observed in the proportions of phenols and sugars,  
27 respectively. An optimum was found at 186 °C using an acid concentration of 1 mol/L and a total  
28 reaction time of 2 min. These conditions maximise the solubilisation of cellulose and hemicellulose  
29 without altering the lignin content of the solid; thus allowing the selective and simultaneous  
30 production of high purity (85 wt.%) lignin together with a rich oligosaccharide (51 C-wt.%) solution.  
31 The acid can be recovered from the sugar mixture, which not only improves the efficiency of the  
32 process but also allows the production of a pure saccharide (92 C-wt.%) product.

33 **Keywords:** microwaves, rapeseed meal, biomass valorisation, lignin, oligosaccharides

34

## 35 **1. Introduction**

36 Rapeseed, the third largest source of vegetable oil in the world, is currently used for the production of  
37 both edible oil and biodiesel [1]. During the processing of rapeseed seeds to produce the oil, around 65  
38 wt.% of the feedstock is converted into a lignocellulosic solid residue called rapeseed meal or  
39 rapeseed cake [2, 3]. This solid material is mainly composed of cellulose, hemicellulose, lignin and  
40 proteins; the precise chemical composition of the residue depending on the type of rapeseed plant and  
41 extraction process [2]. Traditionally, rapeseed meal has been used as a livestock feed due to the  
42 presence of proteins in the residue. However, the increase in biodiesel production has oversaturated  
43 the agricultural market and new processes and alternative strategies need to be developed for the  
44 valorisation of this feedstock [4].

45

46 In this context, two alternative options have normally been considered for the valorisation of rapeseed  
47 meal. The first is the application of different extraction systems to recover valuable products. In this  
48 respect, Purkayastha et al. [5] analysed the effectiveness of several solvents for the extraction of  
49 residual oils and polyphenols from a rapeseed cake at 25°C for 2 h. It was found that non-polar  
50 solvents were the most effective in recovering the residual oil. Terpinic et al. [6] investigated the  
51 extraction of polyphenols from camelina linseed, rapeseed and white mustard using methanol and  
52 ethanol at room temperature for 12 h. They found that the plant material and the extraction solvent not  
53 only significantly influenced the amount of phenols extracted, but also the antioxidant properties of  
54 the extracts. Li et al. [7] investigated the use of pressurised solvent systems to recover phenols,  
55 analysing the effects of the solvent type (ethanol, methanol, 2-propanol, acetone and acetonitrile) and  
56 concentration, temperature (80-200 °C) and time (2-30 min). The use of a 60 vol.% methanol/water  
57 solution at 200 °C for 20 min extracted the highest amount of phenols (93 mg/g).

58

59 The second option relies on the use of thermochemical processes, such as pyrolysis, gasification,  
60 combustion and hydrothermal treatments to produce bio-fuels, energy and value-added chemicals.

61 Özcimen et al. [8] examined the production of bio-oil and bio-char from a rapeseed cake produced  
62 during oil extraction from Brassica Napus. The pyrolysis experiments were performed in a fixed bed  
63 reactor at 500 °C, employing different gas space velocities (50-300 cm<sup>3</sup>/min). Regardless of the space  
64 velocity, around 73% of the rapeseed meal was converted into bio-oil (60%), bio-char (27%) and  
65 permanent gases (13%). The valorisation of this type of cake was also investigated by Ucar et al. [9]  
66 who analysed the effect of the reaction temperature (400-900°C) during the pyrolysis of the residue.  
67 The gas consisted of CO<sub>2</sub>, CO, CH<sub>4</sub> and H<sub>2</sub>S, while the bio-oil was made up of carboxylic acids,  
68 amides and phenols. An increase in the temperature increased and decreased the gas (8-14%) and char  
69 (30-38%) yields, respectively; while the bio-oil yield increased between 400 and 500°C (14-19%) and  
70 slyly decreased with further increasing the temperature up to 900°C. Giannakopoulou et al. [1]  
71 conducted catalytic pressurised pyrolysis experiments of a spent rapeseed meal produced during the  
72 production of biodiesel. Two catalysts (H-ZSM-5 and H-Beta zeolites) and two reactor configurations  
73 (a pressurised pyrolysis unit, and a pressurised pyrolysis unit with catalytic upgrading of the pyrolysis  
74 vapours) were tested. In the process, two liquid phases (aqueous and organic), gases and a solid  
75 residue were obtained. The organic phase was made up of aliphatic and aromatic hydrocarbons,  
76 carboxylic acids, esters, nitriles, amides, poly-phenols and N-heterocyclic compounds. The liquid  
77 phase consisted of a mixture of phenols, ketones, alcohols and heterocyclic and N-heterocyclic  
78 compounds.

79

80 Pinkowska et al. [3] studied the hydrothermolysis of rapeseed meal using sub-critical water for the  
81 recovery of fatty acids and amino acids, examining the effects of the reaction time and temperature on  
82 the process. The maximum yield of amino acids (136 g/kg of rapeseed cake) took place when the solid  
83 was treated at 215°C for 26 min. A further increase in the temperature led to the decomposition of the  
84 amino acids. The maximum fatty acid production (0.91 g/kg) occurred at 246 °C using a reaction time  
85 of 65 min. Briones et al. [4] explored the possibility of co-valorising two biodiesel by-products: crude  
86 glycerol and rapeseed meal. The effects of the mass/solvent ratio, temperature and reaction time on  
87 rapeseed meal valorisation were experimentally investigated. In the process, the cellulose,  
88 hemicellulose and lignin contents of the solid were decomposed, leading to the production a liquid

89 mixture consisting of glycols, carboxylic acids, furans esters and ethers. Egües et al. [2] employed a  
90 two-step process for the production of saccharides from rapeseed meal pellets. Firstly, the  
91 hemicellulose content of the feed was extracted and purified; then, this fraction was converted into  
92 saccharides by auto-hydrolysis or acid hydrolysis. Glucose and xylose were the main sugars identified  
93 in the hydrolysates; their specific amounts depending on the hydrolysis process. In the case of auto-  
94 hydrolysis, they accounted for 23% and 40%, respectively, while their relative amounts were 28% and  
95 37%, when acid hydrolysis was used.

96

97 Another interesting option for the valorisation of rapeseed meal that has not been considered before is  
98 the simultaneous production of saccharides and pure lignin from the solid aiming to build a bio-  
99 refinery concept around this residue. However, the extraction of polysaccharide-free lignin from  
100 biomass is very challenging because lignin is strongly covalent bonded to cellulose and hemicellulose,  
101 which hinders the selective extraction of pure lignin. Therefore, the development of a suitable method  
102 for lignin isolation is of paramount importance for the production of pure lignin from biomass. In this  
103 respect, the two-step Klason acidolysis method is one of the most widespread used [10]. However, its  
104 major drawback is the use of concentrated sulphuric acid, which is not environmentally friendly and  
105 also damages the lignin structure. Another method is the combination of biomass milling, to break the  
106 linkages between lignin and saccharides, followed by solvent extraction using a dioxane-water solvent  
107 system [11]. Though, this latter methodology is considered extremely time-consuming as a reaction  
108 time as long as 3 weeks is needed in some cases. This led to the modification of this latter  
109 methodology using enzymes to increase the lignin yield; nevertheless, the lignin yield was still low  
110 and a high enzyme dosage was needed [10].

111

112 Therefore, more research needs to be conducted for the development of novel and energy efficient  
113 methodologies for lignin production from biomass. As part of this, the use of microwave heating has  
114 recently appeared as a new and promising alternative. Microwave heating is based on the high  
115 frequency rotation of polar molecules, which produces a quicker and higher heating of the species  
116 with higher polarity within the biomass structure [12]. As lignin has a higher aromaticity, i.e. lower

117 polarity, than cellulose and hemicellulose, it is less active during microwave heating [13]. This could  
118 allow the separation of cellulose and hemicellulose from the biomass without significantly altering the  
119 lignin structure; thus allowing a high purity lignin to be produced. In addition, as water is highly  
120 effective in microwave energy absorption, the combination of hydrothermal conditions together with  
121 microwave-assisted heating might be an interesting new technology for the valorisation of rapeseed  
122 meal. To the best of the authors' knowledge, the work conducted using microwave assisted  
123 hydrothermal conditions for the extraction of lignin from biomass is very scarce. In particular, Zhou et  
124 al. [14] used formic acid to extract lignin from birch biomass employing conventional and microwave  
125 heating. A higher amount of delignification was reported when microwave heating was used in the  
126 experiments. Li et al. [15] studied the effect of the temperature (90-109 °C) during the isolation of  
127 lignin from bamboo. The temperature was found to significantly influence the process and the use of  
128 higher temperatures resulted in a greater lignin yield. Zoia et al. [16] conducted microwave assisted  
129 lignin isolation using HCl and reported that their methodology was capable of recovering up to 55 wt.%  
130 of the total lignin present in the material. Long et al. [13, 17] addressed the effects of the temperature  
131 (160-210 °C) and reaction time (5-20 min) during the isolation of lignin from softwood employing  
132 H<sub>2</sub>SO<sub>4</sub>. They found that an increase in both the temperature and reaction time increased the lignin  
133 yield and purity. Maxima for the yield (82 wt.%) and purity (93 wt.%) occurred using a 0.2 mol/L  
134 sulphuric acid solution at 190 °C for 10 minutes. The liquid phase consisted of a mixture of  
135 saccharides, carboxylic acids and furans and was found to have potential to be used in fermentation  
136 processes.

137

138 Given this background, this work addresses the valorisation of rapeseed meal by means of a  
139 microwave-assisted hydrothermal process catalysed by acetic acid, a much safer and greener  
140 alternative to mineral acids, for the simultaneous production of pure lignin and polysaccharide rich  
141 aqueous solutions. In particular, the effects of the temperature (150-210 °C), time (0-1 h) and catalyst  
142 (acetic acid) amount (1-4 mol/L) together with all the possible interactions between these variables on  
143 rapeseed meal valorisation have been thoroughly analysed. Given that the microwave-assisted  
144 hydrothermal valorisation of rapeseed meal has never been reported before and the works dealing with

145 the isolation of lignin from biomass using microwave technology are very scarce, this work represents  
146 a novel and challenging investigation not only for the management and valorisation of rapeseed meal,  
147 but also for the development of a novel, quick and environmentally-friendly methodology for the  
148 production of pure lignin and saccharides from other types of biomass. In addition, the fact that acetic  
149 acid can be directly produced from biomass and the use of an energy efficient microwave-assisted  
150 hydrothermal process convert this process into a green, efficient and sustainable route for biomass  
151 valorisation.

152

## 153 **2. Experimental**

### 154 **2.1 Microwave experiments**

155 A CEM Discover II microwave facility was used for the experiments. The experiments were  
156 conducted in a 30 mL batch reactor using a maximum power of 300W. For each experiment, 0.5 g of  
157 biomass was placed in the reactor along with 15 mL of solvent (CH<sub>3</sub>COOH/H<sub>2</sub>O). Before placing the  
158 reactor inside the microwave unit, the reaction mixture was pre-stirred at room temperature for 2 min.  
159 A heating rate of 1°C/s was used for all the experiments; and therefore, the ramping time (time to  
160 reach the temperature of the experiment) varied between 2 and 3 min. The reaction time shifted  
161 between 0 and 60 min according to the experimental design. After reaction, the reactor was cooled  
162 down from the reaction temperature to 60°C at a rate of 0.5 °C/s. Subsequently, the reactor was opened  
163 and its content, consisting of a mixture of liquid and solid, was transferred to a centrifuge tube.  
164 Centrifugation was used to separate the solid from the liquid. Then, the solid residue obtained after  
165 centrifugation was dried overnight at 105°C and the liquid phase obtained was stored for further  
166 characterisation.

167

### 168 **2.2 Response variables and analytical methods**

169 Several response variables were used to analyse the effect of the operating conditions on the process.  
170 These include the gas, liquid and solid yields and some of the most important properties of the liquid  
171 and the solid products. Table 1 summarises the response variables and the analytical methods used for

172 their calculation. The solid fractions (both the original biomass as well as the solids produced) were  
 173 characterised by means of ultimate and fibre (cellulose, hemicellulose, lignin and protein) analyses,  
 174 and Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC/MS). In addition, the original  
 175 feedstock was also characterised by proximate analysis and Inductively Coupled Plasma Mass  
 176 Spectrometry (ICP-MS) to identify and quantify the amounts of metals. Proximate and ultimate  
 177 analyses were performed according to standard methods (ISO-589-1981 for moisture, ISO-1171-1976  
 178 for ash and ISO-5623-1974 for volatiles). Elemental analysis was carried out using an Exeter  
 179 Analytical (Warwick, UK) CE440 Elemental Analyser, calibrated against acetanilide with a S-benzyl-  
 180 thiuronium chloride internal standard. Fibre characterisation was performed by using the chemical  
 181 titration method described by Hu et al. [18] to determine the amount of cellulose and hemicellulose,  
 182 while the lignin content was determined by the standard TAPPI T222 method. Py-GC/MS results were  
 183 obtained using a CDS Analytical 5250-T Trapping Pyrolysis Auto sampler coupled with an Agilent  
 184 7890 B gas chromatograph equipped with a 5977A MSD mass spectrum unit. The sample was loaded  
 185 into the pyrolysis unit and pyrolysed at 600 °C for 10 s. The volatile materials released were carried  
 186 into the GC/MS unit by nitrogen for analysis.

187

188 Table 1. Response variables. Definitions and analytical techniques used in their determination.

Product	Response variable	Analytical method
<b>Liquid</b>	Liquid yield (%) = $\frac{\text{liquid compounds (g)}}{\text{mass of biomass (g)}} 100 = 100 - (\text{Gas yield} + \text{Solid yield})$	Balance
	Composition (C – wt. %) = $\frac{\sum \text{mass of C of each compound (g)}}{\text{total mass of C in solution (g)}} 100$	GC/MS-FID and HPLC
	C, H, O (wt. %) = $\frac{\text{mass of C, H, O (g)}}{\text{mass of organics (g)}} 100$	Elemental Analysis
	HHV (MJ/kg) = 0.3491 C (wt. %) + 1.1783 H (wt. %) – 0.1034 O (wt. %) – 0.015 N (wt. %) + 0.1005 S (wt. %)	Estimated
<b>Solid</b>	Solid yield (%) = $\frac{\text{mass of solid (g)}}{\text{mass of biomass (g)}} 100$	Gravimetric
	Fibre Composition (wt. %) = $\frac{\text{mass of structural component (g)}}{\text{mass of solid residue (g)}} 100$	Chemical titration, Tappi T222 Method
	HHV (MJ/kg) = 0.3491 C (wt. %) + 1.1783 H (wt. %) – 0.1034 O (wt. %) – 0.015 N (wt. %) + 0.1005 S (wt. %)	Estimated
	C, H, O (wt. %) = $\frac{\text{mass of C, H, O (g)}}{\text{mass of solid (g)}} 100$	Elemental Analysis
	Py GC/MS Composition (area %) = $\frac{\text{area of each compound}}{\text{total area}} 100$	Py-GC/MS
<b>Gas</b>	Gas yield (%) = $\frac{\text{mass of gas (g)}}{\text{mass of biomass (g)}} 100$	Gravimetric

189 wt.% = weight percentage

190 C-wt.% = percentage in carbon basis

191 Protein content (wt. %) = 4.62 · N (wt. %)

192

193

194 High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC/MS-FID) and  
195 elemental analysis (described above) were used for the characterisation of the liquid phase. An Agilent  
196 1260 Infinity HPLC equipped with Agilent Hi- Plex H (300 x 7.7mm, 8µm particle size) and ACE  
197 C18 (250 x 4.6mm, 5µm particle size) columns and 1660 DAD WR UV/UV-VIS and 1660 Infinity  
198 Refractive Index (RI) detectors was used for the HPLC analyses. In addition, an Agilent 7890 GC-  
199 system (model G3440A) equipped with Flame Ionization (FID) and Mass Spectrometry (MS)  
200 detectors was used for the GC analysis of the liquid. In this case, the MS detector was used for  
201 identification while the FID detector was used for the quantification of the reaction products.

202

### 203 **2.3 Experimental design and data analysis**

204 The influence of reaction temperature (150-210°C), acetic concentration in water (1-4 mol/L) and  
205 reaction time (0-60 min) on the process was experimentally investigated. The experiments were  
206 planned according to a 2 level 3-factor Box-Wilson Central Composite Face Centred (CCF,  $\alpha: \pm 1$ )  
207 design. This corresponds to a  $2^k$  factorial design, where k indicates the number of factors studied (in  
208 this case 3 operating variables) and  $2^k$  represents the number of runs (in this case 8) for the simple  
209 factorial design. 8 axial experiments were performed to study non-linear effects and interactions. In  
210 addition, 4 replicates at the centre point (centre of the variation interval of each factor) were carried  
211 out in order to evaluate the experimental error. This experimental design is suitable not only for  
212 studying the influence of each variable (linear and quadratic effects) but also for understanding  
213 possible interactions between variables. The results were analysed with an analysis of variance  
214 (ANOVA) with 95% confidence. In addition, the cause-effect Pareto principle was used to calculate  
215 the relative importance of the operating variables in the response variables. In these analyses, the  
216 lower and upper limits of all the operating variables were normalised from -1 to 1 (coded variables) to  
217 investigate their influence in comparable terms. In the interaction Figures, the evolution of these  
218 variables obtained from the ANOVA analysis of all the experiments performed was represented. In  
219 addition, when possible, some experimental points were added. In the interaction plots developed from  
220 the ANOVA analyses only the upper and lower levels for one of the variables have been represented;

221 however, the whole interval of variation was considered for all the variables, carefully analysed and  
 222 thoroughly discussed.

223

#### 224 **2.4 Rapeseed meal characterisation**

225 The rapeseed meal used in this work was provided by Croda International (Widnes, UK). The most  
 226 important physiochemical properties of the material such as proximate, ultimate, fibre, calorific and  
 227 Py-GC/MS analyses are listed in Table 2. The proximate, fibre and elemental analyses as well as the  
 228 higher heating value (HHV) of the residue are fairly similar to those previously reported in the  
 229 literature [1, 2, 4, 9]. In addition, the lignin content of this particular solid is quite high, which makes it  
 230 suitable for the production of lignin. The pyrolysis GC-MS characterisation results reveal that the solid  
 231 decomposes into hydrocarbons, ketones, aldehydes, carboxylic acids, phenols and sugars. The  
 232 proportion of hydrocarbons in the residue is very high due to the presence of residual oil, which was  
 233 not effectively recovered in the extraction process.

234

235 Table 2. Feedstock characterisation.

<b>Proximate analysis (wt.%)</b>		<b>HHV (MJ/kg)</b>	17.07±0.29
Moisture	7.26	<b>Ash composition (wt.%)</b>	
Ash	1.31	Ca	15.94
Volatiles	45.09	Mg	7.98
Fixed carbon	32.04	K	19.75
<b>Fibre analysis (wt.%)</b>		Na	1.19
Cellulose	12.41±0.33	P	24.47
Hemicellulose	7.16±0.26	S	30.67
Lignin	32.39±2.47	<b>Py-GC/MS characterisation (% area)</b>	
Protein	39.47±1.17	Hydrocarbons	43.59±1.22
<b>Elemental analysis (wt.%)</b>		Ketones	2.30±3.25
C	41.54±0.19	Aldehydes	1.46±2.26
H	6.29±0.17	Carboxylic acids	20.88±2.14
N	6.32±0.19	Phenolic compounds	10.19±0.40
O*	45.86±0.17	Sugars	1.75±2.47

236 \*Oxygen was calculated by difference

237

### 238 **3. Results and discussion**

239 Table 3 lists the operating conditions used in the experiments and the experimental results. These  
 240 include the yields to products (gas, liquid and solid) and the most important properties of the solid and  
 241 liquid fractions; i.e. the fibre and elemental analyses and the Py-GC/MS characterisation for the solid  
 242 fraction and the chemical composition and elemental analysis for the liquid fraction.

Table 3. Operating conditions and experimental results produced during the microwave-assisted hydrothermal treatment of rapeseed meal

Run	1	2	3	4	5	6	7	8	9-12	13	14	15	16	17	18
T (°C)	150	210	150	210	150	210	150	210	180	210	180	180	150	180	180
t (min)	0	0	60	60	0	0	60	60	30	30	0	60	30	30	30
AcH (mol/L)	1	1	1	1	4	4	4	4	2.5	2.5	2.5	2.5	2.5	1	4
<b>GLOBAL YIELDS</b>															
Solid yield (%)	63.99	32.25	33.63	27.50	51.44	23.47	23.66	22.20	26.41±1.58	23.79	26.90	35.97	26.97	29.58	24.33
Gas yield (%)	1.93	2.56	0.00	18.43	1.69	2.92	2.91	12.20	4.04±0.76	12.43	0.00	5.24	2.42	4.43	5.55
Liquid yield (%)	34.08	63.19	66.37	54.07	46.87	73.61	73.43	65.60	69.55±2.06	63.78	64.03	67.87	70.61	65.98	70.12
<b>SOLID PROPERTIES</b>															
<b>Fibre analysis (wt.%)</b>															
Cellulose	18.40	25.55	27.72	25.52	26.49	0.00	8.89	0.00	0.90±0.47	0.00	22.13	0.00	21.87	0.00	0.00
Hemicellulose	28.06	0.00	0.82	0.00	0.00	0.00	0.00	0.00	0.22±0.44	0.00	0.61	0.00	0.90	0.00	0.00
Lignin	25.84	58.54	57.16	63.71	50.18	85.36	77.12	87.58	86.37±0.87	88.05	55.79	86.55	63.02	83.59	83.83
Proteins	27.70	15.91	14.30	10.77	23.32	14.64	14.00	12.42	13.41±0.91	11.95	21.47	13.45	14.21	16.41	16.17
<b>Elemental analysis</b>															
C (wt.%)	46.50	52.21	51.32	59.80	46.81	53.45	54.54	63.25	56.12±0.98	60.92	56.29	57.63	53.03	57.22	57.15
H (wt.%)	6.16	6.16	6.03	6.16	6.14	5.90	6.30	5.97	6.11±0.12	5.81	6.42	5.81	6.21	6.18	6.03
O (wt.%)	37.98	37.98	38.92	41.95	37.70	37.70	36.11	27.87	34.26±0.95	30.55	34.60	30.55	37.45	32.38	33.92
N (wt.%)	3.66	3.66	3.74	5.75	5.11	2.97	3.06	2.93	3.51±0.37	2.73	3.18	2.73	3.33	4.22	2.91
HHV (MJ/kg)	19.10	21.50	20.93	24.87	19.16	21.66	22.68	26.18	23.20±0.46	24.91	21.57	23.68	21.90	23.85	23.50
<b>Py-GC/MS (Area %)</b>															
Hydrocarbons	6.92	8.99	5.89	19.10	10.77	11.69	4.59	12.81	11.69±3.17	20.27	8.99	12.64	10.25	20.12	15.91
Carboxylic acids	72.85	55.97	73.36	36.93	48.30	71.53	74.35	57.32	55.57±4.67	39.60	59.92	42.26	57.91	45.26	55.64
Sugars	10.64	9.46	10.92	5.36	0.01	3.01	11.51	0.45	9.48±0.38	10.89	13.74	10.69	14.48	0.44	6.30
Phenolic compounds	1.81	6.55	1.86	17.70	4.74	1.42	0.67	13.08	7.69±1.29	14.19	2.49	8.04	8.36	18.69	1.89
Furanic compounds	3.02	2.76	0.68	6.56	5.21	5.39	3.11	6.98	3.70±0.44	7.46	6.25	5.61	5.83	4.80	7.79
Nitrogen compounds	2.73	9.56	5.21	4.51	30.98	3.06	0.37	0.82	6.14±2.19	3.07	6.70	14.69	3.17	5.15	5.39
<b>LIQUID PROPERTIES</b>															
<b>Chemical composition (C-wt.%)</b>															
Oligosaccharides DP>6	32.8	47.93	45.79	51.12	42.88	44.08	42.76	45.29	45.25±1.35	44.59	44.92	46.38	44.92	48.60	43.99
Oligosaccharides DP2-DP6	1.20	1.78	2.83	0.00	0.35	0.84	0.54	0.00	0.15±0.01	0.00	1.40	0.06	0.75	0.42	0.00
Saccharides	2.94	6.42	6.08	0.38	0.98	0.80	1.94	0.44	1.89±0.51	0.56	3.05	0.71	2.91	1.90	0.38
Carboxylic acids	62.36	42.05	43.59	45.04	55.74	53.22	53.84	53.56	51.57±1.41	50.36	51.25	50.76	44.57	54.24	44.00
Ketones	0.04	0.57	0.44	0.12	0.00	0.19	0.15	0.04	0.25±0.08	0.01	0.17	0.01	0.22	0.29	0.16
Furans	0.51	0.64	0.89	0.42	0.04	0.73	0.67	0.07	1.98±0.20	0.19	0.11	1.18	0.30	2.69	1.03
Phenols	0.02	0.00	0.00	0.14	0.00	0.00	0.00	0.04	0.01±0.02	0.04	0.00	0.01	0.00	0.02	0.00
Nitrogen Compounds	0.08	0.62	0.39	2.78	0.00	0.13	0.10	0.56	0.60±0.10	0.74	0.00	0.40	0.15	1.51	0.20
<b>Elemental analysis (dry basis)</b>															
C (wt.%)	17.60	21.07	20.30	19.87	61.53	63.60	65.00	62.93	42.83±0.61	41.27	42.20	42.70	43.17	20.87	63.93
H (wt.%)	79.97	76.14	76.98	77.46	34.52	32.55	31.21	33.18	52.60±0.48	54.68	53.72	53.21	52.73	76.36	32.23
O (wt.%)	2.43	2.79	2.72	2.67	3.95	3.85	3.79	3.88	4.07±0.05	4.05	4.08	4.09	4.10	2.77	3.84
HHV (MJ/kg)	0.74	2.77	2.33	2.07	22.56	23.37	23.92	23.11	14.31±0.24	13.53	13.98	14.22	14.45	2.65	23.50

244 **3.1 Effect of the operating conditions on the yields to gas, liquid and solid**

245 The yields of gas, solid and liquid vary by 0-18%, 22-64% and 34-74%, respectively. The relative  
 246 influence of the operating variables on the global yields according to the ANOVA analysis and the  
 247 cause-effect Pareto principle is shown in Table 4. This analysis shows that the reaction time and the  
 248 concentration of acetic acid are the operating variables exerting the highest influence on the solid  
 249 yield. In addition, this response variable is also influenced by the interaction between the time and the  
 250 temperature. The liquid and solid yields are strongly influenced by both the temperature and its  
 251 interaction with the reaction time. This interaction was also found by Long et al. [13], who reported  
 252 that at a certain temperature the effect of the reaction time on the solid yield was negligible. The  
 253 effects of the operating variables and the most important interactions detected with the ANOVA  
 254 analysis are plotted in Figure 1. Specifically, Figure 1 a and b illustrates the effects of the temperature  
 255 for 0 and 60 min reaction time for the lowest (1 mol/L) and the highest (4 mol/L) acetic acid  
 256 concentration, respectively. These effects are also shown for the liquid and solid yields in Figure 1 c-d  
 257 and e-f, respectively.

