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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, CH3COOH) 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 oligossacharide (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 he 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 ºC, 3 min and 1 mol/L $CH_3COOH$

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

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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 ºC), reaction time (0-60 min) and catalyst amount (1-4 mol/L, $CH_3COOH$) 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 ºC 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 oligossacharide (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.

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

## 1. Introduction

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

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

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

Özcimen et al. [8] examined the production of bio-oil and bio-char from a rapeseed cake produced during oil extraction from Brassica Napus. The pyrolysis experiments were performed in a fixed bed reactor at 500 ºC, employing different gas space velocities (50-300 $cm^3$/min). Regardless of the space velocity, around 73% of the rapeseed meal was converted into bio-oil (60%), bio-char (27%) and permanent gases (13%). The valorisation of this type of cake was also investigated by Ucar et al. [9] who analysed the effect of the reaction temperature (400-900ºC) during the pyrolysis of the residue. The gas consisted of $CO_2$, CO, $CH_4$ and $H_2S$, while the bio-oil was made up of carboxylic acids, amides and phenols. An increase in the temperature increased and decreased the gas (8-14%) and char (30-38%) yields, respectively; while the bio-oil yield increased between 400 and 500ºC (14-19%) and slyly decreased with further increasing the temperature up to 900ºC. Giannakopoulou et al. [1] conducted catalytic pressurised pyrolysis experiments of a spent rapeseed meal produced during the production of biodiesel. Two catalysts (H-ZSM-5 and H-Beta zeolites) and two reactor configurations (a pressurised pyrolysis unit, and a pressurised pyrolysis unit with catalytic upgrading of the pyrolysis vapours) were tested. In the process, two liquid phases (aqueous and organic), gases and a solid residue were obtained. The organic phase was made up of aliphatic and aromatic hydrocarbons, carboxylic acids, esters, nitriles, amides, poly-phenols and N-heterocyclic compounds. The liquid phase consisted of a mixture of phenols, ketones, alcohols and heterocyclic and N-heterocyclic compounds.

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

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

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

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

polarity, than cellulose and hemicellulose, it is less active during microwave heating [13]. This could allow the separation of cellulose and hemicellulose from the biomass without significantly altering the lignin structure; thus allowing a high purity lignin to be produced. In addition, as water is highly effective in microwave energy absorption, the combination of hydrothermal conditions together with microwave-assisted heating might be an interesting new technology for the valorisation of rapeseed meal. To the best of the authors' knowledge, the work conducted using microwave assisted hydrothermal conditions for the extraction of lignin from biomass is very scarce. In particular, Zhou et al. [14] used formic acid to extract lignin from birch biomass employing conventional and microwave heating. A higher amount of delignification was reported when microwave heating was used in the experiments. Li et al. [15] studied the effect of the temperature (90-109 ºC) during the isolation of lignin from bamboo. The temperature was found to significantly influence the process and the use of higher temperatures resulted in a greater lignin yield. Zoia et al. [16] conducted microwave assisted lignin isolation using HCl and reported that their methodology was capable of recovering up to 55 wt.% of the total lignin present in the material. Long et al. [13, 17] addressed the effects of the temperature (160-210 ºC) and reaction time (5-20 min) during the isolation of lignin from softwood employing $H_2SO_4$. They found that an increase in both the temperature and reaction time increased the lignin yield and purity. Maxima for the yield (82 wt.%) and purity (93 wt.%) occurred using a 0.2 mol/L sulphuric acid solution at 190 ºC for 10 minutes. The liquid phase consisted of a mixture of saccharides, carboxylic acids and furans and was found to have potential to be used in fermentation processes.

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

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

## 2. Experimental

### 2.1 Microwave experiments

A CEM Discover II microwave facility was used for the experiments. The experiments were conducted in a 30 mL batch reactor using a maximum power of 300W. For each experiment, 0.5 g of biomass was placed in the reactor along with 15 mL of solvent ($CH_3COOH/H_2O$). Before placing the reactor inside the microwave unit, the reaction mixture was pre-stirred at room temperature for 2 min. A heating rate of 1ºC/s was used for all the experiments; and therefore, the ramping time (time to reach the temperature of the experiment) varied between 2 and 3 min. The reaction time shifted between 0 and 60 min according to the experimental design. After reaction, the reactor was cooled down from the reaction temperature to 60ºC at a rate of 0.5 ºC/s. Subsequently, the reactor was opened and its content, consisting of a mixture of liquid and solid, was transferred to a centrifuge tube. Centrifugation was used to separate the solid from the liquid. Then, the solid residue obtained after centrifugation was dried overnight at 105°C and the liquid phase obtained was stored for further characterisation.

### 2.2 Response variables and analytical methods

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

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

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

| Product | Response variable | Analytical method |
|---|---|---|
| **Liquid** | $\text{Liquid yield (\%)} = \frac{\text{liquid compounds (g)}}{\text{mass of biomass (g)}} 100 = 100 - (\text{Gas yield} + \text{Solid yield})$ | Balance |
| | $\text{Composition (C} - \text{wt.\%)} = \frac{\sum \text{mass of C of each compound (g)}}{\text{total mass of C in solution (g)}} 100$ | GC/MS-FID and HPLC |
| | $\text{C, H, O (wt.\%)} = \frac{\text{mass of C, H, O (g)}}{\text{mass of organics (g)}} 100$ | 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 |
| **Solid** | $\text{Solid yield (\%)} = \frac{\text{mass of solid (g)}}{\text{mass of biomass (g)}} 100$ | Gravimetric |
| | $\text{Fibre Composition (wt.\%)} = \frac{\text{mass of structural component (g)}}{\text{mass of solid residue(g)}} 100$ | 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 |
| | $\text{C, H, O (wt.\%)} = \frac{\text{mass of C, H, O (g)}}{\text{mass of solid (g)}} 100$ | Elemental Analysis |
| | $\text{Py GC/MS Composition (area \%)} = \frac{\text{area of each compound}}{\text{total area}} 100$ | Py-GC/MS |
| **Gas** | $\text{Gas yield (\%)} = \frac{\text{mass of gas (g)}}{\text{mass of biomass (g)}} 100$ | Gravimetric |

wt.% = weight percentage
C-wt.% = percentage in carbon basis
Protein content (wt.%) = 4.62·N (wt.%)

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

### 2.3 Experimental design and data analysis

The influence of reaction temperature (150-210°C), acetic concentration in water (1-4 mol/L) and reaction time (0-60 min) on the process was experimentally investigated. The experiments were planned according to a 2 level 3-factor Box-Wilson Central Composite Face Centred (CCF, α: ±1) design. This corresponds to a $2^k$ factorial design, where k indicates the number of factors studied (in this case 3 operating variables) and $2^k$ represents the number of runs (in this case 8) for the simple factorial design. 8 axial experiments were performed to study non-linear effects and interactions. In addition, 4 replicates at the centre point (centre of the variation interval of each factor) were carried out in order to evaluate the experimental error. This experimental design is 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 analysed with 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. In these analyses, the lower and upper limits of all the operating variables were normalised from -1 to 1 (codec variables) to investigate their influence in comparable terms. In the interaction Figures, the evolution of these variables obtained from the ANOVA analysis of all the experiments performed was represented. In addition, when possible, some experimental points were added. In the interaction plots developed from the ANOVA analyses only the upper and lower levels for one of the variables have been represented;

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

### 2.4 Rapeseed meal characterisation

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

Table 2. Feedstock characterisation.

| | | | |
|---|---|---|---|
| **Proximate analysis (wt.%)** | | **HHV (MJ/kg)** | 17.07±0.29 |
| Moisture | 7.26 | **Ash composition (wt.%)** | |
| Ash | 1.31 | Ca | 15.94 |
| Volatiles | 45.09 | Mg | 7.98 |
| Fixed carbon | 32.04 | K | 19.75 |
| **Fibre analysis (wt.%)** | | Na | 1.19 |
| Cellulose | 12.41±0.33 | P | 24.47 |
| Hemicellulose | 7.16±0.26 | S | 30.67 |
| Lignin | 32.39±2.47 | **Py-GC/MS characterisation (% area)** | |
| Protein | 39.47±1.17 | Hydrocarbons | 43.59±1.22 |
| **Elemental analysis (wt.%)** | | 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 |

