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Towards the development of a novel "bamboo-refinery" concept: selective bamboo fractionation by means of a microwave-assisted, acid-catalysed, organosolv process

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Abstract

This work addresses a novel microwave-assisted, acid-catalysed, organosolv (EtOH/H_2O) system for the selective fractionation of bamboo, examining the effects of the temperature (110-190 ºC), solvent system (EtOH/H_2O) and catalyst amount (0-5 vol.% formic acid) on the process. The statistical analysis of the results revealed that the operating variables have a significant influence on bamboo fractionation, allowing the selective production of i) a cellulose-rich solid fraction, ii) a hemicellulose rich water-soluble fraction and iii) a lignin rich solid fraction. The yields of each of these fractions varied between 51-94%, 2-23% and 2-32%, respectively. Increasing temperature exerted a positive effect on bamboo decomposition, increasing the overall bamboo conversion and influencing the effect that the solvent system (EtOH/H_2O) has on the process. At low temperature (110 ºC) the solvent system does not have much influence, while a synergetic interaction between EtOH and H_2O took place at higher temperatures, which allowed better results to be obtained with EtOH/H_2O mixtures than with the pure solvents alone. The effect of the catalyst was relatively weak, being greatest when using a high temperature (190 ºC) and high proportions of water (>85 vol.%) in the solvent system. With respect to the properties of each fraction, the cellulose rich solid fraction was made up of un-reacted cellulose (44-83 wt.%), hemicellulose (0-21 wt.%) and lignin (12-34 wt.%); the water-soluble hemicellulose rich fraction consisted of a mixture of oligomers, sugars, carboxylic acids, ketones and furans; and the solid rich lignin fraction comprised high purity (>95 wt.%) organosolv lignin. The optimisation of the process revealed that by using a temperature of 190 ºC, a solvent system consisting of 45 vol.% EtOH and 55 vol.% H_2O with a concentration of formic acid of 5 vol.% it is possible to fractionate bamboo into a high purity (84 wt.%) cellulose solid fraction, very pure (>95%) organosolv lignin and a rich water-soluble hemicellulose fraction consisting of a mixture of oligomers (27 wt.%), sugars (56 wt.%) and carboxylic acids (14 wt.%); thus converting this process into a very promising method for the selective fractionation of bamboo.

Keywords: microwaves, bamboo, organosolv, biomass fractionation, value-added products

Highlights (85 characters including spaces)

- Microwave-assisted bamboo fractionation into cellulose, hemicellulose and lignin

- Analysis and optimisation of the temperature, EtOH/H_2O system and catalyst amount

- Synergetic effect between EtOH & H_2O during bamboo fractionation at high temperature

- Optimum conditions for bamboo fractionation: 190 ºC, 45% EtOH/H_2O and 5 vol.% HCOOH

1. Introduction

Throughout the world new policies to mitigate global climate change have led researchers to seek new processes, alternative renewable materials and more sustainable strategies to replace the current petroleum-based energy industry with a greener and more environmentally-friendly energy market. As part of this, the bio-refinery concept is gaining increasing attention, as biomass is the only renewable source of carbon that can be converted into gas, liquid and solid products [1]. Unfortunately, the development of a lignocellulosic biorefinery has received some criticism, since energy and chemical production in this way could affect the food supply and total forestry area. To overcome this issue, researchers are looking for fast growing and low “food-conflict” biomasses.

Among all the potential biomass feedstocks, bamboo is regarded as an excellent candidate. Bamboo is a big wooden plant large, which is widely distributed in subtropical temperature zones around the world (latitudes from 46°N to 47°S) [2]. As one of the fastest growing woody plants, bamboo can grow at a rate of 30-60 cm/day to 15-40 m with a culm diameter of 30 cm [2, 3]. Due to its fast-growing property, bamboo generally could produce a higher yield of biomass than other common crops [4]. Typically, bamboo contains 37-47 wt.% cellulose, 15-30 wt.% of hemicellulose and 18-31 wt.% lignin, depending on the species [4]. In the past, bamboo was widely cultivated in Asian countries and used as a raw material for food, handicrafts, paper and construction. In recent years, bamboo has shown a huge potential to be used for the production of biofuel and other value-added chemicals via bioconversion [4-7].