258

259 *Table 4. Relative influence of the operating conditions on the global yields*

Variable	R <sup>2</sup>	I. Term	T	t	C	Tt	TC	tC	TtC	T <sup>2</sup>	t <sup>2</sup>	C <sup>2</sup>	T <sup>2</sup> t	T <sup>2</sup> C	Tt <sup>2</sup>	TC <sup>2</sup>	T <sup>2</sup> t <sup>2</sup>
Solid yield (%)	0.98	26.29	n.s.	-4.54 (16)	-4.19 (12)	6.51 (17)	n.s.	n.s.	n.s.	n.s.	5.14 (15)	n.s.	-3.48 (15)	n.s.	-8.41 (22)	n.s.	3.33 (4)
Liquid yield (%)	0.98	69.10	n.s.	n.s.	n.s.	-9.54 (29)	n.s.	n.s.	n.s.	-5.19 (16)	n.s.	n.s.	5.22 (16)	5.23 (16)	4.46 (13)	5.23	-4.25 (10)
Gas yield (%)	0.97	4.23	-6.21 (30)	n.s.	n.s.	3.24 (22)	-1.06 (7)	n.s.	-1.22 (8)	1.28 (9)	n.s.	n.s.	3.06 (20)	n.s.	9.91 (4)	n.s.	n.s.

260 n.s: Non significant with 95% confidence  
 261 Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C  
 262 Coefficient TtC·T·t·C + Coefficient T<sup>2</sup>·T<sup>2</sup> + Coefficient t<sup>2</sup>·t<sup>2</sup> + Coefficient C<sup>2</sup>·C<sup>2</sup> + Coefficient T<sup>2</sup>t·T·t + Coefficient T<sup>2</sup>C·T<sup>2</sup>·C + Coefficient  
 263 Tt<sup>2</sup>·T·t<sup>2</sup> + Coefficient TC<sup>2</sup>·T·C<sup>2</sup> + Coefficient T<sup>2</sup>t<sup>2</sup>·T<sup>2</sup>·t<sup>2</sup>  
 264 Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Pareto values represent the percentage  
 265 of the orthogonal estimated total value.  
 266

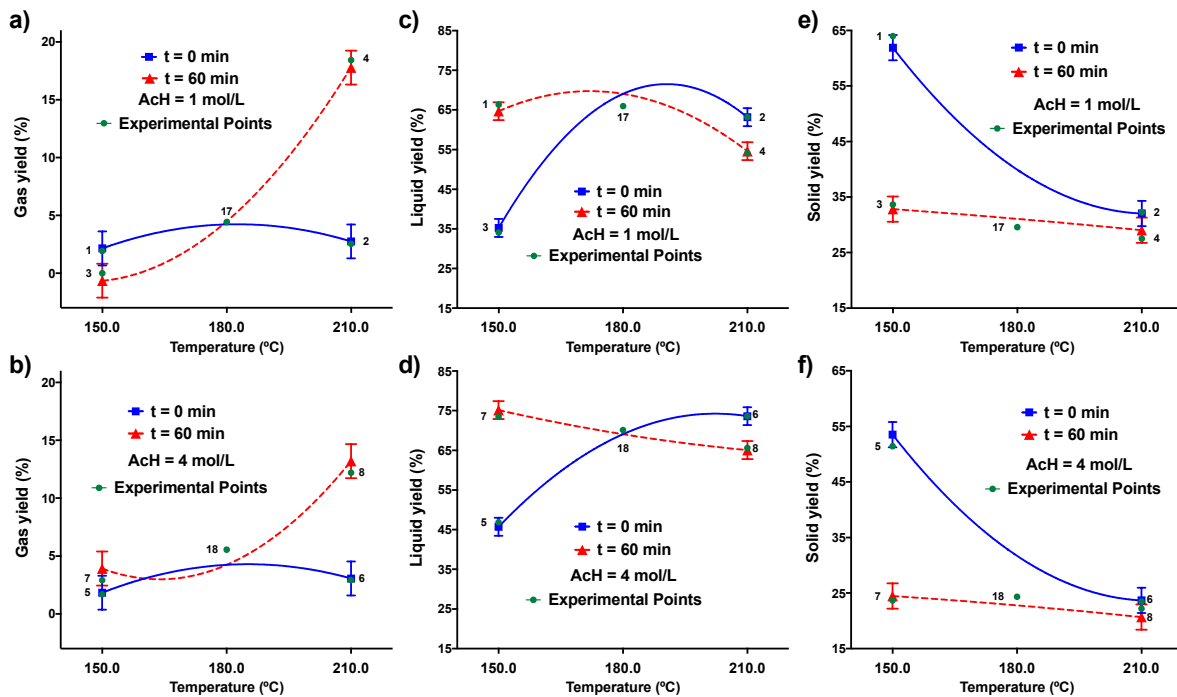
267 The effect of the temperature on the product distribution (yields to gas liquid and solid) depends on the  
 268 reaction time. For a short reaction time (0 min), the temperature does not significantly influence the  
 269 gas yield and a negligible gas formation takes place regardless of the concentration of acetic acid.  
 270 Conversely, the reaction temperature exerts a significant affect on the liquid and solid yields. In  
 271 particular, an initial increase in the temperature from 150 to 190 °C increases the liquid yield and

272 decreases the solid yield, while a further increase in the temperature up to 210 °C does not greatly  
273 modify liquid or solid production. These developments in the liquid and solid yields are accounted for  
274 by the positive kinetic effect of the reaction temperature on biomass solubilisation; thus increasing and  
275 decreasing the liquid yield and solid yield, respectively. In addition, a greater microwave power is also  
276 needed to achieve higher temperatures, thus promoting the breaking of the intramolecular bonds  
277 between cellulose, hemicellulose and lignin [4, 13, 19]. This leads to the solubilisation of the  
278 cellulosic and hemicellulosic matter in the liquid without significantly solubilising the lignin content  
279 of the solid; thus allowing a high purity lignin solid fraction to be produced.

280

281 An increase in the reaction time modifies the effect of the temperature on the product distribution.  
282 Regardless of the concentration of acetic acid, the gas yield is negligible and unaffected by the  
283 reaction time (0-60 min) between 150 and 180 °C. However, an increase in the gas yield occurs  
284 between 190 and 210 °C and when the reaction time increases from 0 to 60 min. The combination of  
285 both high temperatures and long reaction times favours the formation of gases from some of the  
286 species produced during biomass hydrolysis secondary reactions such as carboxylic acids, ketones and  
287 furans through decarboxylation reactions [2, 3]. In addition, gas formation could also be produced  
288 from the thermal decomposition of the proteins present in the solid via deamination [3]. As a result,  
289 for a long reaction time (60 min), an increase in the temperature between 180 to 210 °C produces a  
290 sharp increase in the gas yield. Conversely, the effect of the reaction time on the yields to liquid and  
291 solid is more marked at low temperature (150-190 °C) than at high temperature (190-210 °C). At low  
292 temperature, an increase in the reaction time from 0 to 60 min leads to a sharp increase in the liquid  
293 yield along with a pronounced decrease in the solid yield. This same increment in the reaction time  
294 between 190 and 210 °C slightly decreases the liquid yield; the solid yield remaining unaffected.  
295 These variations make the effect of the temperature on the liquid and solid yields less important. This  
296 development might be accounted for by the long reaction time employed in the experiment, which is  
297 high enough to kinetically control the process. In addition, this also shows the high efficiency of  
298 microwave heating [13, 19-21]. As a result, when a long reaction time (60 min) is used, the effect of  
299 the temperature on the solid yield is negligible. The liquid yield slightly decreases with increasing the

300 temperature due to the sharp increase occurring for the gas yield. This might indicate that part of the  
 301 liquid products is converted into gases if long reaction times and high temperatures are used [3].



302  
 303 Figure 1. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 304 and the highest (4 mol/L) acetic acid concentration for the gas (a and b), liquid (c and d) and solid (e  
 305 and f) yields. Bars are LSD intervals with 95% confidence.  
 306

307 The effect of the concentration of acetic acid on the yields to gas, liquid and solid can be studied by  
 308 comparing Figures 1 a, c and e with b, d and f, respectively. For the gas yield this effect depends on  
 309 the reaction time. For a short reaction time, an increase in the concentration of acetic acid between 1  
 310 and 4 mol/L does not significantly modify the gas yield and a negligible gas formation takes place  
 311 regardless of the concentration of acetic acid used in the experiments. On the contrary, the  
 312 concentration of acetic acid has a significant influence on the gas yield when the reaction time  
 313 increases and different trends for this variable are observed depending on the temperature. When a 60  
 314 min reaction time is used, an increase in the acid concentration from 1 to 4 mol/L leads to an increase  
 315 in the gas yield between 150 and 180 °C, while, this same increase reduces gas formation between 180  
 316 and 210 °C. Acetic acid exerts a significant catalytic effect on the process by producing a greater spread  
 317 of decarboxylation reactions, which leads to an increase in gas formation from biomass secondary  
 318 decomposition products and proteins [2, 3]. At high temperature gas formation decreases probably

319 because the formation of humins and char from the furfural and HMF obtained from sugars at high  
320 temperature [22-24]. The influence of the concentration of acetic acid on the liquid and solid yields  
321 does not depend on the temperature or the reaction time and similar trends are observed regardless the  
322 temperature and time used in the experiments. In general, an increase in the concentration of acetic  
323 acid from 1 to 4 mol/L leads to an increase in the liquid yield and a decrease in the solid yield. The  
324 positive catalytic effect of the acid enhances the dissolution of the cellulose and hemicellulose  
325 fractions [4, 13, 19], which increases the liquid yield and decreases the solid yield. An exception to  
326 this trend occurs at around 190 °C, when long reaction times (60 °C) are used. Under such conditions,  
327 the effect of the concentration of acetic acid on the liquid yield is very weak.

328

### 329 **3.2 Effect of the operating conditions on the solid properties**

330 The solid fraction produced after the microwave-assisted treatment has been characterised by fibre and  
331 elemental analyses and py-GC-MS (Table 1). This fraction consists of the lignin isolated during the  
332 process and contains different amounts of cellulose, hemicellulose and proteins depending on the  
333 operating conditions used in the experiments. The effects of the operating conditions on the properties  
334 of the solid fraction according to the ANOVA and cause-effect Pareto analyses are listed in Table 5.

335

#### 336 *3.2.1 Fibre analysis*

337 The amounts of cellulose, hemicellulose, lignin and proteins in the solid fractions vary as follows: 0-  
338 28%, 0-28%, 26-88% and 11-28%, respectively. The cause effect Pareto analysis (Table 5) reveals  
339 that the temperature (both linear and quadratic factors) and its interaction with the reaction time are  
340 the operating variables exerting the greatest influence on the proportions of cellulose, hemicellulose  
341 and proteins. The relative amount of lignin is strongly influenced by the temperature and the reaction  
342 time. Figure 2 shows the effect of the operating variables and the most important interactions detected  
343 with the ANOVA analysis on the fibre analysis of the solid. Figure 2 a and b illustrates the effects of  
344 the temperature on the proportion of cellulose for 0 and 60 min reaction time for the lowest (1 mol/L)  
345 and the highest (4 mol/L) acetic acid concentration, respectively. These effects are also shown for the  
346 relative amounts of hemicellulose, lignin and proteins in Figure 2 c-d and e-f and g-h, respectively.

347 *Table 5. Relative influence of the operating conditions on the properties of the solid fraction*

Variable	R <sup>2</sup>	I.Term	T	t	C	Tt	TC	tC	TtC	T <sup>2</sup>	t <sup>2</sup>	C <sup>2</sup>	T <sup>2</sup> t	T <sup>2</sup> C	Tt <sup>2</sup>	TC <sup>2</sup>	T <sup>2</sup> t <sup>2</sup>
<b>Fibre analysis (wt.%)</b>																	
Cellulose	1	0.60	11.07 (11)	10.93 (6)	n.s.	1.03 (2)	-5.04 (10)	-3.36 (6)	3.37 (6)	10.46 (17)	10.34 (9)	n.s.	-11.97 (8)	-7.73 (6)	-14.87 (15)	-7.73 (15)	-4.83 (3)
Hemicellulose	1	0.24	-2.92 (11)	n.s.	n.s.	3.40 (12)	3.61 (12)	3.40 (12)	-3.41 (12)	2.84 (7)	n.s.	n.s.	-3.40 (12)	-3.61 (12)	-3.61 (5)	n.s.	3.37 (5)
Lignin	1	86.37	-16.13 (18)	-11.76 (16)	n.s.	-6.35 (9)	0.81 (1)	-0.91 (1)	n.s.	-14.45 (18)	-11.58 (12)	-2.66 (1)	19.96 (5)	11.87 (16)	26.74 (6)	n.s.	5.51 (2)
Proteins	0.99	13.55	4.76 (21)	n.s.	n.s.	1.92 (13)	0.63 (4)	0.88 (6)	n.s.	3.16 (4)	n.s.	2.74 (11)	-3.76 (26)	-3.76 (4)	-7.96 (6)	n.s.	-2.81 (4)
<b>Elemental analysis</b>																	
C (wt.%)	0.99	56.60	-2.32 (28)	-3.94 (26)	n.s.	0.61 (4)	n.s.	0.64 (4)	n.s.	2.00 (14)	n.s.	n.s.	7.69 (7)	1.03 (1)	6.01 (1)	n.s.	-5.12 (11)
H (wt.%)	0.9	6.14	n.s.	0.2 (1)	n.s.	n.s.	-0.09 (25)	n.s.	n.s.	-0.27 (2)	-0.13 (14)	n.s.	-0.19 (10)	n.s.	-0.06 (16)	n.s.	0.36 (32)
O (wt.%)	0.94	33.65	2.03 (9)	3.45 (9)	n.s.	n.s.	-1.90 (11)	-2.53 (16)	n.s.	n.s.	n.s.	n.s.	-4.84 (4)	-1.61 (17)	-3.25 (10)	n.s.	3.86 (20)
N (wt.%)	0.94	3.53	n.s.	n.s.	-0.41 (16)	0.50 (18)	-0.54 (19)	-0.53 (19)	n.s.	-0.57 (8)	-0.50 (3)	n.s.	n.s.	n.s.	n.s.	n.s.	1.40 (16)
HHV (MJ/kg)	0.98	23.11	-1.67 (28)	-0.89 (28)	n.s.	0.32 (5)	0.36 (5)	n.s.	n.s.	n.s.	n.s.	0.57 (9)	2.54 (4)	0.41 (7)	3.21 (1)	n.s.	-1.66 (11)
<b>Py-GC/MS (Area %)</b>																	
Hydrocarbons	0.94	11.78	-5.64 (27)	n.s.	n.s.	2.31 (16)	n.s.	-1.77 (12)	n.s.	2.85 (11)	n.s.	6.24 (2)	n.s.	n.s.	8.69 (6)	n.s.	-10.77 (25)
Carboxylic acids	0.92	51.79	10.16 (18)	7.82 (7)	n.s.	-7.48 (18)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-8.66 (9)	n.s.	-16.05 (4)	n.s.	9.54 (17)
Sugars	1	9.48	1.45 (11)	1.90 (1)	2.93 (9)	-2.31 (12)	n.s.	1.60 (9)	-1.21 (6)	2.81 (4)	3.11 (3)	-6.10 (22)	-1.25 (5)	-5.61 (13)	-3.30 (1)	n.s.	-2.88 (2)
Phenols	0.98	7.98	-5.85 (20)	n.s.	-8.40 (14)	3.32 (16)	-1.40 (7)	n.s.	n.s.	n.s.	n.s.	2.31 (4)	2.38 (12)	7.36 (16)	9.59 (2)	n.s.	-4.35 (10)
Nitrogen compounds	0.96	5.61	n.s.	-4.69 (13)	n.s.	2.60 (11)	-4.20 (17)	-3.78 (15)	4.49 (18)	n.s.	1.90 (8)	n.s.	n.s.	1.65 (7)	-2.67 (11)	n.s.	n.s.

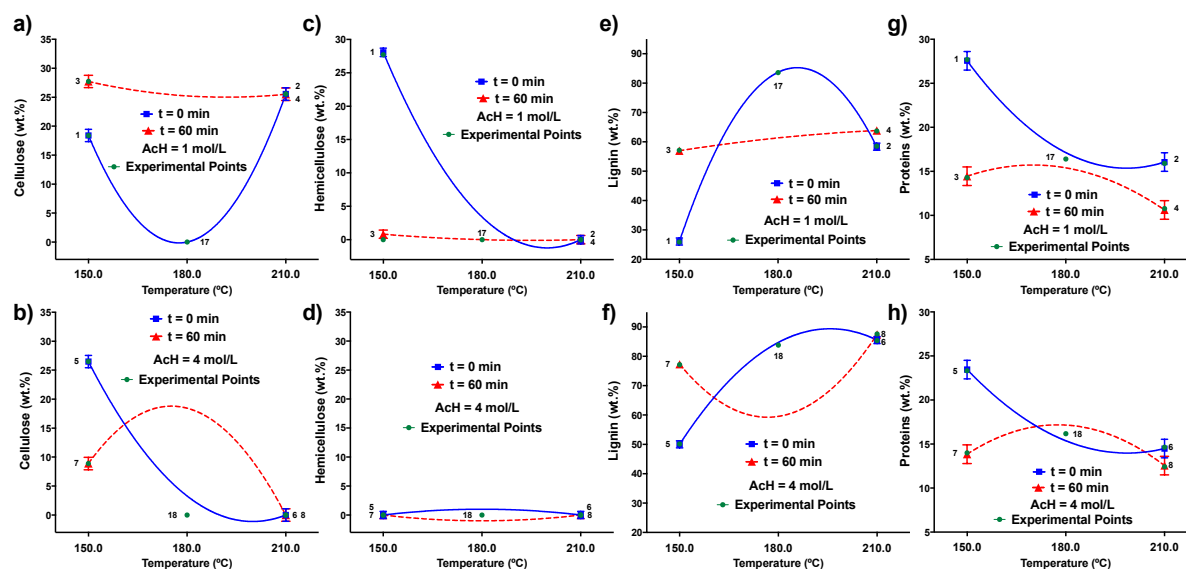
348 n.s.: Non significant with 95% confidence

349 Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C  
 350 Coefficient TtC·T·t·C + Coefficient T<sup>2</sup>·T<sup>2</sup> + Coefficient t<sup>2</sup>·t<sup>2</sup> + Coefficient C<sup>2</sup>·C<sup>2</sup> + Coefficient T<sup>2</sup>t·T·t + Coefficient T<sup>2</sup>C·T<sup>2</sup>·C + Coefficient  
 351 Tt<sup>2</sup>·T·t<sup>2</sup> + Coefficient TC<sup>2</sup>·T·C<sup>2</sup> + Coefficient T<sup>2</sup>t<sup>2</sup>·T<sup>2</sup>·t<sup>2</sup>

352 Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Pareto values represent the percentage  
 353 of the orthogonal estimated total value.

354

355 The effect of the temperature on the fibre analysis of the solid product depends on the reaction time  
 356 and the concentration of acetic acid. For a short reaction time (0 min) when a diluted (1 mol/L) acid  
 357 solution is used (Figure 2 a, c, e and g), an increase in the temperature from 150 to 180 °C leads to a  
 358 sharp decrease in the proportions of cellulose, hemicellulose and proteins together with a pronounced  
 359 increase in the proportion of lignin of the solid, where a maximum is reached. This allows the  
 360 production of relatively high purity lignin (85 wt.%) from rapeseed meal; proteins being the only  
 361 impurity presents in the solid. This development accounts for the solubilisation of the cellulosic and  
 362 hemicellulosic matter without significant lignin solubilisation during microwave hydrothermal  
 363 treatment [13]. In addition, the protein content of the solid decreases due to the decomposition of the  
 364 proteins into liquid and gaseous products via decarboxylation and deamination reactions [2, 3].



365

366 Figure 2. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 367 and the highest (4 mol/L) acetic acid concentration for the proportions of cellulose (a and b),  
 368 hemicellulose (c and d), lignin (e and f) and proteins (g and h). Bars are LSD intervals with 95%  
 369 confidence.

370

371 A further increase in the temperature up to 210 °C increases and decreases the proportion of cellulose  
 372 and lignin, respectively, without altering the relative amounts of hemicellulose and proteins. An  
 373 increase in the temperature might promote the formation of humins and char from the sugars produced  
 374 during the dissolution of cellulose and hemicellulose [22-24]. The formation of these macromolecules  
 375 can occur from the furfural obtained from sugars dehydration, via aldol addition followed by  
 376 condensation or polymerisation [25-31]. Furthermore, sugars monomers can also react with other  
 377 liquid intermediates such as 5-hydroxymethyl-2-furancarboxaldehyde (HMF) by cross-polymerisation  
 378 [25, 26, 28, 30]. The presence of humins and char can interfere with the chemical titration method. In  
 379 particular, humins might have been identified as cellulose in the analysis, thus producing an artificial  
 380 increase in the cellulose content of the solid [18].

381

382 The comparison between Figure 2 a, c, e and g with b, d, f and h shows that an increase in the  
 383 concentration of acetic acid from 1 to 4 mol/L (for 0 min reaction time) decreases the proportion of  
 384 hemicellulose and proteins and increases the relative amount of lignin regardless of the temperature  
 385 (150-210 °C) due to the positive catalytic effect of acetic acid in the process. A similar trend was also  
 386 observed by Long et al. [17] and Zoia et al. [16], who reported the positive catalytic effect of H<sub>2</sub>SO<sub>4</sub>

387 and HCl, respectively, during lignin isolation from biomass. In addition, and very interestingly, in this  
388 work acetic acid also exerts an inhibitory effect on humins and char formation, which allows a  
389 polysaccharide-free lignin, with relatively high purity (88 wt.%) to be produced between 190 and 210  
390 °C.

391

392 The reaction time modifies the effects of the temperature and concentration of acetic acid on the fibre  
393 composition of the solid product. When a diluted acid solution is used, an increase in the reaction time  
394 from 0 to 60 min leads to a decrease in the proportions of hemicellulose and proteins together with a  
395 decrease in the relative amount of cellulose of the solid (Figure 2 a, c, e and g). Long reaction times  
396 favours the solubilisation of cellulose and hemicellulose even at the lowest temperature used in this  
397 work (150 °C) due to the efficiency of microwave heating [13]. However, this also produces the  
398 formation of humins and char from some of the species solubilised in the liquid product, which leads  
399 to an artificial increase in the relative amount of the cellulose content of the solid. The effect of the  
400 reaction time on the proportion of lignin depends on the temperature. While an increase in the reaction  
401 time increases the relative amount of lignin between 150 and 165 °C, this same increment decreases  
402 the proportion of lignin between 165 and 200 °C. At low temperature the formation of humins and  
403 char takes place to a lesser extent, which result in a higher proportion of lignin in the solid.  
404 Conversely, elevated temperatures together with long reaction times increase the production of humins  
405 and char [22-24]. As a result, when a long reaction time is used, the effect of the temperature on the  
406 fibre analysis of the solid is very weak, as the positive kinetic effect of the reaction time can mask the  
407 effect of the temperature.