*Oxygen was calculated by difference

## 3. Results and discussion

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

243 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 (ºC) | 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 |
| **GLOBAL YIELDS** | | | | | | | | | | | | | | | |
| 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 |
| **SOLID PROPERTIES** | | | | | | | | | | | | | | | |
| **Fibre analysis (wt.%)** | | | | | | | | | | | | | | | |
| 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 |
| **Elemental analysis** | | | | | | | | | | | | | | | |
| 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 |
| **Py-GC/MS (Area %)** | | | | | | | | | | | | | | | |
| 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 |
| **LIQUID PROPERTIES** | | | | | | | | | | | | | | | |
| **Chemical composition (C-wt.%)** | | | | | | | | | | | | | | | |
| 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 |
| **Elemental analysis (dry basis)** | | | | | | | | | | | | | | | |
| 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 |

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

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

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

| Variable | $R^2$ | I. Term | T | t | C | Tt | TC | tC | TtC | $T^2$ | $t^2$ | $C^2$ | $T^2t$ | $T^2C$ | $Tt^2$ | $TC^2$ | $T^2t^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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. |

n.s: Non significant with 95% confidence

Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C Coefficient TtC·T·t·C + Coefficient $T^2 \cdot T^2$ + Coefficient $t^2 \cdot t^2$ + Coefficient $C^2 \cdot C^2$ + Coefficient $T^2t \cdot T \cdot t$ + Coefficient $T^2C \cdot T^2 \cdot C$ + Coefficient $Tt^2 \cdot T \cdot t^2$ + Coefficient $TC^2 \cdot T \cdot C^2$ + Coefficient $T^2t^2 \cdot T^2 \cdot t^2$

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

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

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

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

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

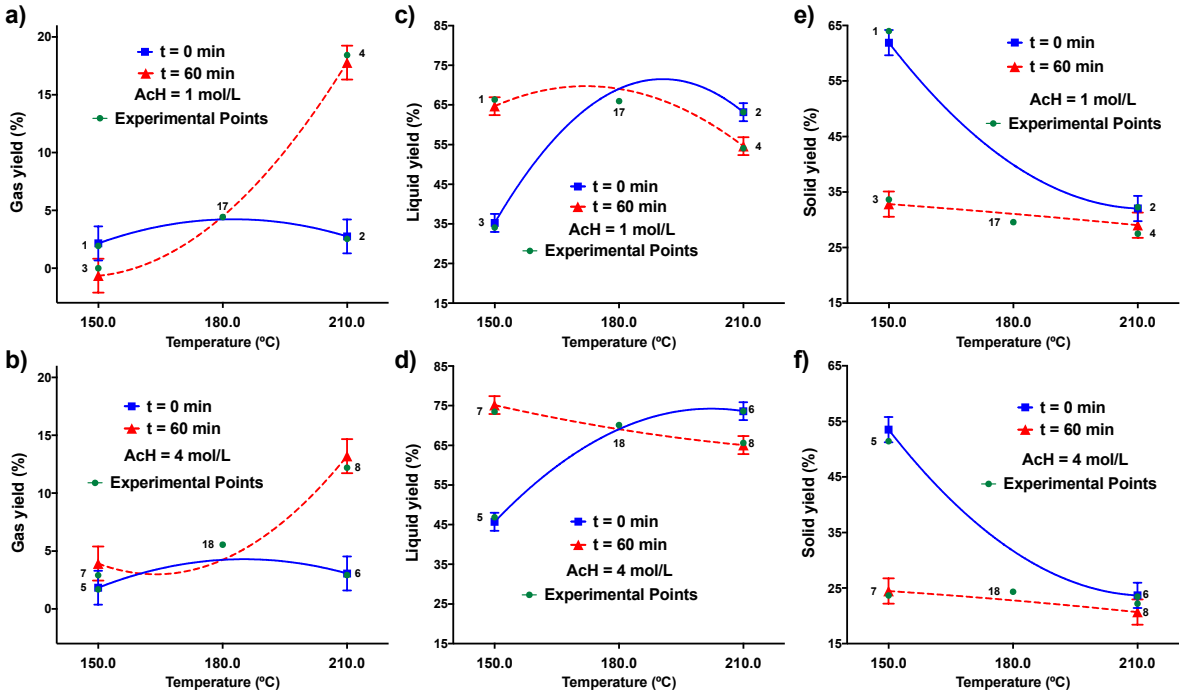


Figure 1. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the gas (a and b), liquid (c and d) and solid (e and f) yields. Bars are LSD intervals with 95% confidence.

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

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

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

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

#### *3.2.1 Fibre analysis*

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

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

| Variable | $R^2$ | I.Term | T | t | C | Tt | TC | tC | TtC | $T^2$ | $t^2$ | $C^2$ | $T^2t$ | $T^2C$ | $Tt^2$ | $TC^2$ | $T^2t^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fibre analysis (wt.%)** | | | | | | | | | | | | | | | | | |
| 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 | -14.87 (6) | -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 | 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) |
| **Elemental analysis** | | | | | | | | | | | | | | | | | |
| 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 | 1.03 (7) | 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) |
| **Py-GC/MS (Area %)** | | | | | | | | | | | | | | | | | |
| 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. (18) | n.s. (9) | 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. |

n.s: Non significant with 95% confidence

Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C Coefficient TtC·T·t·C + Coefficient $T^2 \cdot T^2$ + Coefficient $t^2 \cdot t^2$ + Coefficient $C^2 \cdot C^2$ + Coefficient $T^2t \cdot T \cdot {}^2t$ + Coefficient $T^2C \cdot T^2 \cdot C$ + Coefficient $Tt^2 \cdot T \cdot t^2$ + Coefficient $TC^2 \cdot T \cdot C^2$ + Coefficient $T^2t^2 \cdot T^2 \cdot t^2$

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

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

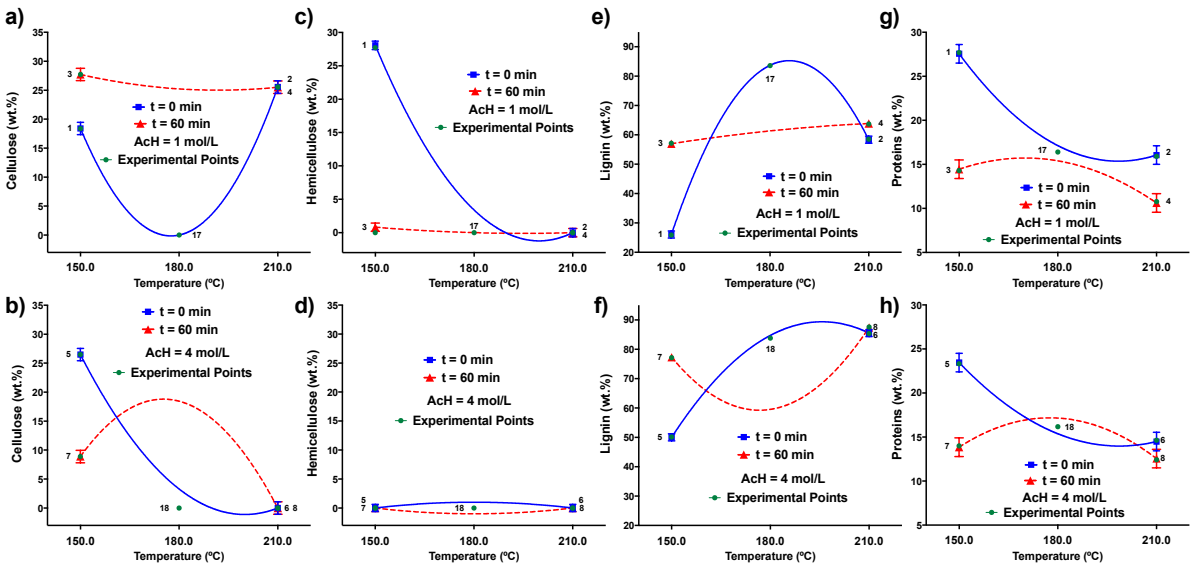


Figure 2. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the proportions of cellulose (a and b), hemicellulose (c and d), lignin (e and f) and proteins (g and h). Bars are LSD intervals with 95% confidence.

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

The comparison between Figure 2 a, c, e and g with b, d, f and h shows that an increase in the concentration of acetic acid from 1 to 4 mol/L (for 0 min reaction time) decreases the proportion of hemicellulose and proteins and increases the relative amount of lignin regardless of the temperature (150-210 ºC) due to the positive catalytic effect of acetic acid in the process. A similar trend was also observed by Long et al. [17] and Zoia et al. [16], who reported the positive catalytic effect of $H_2SO_4$

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 ºC.
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 ºC) 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 ºC, this same increment decreases
402 the proportion of lignin between 165 and 200 ºC. 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.