Bioconversion is believed to be one of the most attractive methods for lignocellulosic biomass utilisation [8, 9]; enzymes and bio-catalytically active cells (fermentation) being the two pathways commonly employed [10]. This biotechnology is mainly used for bioethanol, biodiesel, biobutanol, methane and fine chemicals production [11]. In the past few years, bioconversion technology has undergone dramatic growth, which has increased the attention of researchers and industry. Nevertheless, the intrinsic complexity of biomass hampers the development of this technology, and more research is needed. In particular, within the lignocellulosic biomass structure, cellulose and hemicellulose are intimately associated with lignin in the cell wall, and part of the cellulose is in crystalline status. This hinders enzymes and microbes from achieving biomass digestion and, therefore, the pre-treatment of biomass for its selective fractionation into its main constituents (cellulose, hemicellulose and lignin) is still a challenge.

In the midst of the different strategies for biomass fractionation, the use of water/organic solvent (organosolv) processes is gaining increasing attention. Several works have been conducted with rice straw [12], cotton stalk [13], wheat straw [14-16], hemp hurds [17, 18], corncob [19, 20], maple wood [21], reed [22], corn stover [23] and bamboo [24, 25]. This route is well known for its higher efficiency in removing lignin under acidic condition than other processing methods. Organosolv systems can be classified into two categories: pure organic (simple) and aqueous-organic solvent (multiple) systems. In general, aqueous-organic systems perform better than single solvent systems [26, 27]. The multiple systems consist of a nucleophile agent (H_2O, in the vast majority of the cases, to react with the activated linkages in biomass) and an organic solvent to help dissolve the liberated fragments [20]. This water/organic mixture has a significant influence on biomass depolymerization kinetics and thermodynamics, which affects not only the solubility of biomass but also the dissolution of biomass-derived products. This is of particular interest for the solubilisation of lignin which has both nonpolar and polar functional groups [28]. In addition, a homogeneous acid catalyst is used in some cases to increase the kinetics of biomass depolymerization in organosolv systems and, therefore, help increase the overall efficiency of the process.

Regarding the organic compounds used in H_2O/co-solvent systems, alcohols are the most commonly used as they have a low boiling point and can efficiently fractionate biomass into relatively high purity lignin and cellulose for further applications [29]. In particular, Hu et al. [30] regarded the H_2O/ethanol organosolv system catalysed by oxalic acid as one of the greenest co-solvent systems for biomass fractionation. They reported that by using this orgnaosolv system and employing a temperature of 140°C and a pressure of 2MPa for 1h, it was possible to recover 88 wt.% of the hemicellulose and 89 wt.% of the lignin present in corn stover, the cellulose remaining in the solid residue. Quignard et al. [16] worked on the extraction of lignin from wheat straw by using a H_2O-ethanol organosolv system at 160°C for 2h, examining the effect of different homogeneous Lewis acidic catalysts, such as $FeCl_2$, $CuCl_2$, $FeCl_3$, $Ga(OTf)_3$, and $ZrOCl_2$. It was found that lignin extraction was related to the acidity of the catalyst, with a maximum of 80% lignin conversion. Methanol has also been used in water-organic systems. In particular, Shimizu and Usami [31] used a catalysed (0.2 wt.% of HCl) methanol/H_2O mixture at 170 ºC for 45 min to fractionate pine wood, achieving very high hemicellulose (100%) and lignin (90%) recoveries. In addition, high boiling point alcohols, such as ethylene glycol and glycerol, have also been tested in the presence of H_2O for organosolv processes. This allows a low-pressure treatment to be conducted, but hinders solvent removal; thus decreasing the energy efficiency of the overall treatment [29].

Besides alcohols, other organic solvents have also been tested with positive results for biomass fractionation. For example, the H_2O/γ-valerolactone (GVL) co-solvent system was positively used for the simultaneous separation of lignin from corn stover at 150 °C for 30 min, employing H_2SO_4 as a catalyst, achieving high yields for both lignin (75%) and hemicellulose (72%) [23]. H_2O/tetrahydrofuran (THF) assisted by Na_2CO_3 also showed 94.6% conversion of lignin from corncob residue, at 2 MPa of pressure at 140 °C for 1h, preventing the dissolution of cellulose [20]. Wyman et al. [21] compared the difference between two H_2SO_4 catalysed systems: H_2O/tetrahydrofuran (THF) and pure H_2O for maple wood degradation. The experiments were conducted at temperatures ranging 170-200°C with several reaction times (40-120 min). This comparison revealed that THF could significantly boost the efficiency of organosolv processes due to the good solubility of biomass decomposition products in THF, with more than 90% lignin recovery [21]. Furthermore, several organic acids were also tested in organosolv processes. Sun et al. [25] used an acetic acid-water system catalysed with HCl to extract lignin from bamboo. 95% of lignin and 48% of cellulose were recovered. The optimal delignification degree was achieved with 4 wt.% HCl in a 90 wt.% acetic acid-water solution using a batch reactor at 114°C for 2 h.