408

409 An increase in the concentration of acetic acid from 1 to 4 mol/L when a long reaction time is used  
410 exerts a significant effect on the proportion of cellulose and lignin, without modifying the proportions  
411 of hemicellulose and proteins (Figure 2 a, c e and g vs. b, d f and h, respectively). As described earlier,  
412 the formation of humins and char is inhibited when a concentrated (4 mol/L) solution of acetic acid is  
413 used, and therefore, an increase in the reaction time from 0 to 60 min leads to an increase in the  
414 relative amount of lignin together with a decrease in the proportion of cellulose in the solid product.

415 This is in agreement with the work conducted by van Zandvoort et al. [32], who reported a decrease in  
416 humins formation when increasing the concentration of sulphuric acid during the valorisation of  
417 lignocellulosic biomass. In addition, the effect of the temperature on the proportions of hemicellulose  
418 and proteins is negligible because the effect of the temperature is masked by the positive kinetic effect  
419 of the reaction time, as described earlier. Conversely, the temperature exerts a significant influence on  
420 the proportion of cellulose and lignin when a long reaction time and a concentrated (4 mol/L) solution  
421 of acetic acid are used. Between 150 and 180 °C, the proportion of cellulose and lignin increases and  
422 decreases respectively, while the opposite trend takes place between 180 and 210 °C; i.e. an increase  
423 in the relative amount of lignin together with a decrease in the proportion of cellulose due to the lesser  
424 humins formation occurring when a concentrated acid solution is used.

425

### 426 *3.2.2 Elemental analysis*

427 The relative amounts (wt.%) of C, H, O and N in the solid fraction shifted between 46-63, 5.8-6.4, 28-  
428 42 and 2-6, varying the higher heating value (HHV) of the solid between 19 and 26 MJ/kg. According  
429 to the cause-effect Pareto Analysis, the proportion of C and the HHV of the solid are strongly affected  
430 by the temperature (both linear and quadratic effects) and the reaction time. The interactions of the  
431 temperature with the reaction time and concentration largely influences the relative amounts of H and  
432 O, while the concentration of acetic acid greatly influences the proportion of N in the solid. Figure 3  
433 shows the effect of the operating variables and the most important interactions detected with the  
434 ANOVA analysis on the elemental analysis and HHV of the solid. Figure 3 a and b plots the effects of  
435 the temperature on the relative amount of C for 0 and 60 min reaction time for the lowest (1 mol/L)  
436 and the highest (4 mol/L) acetic acid concentration, respectively. These effects are also shown for the  
437 relative amounts of H, O and N and the HHV in Figure 3 c-d and e-f, g-h and i-j, respectively.

438

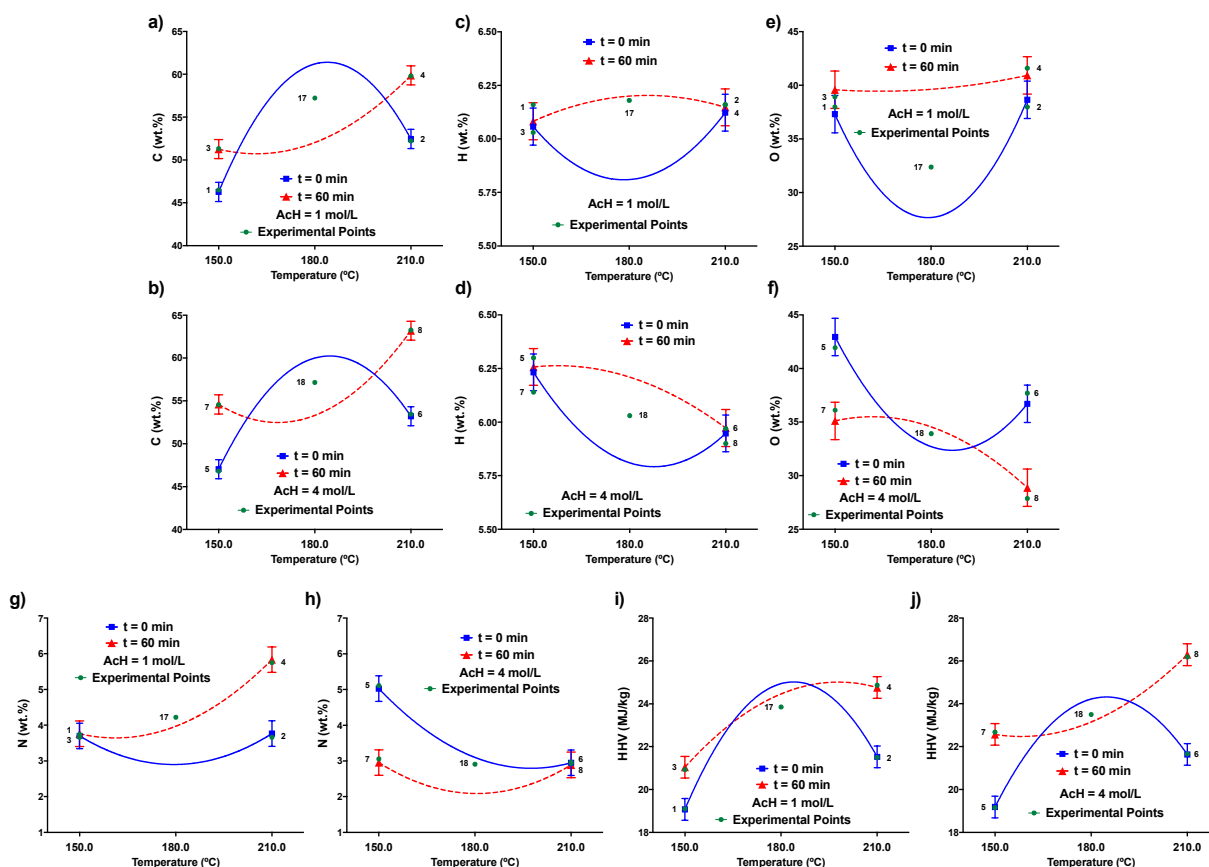
439 The effect of the temperature on the elemental analysis of the solid fraction depends on the reaction  
440 time and the concentration of acetic acid. When a short reaction time is used, the concentration of  
441 acetic acid does not greatly influence the elemental analysis or the HHV of the solid and similar  
442 results are obtained regardless of the acid concentration. In particular, for a short reaction time (0 min),

443 an initial increase in the temperature between 150 and 180 °C increases the proportion of C and the  
444 HHV and decreases the relative amounts of H and O. These variations are accounted for by the  
445 solubilisation of cellulose and hemicellulose, which logically results in a solid product with lower and  
446 higher O and C contents, respectively [33]. In addition, this trend is in good agreement with the results  
447 reported by Long et al. [13]. Conversely, further increasing the temperature up to 210 °C has the  
448 opposite effect; i.e. the amount of C and the HHV decrease and the proportions of H and O in the solid  
449 increase. This development is believed to be the consequence of the formation of humins under these  
450 operating conditions as described earlier. In addition the variations observed in the elemental analysis  
451 of the spent solid are in good agreement with those reported by van Zandvoort et al. [32]. The  
452 temperature does not influence the N content of the solid when short reaction times are used, while a  
453 small increase takes place when the temperature increases from 150 to 210 °C and a long reaction time  
454 is used.

455  
456 The effect of the reaction time depends on the concentration of acetic acid. When a diluted acid  
457 solution (1 mol/L) is used, an increase from 0 to 60 min increases the relative amounts of H, O and N  
458 and decreases the proportion of C in the solid when a temperature ranging from 165 to 195 °C is used.  
459 These developments for the elemental analysis are the consequence of the solubilisation of cellulose  
460 and hemicellulose, which result into a solid fraction with a higher lignin proportion [17, 32]. In  
461 addition, an increase in the reaction time progressively decreases the effect of the temperature on the  
462 elemental analysis and the HHV of the solid product due to the positive kinetic effect of the reaction  
463 time [13]. As a result, when a reaction time of 60 min is used, the reaction temperature does not affect  
464 the proportions of H and O, while a small increase occurs for the relative amounts of C and N when  
465 increasing the temperature from 150 to 210 °C. The HHV slightly increases between 150 and 180 °C  
466 and remains steady with a further increase up to 210 °C.

467  
468 An increase in the concentration of acetic acid when a long reaction time is used modifies the effect of  
469 the temperature on the proportions of H, O and N and the HHV of the solid. In this case, an increase in  
470 the temperature from 150 to 210 °C decreases the proportions of H and O. This leads to a decrease in

471 the HHV of the solid. The positive inhibitory effect of acetic acid on humins and char formation when  
 472 high acid concentrations are used accounts for this circumstance; thus allowing the production of a  
 473 solid with higher lignin purity. As a result, when a concentrated acid solution is used, increasing the  
 474 reaction time from 0 to 60 min when temperatures higher than 180 °C are used produces a decrease in  
 475 the proportion O and increases the HHV of the solid.



476  
 477 Figure 3. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 478 and the highest (4 mol/L) acetic acid concentration for the concentrations of C (a and b), H (c and d),  
 479 O (e and f), N (g and h) and HHV (i and j). Bars are LSD intervals with 95% confidence.  
 480

481  
 482 *3.2.3 Pyrolysis GC-MS characterisation*

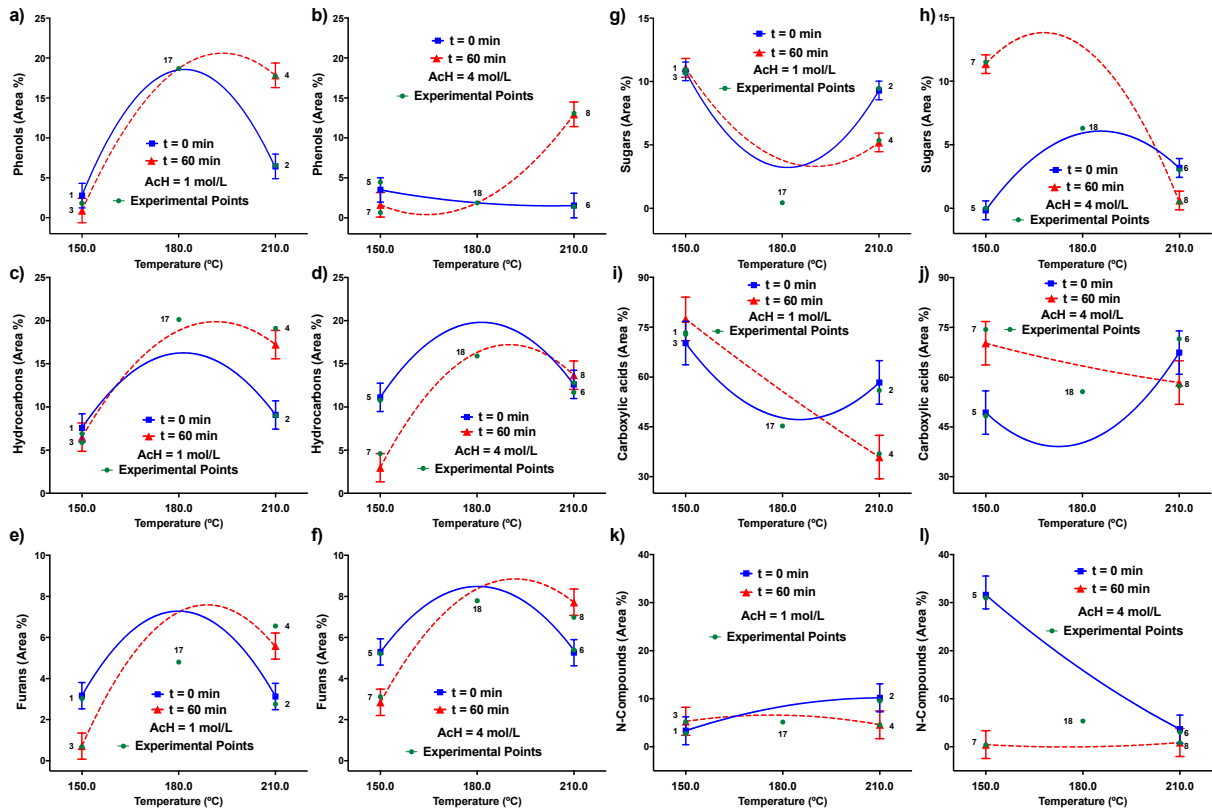
483 It is known that the total amount of compounds produced during the pyrolysis of biomass that can be  
 484 identified by GC-MS usually represents about 20 to 22 wt.% of the total [34], as many lignin-derived  
 485 compounds and proteins cannot be analysed due to their high molecular masses. However, useful  
 486 trends can be retrieved from this analysis, and a comparison can be established. The pyrolysis GC-MS  
 487 characterisation of the solid reveals that the solid product decomposes in a mixture of phenols (1-

488 19%), sugars (0-15%), nitrogen compounds (0-31%), carboxylic acids (37-75%), hydrocarbons (4-  
489 20%) and furans (1-8%). Phenols include phenol, 3-methyl phenol, phenol 2,6-dimethoxy, 2,4-  
490 dimethoxyphenol, catechol and 3-ter-butyl-4-hydroxyanisole. Sugars comprise 1,6 anhydro beta D-  
491 glucopyranose, melezitose and D-mannose. Nitrogen compounds largely include pyridine, pyrrole, 1-  
492 5-dimethyl-1H-Pyrazole and acetamide-1-methyl-1H-pyrrole. Carboxylic acids are made up of n-  
493 hexadecanoic acid and oleic acid while hydrocarbons include linear aliphatic hydrocarbons such as  
494 butane and cyclic hydrocarbons such as R-limonene and 1,3,5-cycloheptatriene. Furans are made up of  
495 furfural, 2-furanmethanol, furan 2- and 3-methyl and furan 2,5-dimethyl.

496  
497 According to the cause-effect Pareto analysis (Table 5), the proportions of hydrocarbons and  
498 carboxylic acids in the liquid are strongly influenced by the temperature and its interaction with the  
499 reaction time. Sugars and phenols are strongly influenced by the temperature, acid concentration and  
500 the interaction between the temperature and the reaction time, while the reaction time and the  
501 interactions of the temperature with both the concentration and reaction time strongly influence the  
502 proportion of nitrogen compounds. Figure 4 a and b plots the effects of the temperature on the relative  
503 amount of phenols for 0 and 60 min reaction time for the lowest (1 mol/L) and the highest (4 mol/L)  
504 acetic acid concentration, respectively. These effects are also shown for the relative amounts of  
505 hydrocarbons, furans, sugars, carboxylic acids and nitrogen compounds in Figure 4 c-d and e-f, g-h, i-j  
506 and k-l, respectively.

507  
508 The effect of the temperature on the Py-GC/MS analysis of the solid fraction depends on the reaction  
509 time and the concentration of acetic acid used in the experiments. For a short reaction time and a low  
510 concentration of acid (0 min and 1 mol/L), an initial increase in the temperature from 150 to 180 °C  
511 increases the proportions of phenols, hydrocarbons and furans and decreases the relative amounts of  
512 sugars and carboxylic acids. Under such conditions, the proportion of N-compounds in the solid is  
513 very low and unaffected by the temperature. The solubilisation of the cellulosic and hemicellulosic  
514 matter of rapeseed meal during the microwave treatment accounts for the decrease in sugars and

515 carboxylic acids in the solid fraction [13]; thus increasing the proportions of phenols and  
 516 hydrocarbons in the solid.  
 517



518  
 519 Figure 4. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 520 and the highest (4 mol/L) acetic acid concentration for the concentrations of phenols (a and b),  
 521 hydrocarbons (c and d), furans (e and f), sugars (g and h), carboxylic acids (i and j) and N-compounds  
 522 (k and l). Bars are LSD intervals with 95% confidence.  
 523

524 In addition, when a diluted acid solution (1 mol/L) is used, the effect of the reaction time on the  
 525 thermal decomposition of the solid fraction depends on the temperature. Between 150 and 190 °C, the  
 526 effect of the reaction time is negligible; however, an increase in the reaction time (from 0 to 60 min)  
 527 between 190 and 210 °C leads to an increase in the relative amount of phenols, hydrocarbons and  
 528 furans together with a substantial decrease in the proportion of sugars and carboxylic acids. At low  
 529 temperatures (150-190°C), the microwave power achieved during the experiments is not sufficient to  
 530 remove the residual cellulose and hemicellulose matter strongly connected with the lignin content in  
 531 rapeseed meal. However, this increase in time at high temperatures (190-210 °C) increases cellulose  
 532 and hemicellulose solubilisation, which leads to the formation of a solid product with a higher

533 proportion of lignin. This increases the proportion of phenols and decreases the relative amount of  
534 sugars in the solid [13, 17]. As a result, when a long reaction time (60 min) is used, the proportions of  
535 phenols, sugars and hydrocarbons remain relatively steady between 190 and 210 °C, while a the  
536 proportions of furans and carboxylic acids decrease slightly and the relative amount of sugars  
537 increases.

538

539 An increase in the concentration of acetic acid from 1 to 4 mol/L (Figures 4 a, c, e, g, i and k v.s. b, d,  
540 f, h, j and l) modifies the effect of the temperature and reaction time on the relative amount of some of  
541 the decomposition products produced during the Py-GC/MS analysis of the solid product. On the one  
542 hand, increasing the concentration of acetic acid in the experiments does not greatly modify the effects  
543 of the temperature or reaction time on proportions of hydrocarbons, furans and carboxylic acids. In all  
544 cases, increasing the concentration of acetic acid from 1 to 4 mol/L increases the proportion of furans  
545 regardless of the reaction time. This increase in the proportion of furans might account for the lesser  
546 production of humins, as these latter compounds can be produced from the auto-condensation of the  
547 former [32]. On the contrary, two different developments occur for the relative amounts of  
548 hydrocarbons and carboxylic acids. While, an increase in the concentration of acetic acid slightly  
549 increases the proportion of hydrocarbons and decreases the relative amount of carboxylic acids when  
550 short reaction times are used; the use of long reaction times produces the opposite effect, i.e. the  
551 relative amount of hydrocarbons and carboxylic acid decreases and decreases, respectively. When  
552 using short reaction times, an increase in the acetic acid concentration might favour the solubilisation  
553 of the fatty acid content of the solid. Conversely, the combination of long reaction times together with  
554 high acid concentrations can increase the solubilisation of hydrocarbons [3, 35, 36].

555

556 On the other hand, the relative amounts of phenols, sugars and N-compounds are strongly influenced  
557 by the concentration of acetic acid used in the experiments and different developments occur  
558 depending on the temperature and the reaction time. For a short reaction time (0 min), an increase in  
559 the concentration of acetic acid reduces the proportion of phenols and sugars and sharply increases the  
560 relative amount of N-compounds, especially between 150 and 180 °C. These variations confirm the

561 positive catalytic effect of acetic acid on the solubilisation of the cellulosic and hemicellulosic  
562 contains of rapeseed meal as described earlier. Conversely, the acid has a lower effect on the  
563 solubilisation and/or removal of the protein contain of the solid, and therefore, a solid product with a  
564 higher proportion of proteins is produced. Under these conditions (0 min and 4 mol/L acetic acid) the  
565 temperature does not significantly influences the concentration of phenols. The proportion of N-  
566 compounds is very high at low temperature and progressively decreases when the temperature  
567 increases up to 210 °C.

568

569 An increase in the reaction time significantly increases the concentration of sugars and decreases the  
570 proportion of N-compounds in the solid. Increasing the temperature and/or the reaction time of the  
571 experiments might produce the degradation of the proteins present in the residue by deamination  
572 (resulting in the formation of ammonia) and decarboxylation (which produces carboxylic acids and  
573 amines) [3, 37-39]. The proportion of phenols is negligible between 150 and 190 °C, and increases  
574 sharply when the temperature increases from 190 to 210 °C due to the lesser humins formation  
575 occurring when a concentrated acid solution is used. As a result, for a long reaction time (60 min), the  
576 temperature does not greatly affect the proportions of phenols and sugars between 150 and 190 °C,  
577 while a sharp increase together a pronounced decrease takes place for the proportion of phenols and  
578 sugars, respectively, between 190 and 210 °C.

579

### 580 **3.3 Effect of the operating conditions on the liquid properties**

#### 581 *3.3.1 Chemical composition*

582 The liquid product consists of a mixture of oligo- (DP2-6 and DP>6) and mono/di- saccharides,  
583 carboxylic acids, ketones, furans and nitrogen compounds; their relative amount (in carbon basis, C-  
584 wt.%) in the liquid product varying as follows: 33-51%, 0-3%, 0-6%, 40-62%, 0-1%, 0-3%, 0-3%.  
585 Saccharides include cellobiose, xylose, glucose, fructose, mannose, arabinose, rhamnose and  
586 levoglucosan. Carboxylic acids comprise lactic, formic, levulinic, glucuronic, galacuronic and acetic  
587 acids. Acetic acid is the major compound for this family as it was initially loaded and used as a  
588 catalyst. In all the cases the amount of this acid was fairly similar to the initial amount initially loaded

589 in the experiments. This indicates that acetic acid decomposition (removal) and secondary reactions  
 590 (production) did not take place to a great extent and/or they compensated for each other. Ketones and  
 591 furans are made of levoglucosenone and 5-hydroxymethyl-2-furancarboxaldehyde (HMF) and furfural,  
 592 respectively. Nitrogen compounds include 3-pyridinol and 6 methyl-3-pyridinol.

593

594 *Table 6. Relative influence of the operating conditions on the properties of the liquid fraction*

Variable	R <sup>2</sup>	I.Term	T	t	C	Tt	TC	tC	TtC	T <sup>2</sup>	t <sup>2</sup>	C <sup>2</sup>	T <sup>2</sup> t	T <sup>2</sup> C	Tt <sup>2</sup>	TC <sup>2</sup>	T <sup>2</sup> t <sup>2</sup>
<b>Chemical composition (C-wt.%)</b>																	
Oligosaccharides DP>6	0.95	45.44	n.s.	n.s.	-2.36 (6)	-1.05 (13)	-2.08 (15)	-1.88 (13)	1.38 (10)	n.s.	n.s.	n.s.	2.15 (15)	1.97 (6)	3.02 (21)	n.s.	-1.35 (7)
Oligosaccharides DP2-6	0.99	0.16	0.70 (10)	0.34 (7)	-0.20 (15)	-0.55 (16)	0.28 (8)	n.s.	0.30 (9)	0.54 (12)	0.24 (10)	n.s.	-0.44 (8)	-0.31 (4)	-0.99 (1)	n.s.	n.s.
Saccharides	0.99	1.01	1.25 (9)	1.10 (7)	-0.76 (19)	-1.31 (17)	n.s.	0.44 (6)	0.98 (13)	0.75 (11)	0.75 (7)	n.s.	-1.39 (5)	-0.70 (4)	-1.73 (4)	n.s.	n.s.
Carboxylic acids	0.94	51.57	-2.52 (11)	n.s.	4.84 (19)	3.00 (15)	2.01 (10)	1.78 (9)	-2.44 (4)	n.s.	n.s.	-2.16 (3)	-2.17 (11)	-1.92 (4)	n.s.	n.s.	1.77 (4)
Ketones	0.94	0.27	0.05 (2)	0.10 (4)	-0.09 (20)	-0.14 (27)	n.s.	n.s.	0.07 (13)	-0.18 (7)	-0.16 (6)	n.s.	-0.11 (6)	n.s.	n.s.	n.s.	0.26 (15)
Furans	0.96	1.76	n.s.	-0.44 (5)	-0.83 (11)	-0.24 (9)	n.s.	n.s.	n.s.	-1.61 (29)	-1.02 (10)	n.s.	0.46 (9)	0.71 (12)	n.s.	n.s.	1.37 (16)
Nitrogen compounds	1	0.35	-0.37 (16)	-0.13 (13)	-0.66 (17)	0.27 (9)	-0.29 (10)	-0.24 (8)	-0.19 (7)	n.s.	-0.08 (1)	0.50 (9)	0.50 (3)	0.27	0.81 (1)	n.s.	-0.19 (1)
<b>Elemental analysis</b>																	
C (wt.%)	1	42.76	0.40 (1)	n.s.	21.71 (90)	-1.01 (4)	-0.39 (1)	n.s.	n.s.	-1.21 (1)	n.s.	n.s.	0.54 (2)	n.s.	n.s.	n.s.	n.s.
H (wt.%)	1	4.08	n.s.	n.s.	0.54 (87)	-0.05 (2)	n.s.	0.09 (1)	0.10 (1)	n.s.	n.s.	-0.77 (4)	-0.12 (2)	0.20 (1)	n.s.	n.s.	-0.17 (1)
O (wt.%)	1	52.89	n.s.	n.s.	-22.07 (90)	1.36 (4)	n.s.	-0.46 (1)	-0.38	1.30 (3)	n.s.	1.39	n.s.	-0.65 (2)	n.s.	-0.65	n.s.
HHV (MJ/kg)	1	14.30	0.28 (1)	n.s.	10.53 (82)	-0.41 (3)	-0.30 (2)	n.s.	n.s.	-0.48 (5)	n.s.	-1.16 (5)	0.32 (2)	n.s.	n.s.	n.s.	n.s.