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

*3.2.2 Elemental analysis*

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

The effect of the temperature on the elemental analysis of the solid fraction depends on the reaction time and the concentration of acetic acid. When a short reaction time is used, the concentration of acetic acid does not greatly influence the elemental analysis or the HHV of the solid and similar 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 ºC 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 ºC 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 ºC 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 ºC 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 ºC. The HHV slightly increases between 150 and 180 ºC
466 and remains steady with a further increase up to 210 ºC.
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 ºC decreases the proportions of H and O. This leads to a decrease in

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

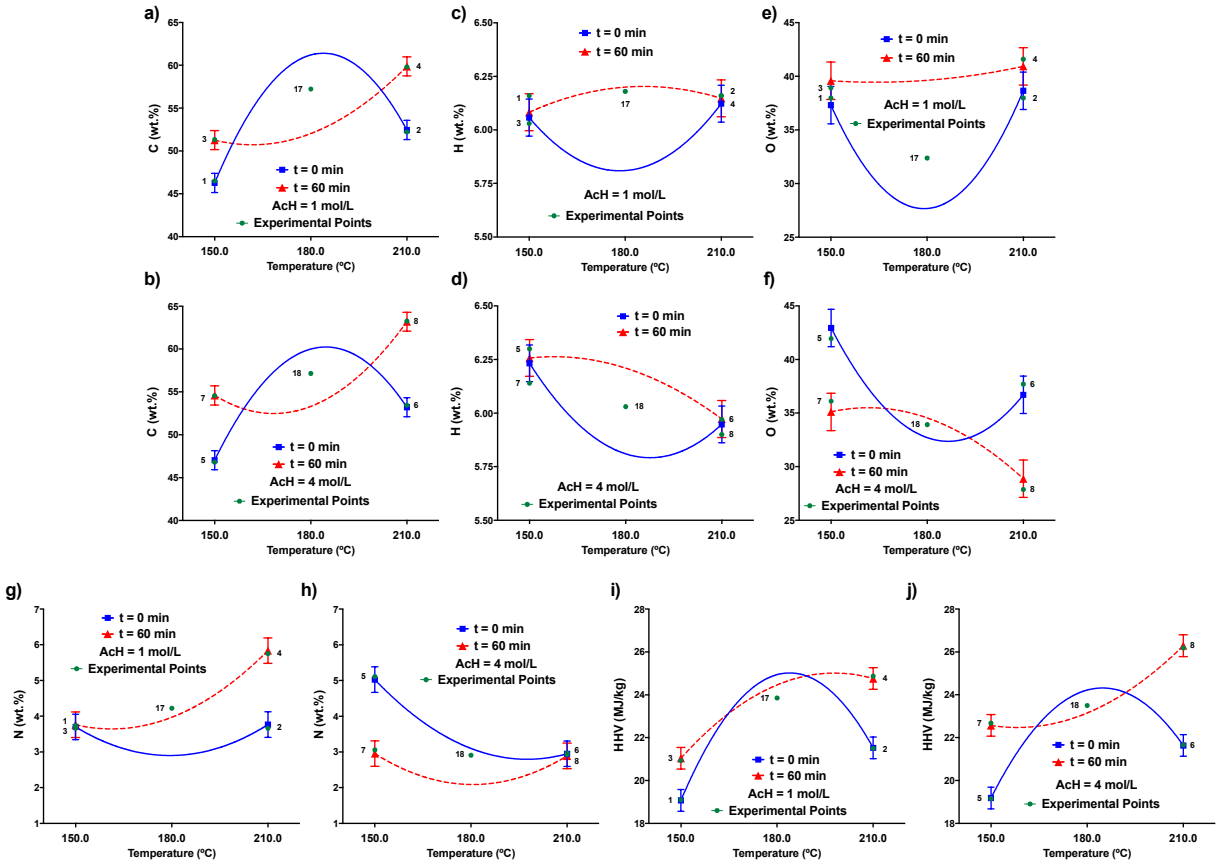


Figure 3. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the concentrations of C (a and b), H (c and d), O (e and f), N (g and h) and HHV (i and j). Bars are LSD intervals with 95% confidence.

*3.2.3 Pyrolysis GC-MS characterisation*

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

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

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

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

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

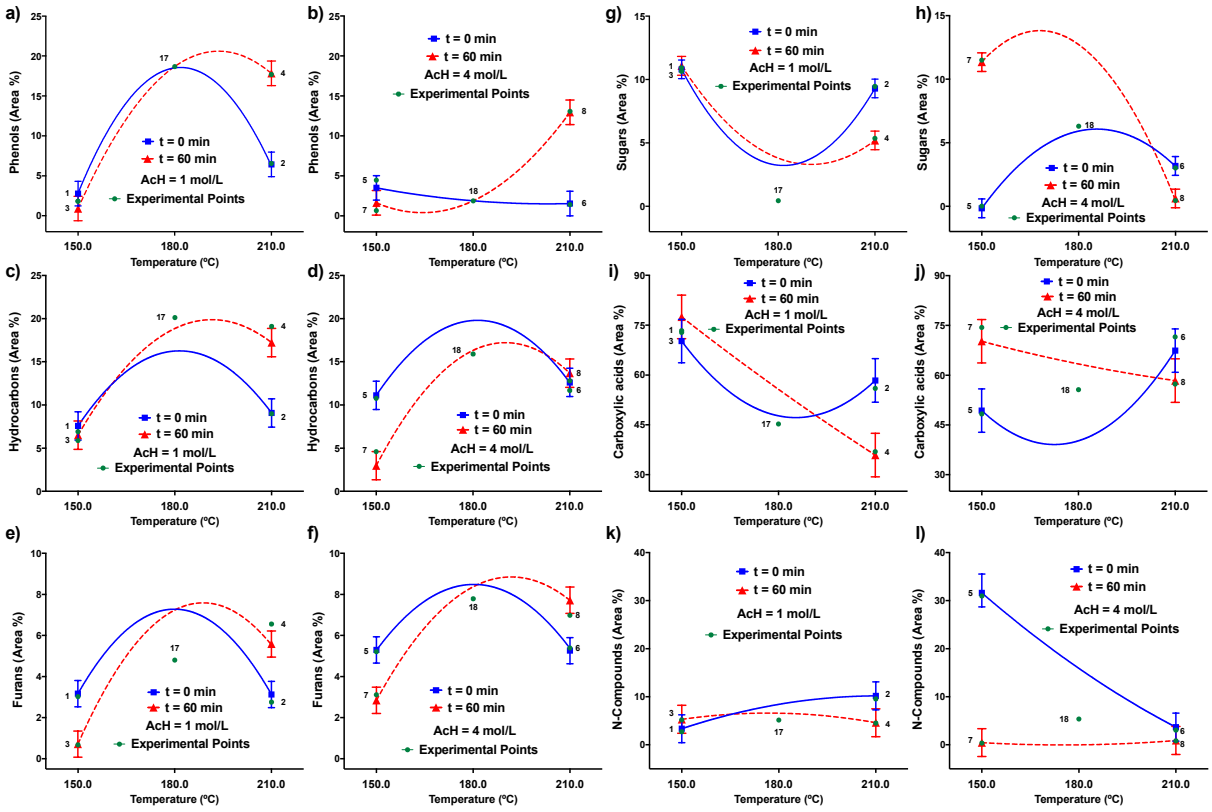


Figure 4. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the concentrations of phenols (a and b), hydrocarbons (c and d), furans (e and f), sugars (g and h), carboxylic acids (i and j) and N-compounds (k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