All these works published to date provide valuable information on the use of different organosolv systems for biomass fractionation. However, the work on analysing bamboo fractionation is very scarce [24, 25, 32-34] and, consequently, more research is needed for the valorisation of this biomass. In addition, these studies showed that the reaction conditions (especially the temperature, solvent system and catalyst) exert a significant influence on biomass fractionation, however parametric studies to thoroughly analyse the specific effect of the variables have never conducted. Therefore, the large number of factors significantly influencing the process increases its intrinsic complexity. Moreover, some interactions between some of these factors could occur so that the effects of some variables may depend on others, resulting in different consequences for the process. These interactions have never been considered in the works addressing organosolv processes for biomass fractionation. In addition, optimum conditions for bamboo fractionation and synergetic effects between solvents have never been analysed in depth. Given this background, this work addresses the microwave-assisted fractionation of bamboo by means of an ethanol/water organosolv process catalysed by formic acid. In particular, the effects of the ethanol/water concentration (0-100 vol.%), catalyst (formic acid) amount (0-5 vol.%) and temperature (110-190 ºC) together with all the possible interactions between these variables on bamboo fractionation have been analysed in depth. Microwave heating represents a potentially faster, more efficient and selective process for the thermal treatment of biomass [35, 36]. As water and ethanol are highly effective in microwave energy absorption, the combination of an organosolv system together with microwave assisted heating offers an interesting new technology for the valorisation not only of bamboo but also of many other types of biomass The fact that the combined effects of the operating variables and their interactions on an organosolv system has never been reported before demonstrate, together with the results provided by the in-depth study and the optimisation and energetic assesetement conducted, that this work represents a novel investigation in this field, which can help to develop a novel biorefinery concept based on bamboo fractionation, “the bamboo-refinery”.

2. Experimental

2.1 Material

Dried bamboo (*Phyllostachys heterocycle cv. pubescens*) was purchased from Anji County, Zhejiang Province, China. The raw bamboo was ground to 300 meshes. After grounding, the bamboo powder was washed by 50°C distilled water and then dried overnight at 105°C. This bamboo powder was characterised by means of proximate, ultimate and fibre (cellulose, hemicellulose and lignin) analyses, calorific value and ash content. Proximate 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, based on carbon, hydrogen and nitrogen content, 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. [24] to determine the amount of cellulose, hemicellulose and lignin in the material. In addition, Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to identify and quantify the amounts of metals.

2.2 Microwave experiments

The experiments were carried out in a CEM-Mars microwave system using a 70 mL scale PrepPlus® reactor copped with a fibre optic temperature sensor. For each experiment, 1.50 g bamboo powder along with 30 mL solvent ($EtOH+H_2O$) and catalyst (formic acid) mixture were loaded into the reactor. Before placing the reactor inside the microwave unit, the reaction mixture was pre-stirred at room temperature for 10 min. A ramping time (time to reach the reaction temperature) and a reaction time of 5 min and 15 min were respectively used for all the experiments. After reaction, the reactor cooled down to room temperature by CEM-Mars default programme. Then, the reactor was opened and its content, consisting of a mixture of liquid and solid, was transferred to a centrifuge tube. After that, the reactor was carefully rinsed with 10 mL of an ethanol/water (50 vol.%) solution for 3 times and transferred to the centrifuge tube to recover all the material. Centrifugation was used to separate the solid from the liquid. Then, the solid residue obtained after centrifugation was rinsed with the 50 vol.% ethanol-water solution until the solution remained clear, and dried overnight at 105°C. The liquid phase obtained was made up of a mixture of ethanol in water along with the hemicellulose and lignin fractions solubilised from bamboo during the microwave experiments. Finally, ethanol was removed from the liquid phase by means of a rotary evaporator, leading to lignin precipitation; thus allowing the separation of hemicellulose (water fraction) and lignin (solid fraction) by filtration. After this separation, part of the rich lignin solid was re-solubilised in ethanol (ethanol fraction) for further analysis. Figure 1 shows a schematic diagram of the fractionation process developed.