595 n.s: Non significant with 95% confidence

596 Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C  
 597 Coefficient TtC·T·t·C + Coefficient T<sup>2</sup>·T<sup>2</sup> + Coefficient t<sup>2</sup>·t<sup>2</sup> + Coefficient C<sup>2</sup>·C<sup>2</sup> + Coefficient T<sup>2</sup>t·T<sup>2</sup>t + Coefficient T<sup>2</sup>C·T<sup>2</sup>C + Coefficient  
 598 T<sup>2</sup>·T·t<sup>2</sup> + Coefficient TC<sup>2</sup>·T·C<sup>2</sup> + Coefficient T<sup>2</sup>t<sup>2</sup>·T<sup>2</sup>t<sup>2</sup>

599 Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Pareto values represent the percentage  
 600 of the orthogonal estimated total value.

601

602 The cause-effect Pareto analysis (Table 6) shows that the proportion of DP>6 oligosaccharides is  
 603 strongly influenced by the interaction between the temperature and reaction time. The temperature  
 604 (linear and quadratic) and concentration followed by the reaction time significantly influence the  
 605 proportions of DP 2-6 oligosaccharides and (mono/di) saccharides. The proportion of carboxylic acids  
 606 is strongly affected by the concentration, while the concentration of furans depends on the temperature,  
 607 acid concentration and the interaction between these two latter variables. The temperature, time and  
 608 concentration have a similar influence on the proportion of nitrogen compounds. Figure 5 shows the  
 609 effect of the operating variables and the most important interactions detected with the ANOVA

610 analysis on the most abundant compounds present in the liquid phase. In particular, Figure 5 a and b  
611 illustrates the effects of the temperature on the proportion of oligosaccharides (DP>6) for 0 and 60  
612 min reaction time for the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentrations,  
613 respectively. These effects are also shown for the relative amounts of oligosaccharides (DP2-6),  
614 saccharides and carboxylic acids in Figure 5 c-d and e-f and g-h, respectively.

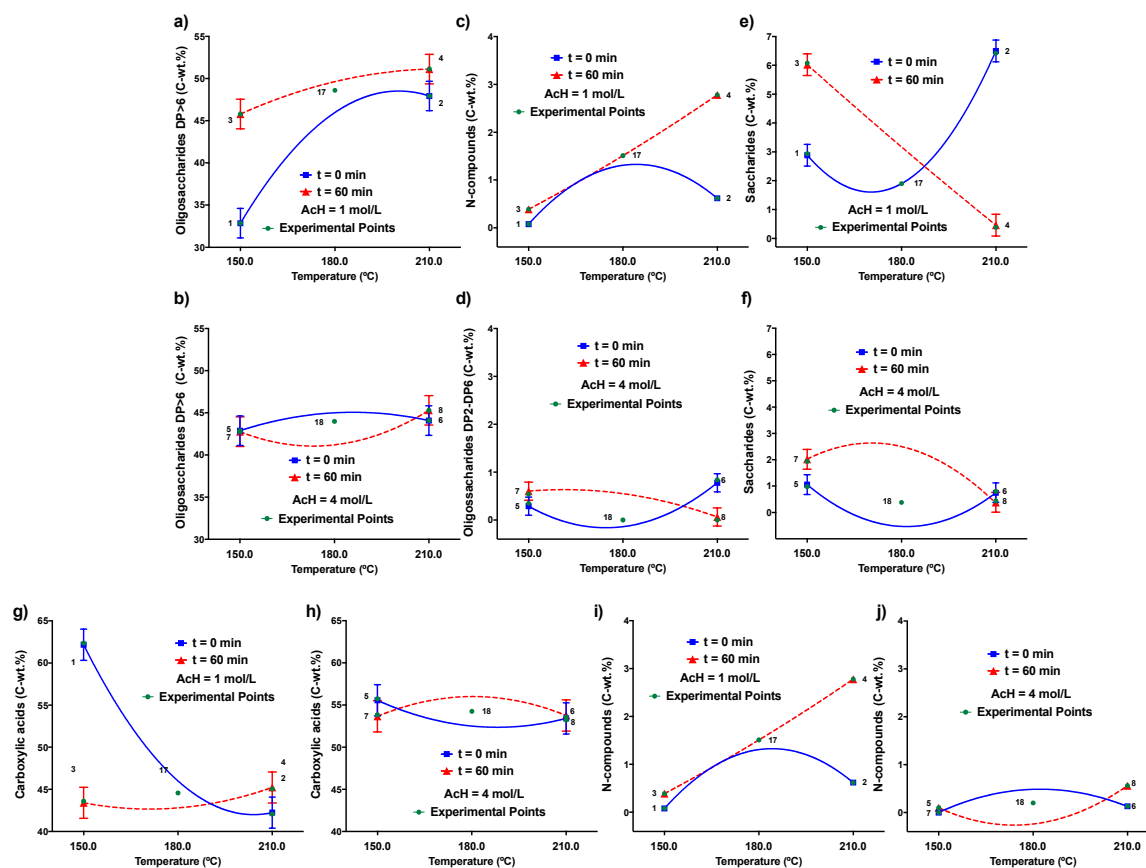
615

616 The effect of the temperature on the chemical composition of the liquid phase depends on the reaction  
617 time and the concentration of acetic acid. In this respect, while the reaction time has a very important  
618 influence for diluted acid solution, it has a negligible influence when the concentration of acetic acid  
619 increases up to 4 mol/L. When a diluted acetic acid concentration (1 mol/L) and short reaction time  
620 are used, an increase in the reaction time from 150 to 190 °C sharply increases and decreases the  
621 relative amounts of oligosaccharides and carboxylic acids, respectively. These developments account  
622 for the progressive dissolution of cellulose and hemicellulose in the liquid, which produces an increase  
623 in the proportion of oligosaccharides and therefore decreases the relative amount of carboxylic acids  
624 (largely the acetic acid used in the experiment). In addition, the proportions of oligosaccharides DP2-6  
625 and saccharides slightly decrease, while the relative amount of N-compounds increases. A further  
626 increase in the temperature up to 210 °C exerts a negligible effect on the proportions of  
627 oligosaccharides and carboxylic acids, while the relative amounts of DP2-6 oligosaccharides and  
628 saccharides increase slightly and the N-compounds decreases. The decomposition of a small amount  
629 of oligosaccharides into saccharides when the temperature increases and the progressive  
630 transformation of N-compounds into gases at high temperature [3, 37-39] can explain these trends.

631

632 An increase in the reaction time when a diluted acid solution (1 mol/L) is used has two different  
633 consequences for the composition of the liquid phase depending on the temperature. On the one hand,  
634 at temperatures lower than 190 °C, an increase in the reaction time from 0 to 60 min significantly  
635 increases the proportions of oligo (DP>6 and DP 2-6) saccharides and decreases the relative amount of  
636 carboxylic acids, without modifying the relative amount of N-compounds. An increase in the reaction

637 time produces a greater spread of the hydrolysis reactions, which experimentally increases the  
 638 proportion of cellulose and hemicellulose derived species in the liquid; thus decreasing the relative  
 639 amount of carboxylic acid (mostly acetic acid). On the other hand, when a temperature between 190  
 640 and 210 °C is used, this same increase in time does not modify the proportions of DP>6  
 641 oligosaccharides or carboxylic acids. These variations are the consequence of the weaker influence of  
 642 the temperature in the hydrolysis of saccharides when long reaction times are used [13]. In addition,  
 643 the proportions of DP2-6 oligosaccharides and saccharides decrease and the relative amount of N-  
 644 compounds increases due to the solubilisation of proteins in the liquid when long reaction times are  
 645 used [3, 37-39]. This lower influence of the temperature on the composition of the liquid phase  
 646 produces that an increase in the temperature from 150 to 210 °C does not greatly modify the  
 647 proportions of oligosaccharides (DP>6) or carboxylic acids when a 60 min reaction time is used.



648  
 649 Figure 5. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 650 and the highest (4 mol/L) acetic acid concentration for the concentrations of oligosaccharides DP>6 (a  
 651 and b), oligosaccharides DP 2-6 (c and d), saccharides (e and f), carboxylic acids (g and h) and N-  
 652 compounds (i and j). Bars are LSD intervals with 95% confidence.

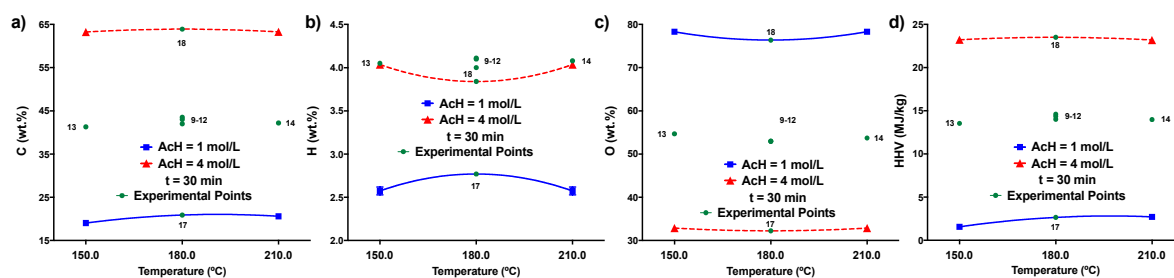
653 An increase in the concentration of acid (Figures 5 a, c, e and g vs. b, d, f and h) diminishes the  
 654 influence of the temperature and reaction time on the composition of the liquid phase as stated earlier.  
 655 For a 4 mol/L acetic acid concentration, oligosaccharides and carboxylic acids are the most abundant  
 656 compounds in the liquid phase, their composition being around 43 C-wt.% and 55 C-wt.%,  
 657 respectively, regardless of the temperature and reaction time. Furthermore, the relative amounts of  
 658 oligosaccharides (DP2-6) and saccharides are very low (< 2%) and the effects of the temperature and  
 659 reaction time, although being statistically significant are not important from a practical point of view.

660

### 661 3.3.2 Elemental analysis and HHV

662 The concentrations of C, H and O (in dry basis) in the liquid fraction vary by 18-65%, 31-80% and 2-  
 663 4%, respectively. This varies the HHV of the liquid between 1 and 24 MJ/kg of dried suspension. The  
 664 elemental analysis and HHV of the liquid is mostly influenced by the concentration of acetic acid  
 665 (with an influence higher than 82% for all these variables); the effects of the temperature and reaction  
 666 time being either statistically insignificant or negligible from a practical point of view. Figure 6 shows  
 667 the effect of the concentration on the elemental analysis and HHV of the liquid for a 30 min reaction  
 668 time as a function of the temperature.

669



670

671 Figure 6. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 672 and the highest (4 mol/L) acetic acid concentration for the concentrations of C (a), H (b), O (c) and  
 673 HHV (d) at 30 min reaction time. Bars are LSD intervals with 95% confidence.

674

675 Regardless of the temperature or reaction time, the concentration of acetic acid exerts the same  
 676 influence on the elemental analysis and HHV of the liquid phase. Increasing the concentration of  
 677 acetic acid from 1 to 4 mol/L leads to an increase in the proportions of C and H together with a  
 678 decrease in the O content of the liquid. This produces a substantial increase in the HHV of the liquid.

679 An increase in the concentration of acetic acid increases the solubilisation of rapeseed meal due to the  
 680 positive catalytic effect of the acid in the process. The original biomass has similar C and H contents  
 681 than acetic acid but a lower proportion of O. Therefore, the progressive addition of acetic acid in the  
 682 liquid phase prior to the experiment produces an increase in the proportions of C and H together with a  
 683 decrease in the relative amount of O of the hydrolysates.

684

### 685 3.4 Theoretical optimisation

686 Optimum conditions were sought for the selective co-production of high purity lignin and soluble  
 687 oligosaccharides from rapeseed meal making use of the experimental models developed. The predicted  
 688  $R^2$  of all the models are greater than 0.90, which allows their use for prediction purposes within the  
 689 range of study considered in this work.

690

691 Table 7. Theoretical optimisation: operating conditions and response variables.

692

Variables	Objective	Interval of variation	Relative importance (1-5)	Optimum Theoretical	Optimum Experimental
T (°C)	minimise	150-210	1	186	
t (min)	minimise	0-60	5	0	
CH <sub>3</sub> COOH (mol/L)	minimise	1-4	3	1	
Gas yield (%)	none	0-100		2	2
Liquid yield (%)	none	0-100		62	63
Solid yield (%)	none	0-100		36	35
<b>Solid fibre and elemental analyses</b>					
Cellulose (wt.%)	minimise	0-100	5	0	0
Hemicellulose (wt.%)	minimise	0-100	5	0	0
Lignin (wt.%)	maximise	0-100	5	85	86
Proteins (wt.%)	minimise	0-100	5	16	14
C (wt.%)	none	0-100		61	62
H (wt.%)	none	0-100		6	6
O (wt.%)	none	0-100		28	29
N (wt.%)	none	0-100		3	3
<b>Liquid composition (C-wt.%)</b>					
Oligosaccharides (DP>6)	none	0-100		47	49
Oligosaccharides (DP 2-6)	none	0-100		2	2
Saccharides	none	0-100		2	2
Ketones	none	0-100		0	0
Furans	none	0-100		2	2
Carboxylic acids	none	0-100		44	43
N-compounds	none	0-100		2	2

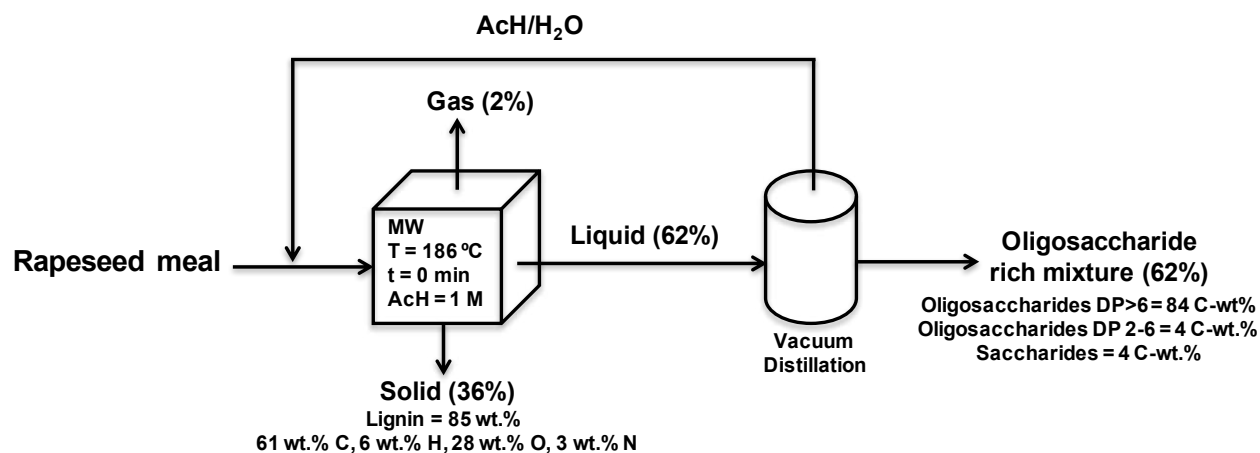
693

694 The optimisation comprises the maximisation of the lignin content together with the minimisation of  
 695 the proportions of cellulose, hemicellulose and proteins in the solid. In addition, the temperature, time  
 696 and acetic acid concentration were also minimised. To meet this objective, a solution that strikes a  
 697 compromise between the optimum values for all the response variables was sought and a relative

698 importance (from 1 to 5) was given to each of the objectives in order to come up with a solution that  
 699 satisfies all the criteria. Table 7 lists the relative importance assigned to each variable as well as the  
 700 criteria used in the whole optimisation.

701  
 702 Taking these conditions into account, the optimisation predicts an optimum at 186 °C using a  
 703 concentration of acetic acid of 1 mol/L for a total reaction time of 2 min; i.e. only the ramping time (2  
 704 min ramping with a holding time of 0 min). Under such conditions, it is possible to selectively convert  
 705 36% of the original feedstock into relatively high purity (85 wt.%) lignin; the rest (63%) being  
 706 converted into a mixture of soluble oligosaccharides containing the acetic acid used in the experiment.  
 707 This indicates that all the lignin and the vast majority of the proteins initially presented in the biomass  
 708 remained in the solid during the isolation process. The elemental analysis of the lignin produced  
 709 (without taking the N content into account) is very similar to the results reported by other authors  
 710 addressing lignin isolation from other types biomass [13, 14, 17]. In addition, to increase the  
 711 effectiveness and sustainability of this process, acetic acid can be recovered from the oligosaccharide  
 712 solution by vacuum distillation (for example) and used again for further experiments. This strategy,  
 713 shown in Figure 7, allows the simultaneous production of sugar-free, relatively high purity lignin (85  
 714 wt.%) along a sugar rich solid fraction comprising oligo- and mono/di-saccharides (92 C-wt.%) with  
 715 several applications in the chemical and biological industries.

716



717  
 718  
 719 Figure 7. Schematic diagram for the simultaneous production of lignin and oligosaccharides  
 720

#### 721 4. Conclusions

722 This work addresses a novel microwave-assisted, acid catalysed process for the selective production of  
723 lignin and oligosaccharides from rapeseed meal, analysing the effects of the operating conditions on  
724 the yields and the most important properties of each fraction. The most important conclusions are  
725 summarised as follows.

726 1. The gas, liquid and solid yield are significantly influenced by the operating conditions, their yields  
727 varying by 0-18%, 22-64% and 34-74%, respectively. Increasing the temperature or time increased the  
728 liquid yield and decreased the solid yield due to the progressive solubilisation of the cellulosic and  
729 hemicellulosic contents of the original feedstock, which resulted into the production of a rich lignin  
730 solid in some cases. Acetic acid exerted a positive catalytic effect on the process promoting cellulose  
731 and hemicellulose solubilisation and preventing the formation of humins.

732 2. The solid fraction consisted of high purity lignin (26-88 wt.%) together with unreacted cellulose (0-  
733 28 wt.%), hemicellulose (0-28 wt.%) and proteins (11-28 wt.%). An increase in the temperature or  
734 reaction time decreased the amount of cellulose and hemicellulose and increased the lignin purity of  
735 the solid when temperatures lower than 190 °C were used. A further increase up to 210 °C led to a  
736 decrease in the lignin content of the solid due to the formation of humins. However, acetic acid  
737 displayed an inhibitory effect on humins formation, which allowed high temperatures and reaction  
738 times to be used when using concentrated acid solutions.

739 3. The relative amounts (wt.%) of C, H, O and N in the solid fraction shifted between 46-63, 5.8-6.4,  
740 28-42 and 2-6%, respectively. Py-GC/MS characterisation revealed that the solid product decomposed  
741 into a mixture of phenols (1-19%), sugars (0-15%), nitrogen compounds (0-31%), carboxylic acids  
742 (37-75%), hydrocarbons (4-20%) and furans (1-8%). The progressive solubilisation of the cellulose  
743 and hemicellulose during the reaction produced an increase in the C content together with a decrease  
744 in the proportions of H and O of the solid. This also increased and decreased in the proportions of  
745 phenols and sugars, respectively.

746 4. The liquid phase was made up of oligo- (DP2-6 and DP>6) and mono/di-saccharides, carboxylic  
747 acids, ketones, furans and nitrogen compounds. Their relative amount (in carbon basis, C-wt.%) varied  
748 by: 33-51%, 0-3%, 0-6%, 40-62%, 0-1%, 0-3%, 0-3%. DP>6 oligosaccharides and carboxylic acids

749 were strongly influenced by the operating conditions, while the variations observed for the other  
750 species were less important. An increase in the temperature and reaction time led to an increase in the  
751 proportion of oligosaccharides and decreased the relative amount of carboxylic acids in the liquid.

752 5. An optimum for this process was found at 186 °C using a concentration of acetic acid of 1 mol/L  
753 and employing a total reaction time as short as 2 min. These conditions maximise the solubilisation of  
754 cellulose and hemicellulose and minimise lignin solubilisation; thus allowing the selective and  
755 simultaneous production of a rich (85 wt.%) lignin solid and a oligosaccharide rich water solution. In  
756 addition, acetic acid could be recovered from the sugar mixture, which not only can improve the  
757 economy and efficiency of the process but also it allows the production of high purity saccharides (92  
758 C-wt.%) with many applications in both the chemical and biological industries.

759

## 760 **Acknowledgements**

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764

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870

Dear Professor Keat Teong Lee,

We are very grateful for your e-mail and the comments of the reviewers, which we consider clearly contribute to the improvement of the work.

We have revised the work taking into consideration all the suggestions made. An itemised list of the changes made in the revised manuscript and our response to all the issues raised by the reviewers is attached below.

### **Reviewer #1**

**1. Page 7, Table 1, the liquid yield equals to 100-(Gas yield+Solid yield), but not "Liquid yield".**

We agree with the reviewer's concern and Table 1 has been modified accordingly.

**2. The amount of acetic acid in the liquid product was not measured so that we could not make sure that the acetic acid worked as catalyst during the microwave-assisted hydrothermal process.**

The amount of acetic acid in the liquids after the treatment was measured by HPLC. In all the cases the amount of this acid was fairly similar to the initial amount initially loaded in the experiments. This indicates that acetic acid decomposition (removal) and sugars decomposition secondary reactions (production) did not take place to a great extent and/or they compensated for each other. In addition, it was found that an increase in the concentration of acetic acid not only did increase the solubilisation of the cellulosic and hemicellulosic content of rapeseed meal in the liquid but also helped to prevent the formation of humins. Therefore, these two developments indicate that acetic acid played an important catalytic role in the process.

All this information has been included in the revised version of the manuscript to avoid any possible misunderstanding and help the potential readerships to gain a deeper insight into the process.

**3. Figure 5 is missing. Page 13, line 303, the figure number is missing.**

We agree with the reviewer's concern. All the figures are now correctly identified and subsequently numbered.

**4. The optimum condition should be explained clearly. In other words, the holding time of 0 mins should be introduced more precisely in the experimental section. It would be better to mention the time required for heating up to the target temperature.**

We agree with the reviewer's concern and this information is clearly stated in the revised version of the manuscript.

**5. The third highlight should be rewritten or even removed.**

We agree with the reviewer's suggestion and this highlight has been removed.

## **Reviewer #2**

### **1. Page 29: "Table 5" should be "Table 7"**

We agree with the reviewer's concern and the manuscript has been modified accordingly.

### **2. It should be pointed out that microwave method and microwave-assisted hydrothermal method are different methods. Therefore, authors should indicate the difference between these methods, and indicate the advantages of microwave-assisted hydrothermal method, compared with microwave method.**

We strongly agree with the reviewer's concern. Microwave-assisted hydrothermal treatment is a process that uses microwaves as the heating mechanism to achieve hydrothermal conditions using water as the solvent. As water is highly effective in microwave energy absorption, the combination of hydrothermal conditions together with microwave assisted heating has recently appeared as an interesting new technology for the valorisation of biomass.

This information has been included in the revised version of the manuscript to avoid any possible misunderstanding and to clearly state the differences between microwave heating and microwave-assisted hydrothermal treatment.

## **Reviewer #3**

### **1. The second option, for the valorisation of rapeseed meal, was primary rather than the first, therefore, the review of literatures about the first option ought to be compressed in second paragraph of the Introduction.**

We agree with the reviewer's suggestion and the second paragraph has been considerably reduced.

### **2. Nowadays, it is difficult for microwave heating to be applied in the large-scale (industry) (Page 7, Line 117-120).**

We agree with the reviewer's concern and the manuscript has been modified accordingly.

### **3. Detailed temperature profile of heating and cooling conditions was indispensable in Experimental.**

For the reactions, a heating rate of 1°C/s was used for the experiments. This varied the ramping time (time to reach the temperature of the experiment) between 2 and 3 min depending on the temperature of the experiment. The reaction time shifted between 0 and 60 min according to the experimental design. After reaction, the reactor was cooled down to 60 °C at a rate of around 0.5 °C/s.

All this information has been included in the revised version of the manuscript.