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

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

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

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

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

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

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

*3.3.1 Chemical composition*

The liquid product consists of a mixture of oligo- (DP2-6 and DP>6) and mono/di- saccharides, carboxylic acids, ketones, furans and nitrogen compounds; their relative amount (in carbon basis, C-wt.%) in the liquid product varying as follows: 33-51%, 0-3%, 0-6%, 40-62%, 0-1%, 0-3%, 0-3%. Saccharides include cellobiose, xylose, glucose, fructose, mannose, arabinose, rhamnose and levoglucosan. Carboxylic acids comprise lactic, formic, levulinic, glucuronic, galacuronic and acetic acids. Acetic acid is the major compound for this family as it was initially loaded and used as a 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-pyrinidol and 6 methyl-3-pyrinidol.
593
594 *Table 6. Relative influence of the operating conditions on the properties of the liquid fraction*

| Variable | $R^2$ | I.Term | T | t | C | Tt | TC | tC | TtC | $T^2$ | $t^2$ | $C^2$ | $T^2t$ | $T^2C$ | $Tt^2$ | $TC^2$ | $T^2t^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chemical composition (C-wt.%)** | | | | | | | | | | | | | | | | | |
| 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) |
| **Elemental analysis** | | | | | | | | | | | | | | | | | |
| 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. (1) | -0.46 | -0.38 | 1.30 (3) | n.s. | 1.39 | n.s. (2) | -0.65 | 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 $T^2 \cdot T^2$ + Coefficient $t^2 \cdot t^2$ + Coefficient $C^2 \cdot C^2$ + Coefficient $T^2t \cdot T \cdot {}^2t$ + Coefficient $T^2C \cdot T^2 \cdot C$ + Coefficient
598 $Tt^2 \cdot T \cdot t^2$ + Coefficient $TC^2 \cdot T \cdot C^2$ + Coefficient $T^2t^2 \cdot T^2 \cdot t^2$
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 ºC 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 ºC 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 ºC, 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

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

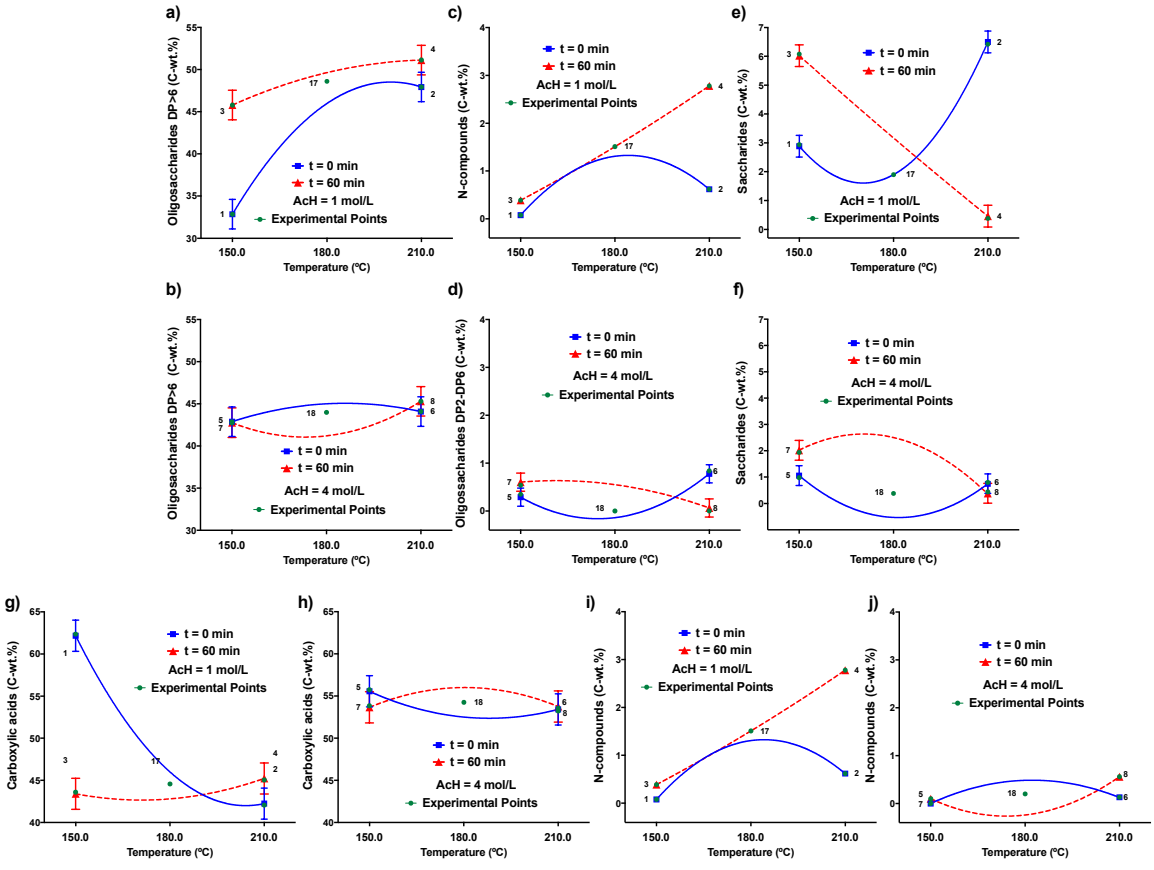


Figure 5. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the concentrations of oligossacharides DP>6 (a and b), oligosaccharides DP 2-6 (c and d), saccharides (e and f), carboxylic acids (g and h) and N-compounds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

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

*3.3.2 Elemental analysis and HHV*

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

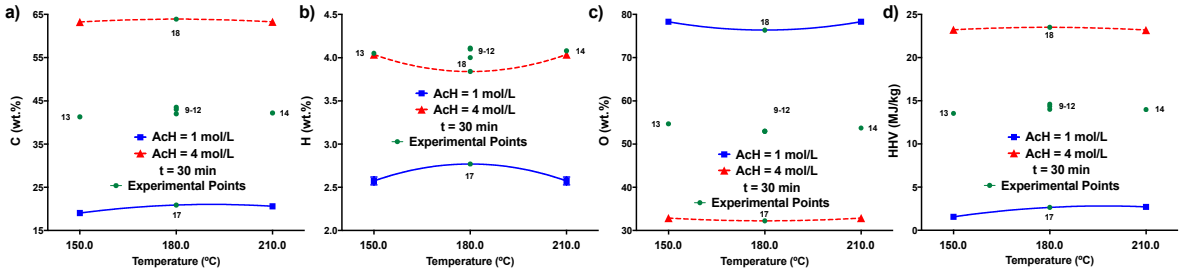


Figure 6. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the concentrations of C (a), H (b), O (c) and HHV (d) at 30 min reaction time. Bars are LSD intervals with 95% confidence.

Regardless of the temperature or reaction time, the concentration of acetic acid exerts the same influence on the elemental analysis and HHV of the liquid phase. Increasing the concentration of acetic acid from 1 to 4 mol/L leads to an increase in the proportions of C and H together with a 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 (ºC) | minimise | 150-210 | 1 | 186 | |
| t (min) | minimise | 0-60 | 5 | 0 | |
| $CH_3COOH$ (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 |
| **Solid fibre and elemental analyses** | | | | | |
| 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 |
| **Liquid composition (C-wt.%)** | | | | | |
| 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

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

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

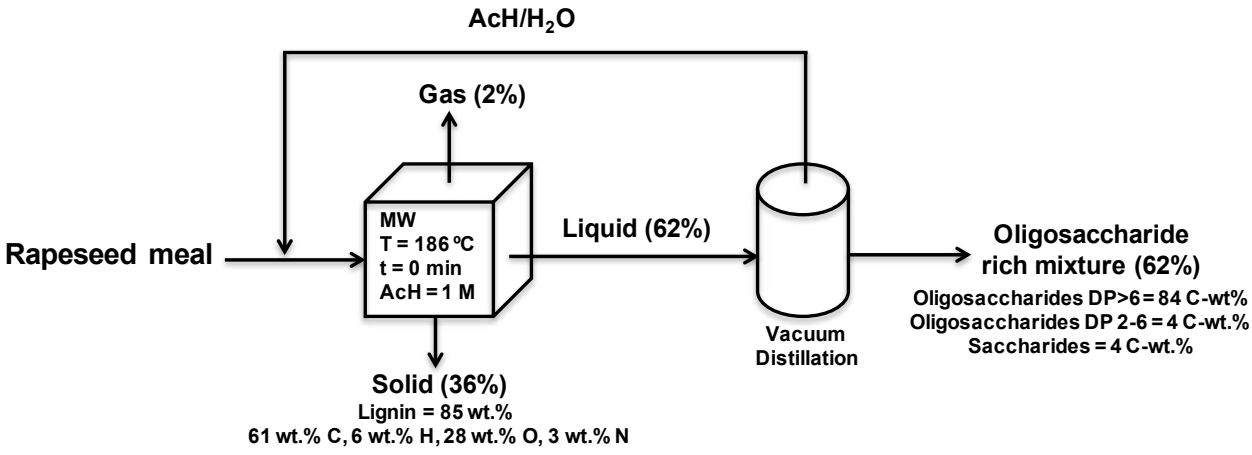


Figure 7. Schematic diagram for the simultaneous production of lignin and oligosaccharides

## 4. Conclusions

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

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

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

3. 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 characterisation revealed that the solid product decomposed into a mixture of phenols (1-19%), sugars (0-15%), nitrogen compounds (0-31%), carboxylic acids (37-75%), hydrocarbons (4-20%) and furans (1-8%). The progressive solubilisation of the cellulose and hemicellulose during the reaction produced an increase in the C content together with a decrease in the proportions of H and O of the solid. This also increased and decreased in the proportions of phenols and sugars, respectively.

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

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

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

**Acknowledgements**

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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 1ºC/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 ºC at a rate of around 0.5 ºC/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

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

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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 ºC), reaction time (0-60 min) and catalyst amount (1-4 mol/L, $CH_3COOH$) 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 ºC 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 oligossacharide (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.

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

## 1. Introduction

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

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

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

Özcimen et al. [8] examined the production of bio-oil and bio-char from a rapeseed cake produced during oil extraction from Brassica Napus. The pyrolysis experiments were performed in a fixed bed reactor at 500 ºC, employing different gas space velocities (50-300 $cm^3$/min). Regardless of the space velocity, around 73% of the rapeseed meal was converted into bio-oil (60%), bio-char (27%) and permanent gases (13%). The valorisation of this type of cake was also investigated by Ucar et al. [9] who analysed the effect of the reaction temperature (400-900ºC) during the pyrolysis of the residue. The gas consisted of $CO_2$, CO, $CH_4$ and $H_2S$, while the bio-oil was made up of carboxylic acids, amides and phenols. An increase in the temperature increased and decreased the gas (8-14%) and char (30-38%) yields, respectively; while the bio-oil yield increased between 400 and 500ºC (14-19%) and slyly decreased with further increasing the temperature up to 900ºC. Giannakopoulou et al. [1] conducted catalytic pressurised pyrolysis experiments of a spent rapeseed meal produced during the production of biodiesel. Two catalysts (H-ZSM-5 and H-Beta zeolites) and two reactor configurations (a pressurised pyrolysis unit, and a pressurised pyrolysis unit with catalytic upgrading of the pyrolysis vapours) were tested. In the process, two liquid phases (aqueous and organic), gases and a solid residue were obtained. The organic phase was made up of aliphatic and aromatic hydrocarbons, carboxylic acids, esters, nitriles, amides, poly-phenols and N-heterocyclic compounds. The liquid phase consisted of a mixture of phenols, ketones, alcohols and heterocyclic and N-heterocyclic compounds.

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

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

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

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

polarity, than cellulose and hemicellulose, it is less active during microwave heating [13]. This could allow the separation of cellulose and hemicellulose from the biomass without significantly altering the lignin structure; thus allowing a high purity lignin to be produced. In addition, as water is highly effective in microwave energy absorption, the combination of hydrothermal conditions together with microwave-assisted heating might be an interesting new technology for the valorisation of rapeseed meal. To the best of the authors' knowledge, the work conducted using microwave assisted hydrothermal conditions for the extraction of lignin from biomass is very scarce. In particular, Zhou et al. [14] used formic acid to extract lignin from birch biomass employing conventional and microwave heating. A higher amount of delignification was reported when microwave heating was used in the experiments. Li et al. [15] studied the effect of the temperature (90-109 ºC) during the isolation of lignin from bamboo. The temperature was found to significantly influence the process and the use of higher temperatures resulted in a greater lignin yield. Zoia et al. [16] conducted microwave assisted lignin isolation using HCl and reported that their methodology was capable of recovering up to 55 wt.% of the total lignin present in the material. Long et al. [13, 17] addressed the effects of the temperature (160-210 ºC) and reaction time (5-20 min) during the isolation of lignin from softwood employing $H_2SO_4$. They found that an increase in both the temperature and reaction time increased the lignin yield and purity. Maxima for the yield (82 wt.%) and purity (93 wt.%) occurred using a 0.2 mol/L sulphuric acid solution at 190 ºC for 10 minutes. The liquid phase consisted of a mixture of saccharides, carboxylic acids and furans and was found to have potential to be used in fermentation processes.

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

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

## 2. Experimental

### 2.1 Microwave experiments

A CEM Discover II microwave facility was used for the experiments. The experiments were conducted in a 30 mL batch reactor using a maximum power of 300W. For each experiment, 0.5 g of biomass was placed in the reactor along with 15 mL of solvent ($CH_3COOH/H_2O$). Before placing the reactor inside the microwave unit, the reaction mixture was pre-stirred at room temperature for 2 min. A heating rate of 1ºC/s was used for all the experiments; and therefore, the ramping time (time to reach the temperature of the experiment) varied between 2 and 3 min. The reaction time shifted between 0 and 60 min according to the experimental design. After reaction, the reactor was cooled down from the reaction temperature to 60ºC at a rate of 0.5 ºC/s. Subsequently, the reactor was opened and its content, consisting of a mixture of liquid and solid, was transferred to a centrifuge tube. Centrifugation was used to separate the solid from the liquid. Then, the solid residue obtained after centrifugation was dried overnight at 105°C and the liquid phase obtained was stored for further characterisation.

### 2.2 Response variables and analytical methods

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

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

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

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

wt.% = weight percentage
C-wt.% = percentage in carbon basis
Protein content (wt.%) = 4.62·N (wt.%)

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, 8um particle size) and ACE
197 C18 (250 x 4.6mm, 5um 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, α: ±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 (codec 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;

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

### 2.4 Rapeseed meal characterisation

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

Table 2. Feedstock characterisation.

| | | | |
|---|---|---|---|
| **Proximate analysis (wt.%)** | | **HHV (MJ/kg)** | 17.07±0.29 |
| Moisture | 7.26 | **Ash composition (wt.%)** | |
| Ash | 1.31 | Ca | 15.94 |
| Volatiles | 45.09 | Mg | 7.98 |
| Fixed carbon | 32.04 | K | 19.75 |
| **Fibre analysis (wt.%)** | | Na | 1.19 |
| Cellulose | 12.41±0.33 | P | 24.47 |
| Hemicellulose | 7.16±0.26 | S | 30.67 |
| Lignin | 32.39±2.47 | **Py-GC/MS characterisation (% area)** | |
| Protein | 39.47±1.17 | Hydrocarbons | 43.59±1.22 |
| **Elemental analysis (wt.%)** | | 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 |

*Oxygen was calculated by difference

## 3. Results and discussion

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

243 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 (ºC) | 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 |
| **GLOBAL YIELDS** | | | | | | | | | | | | | | | |
| 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 |
| **SOLID PROPERTIES** | | | | | | | | | | | | | | | |
| **Fibre analysis (wt.%)** | | | | | | | | | | | | | | | |
| 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 |
| **Elemental analysis** | | | | | | | | | | | | | | | |
| 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 |
| **Py-GC/MS (Area %)** | | | | | | | | | | | | | | | |
| 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 |
| **LIQUID PROPERTIES** | | | | | | | | | | | | | | | |
| **Chemical composition (C-wt.%)** | | | | | | | | | | | | | | | |
| 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 |
| **Elemental analysis (dry basis)** | | | | | | | | | | | | | | | |
| 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 |

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

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

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

| Variable | $R^2$ | I. Term | T | t | C | Tt | TC | tC | TtC | $T^2$ | $t^2$ | $C^2$ | $T^2t$ | $T^2C$ | $Tt^2$ | $TC^2$ | $T^2t^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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. |

n.s: Non significant with 95% confidence

Response = I. Term + Coefficient $T \cdot T$ + Coefficient $t \cdot t$ + Coefficient $C \cdot C$ + Coefficient $Tt \cdot T \cdot t$ + Coefficient $TC \cdot T \cdot C$ Coefficient $tC \cdot t \cdot C$ Coefficient $TtC \cdot T \cdot t \cdot C$ + Coefficient $T^2 \cdot T^2$ + Coefficient $t^2 \cdot t^2$ + Coefficient $C^2 \cdot C^2$ + Coefficient $T^2t \cdot T \cdot {}^2t$ + Coefficient $T^2C \cdot T^2 \cdot C$ + Coefficient $Tt^2 \cdot T \cdot t^2$ + Coefficient $TC^2 \cdot T \cdot C^2$ + Coefficient $T^2t^2 \cdot T^2 \cdot t^2$