**4. There were only two levels of acetic concentration and reaction time, thus the analyses of tendencies were lacked of persuasion.**

The experiments were planned according to a 2 level 3-factor Box-Wilson Central Composite Face Centred (CCF,  $\alpha: \pm 1$ ) design. These experiments were designed to analyse the full interval of variation for the operating variables and are suitable not only for studying the influence of each variable (linear and quadratic effects) but also for understanding possible interactions between variables. The results were rigorously analysed by means of an analysis of variance (ANOVA) with 95% confidence. In addition, the cause-effect Pareto principle was used to calculate the relative importance of the operating variables in the response variables. All these results are shown in the ANOVA tables.

To explain and discuss the effects of the operating variables and interactions, the evolutions of these variables obtained from the ANOVA analysis of all the experiments performed were graphically represented in the interaction plots. In addition, when possible, some experimental points were added. To concisely and clearly explain the effects and interactions in these plots, it is a well-accepted and well-established common practice to represent only two intervals (typically the upper and the lower) for one of the variables to show and explain the interactions occurring between both variables. However, it must be borne in mind that the full intervals of variation for all the variables have been thoroughly investigated and analysed. The interaction plots were carefully developed to gain a complete and clear insight into the process.

Given this information, we strongly believe that the ANOVA analyses (Tables) and the interaction plots developed for this work thoroughly describe and clearly explain the most important effects of the operating conditions on the process. In addition, the fact that they were developed from the ANOVA analyses (provided) make it possible that any readership (if interested) can develop their own graphs for their own purposes. The manuscript has been modified to include all this information and clearly state the utility of all the data included in the tables and figures, which can be of great value for the potential readerships of the journal.

We believe that the concerns of the reviewers have been adequately answered, and that the work has been considerably improved. We hope that the work may be published in Energy Conversion and Management.

Yours sincerely,

Dr. Javier Remón  
Prof. James H. Clark

1 **Simultaneous production of lignin and polysaccharide rich aqueous solutions by microwave-**  
2 **assisted hydrothermal treatment of rapeseed meal**

3  
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9  
10 **Abstract**

11 This work addresses a novel and green process for the co-production of lignin and oligosaccharides  
12 from rapeseed meal, examining the effects of the temperature (150-210 °C), reaction time (0-60 min)  
13 and catalyst amount (1-4 mol/L, CH<sub>3</sub>COOH) on the process. The yields to gas, liquid and solid varied  
14 by 0-18%, 22-64% and 34-74%, respectively. The solid consisted of high purity lignin (26-88 wt.%)  
15 together with unreacted cellulose (0-28 wt.%), hemicellulose (0-28 wt.%) and proteins (11-28 wt.%).  
16 Increasing the temperature and/or reaction time produced an increase in the liquid yield and a decrease  
17 in the solid yield due to the solubilisation of the cellulosic and hemicellulosic contents of the  
18 feedstock. Acetic acid exerted a positive catalytic effect, promoting the solubilisation of cellulose and  
19 hemicellulose and preventing humins formation. The relative amounts (wt.%) of C, H, O and N in the  
20 solid fraction shifted between 46-63, 5.8-6.4, 28-42 and 2-6, respectively. Py-GC/MS analysis  
21 revealed that the solid decomposed into phenols (1-19%), sugars (0-15%), N-compounds (0-31%),  
22 carboxylic acids (37-75%), hydrocarbons (4-20%) and furans (1-8%). The liquid phase comprised  
23 oligo- and mono/di-saccharides (33-51 C-wt.%, 0-3 C-wt.% and 0-6 C-wt.%) and carboxylic acids  
24 (40-62 C-wt.%). The progressive solubilisation of cellulose and hemicellulose produced an increase in  
25 the proportion of C together with a decrease in the amounts of H and O in the solid product, which  
26 also accounted for the increase and decrease observed in the proportions of phenols and sugars,  
27 respectively. An optimum was found at 186 °C using an acid concentration of 1 mol/L and a **total**  
28 **reaction time of 2 min.** These conditions maximise the solubilisation of cellulose and hemicellulose  
29 without altering the lignin content of the solid; thus allowing the selective and simultaneous  
30 production of high purity (85 wt.%) lignin together with a rich oligosaccharide (51 C-wt.%) solution.  
31 The acid can be recovered from the sugar mixture, which not only improves the efficiency of the  
32 process but also allows the production of a pure saccharide (92 C-wt.%) product.

33 **Keywords:** microwaves, rapeseed meal, biomass valorisation, lignin, oligosaccharides

34

## 35 **1. Introduction**

36 Rapeseed, the third largest source of vegetable oil in the world, is currently used for the production of  
37 both edible oil and biodiesel [1]. During the processing of rapeseed seeds to produce the oil, around 65  
38 wt.% of the feedstock is converted into a lignocellulosic solid residue called rapeseed meal or  
39 rapeseed cake [2, 3]. This solid material is mainly composed of cellulose, hemicellulose, lignin and  
40 proteins; the precise chemical composition of the residue depending on the type of rapeseed plant and  
41 extraction process [2]. Traditionally, rapeseed meal has been used as a livestock feed due to the  
42 presence of proteins in the residue. However, the increase in biodiesel production has oversaturated  
43 the agricultural market and new processes and alternative strategies need to be developed for the  
44 valorisation of this feedstock [4].

45

46 In this context, two alternative options have normally been considered for the valorisation of rapeseed  
47 meal. The first is the application of different extraction systems to recover valuable products. In this  
48 respect, Purkayastha et al. [5] analysed the effectiveness of several solvents for the extraction of  
49 residual oils and polyphenols from a rapeseed cake at 25°C for 2 h. It was found that non-polar  
50 solvents were the most effective in recovering the residual oil. Terpinic et al. [6] investigated the  
51 extraction of polyphenols from camelina linseed, rapeseed and white mustard using methanol and  
52 ethanol at room temperature for 12 h. They found that the plant material and the extraction solvent not  
53 only significantly influenced the amount of phenols extracted, but also the antioxidant properties of  
54 the extracts. Li et al. [7] investigated the use of pressurised solvent systems to recover phenols,  
55 analysing the effects of the solvent type (ethanol, methanol, 2-propanol, acetone and acetonitrile) and  
56 concentration, temperature (80-200 °C) and time (2-30 min). The use of a 60 vol.% methanol/water  
57 solution at 200 °C for 20 min extracted the highest amount of phenols (93 mg/g).

58

59 The second option relies on the use of thermochemical processes, such as pyrolysis, gasification,  
60 combustion and hydrothermal treatments to produce bio-fuels, energy and value-added chemicals.

61 Özcimen et al. [8] examined the production of bio-oil and bio-char from a rapeseed cake produced  
62 during oil extraction from Brassica Napus. The pyrolysis experiments were performed in a fixed bed  
63 reactor at 500 °C, employing different gas space velocities (50-300 cm<sup>3</sup>/min). Regardless of the space  
64 velocity, around 73% of the rapeseed meal was converted into bio-oil (60%), bio-char (27%) and  
65 permanent gases (13%). The valorisation of this type of cake was also investigated by Ucar et al. [9]  
66 who analysed the effect of the reaction temperature (400-900°C) during the pyrolysis of the residue.  
67 The gas consisted of CO<sub>2</sub>, CO, CH<sub>4</sub> and H<sub>2</sub>S, while the bio-oil was made up of carboxylic acids,  
68 amides and phenols. An increase in the temperature increased and decreased the gas (8-14%) and char  
69 (30-38%) yields, respectively; while the bio-oil yield increased between 400 and 500°C (14-19%) and  
70 slyly decreased with further increasing the temperature up to 900°C. Giannakopoulou et al. [1]  
71 conducted catalytic pressurised pyrolysis experiments of a spent rapeseed meal produced during the  
72 production of biodiesel. Two catalysts (H-ZSM-5 and H-Beta zeolites) and two reactor configurations  
73 (a pressurised pyrolysis unit, and a pressurised pyrolysis unit with catalytic upgrading of the pyrolysis  
74 vapours) were tested. In the process, two liquid phases (aqueous and organic), gases and a solid  
75 residue were obtained. The organic phase was made up of aliphatic and aromatic hydrocarbons,  
76 carboxylic acids, esters, nitriles, amides, poly-phenols and N-heterocyclic compounds. The liquid  
77 phase consisted of a mixture of phenols, ketones, alcohols and heterocyclic and N-heterocyclic  
78 compounds.

79

80 Pinkowska et al. [3] studied the hydrothermolysis of rapeseed meal using sub-critical water for the  
81 recovery of fatty acids and amino acids, examining the effects of the reaction time and temperature on  
82 the process. The maximum yield of amino acids (136 g/kg of rapeseed cake) took place when the solid  
83 was treated at 215°C for 26 min. A further increase in the temperature led to the decomposition of the  
84 amino acids. The maximum fatty acid production (0.91 g/kg) occurred at 246 °C using a reaction time  
85 of 65 min. Briones et al. [4] explored the possibility of co-valorising two biodiesel by-products: crude  
86 glycerol and rapeseed meal. The effects of the mass/solvent ratio, temperature and reaction time on  
87 rapeseed meal valorisation were experimentally investigated. In the process, the cellulose,  
88 hemicellulose and lignin contents of the solid were decomposed, leading to the production a liquid

89 mixture consisting of glycols, carboxylic acids, furans esters and ethers. Egües et al. [2] employed a  
90 two-step process for the production of saccharides from rapeseed meal pellets. Firstly, the  
91 hemicellulose content of the feed was extracted and purified; then, this fraction was converted into  
92 saccharides by auto-hydrolysis or acid hydrolysis. Glucose and xylose were the main sugars identified  
93 in the hydrolysates; their specific amounts depending on the hydrolysis process. In the case of auto-  
94 hydrolysis, they accounted for 23% and 40%, respectively, while their relative amounts were 28% and  
95 37%, when acid hydrolysis was used.

96

97 Another interesting option for the valorisation of rapeseed meal that has not been considered before is  
98 the simultaneous production of saccharides and pure lignin from the solid aiming to build a bio-  
99 refinery concept around this residue. However, the extraction of polysaccharide-free lignin from  
100 biomass is very challenging because lignin is strongly covalent bonded to cellulose and hemicellulose,  
101 which hinders the selective extraction of pure lignin. Therefore, the development of a suitable method  
102 for lignin isolation is of paramount importance for the production of pure lignin from biomass. In this  
103 respect, the two-step Klason acidolysis method is one of the most widespread used [10]. However, its  
104 major drawback is the use of concentrated sulphuric acid, which is not environmentally friendly and  
105 also damages the lignin structure. Another method is the combination of biomass milling, to break the  
106 linkages between lignin and saccharides, followed by solvent extraction using a dioxane-water solvent  
107 system [11]. Though, this latter methodology is considered extremely time-consuming as a reaction  
108 time as long as 3 weeks is needed in some cases. This led to the modification of this latter  
109 methodology using enzymes to increase the lignin yield; nevertheless, the lignin yield was still low  
110 and a high enzyme dosage was needed [10].

111

112 Therefore, more research needs to be conducted for the development of novel and energy efficient  
113 methodologies for lignin production from biomass. As part of this, the use of microwave heating has  
114 recently appeared as a new and promising alternative. Microwave heating is based on the high  
115 frequency rotation of polar molecules, which produces a quicker and higher heating of the species  
116 with higher polarity within the biomass structure [12]. As lignin has a higher aromaticity, i.e. lower

117 polarity, than cellulose and hemicellulose, it is less active during microwave heating [13]. This could  
118 allow the separation of cellulose and hemicellulose from the biomass without significantly altering the  
119 lignin structure; thus allowing a high purity lignin to be produced. In addition, as water is highly  
120 effective in microwave energy absorption, the combination of hydrothermal conditions together with  
121 microwave-assisted heating might be an interesting new technology for the valorisation of rapeseed  
122 meal. To the best of the authors' knowledge, the work conducted using microwave assisted  
123 hydrothermal conditions for the extraction of lignin from biomass is very scarce. In particular, Zhou et  
124 al. [14] used formic acid to extract lignin from birch biomass employing conventional and microwave  
125 heating. A higher amount of delignification was reported when microwave heating was used in the  
126 experiments. Li et al. [15] studied the effect of the temperature (90-109 °C) during the isolation of  
127 lignin from bamboo. The temperature was found to significantly influence the process and the use of  
128 higher temperatures resulted in a greater lignin yield. Zoia et al. [16] conducted microwave assisted  
129 lignin isolation using HCl and reported that their methodology was capable of recovering up to 55 wt.%  
130 of the total lignin present in the material. Long et al. [13, 17] addressed the effects of the temperature  
131 (160-210 °C) and reaction time (5-20 min) during the isolation of lignin from softwood employing  
132 H<sub>2</sub>SO<sub>4</sub>. They found that an increase in both the temperature and reaction time increased the lignin  
133 yield and purity. Maxima for the yield (82 wt.%) and purity (93 wt.%) occurred using a 0.2 mol/L  
134 sulphuric acid solution at 190 °C for 10 minutes. The liquid phase consisted of a mixture of  
135 saccharides, carboxylic acids and furans and was found to have potential to be used in fermentation  
136 processes.

137

138 Given this background, this work addresses the valorisation of rapeseed meal by means of a  
139 microwave-assisted hydrothermal process catalysed by acetic acid, a much safer and greener  
140 alternative to mineral acids, for the simultaneous production of pure lignin and polysaccharide rich  
141 aqueous solutions. In particular, the effects of the temperature (150-210 °C), time (0-1 h) and catalyst  
142 (acetic acid) amount (1-4 mol/L) together with all the possible interactions between these variables on  
143 rapeseed meal valorisation have been thoroughly analysed. Given that the microwave-assisted  
144 hydrothermal valorisation of rapeseed meal has never been reported before and the works dealing with

145 the isolation of lignin from biomass using microwave **technology** are very scarce, this work represents  
146 a novel and challenging investigation not only for the management and valorisation of rapeseed meal,  
147 but also for the development of a novel, quick and environmentally-friendly methodology for the  
148 production of pure lignin and saccharides from other types of biomass. In addition, the fact that acetic  
149 acid can be directly produced from biomass and the use of an energy efficient **microwave-assisted**  
150 **hydrothermal process** convert this process into a green, efficient and sustainable route for biomass  
151 valorisation.

152

## 153 **2. Experimental**

### 154 **2.1 Microwave experiments**

155 A CEM Discover II microwave facility was used for the experiments. The experiments were  
156 conducted in a 30 mL batch reactor using a maximum power of 300W. For each experiment, 0.5 g of  
157 biomass was placed in the reactor along with 15 mL of solvent (CH<sub>3</sub>COOH/H<sub>2</sub>O). Before placing the  
158 reactor inside the microwave unit, the reaction mixture was pre-stirred at room temperature for 2 min.  
159 **A heating rate of 1°C/s was used for all the experiments; and therefore, the ramping time (time to**  
160 **reach the temperature of the experiment) varied between 2 and 3 min. The reaction time shifted**  
161 **between 0 and 60 min according to the experimental design. After reaction, the reactor was cooled**  
162 **down from the reaction temperature to 60°C at a rate of 0.5 °C/s.** Subsequently, the reactor was opened  
163 and its content, consisting of a mixture of liquid and solid, was transferred to a centrifuge tube.  
164 Centrifugation was used to separate the solid from the liquid. Then, the solid residue obtained after  
165 centrifugation was dried overnight at 105°C and the liquid phase obtained was stored for further  
166 characterisation.

167

### 168 **2.2 Response variables and analytical methods**

169 Several response variables were used to analyse the effect of the operating conditions on the process.  
170 These include the gas, liquid and solid yields and some of the most important properties of the liquid  
171 and the solid products. Table 1 summarises the response variables and the analytical methods used for

172 their calculation. The solid fractions (both the original biomass as well as the solids produced) were  
 173 characterised by means of ultimate and fibre (cellulose, hemicellulose, lignin and protein) analyses,  
 174 and Pyrolysis Gas Chromatography Mass Spectrometry (Py-GC/MS). In addition, the original  
 175 feedstock was also characterised by proximate analysis and Inductively Coupled Plasma Mass  
 176 Spectrometry (ICP-MS) to identify and quantify the amounts of metals. Proximate and ultimate  
 177 analyses were performed according to standard methods (ISO-589-1981 for moisture, ISO-1171-1976  
 178 for ash and ISO-5623-1974 for volatiles). Elemental analysis was carried out using an Exeter  
 179 Analytical (Warwick, UK) CE440 Elemental Analyser, calibrated against acetanilide with a S-benzyl-  
 180 thiuronium chloride internal standard. Fibre characterisation was performed by using the chemical  
 181 titration method described by Hu et al. [18] to determine the amount of cellulose and hemicellulose,  
 182 while the lignin content was determined by the standard TAPPI T222 method. Py-GC/MS results were  
 183 obtained using a CDS Analytical 5250-T Trapping Pyrolysis Auto sampler coupled with an Agilent  
 184 7890 B gas chromatograph equipped with a 5977A MSD mass spectrum unit. The sample was loaded  
 185 into the pyrolysis unit and pyrolysed at 600 °C for 10 s. The volatile materials released were carried  
 186 into the GC/MS unit by nitrogen for analysis.

187

188 Table 1. Response variables. Definitions and analytical techniques used in their determination.

Product	Response variable	Analytical method
Liquid	Liquid yield (%) = $\frac{\text{liquid compounds (g)}}{\text{mass of biomass (g)}} 100 = 100 - (\text{Gas yield} + \text{Solid yield})$	Balance
	Composition (C – wt. %) = $\frac{\sum \text{mass of C of each compound (g)}}{\text{total mass of C in solution (g)}} 100$	GC/MS-FID and HPLC
	C, H, O (wt. %) = $\frac{\text{mass of C, H, O (g)}}{\text{mass of organics (g)}} 100$	Elemental Analysis
	HHV (MJ/kg) = $0.3491 \text{ C (wt. \%)} + 1.1783 \text{ H (wt. \%)} - 0.1034 \text{ O (wt. \%)} - 0.015 \text{ N (wt. \%)} + 0.1005 \text{ S (wt. \%)}$	Estimated
Solid	Solid yield (%) = $\frac{\text{mass of solid (g)}}{\text{mass of biomass (g)}} 100$	Gravimetric
	Fibre Composition (wt. %) = $\frac{\text{mass of structural component (g)}}{\text{mass of solid residue (g)}} 100$	Chemical titration, Tappi T222 Method
	HHV (MJ/kg) = $0.3491 \text{ C (wt. \%)} + 1.1783 \text{ H (wt. \%)} - 0.1034 \text{ O (wt. \%)} - 0.015 \text{ N (wt. \%)} + 0.1005 \text{ S (wt. \%)}$	Estimated
	C, H, O (wt. %) = $\frac{\text{mass of C, H, O (g)}}{\text{mass of solid (g)}} 100$	Elemental Analysis
	Py GC/MS Composition (area %) = $\frac{\text{area of each compound}}{\text{total area}} 100$	Py-GC/MS
Gas	Gas yield (%) = $\frac{\text{mass of gas (g)}}{\text{mass of biomass (g)}} 100$	Gravimetric

189 wt.% = weight percentage

190 C-wt.% = percentage in carbon basis

191 Protein content (wt.%) =  $4.62 \cdot \text{N (wt. \%)}$

192

193

194 High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC/MS-FID) and  
195 elemental analysis (described above) were used for the characterisation of the liquid phase. An Agilent  
196 1260 Infinity HPLC equipped with Agilent Hi- Plex H (300 x 7.7mm, 8µm particle size) and ACE  
197 C18 (250 x 4.6mm, 5µm particle size) columns and 1660 DAD WR UV/UV-VIS and 1660 Infinity  
198 Refractive Index (RI) detectors was used for the HPLC analyses. In addition, an Agilent 7890 GC-  
199 system (model G3440A) equipped with Flame Ionization (FID) and Mass Spectrometry (MS)  
200 detectors was used for the GC analysis of the liquid. In this case, the MS detector was used for  
201 identification while the FID detector was used for the quantification of the reaction products.

202

### 203 **2.3 Experimental design and data analysis**

204 The influence of reaction temperature (150-210°C), acetic concentration in water (1-4 mol/L) and  
205 reaction time (0-60 min) on the process was experimentally investigated. The experiments were  
206 planned according to a 2 level 3-factor Box-Wilson Central Composite Face Centred (CCF,  $\alpha: \pm 1$ )  
207 design. This corresponds to a  $2^k$  factorial design, where k indicates the number of factors studied (in  
208 this case 3 operating variables) and  $2^k$  represents the number of runs (in this case 8) for the simple  
209 factorial design. 8 axial experiments were performed to study non-linear effects and interactions. In  
210 addition, 4 replicates at the centre point (centre of the variation interval of each factor) were carried  
211 out in order to evaluate the experimental error. This experimental design is suitable not only for  
212 studying the influence of each variable (linear and quadratic effects) but also for understanding  
213 possible interactions between variables. The results were analysed with an analysis of variance  
214 (ANOVA) with 95% confidence. In addition, the cause-effect Pareto principle was used to calculate  
215 the relative importance of the operating variables in the response variables. In these analyses, the  
216 lower and upper limits of all the operating variables were normalised from -1 to 1 (coded variables) to  
217 investigate their influence in comparable terms. In the interaction Figures, the evolution of these  
218 variables obtained from the ANOVA analysis of all the experiments performed was represented. In  
219 addition, when possible, some experimental points were added. **In the interaction plots developed from**  
220 **the ANOVA analyses only the upper and lower levels for one of the variables have been represented;**

221 however, the whole interval of variation was considered for all the variables, carefully analysed and  
 222 thoroughly discussed.

223

#### 224 2.4 Rapeseed meal characterisation

225 The rapeseed meal used in this work was provided by Croda International (Widnes, UK). The most  
 226 important physiochemical properties of the material such as proximate, ultimate, fibre, calorific and  
 227 Py-GC/MS analyses are listed in Table 2. The proximate, fibre and elemental analyses as well as the  
 228 higher heating value (HHV) of the residue are fairly similar to those previously reported in the  
 229 literature [1, 2, 4, 9]. In addition, the lignin content of this particular solid is quite high, which makes it  
 230 suitable for the production of lignin. The pyrolysis GC-MS characterisation results reveal that the solid  
 231 decomposes into hydrocarbons, ketones, aldehydes, carboxylic acids, phenols and sugars. The  
 232 proportion of hydrocarbons in the residue is very high due to the presence of residual oil, which was  
 233 not effectively recovered in the extraction process.

234

235 Table 2. Feedstock characterisation.

<b>Proximate analysis (wt.%)</b>		<b>HHV (MJ/kg)</b>	17.07±0.29
Moisture	7.26	<b>Ash composition (wt.%)</b>	
Ash	1.31	Ca	15.94
Volatiles	45.09	Mg	7.98
Fixed carbon	32.04	K	19.75
<b>Fibre analysis (wt.%)</b>		Na	1.19
Cellulose	12.41±0.33	P	24.47
Hemicellulose	7.16±0.26	S	30.67
Lignin	32.39±2.47	<b>Py-GC/MS characterisation (% area)</b>	
Protein	39.47±1.17	Hydrocarbons	43.59±1.22
<b>Elemental analysis (wt.%)</b>		Ketones	2.30±3.25
C	41.54±0.19	Aldehydes	1.46±2.26
H	6.29±0.17	Carboxylic acids	20.88±2.14
N	6.32±0.19	Phenolic compounds	10.19±0.40
O*	45.86±0.17	Sugars	1.75±2.47

236 \*Oxygen was calculated by difference

237

#### 238 3. Results and discussion

239 Table 3 lists the operating conditions used in the experiments and the experimental results. These  
 240 include the yields to products (gas, liquid and solid) and the most important properties of the solid and  
 241 liquid fractions; i.e. the fibre and elemental analyses and the Py-GC/MS characterisation for the solid  
 242 fraction and the chemical composition and elemental analysis for the liquid fraction.