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

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

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

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

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

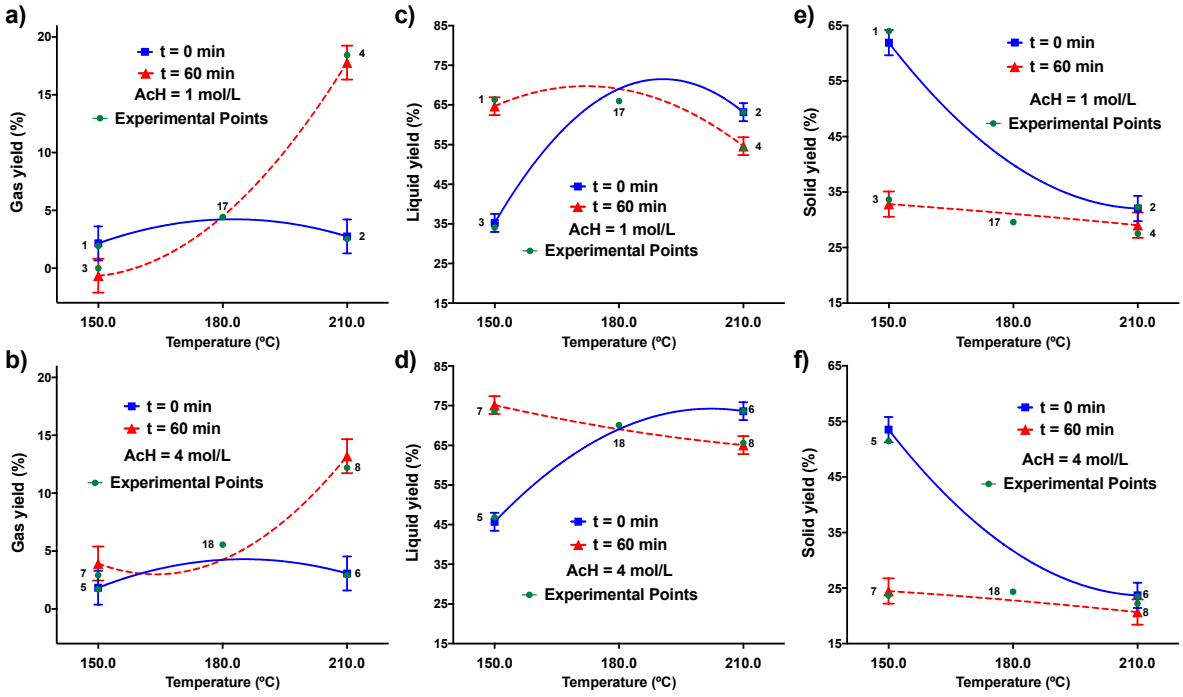


Figure 1. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the gas (a and b), liquid (c and d) and solid (e and f) yields. Bars are LSD intervals with 95% confidence.

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

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

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

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

#### *3.2.1 Fibre analysis*

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

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

| Variable | $R^2$ | I.Term | T | t | C | Tt | TC | tC | TtC | $T^2$ | $t^2$ | $C^2$ | $T^2t$ | $T^2C$ | $Tt^2$ | $TC^2$ | $T^2t^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fibre analysis (wt.%)** | | | | | | | | | | | | | | | | | |
| 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 | -14.87 (6) | -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 | 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) |
| **Elemental analysis** | | | | | | | | | | | | | | | | | |
| 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 | 1.03 (7) | 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) |
| **Py-GC/MS (Area %)** | | | | | | | | | | | | | | | | | |
| 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. (18) | n.s. (9) | 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. |

n.s: Non significant with 95% confidence

Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C Coefficient TtC·T·t·C + Coefficient $T^2 \cdot T^2$ + Coefficient $t^2 \cdot t^2$ + Coefficient $C^2 \cdot C^2$ + Coefficient $T^2t \cdot T \cdot {}^2t$ + Coefficient $T^2C \cdot T^2 \cdot C$ + Coefficient $Tt^2 \cdot T \cdot t^2$ + Coefficient $TC^2 \cdot T \cdot C^2$ + Coefficient $T^2t^2 \cdot T^2 \cdot t^2$

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

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

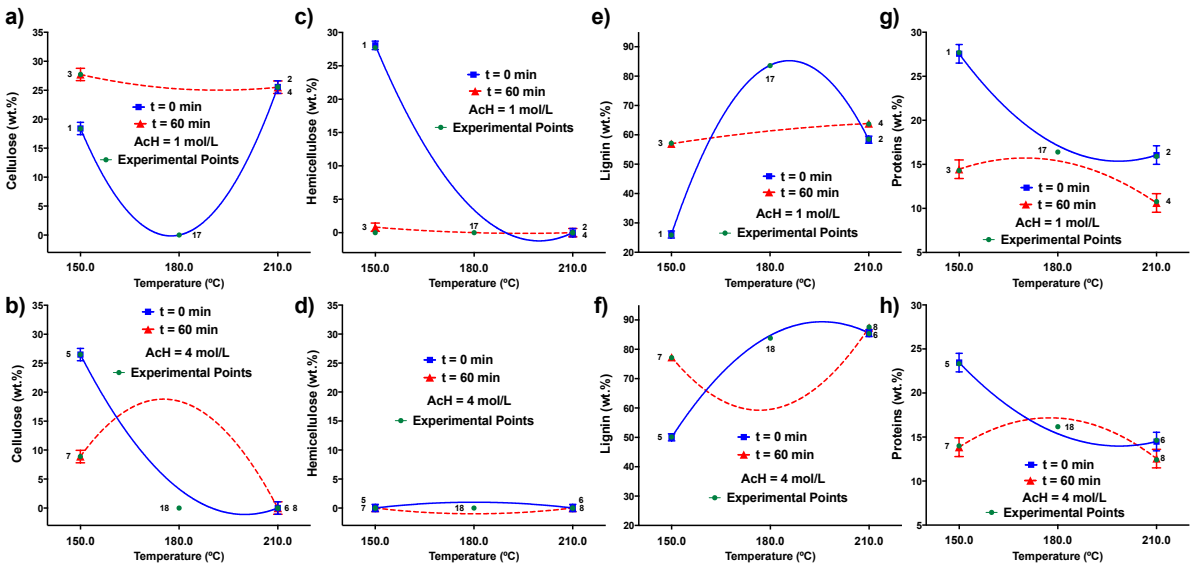


Figure 2. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the proportions of cellulose (a and b), hemicellulose (c and d), lignin (e and f) and proteins (g and h). Bars are LSD intervals with 95% confidence.

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

The comparison between Figure 2 a, c, e and g with b, d, f and h shows that an increase in the concentration of acetic acid from 1 to 4 mol/L (for 0 min reaction time) decreases the proportion of hemicellulose and proteins and increases the relative amount of lignin regardless of the temperature (150-210 ºC) due to the positive catalytic effect of acetic acid in the process. A similar trend was also observed by Long et al. [17] and Zoia et al. [16], who reported the positive catalytic effect of $H_2SO_4$

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

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

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

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

*3.2.2 Elemental analysis*

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

The effect of the temperature on the elemental analysis of the solid fraction depends on the reaction time and the concentration of acetic acid. When a short reaction time is used, the concentration of acetic acid does not greatly influence the elemental analysis or the HHV of the solid and similar 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 ºC 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 ºC 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 ºC 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 ºC 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 ºC. The HHV slightly increases between 150 and 180 ºC
466 and remains steady with a further increase up to 210 ºC.
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 ºC decreases the proportions of H and O. This leads to a decrease in

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

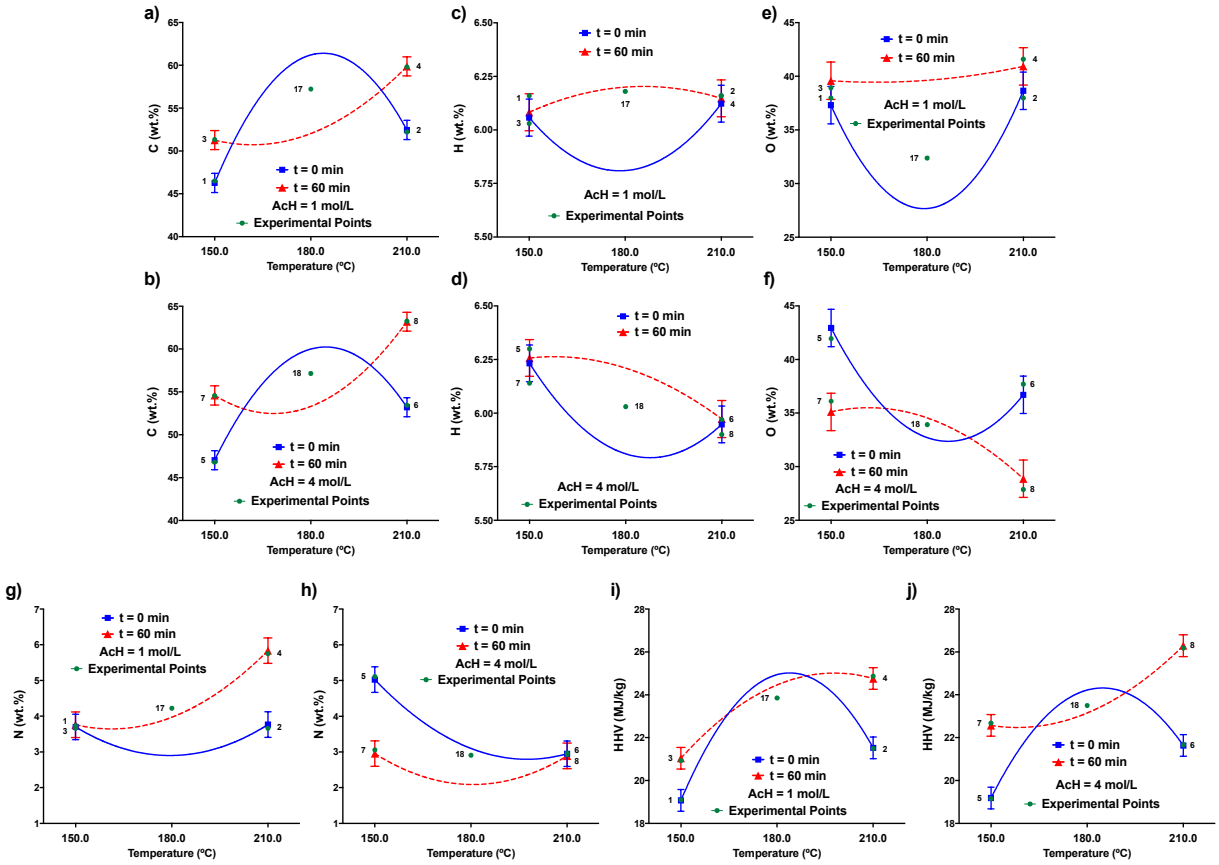


Figure 3. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the concentrations of C (a and b), H (c and d), O (e and f), N (g and h) and HHV (i and j). Bars are LSD intervals with 95% confidence.

*3.2.3 Pyrolysis GC-MS characterisation*

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

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

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

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

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

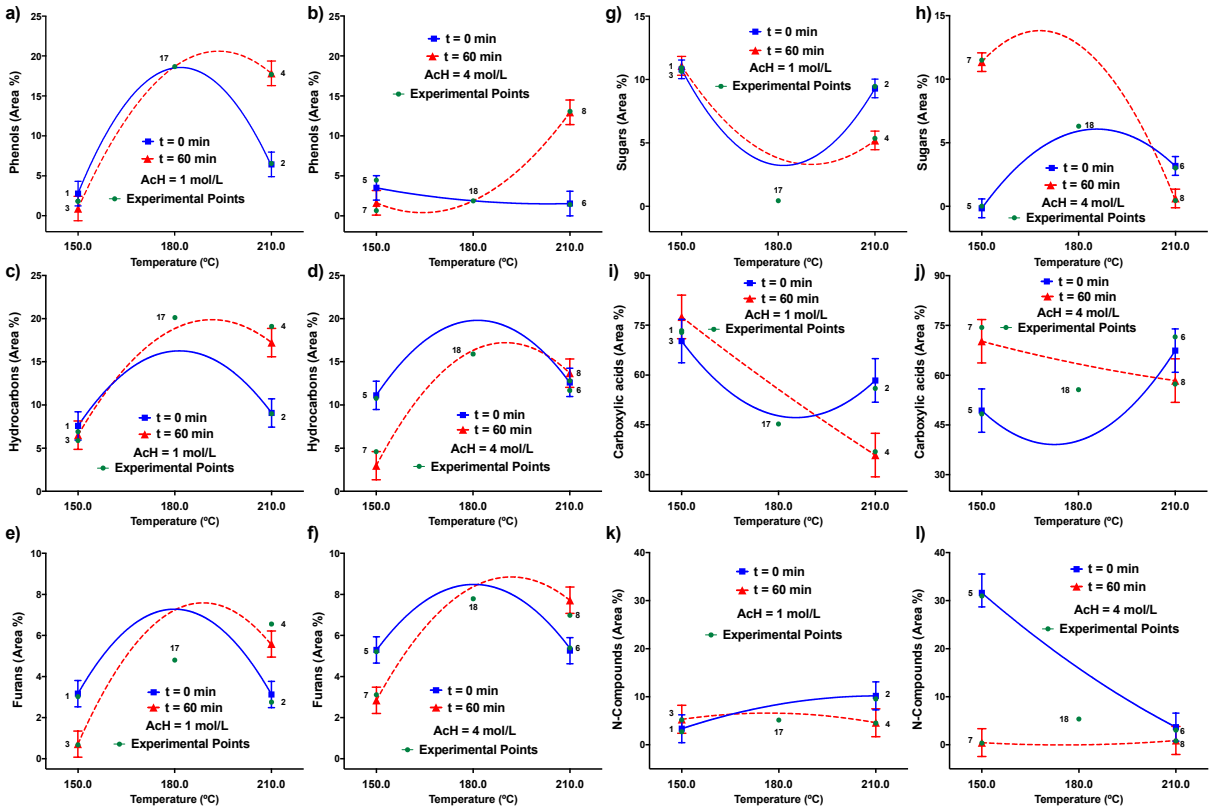


Figure 4. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the concentrations of phenols (a and b), hydrocarbons (c and d), furans (e and f), sugars (g and h), carboxylic acids (i and j) and N-compounds (k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

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

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

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

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

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

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

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

#### *3.3.1 Chemical composition*

The liquid product consists of a mixture of oligo- (DP2-6 and DP>6) and mono/di- saccharides, carboxylic acids, ketones, furans and nitrogen compounds; their relative amount (in carbon basis, C-wt.%) in the liquid product varying as follows: 33-51%, 0-3%, 0-6%, 40-62%, 0-1%, 0-3%, 0-3%. Saccharides include cellobiose, xylose, glucose, fructose, mannose, arabinose, rhamnose and levoglucosan. Carboxylic acids comprise lactic, formic, levulinic, glucuronic, galacuronic and acetic acids. Acetic acid is the major compound for this family as it was initially loaded and used as a catalyst. 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 secondary reactions (production) did not take place to a great extent and/or they compensated for each other. Ketones and furans are made of levoglucosenone and 5-hydroxymethyl-2-furancarboxaldehyde (HMF) and furfural, respectively. Nitrogen compounds include 3-pyrinidol and 6 methyl-3-pyrinidol.

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

| Variable | $R^2$ | I.Term | T | t | C | Tt | TC | tC | TtC | $T^2$ | $t^2$ | $C^2$ | $T^2t$ | $T^2C$ | $Tt^2$ | $TC^2$ | $T^2t^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chemical composition (C-wt.%)** | | | | | | | | | | | | | | | | | |
| 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) |
| **Elemental analysis** | | | | | | | | | | | | | | | | | |
| 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. (1) | -0.46 | -0.38 | 1.30 (3) | n.s. | 1.39 | n.s. (2) | -0.65 | 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. |

n.s: Non significant with 95% confidence

Response = I. Term + Coefficient T·T + Coefficient t·t + Coefficient C·C + Coefficient Tt·T·t + Coefficient TC·T·C Coefficient tC·t·C Coefficient TtC·T·t·C + Coefficient $T^2 \cdot T^2$ + Coefficient $t^2 \cdot t^2$ + Coefficient $C^2 \cdot C^2$ + Coefficient $T^2t \cdot T \cdot {}^2t$ + Coefficient $T^2C \cdot T^2 \cdot C$ + Coefficient $Tt^2 \cdot T \cdot t^2$ + Coefficient $TC^2 \cdot T \cdot C^2$ + Coefficient $T^2t^2 \cdot T^2 \cdot t^2$