Table 3. Operating conditions and experimental results produced during the microwave-assisted hydrothermal treatment of rapeseed meal

Run	1	2	3	4	5	6	7	8	9-12	13	14	15	16	17	18
T (°C)	150	210	150	210	150	210	150	210	180	210	180	180	150	180	180
t (min)	0	0	60	60	0	0	60	60	30	30	0	60	30	30	30
AcH (mol/L)	1	1	1	1	4	4	4	4	2.5	2.5	2.5	2.5	2.5	1	4
<b>GLOBAL YIELDS</b>															
Solid yield (%)	63.99	32.25	33.63	27.50	51.44	23.47	23.66	22.20	26.41±1.58	23.79	26.90	35.97	26.97	29.58	24.33
Gas yield (%)	1.93	2.56	0.00	18.43	1.69	2.92	2.91	12.20	4.04±0.76	12.43	0.00	5.24	2.42	4.43	5.55
Liquid yield (%)	34.08	63.19	66.37	54.07	46.87	73.61	73.43	65.60	69.55±2.06	63.78	64.03	67.87	70.61	65.98	70.12
<b>SOLID PROPERTIES</b>															
<b>Fibre analysis (wt.%)</b>															
Cellulose	18.40	25.55	27.72	25.52	26.49	0.00	8.89	0.00	0.90±0.47	0.00	22.13	0.00	21.87	0.00	0.00
Hemicellulose	28.06	0.00	0.82	0.00	0.00	0.00	0.00	0.00	0.22±0.44	0.00	0.61	0.00	0.90	0.00	0.00
Lignin	25.84	58.54	57.16	63.71	50.18	85.36	77.12	87.58	86.37±0.87	88.05	55.79	86.55	63.02	83.59	83.83
Proteins	27.70	15.91	14.30	10.77	23.32	14.64	14.00	12.42	13.41±0.91	11.95	21.47	13.45	14.21	16.41	16.17
<b>Elemental analysis</b>															
C (wt.%)	46.50	52.21	51.32	59.80	46.81	53.45	54.54	63.25	56.12±0.98	60.92	56.29	57.63	53.03	57.22	57.15
H (wt.%)	6.16	6.16	6.03	6.16	6.14	5.90	6.30	5.97	6.11±0.12	5.81	6.42	5.81	6.21	6.18	6.03
O (wt.%)	37.98	37.98	38.92	41.95	37.70	37.70	36.11	27.87	34.26±0.95	30.55	34.60	30.55	37.45	32.38	33.92
N (wt.%)	3.66	3.66	3.74	5.75	5.11	2.97	3.06	2.93	3.51±0.37	2.73	3.18	2.73	3.33	4.22	2.91
HHV (MJ/kg)	19.10	21.50	20.93	24.87	19.16	21.66	22.68	26.18	23.20±0.46	24.91	21.57	23.68	21.90	23.85	23.50
<b>Py-GC/MS (Area %)</b>															
Hydrocarbons	6.92	8.99	5.89	19.10	10.77	11.69	4.59	12.81	11.69±3.17	20.27	8.99	12.64	10.25	20.12	15.91
Carboxylic acids	72.85	55.97	73.36	36.93	48.30	71.53	74.35	57.32	55.57±4.67	39.60	59.92	42.26	57.91	45.26	55.64
Sugars	10.64	9.46	10.92	5.36	0.01	3.01	11.51	0.45	9.48±0.38	10.89	13.74	10.69	14.48	0.44	6.30
Phenolic compounds	1.81	6.55	1.86	17.70	4.74	1.42	0.67	13.08	7.69±1.29	14.19	2.49	8.04	8.36	18.69	1.89
Furanic compounds	3.02	2.76	0.68	6.56	5.21	5.39	3.11	6.98	3.70±0.44	7.46	6.25	5.61	5.83	4.80	7.79
Nitrogen compounds	2.73	9.56	5.21	4.51	30.98	3.06	0.37	0.82	6.14±2.19	3.07	6.70	14.69	3.17	5.15	5.39
<b>LIQUID PROPERTIES</b>															
<b>Chemical composition (C-wt.%)</b>															
Oligosaccharides DP>6	32.8	47.93	45.79	51.12	42.88	44.08	42.76	45.29	45.25±1.35	44.59	44.92	46.38	44.92	48.60	43.99
Oligosaccharides DP2-DP6	1.20	1.78	2.83	0.00	0.35	0.84	0.54	0.00	0.15±0.01	0.00	1.40	0.06	0.75	0.42	0.00
Saccharides	2.94	6.42	6.08	0.38	0.98	0.80	1.94	0.44	1.89±0.51	0.56	3.05	0.71	2.91	1.90	0.38
Carboxylic acids	62.36	42.05	43.59	45.04	55.74	53.22	53.84	53.56	51.57±1.41	50.36	51.25	50.76	44.57	54.24	44.00
Ketones	0.04	0.57	0.44	0.12	0.00	0.19	0.15	0.04	0.25±0.08	0.01	0.17	0.01	0.22	0.29	0.16
Furans	0.51	0.64	0.89	0.42	0.04	0.73	0.67	0.07	1.98±0.20	0.19	0.11	1.18	0.30	2.69	1.03
Phenols	0.02	0.00	0.00	0.14	0.00	0.00	0.00	0.04	0.01±0.02	0.04	0.00	0.01	0.00	0.02	0.00
Nitrogen Compounds	0.08	0.62	0.39	2.78	0.00	0.13	0.10	0.56	0.60±0.10	0.74	0.00	0.40	0.15	1.51	0.20
<b>Elemental analysis (dry basis)</b>															
C (wt.%)	17.60	21.07	20.30	19.87	61.53	63.60	65.00	62.93	42.83±0.61	41.27	42.20	42.70	43.17	20.87	63.93
H (wt.%)	79.97	76.14	76.98	77.46	34.52	32.55	31.21	33.18	52.60±0.48	54.68	53.72	53.21	52.73	76.36	32.23
O (wt.%)	2.43	2.79	2.72	2.67	3.95	3.85	3.79	3.88	4.07±0.05	4.05	4.08	4.09	4.10	2.77	3.84
HHV (MJ/kg)	0.74	2.77	2.33	2.07	22.56	23.37	23.92	23.11	14.31±0.24	13.53	13.98	14.22	14.45	2.65	23.50

244 **3.1 Effect of the operating conditions on the yields to gas, liquid and solid**

245 The yields of gas, solid and liquid vary by 0-18%, 22-64% and 34-74%, respectively. The relative  
 246 influence of the operating variables on the global yields according to the ANOVA analysis and the  
 247 cause-effect Pareto principle is shown in Table 4. This analysis shows that the reaction time and the  
 248 concentration of acetic acid are the operating variables exerting the highest influence on the solid  
 249 yield. In addition, this response variable is also influenced by the interaction between the time and the  
 250 temperature. The liquid and solid yields are strongly influenced by both the temperature and its  
 251 interaction with the reaction time. This interaction was also found by Long et al. [13], who reported  
 252 that at a certain temperature the effect of the reaction time on the solid yield was negligible. The  
 253 effects of the operating variables and the most important interactions detected with the ANOVA  
 254 analysis are plotted in Figure 1. Specifically, Figure 1 a and b illustrates the effects of the temperature  
 255 for 0 and 60 min reaction time for the lowest (1 mol/L) and the highest (4 mol/L) acetic acid  
 256 concentration, respectively. These effects are also shown for the liquid and solid yields in Figure 1 c-d  
 257 and e-f, respectively.

258

259 *Table 4. Relative influence of the operating conditions on the global yields*

Variable	R <sup>2</sup>	I. Term	T	t	C	Tt	TC	tC	TtC	T <sup>2</sup>	t <sup>2</sup>	C <sup>2</sup>	T <sup>2</sup> t	T <sup>2</sup> C	Tt <sup>2</sup>	TC <sup>2</sup>	T <sup>2</sup> t <sup>2</sup>
Solid yield (%)	0.98	26.29	n.s.	-4.54 (16)	-4.19 (12)	6.51 (17)	n.s.	n.s.	n.s.	n.s.	5.14 (15)	n.s.	-3.48 (15)	n.s.	-8.41 (22)	n.s.	3.33 (4)
Liquid yield (%)	0.98	69.10	n.s.	n.s.	n.s.	-9.54 (29)	n.s.	n.s.	n.s.	-5.19 (16)	n.s.	n.s.	5.22 (16)	5.23 (16)	4.46 (13)	5.23	-4.25 (10)
Gas yield (%)	0.97	4.23	-6.21 (30)	n.s.	n.s.	3.24 (22)	-1.06 (7)	n.s.	-1.22 (8)	1.28 (9)	n.s.	n.s.	3.06 (20)	n.s.	9.91 (4)	n.s.	n.s.

260 n.s: Non significant with 95% confidence  
 261 Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C  
 262 Coefficient TtC·T·t·C + Coefficient T<sup>2</sup>·T<sup>2</sup> + Coefficient t<sup>2</sup>·t<sup>2</sup> + Coefficient C<sup>2</sup>·C<sup>2</sup> + Coefficient T<sup>2</sup>t·T·t + Coefficient T<sup>2</sup>C·T<sup>2</sup>·C + Coefficient  
 263 Tt<sup>2</sup>·T·t<sup>2</sup> + Coefficient TC<sup>2</sup>·T·C<sup>2</sup> + Coefficient T<sup>2</sup>t<sup>2</sup>·T<sup>2</sup>·t<sup>2</sup>  
 264 Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Pareto values represent the percentage  
 265 of the orthogonal estimated total value.  
 266

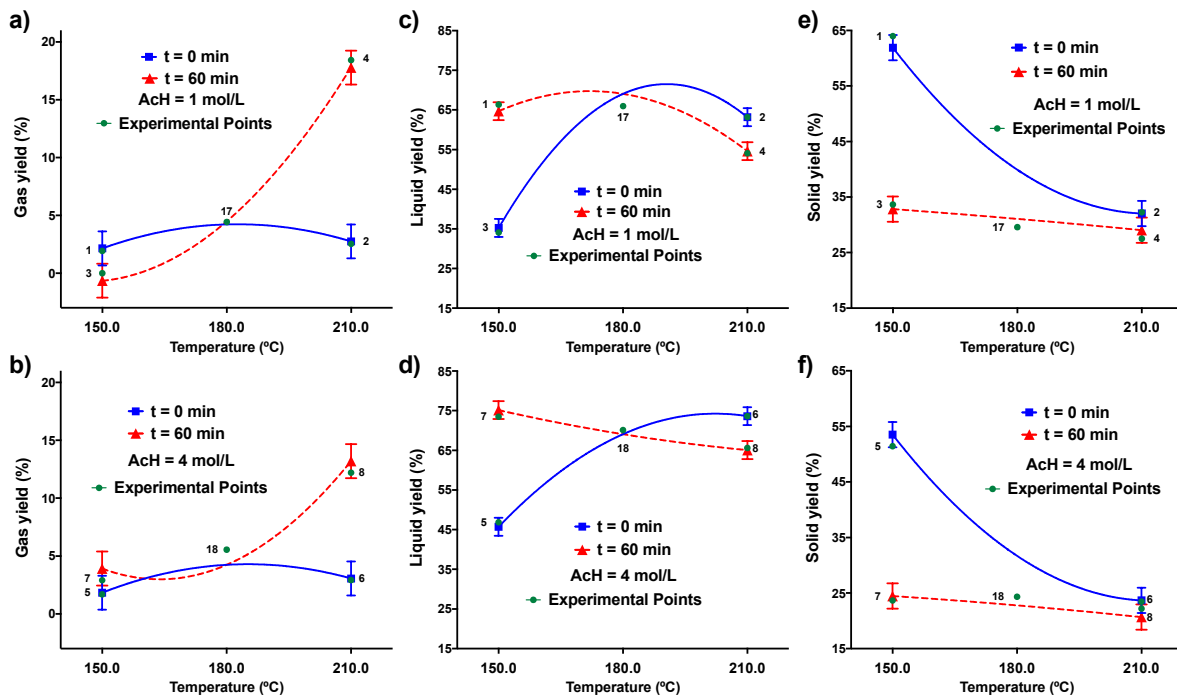
267 The effect of the temperature on the product distribution (yields to gas liquid and solid) depends on the  
 268 reaction time. For a short reaction time (0 min), the temperature does not significantly influence the  
 269 gas yield and a negligible gas formation takes place regardless of the concentration of acetic acid.  
 270 Conversely, the reaction temperature exerts a significant affect on the liquid and solid yields. In  
 271 particular, an initial increase in the temperature from 150 to 190 °C increases the liquid yield and

272 decreases the solid yield, while a further increase in the temperature up to 210 °C does not greatly  
273 modify liquid or solid production. These developments in the liquid and solid yields are accounted for  
274 by the positive kinetic effect of the reaction temperature on biomass solubilisation; thus increasing and  
275 decreasing the liquid yield and solid yield, respectively. In addition, a greater microwave power is also  
276 needed to achieve higher temperatures, thus promoting the breaking of the intramolecular bonds  
277 between cellulose, hemicellulose and lignin [4, 13, 19]. This leads to the solubilisation of the  
278 cellulosic and hemicellulosic matter in the liquid without significantly solubilising the lignin content  
279 of the solid; thus allowing a high purity lignin solid fraction to be produced.

280

281 An increase in the reaction time modifies the effect of the temperature on the product distribution.  
282 Regardless of the concentration of acetic acid, the gas yield is negligible and unaffected by the  
283 reaction time (0-60 min) between 150 and 180 °C. However, an increase in the gas yield occurs  
284 between 190 and 210 °C and when the reaction time increases from 0 to 60 min. The combination of  
285 both high temperatures and long reaction times favours the formation of gases from some of the  
286 species produced during biomass hydrolysis secondary reactions such as carboxylic acids, ketones and  
287 furans through decarboxylation reactions [2, 3]. In addition, gas formation could also be produced  
288 from the thermal decomposition of the proteins present in the solid via deamination [3]. As a result,  
289 for a long reaction time (60 min), an increase in the temperature between 180 to 210 °C produces a  
290 sharp increase in the gas yield. Conversely, the effect of the reaction time on the yields to liquid and  
291 solid is more marked at low temperature (150-190 °C) than at high temperature (190-210 °C). At low  
292 temperature, an increase in the reaction time from 0 to 60 min leads to a sharp increase in the liquid  
293 yield along with a pronounced decrease in the solid yield. This same increment in the reaction time  
294 between 190 and 210 °C slightly decreases the liquid yield; the solid yield remaining unaffected.  
295 These variations make the effect of the temperature on the liquid and solid yields less important. This  
296 development might be accounted for by the long reaction time employed in the experiment, which is  
297 high enough to kinetically control the process. In addition, this also shows the high efficiency of  
298 microwave heating [13, 19-21]. As a result, when a long reaction time (60 min) is used, the effect of  
299 the temperature on the solid yield is negligible. The liquid yield slightly decreases with increasing the

300 temperature due to the sharp increase occurring for the gas yield. This might indicate that part of the  
 301 liquid products is converted into gases if long reaction times and high temperatures are used [3].



302  
 303 Figure 1. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 304 and the highest (4 mol/L) acetic acid concentration for the gas (a and b), liquid (c and d) and solid (e  
 305 and f) yields. Bars are LSD intervals with 95% confidence.  
 306

307 The effect of the concentration of acetic acid on the yields to gas, liquid and solid can be studied by  
 308 comparing Figures 1 a, c and e with b, d and f, respectively. For the gas yield this effect depends on  
 309 the reaction time. For a short reaction time, an increase in the concentration of acetic acid between 1  
 310 and 4 mol/L does not significantly modify the gas yield and a negligible gas formation takes place  
 311 regardless of the concentration of acetic acid used in the experiments. On the contrary, the  
 312 concentration of acetic acid has a significant influence on the gas yield when the reaction time  
 313 increases and different trends for this variable are observed depending on the temperature. When a 60  
 314 min reaction time is used, an increase in the acid concentration from 1 to 4 mol/L leads to an increase  
 315 in the gas yield between 150 and 180 °C, while, this same increase reduces gas formation between 180  
 316 and 210 °C. Acetic acid exert a significant catalytic effect on the process by producing a greater spread  
 317 of decarboxylation reactions, which leads to an increase in gas formation from biomass secondary  
 318 decomposition products and proteins [2, 3]. At high temperature gas formation decreases probably

319 because the formation of humins and char from the furfural and HMF obtained from sugars at high  
320 temperature [22-24]. The influence of the concentration of acetic acid on the liquid and solid yields  
321 does not depend on the temperature or the reaction time and similar trends are observed regardless the  
322 temperature and time used in the experiments. In general, an increase in the concentration of acetic  
323 acid from 1 to 4 mol/L leads to an increase in the liquid yield and a decrease in the solid yield. The  
324 positive catalytic effect of the acid enhances the dissolution of the cellulose and hemicellulose  
325 fractions [4, 13, 19], which increases the liquid yield and decreases the solid yield. An exception to  
326 this trend occurs at around 190 °C, when long reaction times (60 °C) are used. Under such conditions,  
327 the effect of the concentration of acetic acid on the liquid yield is very weak.

328

### 329 **3.2 Effect of the operating conditions on the solid properties**

330 The solid fraction produced after the microwave-assisted treatment has been characterised by fibre and  
331 elemental analyses and py-GC-MS (Table 1). This fraction consists of the lignin isolated during the  
332 process and contains different amounts of cellulose, hemicellulose and proteins depending on the  
333 operating conditions used in the experiments. The effects of the operating conditions on the properties  
334 of the solid fraction according to the ANOVA and cause-effect Pareto analyses are listed in Table 5.

335

#### 336 *3.2.1 Fibre analysis*

337 The amounts of cellulose, hemicellulose, lignin and proteins in the solid fractions vary as follows: 0-  
338 28%, 0-28%, 26-88% and 11-28%, respectively. The cause effect Pareto analysis (Table 5) reveals  
339 that the temperature (both linear and quadratic factors) and its interaction with the reaction time are  
340 the operating variables exerting the greatest influence on the proportions of cellulose, hemicellulose  
341 and proteins. The relative amount of lignin is strongly influenced by the temperature and the reaction  
342 time. Figure 2 shows the effect of the operating variables and the most important interactions detected  
343 with the ANOVA analysis on the fibre analysis of the solid. Figure 2 a and b illustrates the effects of  
344 the temperature on the proportion of cellulose for 0 and 60 min reaction time for the lowest (1 mol/L)  
345 and the highest (4 mol/L) acetic acid concentration, respectively. These effects are also shown for the  
346 relative amounts of hemicellulose, lignin and proteins in Figure 2 c-d and e-f and g-h, respectively.

347 *Table 5. Relative influence of the operating conditions on the properties of the solid fraction*

Variable	R <sup>2</sup>	I.Term	T	t	C	Tt	TC	tC	TtC	T <sup>2</sup>	t <sup>2</sup>	C <sup>2</sup>	T <sup>2</sup> t	T <sup>2</sup> C	Tt <sup>2</sup>	TC <sup>2</sup>	T <sup>2</sup> t <sup>2</sup>
<b>Fibre analysis (wt.%)</b>																	
Cellulose	1	0.60	11.07 (11)	10.93 (6)	n.s.	1.03 (2)	-5.04 (10)	-3.36 (6)	3.37 (6)	10.46 (17)	10.34 (9)	n.s.	-11.97 (8)	-7.73 (6)	-14.87 (15)	-7.73 (15)	-4.83 (3)
Hemicellulose	1	0.24	-2.92 (11)	n.s.	n.s.	3.40 (12)	3.61 (12)	3.40 (12)	-3.41 (12)	2.84 (7)	n.s.	n.s.	-3.40 (12)	-3.61 (12)	-3.61 (5)	n.s.	3.37 (5)
Lignin	1	86.37	-16.13 (18)	-11.76 (16)	n.s.	-6.35 (9)	0.81 (1)	-0.91 (1)	n.s.	-14.45 (18)	-11.58 (12)	-2.66 (1)	19.96 (5)	11.87 (16)	26.74 (6)	n.s.	5.51 (2)
Proteins	0.99	13.55	4.76 (21)	n.s.	n.s.	1.92 (13)	0.63 (4)	0.88 (6)	n.s.	3.16 (4)	n.s.	2.74 (11)	-3.76 (26)	-3.76 (4)	-7.96 (6)	n.s.	-2.81 (4)
<b>Elemental analysis</b>																	
C (wt.%)	0.99	56.60	-2.32 (28)	-3.94 (26)	n.s.	0.61 (4)	n.s.	0.64	n.s.	2.00 (14)	n.s.	n.s.	7.69 (7)	1.03 (1)	6.01 (1)	n.s.	-5.12 (11)
H (wt.%)	0.9	6.14	n.s.	0.2 (1)	n.s.	n.s.	-0.09 (25)	n.s.	n.s.	-0.27 (2)	-0.13 (14)	n.s.	-0.19 (10)	n.s.	-0.06 (16)	n.s.	0.36 (32)
O (wt.%)	0.94	33.65	2.03	3.45 (9)	n.s.	n.s.	-1.90 (11)	-2.53 (16)	n.s.	n.s.	n.s.	n.s.	-4.84 (4)	-1.61 (17)	-3.25 (10)	n.s.	3.86 (20)
N (wt.%)	0.94	3.53	n.s.	n.s.	-0.41 (16)	0.50 (18)	-0.54 (19)	-0.53 (19)	n.s.	-0.57 (8)	-0.50 (3)	n.s.	n.s.	n.s.	n.s.	n.s.	1.40 (16)
HHV (MJ/kg)	0.98	23.11	-1.67 (28)	-0.89 (28)	n.s.	0.32 (5)	0.36	n.s.	n.s.	n.s.	n.s.	0.57 (9)	2.54 (4)	0.41 (7)	3.21 (1)	n.s.	-1.66 (11)
<b>Py-GC/MS (Area %)</b>																	
Hydrocarbons	0.94	11.78	-5.64 (27)	n.s.	n.s.	2.31 (16)	n.s.	-1.77 (12)	n.s.	2.85 (11)	n.s.	6.24 (2)	n.s.	n.s.	8.69 (6)	n.s.	-10.77 (25)
Carboxylic acids	0.92	51.79	10.16 (18)	7.82 (7)	n.s.	-7.48 (18)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-8.66 (9)	n.s.	-16.05 (4)	n.s.	9.54 (17)
Sugars	1	9.48	1.45 (11)	1.90 (1)	2.93 (9)	-2.31 (12)	n.s.	1.60 (9)	-1.21 (6)	2.81 (4)	3.11 (3)	-6.10 (22)	-1.25 (5)	-5.61 (13)	-3.30 (1)	n.s.	-2.88 (2)
Phenols	0.98	7.98	-5.85 (20)	n.s.	-8.40 (14)	3.32 (16)	-1.40 (7)	n.s.	n.s.	n.s.	n.s.	2.31 (4)	2.38 (12)	7.36 (16)	9.59 (2)	n.s.	-4.35 (10)
Nitrogen compounds	0.96	5.61	n.s.	-4.69 (13)	n.s.	2.60 (11)	-4.20 (17)	-3.78 (15)	4.49 (18)	n.s.	1.90 (8)	n.s.	n.s.	1.65 (7)	-2.67 (11)	n.s.	n.s.

348 n.s.: Non significant with 95% confidence

349 Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C

350 Coefficient TtC·T·t·C + Coefficient T<sup>2</sup>·T<sup>2</sup> + Coefficient t<sup>2</sup>·t<sup>2</sup> + Coefficient C<sup>2</sup>·C<sup>2</sup> + Coefficient T<sup>2</sup>t·T·t + Coefficient T<sup>2</sup>C·T<sup>2</sup>·C + Coefficient

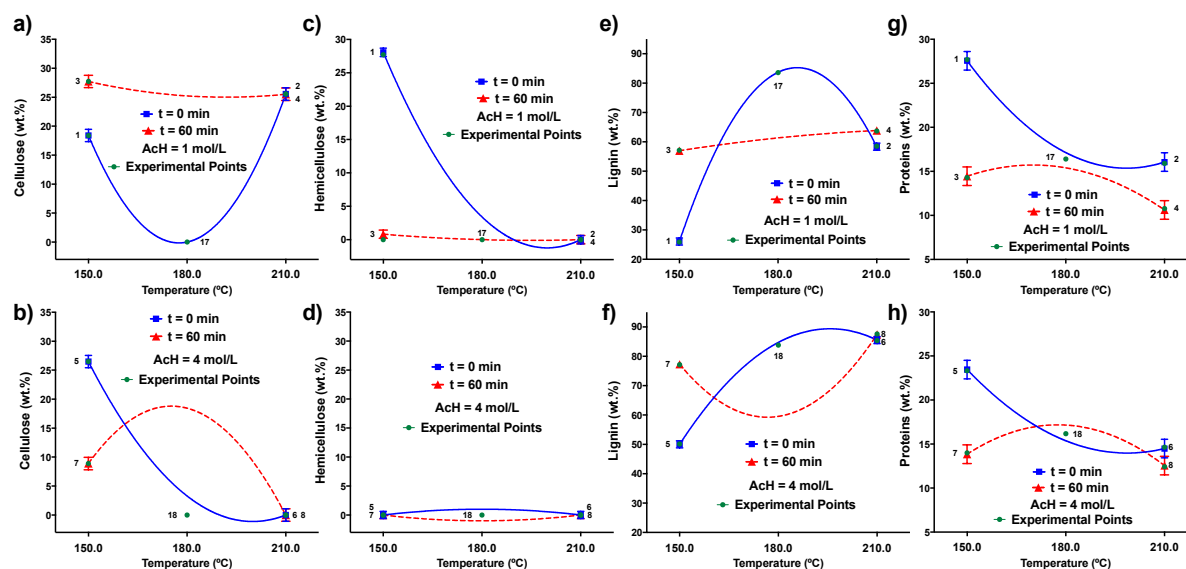
351 Tt<sup>2</sup>·T·t<sup>2</sup> + Coefficient TC<sup>2</sup>·T·C<sup>2</sup> + Coefficient T<sup>2</sup>t<sup>2</sup>·T<sup>2</sup>·t<sup>2</sup>

352 Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Pareto values represent the percentage

353 of the orthogonal estimated total value.