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

The cause-effect Pareto analysis (Table 6) shows that the proportion of DP>6 oligosaccharides is strongly influenced by the interaction between the temperature and reaction time. The temperature (linear and quadratic) and concentration followed by the reaction time significantly influence the proportions of DP 2-6 oligosaccharides and (mono/di) saccharides. The proportion of carboxylic acids is strongly affected by the concentration, while the concentration of furans depends on the temperature, acid concentration and the interaction between these two latter variables. The temperature, time and concentration have a similar influence on the proportion of nitrogen compounds. Figure 5 shows the 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 ºC 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 ºC 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 ºC, 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

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

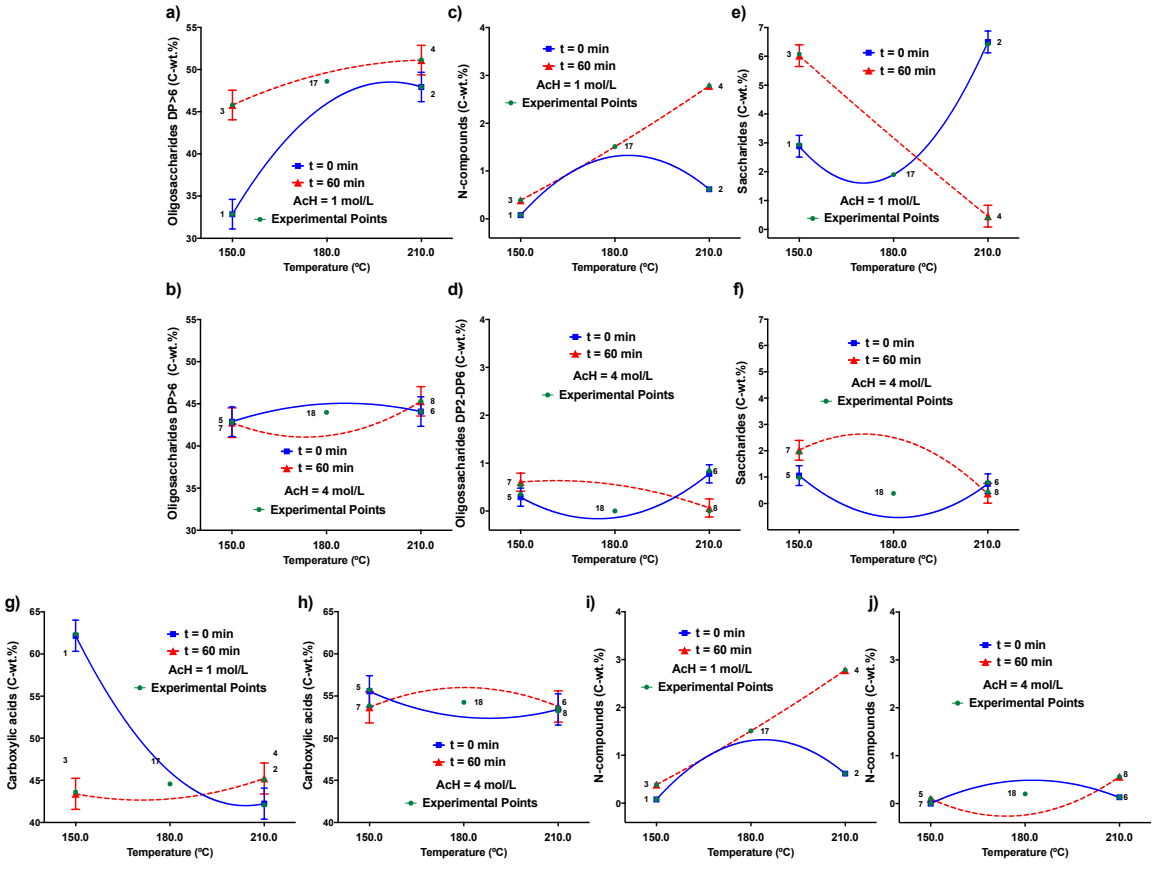


Figure 5. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the concentrations of oligossacharides DP>6 (a and b), oligosaccharides DP 2-6 (c and d), saccharides (e and f), carboxylic acids (g and h) and N-compounds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

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

*3.3.2 Elemental analysis and HHV*

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

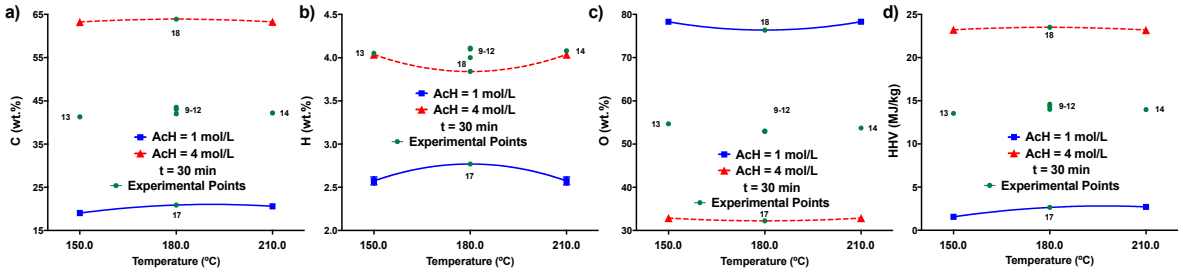


Figure 6. Interaction plots between the temperature and the reaction time with the lowest (1 mol/L) and the highest (4 mol/L) acetic acid concentration for the concentrations of C (a), H (b), O (c) and HHV (d) at 30 min reaction time. Bars are LSD intervals with 95% confidence.

Regardless of the temperature or reaction time, the concentration of acetic acid exerts the same influence on the elemental analysis and HHV of the liquid phase. Increasing the concentration of acetic acid from 1 to 4 mol/L leads to an increase in the proportions of C and H together with a 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 (ºC) | minimise | 150-210 | 1 | 186 | |
| t (min) | minimise | 0-60 | 5 | 0 | |
| $CH_3COOH$ (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 |
| **Solid fibre and elemental analyses** | | | | | |
| 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 |
| **Liquid composition (C-wt.%)** | | | | | |
| 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

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

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

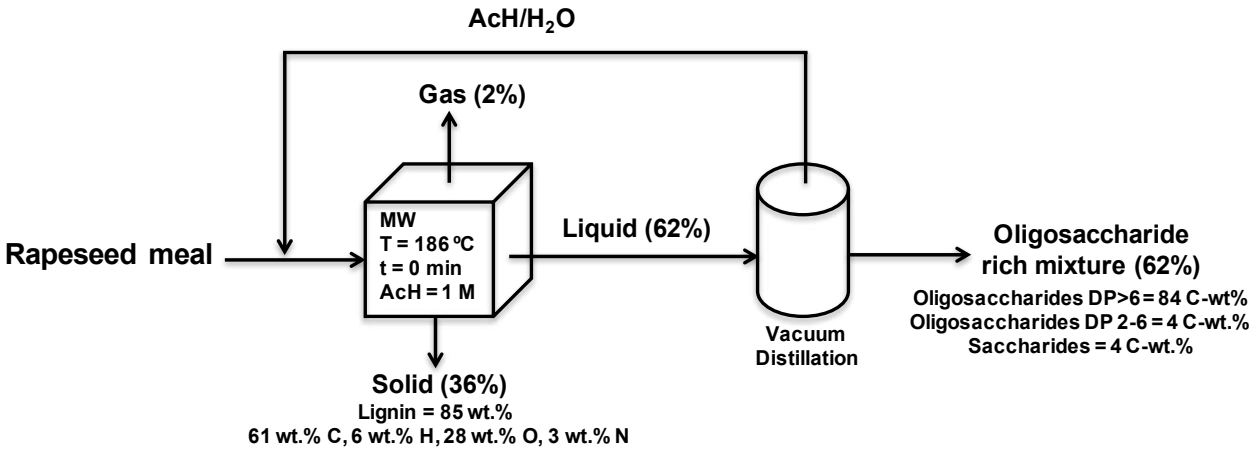


Figure 7. Schematic diagram for the simultaneous production of lignin and oligosaccharides

## 4. Conclusions

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

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

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

3. 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 characterisation revealed that the solid product decomposed into a mixture of phenols (1-19%), sugars (0-15%), nitrogen compounds (0-31%), carboxylic acids (37-75%), hydrocarbons (4-20%) and furans (1-8%). The progressive solubilisation of the cellulose and hemicellulose during the reaction produced an increase in the C content together with a decrease in the proportions of H and O of the solid. This also increased and decreased in the proportions of phenols and sugars, respectively.

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

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

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

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