354

355 The effect of the temperature on the fibre analysis of the solid product depends on the reaction time  
 356 and the concentration of acetic acid. For a short reaction time (0 min) when a diluted (1 mol/L) acid  
 357 solution is used (Figure 2 a, c, e and g), an increase in the temperature from 150 to 180 °C leads to a  
 358 sharp decrease in the proportions of cellulose, hemicellulose and proteins together with a pronounced  
 359 increase in the proportion of lignin of the solid, where a maximum is reached. This allows the  
 360 production of relatively high purity lignin (85 wt.%) from rapeseed meal; proteins being the only  
 361 impurity presents in the solid. This development accounts for the solubilisation of the cellulosic and  
 362 hemicellulosic matter without significant lignin solubilisation during microwave **hydrothermal**  
 363 **treatment** [13]. In addition, the protein content of the solid decreases due to the decomposition of the  
 364 proteins into liquid and gaseous products via decarboxylation and deamination reactions [2, 3].



365

366 Figure 2. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 367 and the highest (4 mol/L) acetic acid concentration for the proportions of cellulose (a and b),  
 368 hemicellulose (c and d), lignin (e and f) and proteins (g and h). Bars are LSD intervals with 95%  
 369 confidence.

370

371 A further increase in the temperature up to 210 °C increases and decreases the proportion of cellulose  
 372 and lignin, respectively, without altering the relative amounts of hemicellulose and proteins. An  
 373 increase in the temperature might promote the formation of humins and char from the sugars produced  
 374 during the dissolution of cellulose and hemicellulose [22-24]. The formation of these macromolecules  
 375 can occur from the furfural obtained from sugars dehydration, via aldol addition followed by  
 376 condensation or polymerisation [25-31]. Furthermore, sugars monomers can also react with other  
 377 liquid intermediates such as 5-hydroxymethyl-2-furancarboxaldehyde (HMF) by cross-polymerisation  
 378 [25, 26, 28, 30]. The presence of humins and char can interfere with the chemical titration method. In  
 379 particular, humins might have been identified as cellulose in the analysis, thus producing an artificial  
 380 increase in the cellulose content of the solid [18].

381

382 The comparison between Figure 2 a, c, e and g with b, d, f and h shows that an increase in the  
 383 concentration of acetic acid from 1 to 4 mol/L (for 0 min reaction time) decreases the proportion of  
 384 hemicellulose and proteins and increases the relative amount of lignin regardless of the temperature  
 385 (150-210 °C) due to the positive catalytic effect of acetic acid in the process. A similar trend was also  
 386 observed by Long et al. [17] and Zoia et al. [16], who reported the positive catalytic effect of H<sub>2</sub>SO<sub>4</sub>

387 and HCl, respectively, during lignin isolation from biomass. In addition, and very interestingly, in this  
388 work acetic acid also exerts an inhibitory effect on humins and char formation, which allows a  
389 polysaccharide-free lignin, with relatively high purity (88 wt.%) to be produced between 190 and 210  
390 °C.

391

392 The reaction time modifies the effects of the temperature and concentration of acetic acid on the fibre  
393 composition of the solid product. When a diluted acid solution is used, an increase in the reaction time  
394 from 0 to 60 min leads to a decrease in the proportions of hemicellulose and proteins together with a  
395 decrease in the relative amount of cellulose of the solid (Figure 2 a, c, e and g). Long reaction times  
396 favours the solubilisation of cellulose and hemicellulose even at the lowest temperature used in this  
397 work (150 °C) due to the efficiency of microwave heating [13]. However, this also produces the  
398 formation of humins and char from some of the species solubilised in the liquid product, which leads  
399 to an artificial increase in the relative amount of the cellulose content of the solid. The effect of the  
400 reaction time on the proportion of lignin depends on the temperature. While an increase in the reaction  
401 time increases the relative amount of lignin between 150 and 165 °C, this same increment decreases  
402 the proportion of lignin between 165 and 200 °C. At low temperature the formation of humins and  
403 char takes place to a lesser extent, which result in a higher proportion of lignin in the solid.  
404 Conversely, elevated temperatures together with long reaction times increase the production of humins  
405 and char [22-24]. As a result, when a long reaction time is used, the effect of the temperature on the  
406 fibre analysis of the solid is very weak, as the positive kinetic effect of the reaction time can mask the  
407 effect of the temperature.

408

409 An increase in the concentration of acetic acid from 1 to 4 mol/L when a long reaction time is used  
410 exerts a significant effect on the proportion of cellulose and lignin, without modifying the proportions  
411 of hemicellulose and proteins (Figure 2 a, c e and g vs. b, d f and h, respectively). As described earlier,  
412 the formation of humins and char is inhibited when a concentrated (4 mol/L) solution of acetic acid is  
413 used, and therefore, an increase in the reaction time from 0 to 60 min leads to an increase in the  
414 relative amount of lignin together with a decrease in the proportion of cellulose in the solid product.

415 This is in agreement with the work conducted by van Zandvoort et al. [32], who reported a decrease in  
416 humins formation when increasing the concentration of sulphuric acid during the valorisation of  
417 lignocellulosic biomass. In addition, the effect of the temperature on the proportions of hemicellulose  
418 and proteins is negligible because the effect of the temperature is masked by the positive kinetic effect  
419 of the reaction time, as described earlier. Conversely, the temperature exerts a significant influence on  
420 the proportion of cellulose and lignin when a long reaction time and a concentrated (4 mol/L) solution  
421 of acetic acid are used. Between 150 and 180 °C, the proportion of cellulose and lignin increases and  
422 decreases respectively, while the opposite trend takes place between 180 and 210 °C; i.e. an increase  
423 in the relative amount of lignin together with a decrease in the proportion of cellulose due to the lesser  
424 humins formation occurring when a concentrated acid solution is used.

425

### 426 *3.2.2 Elemental analysis*

427 The relative amounts (wt.%) of C, H, O and N in the solid fraction shifted between 46-63, 5.8-6.4, 28-  
428 42 and 2-6, varying the higher heating value (HHV) of the solid between 19 and 26 MJ/kg. According  
429 to the cause-effect Pareto Analysis, the proportion of C and the HHV of the solid are strongly affected  
430 by the temperature (both linear and quadratic effects) and the reaction time. The interactions of the  
431 temperature with the reaction time and concentration largely influences the relative amounts of H and  
432 O, while the concentration of acetic acid greatly influences the proportion of N in the solid. Figure 3  
433 shows the effect of the operating variables and the most important interactions detected with the  
434 ANOVA analysis on the elemental analysis and HHV of the solid. Figure 3 a and b plots the effects of  
435 the temperature on the relative amount of C for 0 and 60 min reaction time for the lowest (1 mol/L)  
436 and the highest (4 mol/L) acetic acid concentration, respectively. These effects are also shown for the  
437 relative amounts of H, O and N and the HHV in Figure 3 c-d and e-f, g-h and i-j, respectively.

438

439 The effect of the temperature on the elemental analysis of the solid fraction depends on the reaction  
440 time and the concentration of acetic acid. When a short reaction time is used, the concentration of  
441 acetic acid does not greatly influence the elemental analysis or the HHV of the solid and similar  
442 results are obtained regardless of the acid concentration. In particular, for a short reaction time (0 min),

443 an initial increase in the temperature between 150 and 180 °C increases the proportion of C and the  
444 HHV and decreases the relative amounts of H and O. These variations are accounted for by the  
445 solubilisation of cellulose and hemicellulose, which logically results in a solid product with lower and  
446 higher O and C contents, respectively [33]. In addition, this trend is in good agreement with the results  
447 reported by Long et al. [13]. Conversely, further increasing the temperature up to 210 °C has the  
448 opposite effect; i.e. the amount of C and the HHV decrease and the proportions of H and O in the solid  
449 increase. This development is believed to be the consequence of the formation of humins under these  
450 operating conditions as described earlier. In addition the variations observed in the elemental analysis  
451 of the spent solid are in good agreement with those reported by van Zandvoort et al. [32]. The  
452 temperature does not influence the N content of the solid when short reaction times are used, while a  
453 small increase takes place when the temperature increases from 150 to 210 °C and a long reaction time  
454 is used.

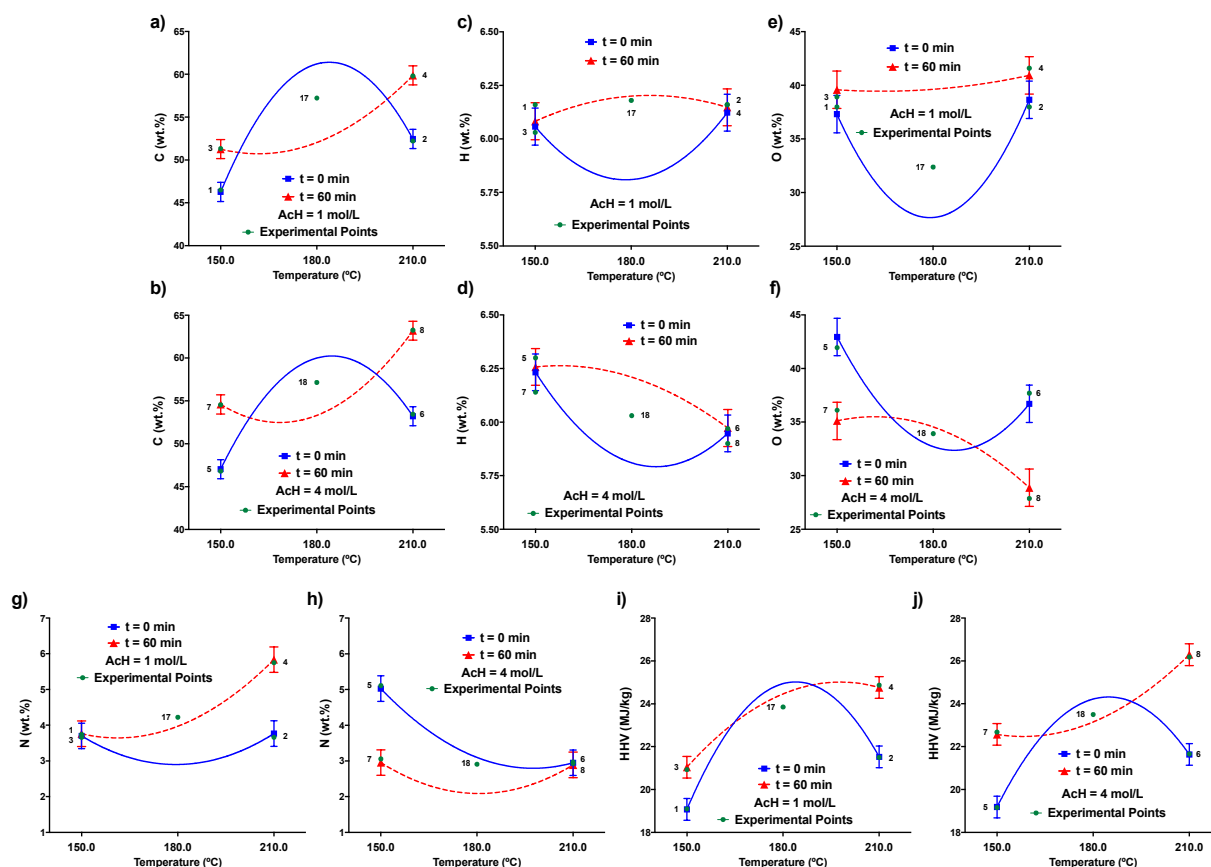
455

456 The effect of the reaction time depends on the concentration of acetic acid. When a diluted acid  
457 solution (1 mol/L) is used, an increase from 0 to 60 min increases the relative amounts of H, O and N  
458 and decreases the proportion of C in the solid when a temperature ranging from 165 to 195 °C is used.  
459 These developments for the elemental analysis are the consequence of the solubilisation of cellulose  
460 and hemicellulose, which result into a solid fraction with a higher lignin proportion [17, 32]. In  
461 addition, an increase in the reaction time progressively decreases the effect of the temperature on the  
462 elemental analysis and the HHV of the solid product due to the positive kinetic effect of the reaction  
463 time [13]. As a result, when a reaction time of 60 min is used, the reaction temperature does not affect  
464 the proportions of H and O, while a small increase occurs for the relative amounts of C and N when  
465 increasing the temperature from 150 to 210 °C. The HHV slightly increases between 150 and 180 °C  
466 and remains steady with a further increase up to 210 °C.

467

468 An increase in the concentration of acetic acid when a long reaction time is used modifies the effect of  
469 the temperature on the proportions of H, O and N and the HHV of the solid. In this case, an increase in  
470 the temperature from 150 to 210 °C decreases the proportions of H and O. This leads to a decrease in

471 the HHV of the solid. The positive inhibitory effect of acetic acid on humins and char formation when  
 472 high acid concentrations are used accounts for this circumstance; thus allowing the production of a  
 473 solid with higher lignin purity. As a result, when a concentrated acid solution is used, increasing the  
 474 reaction time from 0 to 60 min when temperatures higher than 180 °C are used produces a decrease in  
 475 the proportion O and increases the HHV of the solid.



476  
 477 Figure 3. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 478 and the highest (4 mol/L) acetic acid concentration for the concentrations of C (a and b), H (c and d),  
 479 O (e and f), N (g and h) and HHV (i and j). Bars are LSD intervals with 95% confidence.  
 480

481  
 482 *3.2.3 Pyrolysis GC-MS characterisation*

483 It is known that the total amount of compounds produced during the pyrolysis of biomass that can be  
 484 identified by GC-MS usually represents about 20 to 22 wt.% of the total [34], as many lignin-derived  
 485 compounds and proteins cannot be analysed due to their high molecular masses. However, useful  
 486 trends can be retrieved from this analysis, and a comparison can be established. The pyrolysis GC-MS  
 487 characterisation of the solid reveals that the solid product decomposes in a mixture of phenols (1-

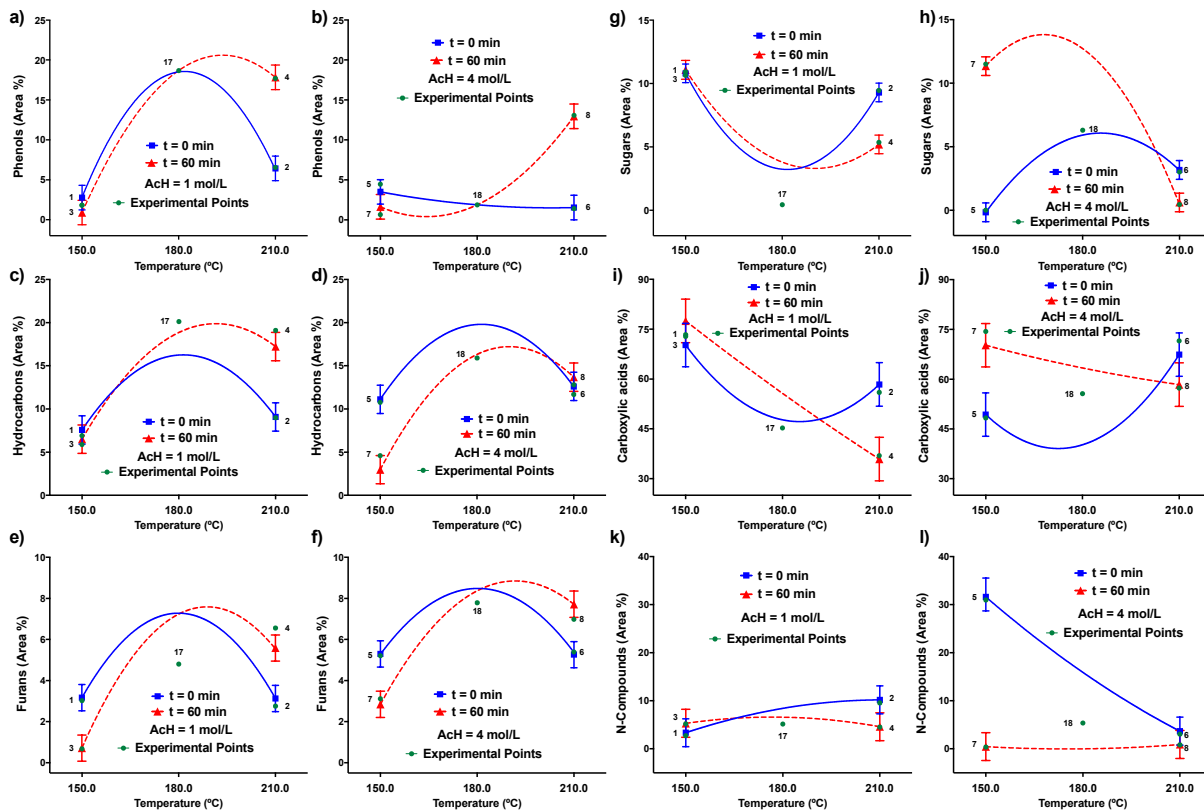
488 19%), sugars (0-15%), nitrogen compounds (0-31%), carboxylic acids (37-75%), hydrocarbons (4-  
489 20%) and furans (1-8%). Phenols include phenol, 3-methyl phenol, phenol 2,6-dimethoxy, 2,4-  
490 dimethoxyphenol, catechol and 3-ter-butyl-4-hydroxyanisole. Sugars comprise 1,6 anhydro beta D-  
491 glucopyranose, melezitose and D-mannose. Nitrogen compounds largely include pyridine, pyrrole, 1-  
492 5-dimethyl-1H-Pyrazole and acetamide-1-methyl-1H-pyrrole. Carboxylic acids are made up of n-  
493 hexadecanoic acid and oleic acid while hydrocarbons include linear aliphatic hydrocarbons such as  
494 butane and cyclic hydrocarbons such as R-limonene and 1,3,5-cycloheptatriene. Furans are made up of  
495 furfural, 2-furanmethanol, furan 2- and 3-methyl and furan 2,5-dimethyl.

496  
497 According to the cause-effect Pareto analysis (Table 5), the proportions of hydrocarbons and  
498 carboxylic acids in the liquid are strongly influenced by the temperature and its interaction with the  
499 reaction time. Sugars and phenols are strongly influenced by the temperature, acid concentration and  
500 the interaction between the temperature and the reaction time, while the reaction time and the  
501 interactions of the temperature with both the concentration and reaction time strongly influence the  
502 proportion of nitrogen compounds. Figure 4 a and b plots the effects of the temperature on the relative  
503 amount of phenols for 0 and 60 min reaction time for the lowest (1 mol/L) and the highest (4 mol/L)  
504 acetic acid concentration, respectively. These effects are also shown for the relative amounts of  
505 hydrocarbons, furans, sugars, carboxylic acids and nitrogen compounds in Figure 4 c-d and e-f, g-h, i-j  
506 and k-l, respectively.

507  
508 The effect of the temperature on the Py-GC/MS analysis of the solid fraction depends on the reaction  
509 time and the concentration of acetic acid used in the experiments. For a short reaction time and a low  
510 concentration of acid (0 min and 1 mol/L), an initial increase in the temperature from 150 to 180 °C  
511 increases the proportions of phenols, hydrocarbons and furans and decreases the relative amounts of  
512 sugars and carboxylic acids. Under such conditions, the proportion of N-compounds in the solid is  
513 very low and unaffected by the temperature. The solubilisation of the cellulosic and hemicellulosic  
514 matter of rapeseed meal during the microwave treatment accounts for the decrease in sugars and

515 carboxylic acids in the solid fraction [13]; thus increasing the proportions of phenols and  
 516 hydrocarbons in the solid.

517



518

519 Figure 4. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 520 and the highest (4 mol/L) acetic acid concentration for the concentrations of phenols (a and b),  
 521 hydrocarbons (c and d), furans (e and f), sugars (g and h), carboxylic acids (i and j) and N-compounds  
 522 (k and l). Bars are LSD intervals with 95% confidence.

523

524 In addition, when a diluted acid solution (1 mol/L) is used, the effect of the reaction time on the  
 525 thermal decomposition of the solid fraction depends on the temperature. Between 150 and 190 °C, the  
 526 effect of the reaction time is negligible; however, an increase in the reaction time (from 0 to 60 min)  
 527 between 190 and 210 °C leads to an increase in the relative amount of phenols, hydrocarbons and  
 528 furans together with a substantial decrease in the proportion of sugars and carboxylic acids. At low  
 529 temperatures (150-190°C), the microwave power achieved during the experiments is not sufficient to  
 530 remove the residual cellulose and hemicellulose matter strongly connected with the lignin content in  
 531 rapeseed meal. However, this increase in time at high temperatures (190-210 °C) increases cellulose  
 532 and hemicellulose solubilisation, which leads to the formation of a solid product with a higher

533 proportion of lignin. This increases the proportion of phenols and decreases the relative amount of  
534 sugars in the solid [13, 17]. As a result, when a long reaction time (60 min) is used, the proportions of  
535 phenols, sugars and hydrocarbons remain relatively steady between 190 and 210 °C, while a the  
536 proportions of furans and carboxylic acids decrease slightly and the relative amount of sugars  
537 increases.

538

539 An increase in the concentration of acetic acid from 1 to 4 mol/L (Figures 4 a, c, e, g, i and k v.s. b, d,  
540 f, h, j and l) modifies the effect of the temperature and reaction time on the relative amount of some of  
541 the decomposition products produced during the Py-GC/MS analysis of the solid product. On the one  
542 hand, increasing the concentration of acetic acid in the experiments does not greatly modify the effects  
543 of the temperature or reaction time on proportions of hydrocarbons, furans and carboxylic acids. In all  
544 cases, increasing the concentration of acetic acid from 1 to 4 mol/L increases the proportion of furans  
545 regardless of the reaction time. This increase in the proportion of furans might account for the lesser  
546 production of humins, as these latter compounds can be produced from the auto-condensation of the  
547 former [32]. On the contrary, two different developments occur for the relative amounts of  
548 hydrocarbons and carboxylic acids. While, an increase in the concentration of acetic acid slightly  
549 increases the proportion of hydrocarbons and decreases the relative amount of carboxylic acids when  
550 short reaction times are used; the use of long reaction times produces the opposite effect, i.e. the  
551 relative amount of hydrocarbons and carboxylic acid decreases and decreases, respectively. When  
552 using short reaction times, an increase in the acetic acid concentration might favour the solubilisation  
553 of the fatty acid content of the solid. Conversely, the combination of long reaction times together with  
554 high acid concentrations can increase the solubilisation of hydrocarbons [3, 35, 36].

555

556 On the other hand, the relative amounts of phenols, sugars and N-compounds are strongly influenced  
557 by the concentration of acetic acid used in the experiments and different developments occur  
558 depending on the temperature and the reaction time. For a short reaction time (0 min), an increase in  
559 the concentration of acetic acid reduces the proportion of phenols and sugars and sharply increases the  
560 relative amount of N-compounds, especially between 150 and 180 °C. These variations confirm the

561 positive catalytic effect of acetic acid on the solubilisation of the cellulosic and hemicellulosic  
562 contains of rapeseed meal as described earlier. Conversely, the acid has a lower effect on the  
563 solubilisation and/or removal of the protein contain of the solid, and therefore, a solid product with a  
564 higher proportion of proteins is produced. Under these conditions (0 min and 4 mol/L acetic acid) the  
565 temperature does not significantly influences the concentration of phenols. The proportion of N-  
566 compounds is very high at low temperature and progressively decreases when the temperature  
567 increases up to 210 °C.

568

569 An increase in the reaction time significantly increases the concentration of sugars and decreases the  
570 proportion of N-compounds in the solid. Increasing the temperature and/or the reaction time of the  
571 experiments might produce the degradation of the proteins present in the residue by deamination  
572 (resulting in the formation of ammonia) and decarboxylation (which produces carboxylic acids and  
573 amines) [3, 37-39]. The proportion of phenols is negligible between 150 and 190 °C, and increases  
574 sharply when the temperature increases from 190 to 210 °C due to the lesser humins formation  
575 occurring when a concentrated acid solution is used. As a result, for a long reaction time (60 min), the  
576 temperature does not greatly affect the proportions of phenols and sugars between 150 and 190 °C,  
577 while a sharp increase together a pronounced decrease takes place for the proportion of phenols and  
578 sugars, respectively, between 190 and 210 °C.

579

### 580 **3.3 Effect of the operating conditions on the liquid properties**

#### 581 *3.3.1 Chemical composition*

582 The liquid product consists of a mixture of oligo- (DP2-6 and DP>6) and mono/di- saccharides,  
583 carboxylic acids, ketones, furans and nitrogen compounds; their relative amount (in carbon basis, C-  
584 wt.%) in the liquid product varying as follows: 33-51%, 0-3%, 0-6%, 40-62%, 0-1%, 0-3%, 0-3%.  
585 Saccharides include cellobiose, xylose, glucose, fructose, mannose, arabinose, rhamnose and  
586 levoglucosan. Carboxylic acids comprise lactic, formic, levulinic, glucuronic, galacuronic and acetic  
587 acids. Acetic acid is the major compound for this family as it was initially loaded and used as a  
588 catalyst. In all the cases the amount of this acid was fairly similar to the initial amount initially loaded

589 in the experiments. This indicates that acetic acid decomposition (removal) and secondary reactions  
 590 (production) did not take place to a great extent and/or they compensated for each other. Ketones and  
 591 furans are made of levoglucosenone and 5-hydroxymethyl-2-furancarboxaldehyde (HMF) and furfural,  
 592 respectively. Nitrogen compounds include 3-pyridinol and 6 methyl-3-pyridinol.

593

594 Table 6. Relative influence of the operating conditions on the properties of the liquid fraction

Variable	R <sup>2</sup>	I.Term	T	t	C	Tt	TC	tC	TtC	T <sup>2</sup>	t <sup>2</sup>	C <sup>2</sup>	T <sup>2</sup> t	T <sup>2</sup> C	Tt <sup>2</sup>	TC <sup>2</sup>	T <sup>2</sup> t <sup>2</sup>
<b>Chemical composition (C-wt.%)</b>																	
Oligosaccharides DP>6	0.95	45.44	n.s.	n.s.	-2.36 (6)	-1.05 (13)	-2.08 (15)	-1.88 (13)	1.38 (10)	n.s.	n.s.	n.s.	2.15 (15)	1.97 (6)	3.02 (21)	n.s.	-1.35 (7)
Oligosaccharides DP2-6	0.99	0.16	0.70 (10)	0.34 (7)	-0.20 (15)	-0.55 (16)	0.28 (8)	n.s.	0.30 (9)	0.54 (12)	0.24 (10)	n.s.	-0.44 (8)	-0.31 (4)	-0.99 (1)	n.s.	n.s.
Saccharides	0.99	1.01	1.25 (9)	1.10 (7)	-0.76 (19)	-1.31 (17)	n.s.	0.44 (6)	0.98 (13)	0.75 (11)	0.75 (7)	n.s.	-1.39 (5)	-0.70 (4)	-1.73 (4)	n.s.	n.s.
Carboxylic acids	0.94	51.57	-2.52 (11)	n.s.	4.84 (19)	3.00 (15)	2.01 (10)	1.78 (9)	-2.44 (4)	n.s.	n.s.	-2.16 (3)	-2.17 (11)	-1.92 (4)	n.s.	n.s.	1.77 (4)
Ketones	0.94	0.27	0.05 (2)	0.10 (4)	-0.09 (20)	-0.14 (27)	n.s.	n.s.	0.07 (13)	-0.18 (7)	-0.16 (6)	n.s.	-0.11 (6)	n.s.	n.s.	n.s.	0.26 (15)
Furans	0.96	1.76	n.s.	-0.44 (5)	-0.83 (11)	-0.24 (9)	n.s.	n.s.	n.s.	-1.61 (29)	-1.02 (10)	n.s.	0.46 (9)	0.71 (12)	n.s.	n.s.	1.37 (16)
Nitrogen compounds	1	0.35	-0.37 (16)	-0.13 (13)	-0.66 (17)	0.27 (9)	-0.29 (10)	-0.24 (8)	-0.19 (7)	n.s.	-0.08 (1)	0.50 (9)	0.50 (3)	0.27	0.81 (1)	n.s.	-0.19 (1)
<b>Elemental analysis</b>																	
C (wt.%)	1	42.76	0.40 (1)	n.s.	21.71 (90)	-1.01 (4)	-0.39 (1)	n.s.	n.s.	-1.21 (1)	n.s.	n.s.	0.54 (2)	n.s.	n.s.	n.s.	n.s.
H (wt.%)	1	4.08	n.s.	n.s.	0.54 (87)	-0.05 (2)	n.s.	0.09 (1)	0.10 (1)	n.s.	n.s.	-0.77 (4)	-0.12 (2)	0.20 (1)	n.s.	n.s.	-0.17 (1)
O (wt.%)	1	52.89	n.s.	n.s.	-22.07 (90)	1.36 (4)	n.s.	-0.46 (1)	-0.38	1.30 (3)	n.s.	1.39	n.s.	-0.65 (2)	n.s.	-0.65	n.s.
HHV (MJ/kg)	1	14.30	0.28 (1)	n.s.	10.53 (82)	-0.41 (3)	-0.30 (2)	n.s.	n.s.	-0.48 (5)	n.s.	-1.16 (5)	0.32 (2)	n.s.	n.s.	n.s.	n.s.

595 n.s: Non significant with 95% confidence

596 Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·Tt + Coefficient TC·TC Coefficient tC·tC  
 597 Coefficient TtC·Tt·C + Coefficient T<sup>2</sup>·T<sup>2</sup> + Coefficient t<sup>2</sup>·t<sup>2</sup> + Coefficient C<sup>2</sup>·C<sup>2</sup> + Coefficient T<sup>2</sup>t·T<sup>2</sup>t + Coefficient T<sup>2</sup>C·T<sup>2</sup>C + Coefficient  
 598 T<sup>2</sup>·T·t<sup>2</sup> + Coefficient TC<sup>2</sup>·T·C<sup>2</sup> + Coefficient T<sup>2</sup>t<sup>2</sup>·T<sup>2</sup>t<sup>2</sup>

599 Numbers in brackets indicate the percentage Pareto influence of each factor on the response variable. Pareto values represent the percentage  
 600 of the orthogonal estimated total value.

601

602 The cause-effect Pareto analysis (Table 6) shows that the proportion of DP>6 oligosaccharides is  
 603 strongly influenced by the interaction between the temperature and reaction time. The temperature  
 604 (linear and quadratic) and concentration followed by the reaction time significantly influence the  
 605 proportions of DP 2-6 oligosaccharides and (mono/di) saccharides. The proportion of carboxylic acids  
 606 is strongly affected by the concentration, while the concentration of furans depends on the temperature,  
 607 acid concentration and the interaction between these two latter variables. The temperature, time and  
 608 concentration have a similar influence on the proportion of nitrogen compounds. Figure 5 shows the  
 609 effect of the operating variables and the most important interactions detected with the ANOVA

610 analysis on the most abundant compounds present in the liquid phase. In particular, Figure 5 a and b  
611 illustrates the effects of the temperature on the proportion of oligosaccharides (DP>6) for 0 and 60  
612 min reaction time for the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentrations,  
613 respectively. These effects are also shown for the relative amounts of oligosaccharides (DP2-6),  
614 saccharides and carboxylic acids in Figure 5 c-d and e-f and g-h, respectively.

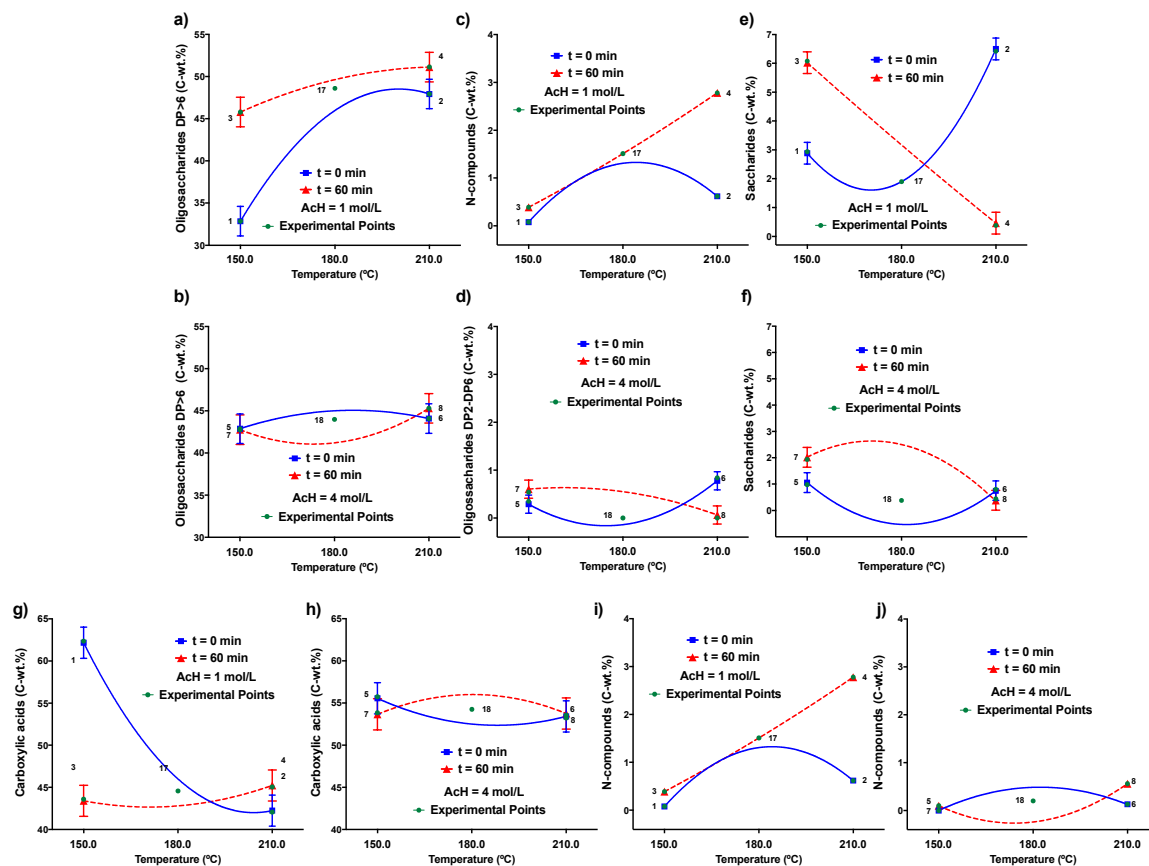
615

616 The effect of the temperature on the chemical composition of the liquid phase depends on the reaction  
617 time and the concentration of acetic acid. In this respect, while the reaction time has a very important  
618 influence for diluted acid solution, it has a negligible influence when the concentration of acetic acid  
619 increases up to 4 mol/L. When a diluted acetic acid concentration (1 mol/L) and short reaction time  
620 are used, an increase in the reaction time from 150 to 190 °C sharply increases and decreases the  
621 relative amounts of oligosaccharides and carboxylic acids, respectively. These developments account  
622 for the progressive dissolution of cellulose and hemicellulose in the liquid, which produces an increase  
623 in the proportion of oligosaccharides and therefore decreases the relative amount of carboxylic acids  
624 (largely the acetic acid used in the experiment). In addition, the proportions of oligosaccharides DP2-6  
625 and saccharides slightly decrease, while the relative amount of N-compounds increases. A further  
626 increase in the temperature up to 210 °C exerts a negligible effect on the proportions of  
627 oligosaccharides and carboxylic acids, while the relative amounts of DP2-6 oligosaccharides and  
628 saccharides increase slightly and the N-compounds decreases. The decomposition of a small amount  
629 of oligosaccharides into saccharides when the temperature increases and the progressive  
630 transformation of N-compounds into gases at high temperature [3, 37-39] can explain these trends.

631

632 An increase in the reaction time when a diluted acid solution (1 mol/L) is used has two different  
633 consequences for the composition of the liquid phase depending on the temperature. On the one hand,  
634 at temperatures lower than 190 °C, an increase in the reaction time from 0 to 60 min significantly  
635 increases the proportions of oligo (DP>6 and DP 2-6) saccharides and decreases the relative amount of  
636 carboxylic acids, without modifying the relative amount of N-compounds. An increase in the reaction

637 time produces a greater spread of the hydrolysis reactions, which experimentally increases the  
 638 proportion of cellulose and hemicellulose derived species in the liquid; thus decreasing the relative  
 639 amount of carboxylic acid (mostly acetic acid). On the other hand, when a temperature between 190  
 640 and 210 °C is used, this same increase in time does not modify the proportions of DP>6  
 641 oligosaccharides or carboxylic acids. These variations are the consequence of the weaker influence of  
 642 the temperature in the hydrolysis of saccharides when long reaction times are used [13]. In addition,  
 643 the proportions of DP2-6 oligosaccharides and saccharides decrease and the relative amount of N-  
 644 compounds increases due to the solubilisation of proteins in the liquid when long reaction times are  
 645 used [3, 37-39]. This lower influence of the temperature on the composition of the liquid phase  
 646 produces that an increase in the temperature from 150 to 210 °C does not greatly modify the  
 647 proportions of oligosaccharides (DP>6) or carboxylic acids when a 60 min reaction time is used.



648

649 **Figure 5.** Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 650 and the highest (4 mol/L) acetic acid concentration for the concentrations of oligosaccharides DP>6 (a  
 651 and b), oligosaccharides DP 2-6 (c and d), saccharides (e and f), carboxylic acids (g and h) and N-  
 652 compounds (i and j). Bars are LSD intervals with 95% confidence.

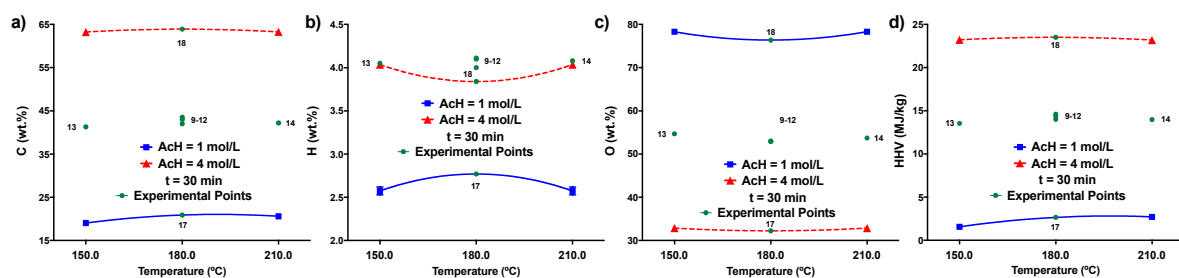
653 An increase in the concentration of acid (Figures 5 a, c, e and g vs. b, d, f and h) diminishes the  
 654 influence of the temperature and reaction time on the composition of the liquid phase as stated earlier.  
 655 For a 4 mol/L acetic acid concentration, oligosaccharides and carboxylic acids are the most abundant  
 656 compounds in the liquid phase, their composition being around 43 C-wt.% and 55 C-wt.%,  
 657 respectively, regardless of the temperature and reaction time. Furthermore, the relative amounts of  
 658 oligosaccharides (DP2-6) and saccharides are very low (< 2%) and the effects of the temperature and  
 659 reaction time, although being statistically significant are not important from a practical point of view.

660

### 661 3.3.2 Elemental analysis and HHV

662 The concentrations of C, H and O (in dry basis) in the liquid fraction vary by 18-65%, 31-80% and 2-  
 663 4%, respectively. This varies the HHV of the liquid between 1 and 24 MJ/kg of dried suspension. The  
 664 elemental analysis and HHV of the liquid is mostly influenced by the concentration of acetic acid  
 665 (with an influence higher than 82% for all these variables); the effects of the temperature and reaction  
 666 time being either statistically insignificant or negligible from a practical point of view. Figure 6 shows  
 667 the effect of the concentration on the elemental analysis and HHV of the liquid for a 30 min reaction  
 668 time as a function of the temperature.

669



670

671 Figure 6. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L)  
 672 and the highest (4 mol/L) acetic acid concentration for the concentrations of C (a), H (b), O (c) and  
 673 HHV (d) at 30 min reaction time. Bars are LSD intervals with 95% confidence.

674

675 Regardless of the temperature or reaction time, the concentration of acetic acid exerts the same  
 676 influence on the elemental analysis and HHV of the liquid phase. Increasing the concentration of  
 677 acetic acid from 1 to 4 mol/L leads to an increase in the proportions of C and H together with a  
 678 decrease in the O content of the liquid. This produces a substantial increase in the HHV of the liquid.

679 An increase in the concentration of acetic acid increases the solubilisation of rapeseed meal due to the  
 680 positive catalytic effect of the acid in the process. The original biomass has similar C and H contents  
 681 than acetic acid but a lower proportion of O. Therefore, the progressive addition of acetic acid in the  
 682 liquid phase prior to the experiment produces an increase in the proportions of C and H together with a  
 683 decrease in the relative amount of O of the hydrolysates.

684

### 685 3.4 Theoretical optimisation

686 Optimum conditions were sought for the selective co-production of high purity lignin and soluble  
 687 oligosaccharides from rapeseed meal making use of the experimental models developed. The predicted  
 688  $R^2$  of all the models are greater than 0.90, which allows their use for prediction purposes within the  
 689 range of study considered in this work.

690

691 Table 7. Theoretical optimisation: operating conditions and response variables.

692

Variables	Objective	Interval of variation	Relative importance (1-5)	Optimum Theoretical	Optimum Experimental
T (°C)	minimise	150-210	1	186	
t (min)	minimise	0-60	5	0	
CH <sub>3</sub> COOH (mol/L)	minimise	1-4	3	1	
Gas yield (%)	none	0-100		2	2
Liquid yield (%)	none	0-100		62	63
Solid yield (%)	none	0-100		36	35
<b>Solid fibre and elemental analyses</b>					
Cellulose (wt.%)	minimise	0-100	5	0	0
Hemicellulose (wt.%)	minimise	0-100	5	0	0
Lignin (wt.%)	maximise	0-100	5	85	86
Proteins (wt.%)	minimise	0-100	5	16	14
C (wt.%)	none	0-100		61	62
H (wt.%)	none	0-100		6	6
O (wt.%)	none	0-100		28	29
N (wt.%)	none	0-100		3	3
<b>Liquid composition (C-wt.%)</b>					
Oligosaccharides (DP>6)	none	0-100		47	49
Oligosaccharides (DP 2-6)	none	0-100		2	2
Saccharides	none	0-100		2	2
Ketones	none	0-100		0	0
Furans	none	0-100		2	2
Carboxylic acids	none	0-100		44	43
N-compounds	none	0-100		2	2

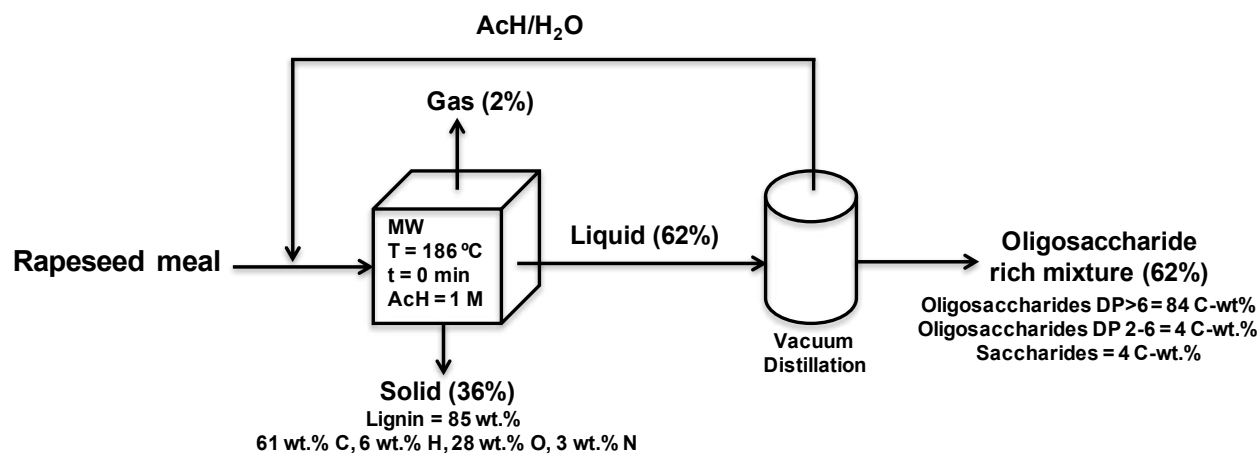
693

694 The optimisation comprises the maximisation of the lignin content together with the minimisation of  
 695 the proportions of cellulose, hemicellulose and proteins in the solid. In addition, the temperature, time  
 696 and acetic acid concentration were also minimised. To meet this objective, a solution that strikes a  
 697 compromise between the optimum values for all the response variables was sought and a relative

698 importance (from 1 to 5) was given to each of the objectives in order to come up with a solution that  
 699 satisfies all the criteria. Table 7 lists the relative importance assigned to each variable as well as the  
 700 criteria used in the whole optimisation.

701  
 702 Taking these conditions into account, the optimisation predicts an optimum at 186 °C using a  
 703 concentration of acetic acid of 1 mol/L for a **total reaction time of 2 min; i.e. only the ramping time (2**  
 704 **min ramping with a holding time of 0 min)**. Under such conditions, it is possible to selectively convert  
 705 36% of the original feedstock into relatively high purity (85 wt.%) lignin; the rest (63%) being  
 706 converted into a mixture of soluble oligosaccharides containing the acetic acid used in the experiment.  
 707 This indicates that all the lignin and the vast majority of the proteins initially presented in the biomass  
 708 remained in the solid during the isolation process. The elemental analysis of the lignin produced  
 709 (without taking the N content into account) is very similar to the results reported by other authors  
 710 addressing lignin isolation from other types biomass [13, 14, 17]. In addition, to increase the  
 711 effectiveness and sustainability of this process, acetic acid can be recovered from the oligosaccharide  
 712 solution by vacuum distillation (for example) and used again for further experiments. This strategy,  
 713 shown in Figure 7, allows the simultaneous production of sugar-free, relatively high purity lignin (85  
 714 wt.%) along a sugar rich solid fraction comprising oligo- and mono/di-saccharides (92 C-wt.%) with  
 715 several applications in the chemical and biological industries.

716



717  
 718  
 719 Figure 7. Schematic diagram for the simultaneous production of lignin and oligosaccharides  
 720

#### 721 4. Conclusions

722 This work addresses a novel microwave-assisted, acid catalysed process for the selective production of  
723 lignin and oligosaccharides from rapeseed meal, analysing the effects of the operating conditions on  
724 the yields and the most important properties of each fraction. The most important conclusions are  
725 summarised as follows.

726 1. The gas, liquid and solid yield are significantly influenced by the operating conditions, their yields  
727 varying by 0-18%, 22-64% and 34-74%, respectively. Increasing the temperature or time increased the  
728 liquid yield and decreased the solid yield due to the progressive solubilisation of the cellulosic and  
729 hemicellulosic contents of the original feedstock, which resulted into the production of a rich lignin  
730 solid in some cases. Acetic acid exerted a positive catalytic effect on the process promoting cellulose  
731 and hemicellulose solubilisation and preventing the formation of humins.

732 2. The solid fraction consisted of high purity lignin (26-88 wt.%) together with unreacted cellulose (0-  
733 28 wt.%), hemicellulose (0-28 wt.%) and proteins (11-28 wt.%). An increase in the temperature or  
734 reaction time decreased the amount of cellulose and hemicellulose and increased the lignin purity of  
735 the solid when temperatures lower than 190 °C were used. A further increase up to 210 °C led to a  
736 decrease in the lignin content of the solid due to the formation of humins. However, acetic acid  
737 displayed an inhibitory effect on humins formation, which allowed high temperatures and reaction  
738 times to be used when using concentrated acid solutions.

739 3. The relative amounts (wt.%) of C, H, O and N in the solid fraction shifted between 46-63, 5.8-6.4,  
740 28-42 and 2-6%, respectively. Py-GC/MS characterisation revealed that the solid product decomposed  
741 into a mixture of phenols (1-19%), sugars (0-15%), nitrogen compounds (0-31%), carboxylic acids  
742 (37-75%), hydrocarbons (4-20%) and furans (1-8%). The progressive solubilisation of the cellulose  
743 and hemicellulose during the reaction produced an increase in the C content together with a decrease  
744 in the proportions of H and O of the solid. This also increased and decreased in the proportions of  
745 phenols and sugars, respectively.

746 4. The liquid phase was made up of oligo- (DP2-6 and DP>6) and mono/di-saccharides, carboxylic  
747 acids, ketones, furans and nitrogen compounds. Their relative amount (in carbon basis, C-wt.%) varied  
748 by: 33-51%, 0-3%, 0-6%, 40-62%, 0-1%, 0-3%, 0-3%. DP>6 oligosaccharides and carboxylic acids

749 were strongly influenced by the operating conditions, while the variations observed for the other  
750 species were less important. An increase in the temperature and reaction time led to an increase in the  
751 proportion of oligosaccharides and decreased the relative amount of carboxylic acids in the liquid.

752 5. An optimum for this process was found at 186 °C using a concentration of acetic acid of 1 mol/L  
753 and employing a total reaction time as short as 2 min. These conditions maximise the solubilisation of  
754 cellulose and hemicellulose and minimise lignin solubilisation; thus allowing the selective and  
755 simultaneous production of a rich (85 wt.%) lignin solid and a oligosaccharide rich water solution. In  
756 addition, acetic acid could be recovered from the sugar mixture, which not only can improve the  
757 economy and efficiency of the process but also it allows the production of high purity saccharides (92  
758 C-wt.%) with many applications in both the chemical and biological industries.

759

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764

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