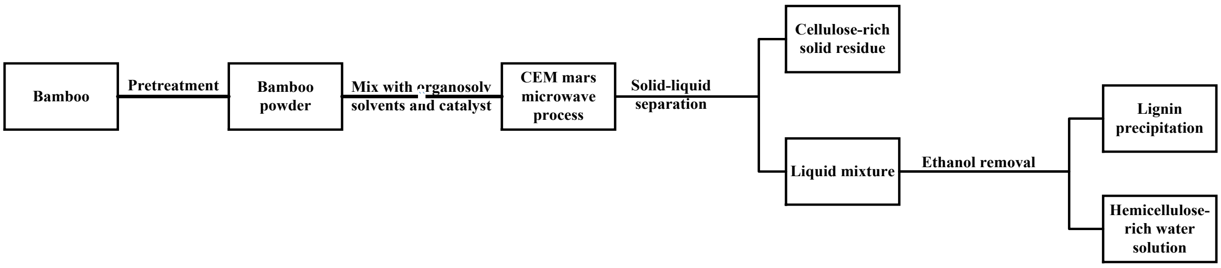

Figure 1. Fractionation process schematic diagram

2.3 Response variables and analytical methods

Several response variables were used to analyse the effect of the operating conditions on the process. These include the overall bamboo conversion, the gas yields as well as the yields and the most important properties of the three fractions produced: i) a cellulose-rich solid fraction, ii) a hemicellulose rich water-soluble fraction and iii) a lignin rich solid fraction. Table 1 summarises the response variables and the analytical methods used for their calculation.

The solid residue produced after centrifugation was used to determine the conversion of cellulose, hemicellulose and lignin by using the chemical titration method described by Hu et al. [24]. This calculation was also used to analyse the relative amounts of cellulose, hemicellulose and lignin in the cellulose rich fraction. In addition, this solid fraction was also analysed by X-ray Powder Diffraction (XRD) and Scanning Electron Microscopy (SEM). The XRD patterns were obtained from a Bruker AXS-D8 Advance diffractometer with a Kristalloflex 760 X-ray generator, which generates focused and monochromatized Kα X-rays from a Cu source. The samples were carefully milled into fine powders and placed evenly on a small round hole bevelled out of an aluminium sample holder. Scans were recorded across the range of 5-80 2θ over 10 minutes with a 40kV and 40mA current. Evaluation programme EVA and the Bruker CDS database were used to identify the phases present in the samples. SEM pictures were taken with an INSPECT F, at an acceleration voltage of 20KV. Samples were coated with gold using a vacuum sputter-coater to improve the conductivity of the samples and the quality of the SEM pictures.

The water liquid phase was analysed by high-performance liquid chromatography (HPLC) and Total Organic Carbon (TOC), while the ethanol liquid phase containing ethanol-soluble lignin was characterised by GPC. HPLC analyses were conducted with an Agilent Technologies 1200 series HPLC system employing a XDB-C18 column and a UV detector. A Waters GPC device equipped with a 2414 refractive index detector was used with Waters Strygel columns HT4 and HT3 in series. Empower III GPC software was used for running the GPC instrument and for calculations. Both the columns and the RI detector were maintained at 45°C for analysis. TOC analyses were conducted in a Vario TOC Cube Analyser.

Table 1. Response variable and analytical methods used for their determination

Response variables	**Analytical method**
Overall results	
$\text{Overall conversion (\%)} = \left(1 - \frac{\text{mass of solid residue}}{\text{mass of bamboo}}\right)100$	Mass difference
$\text{Hemicellulose rich fraction yield (\%)} = \left(1 - \frac{\text{mass of gas (g)} + \text{mass of solid residue (g)}}{\text{mass of bamboo (g)}}\right)100$	Mass difference
$\text{Cellulose rich fraction yield (\%)} = \frac{\text{mass of solid residue (g)}}{\text{mass of bamboo (g)}}100$	Mass difference
$\text{Lignin rich fraction yield (\%)} = \frac{\text{mass of lignin (g)}}{\text{mass of bamboo (g)}}100$	
$\text{Gas yield (\%)} = \frac{\text{mass of gas (g)}}{\text{mass of bamboo (g)}}100$	Mass difference
Cellulose rich fraction phase properties	
$\text{Composition (wt.\%)} = \frac{\text{mass of structural component (g)}}{\text{mass of solid residue(g)}}100$	Chemical titration
Lignin rich fraction phase properties	
Molecular weight (g/mol)	GPC (Gel permeation chromatography)
Hemicellulose rich fraction phase properties	
TOC (Total Organic Carbon)	TC-IC (Total Carbon- Inorganic Carbon) measurement
$\text{Composition (C} - \text{wt.\%)} = \frac{\sum \text{mass of C of each compound (g)}}{\text{total mass of C in solution (g)}}100$	HPLC (High performance liquid chromatography)

wt.% = weight percentage

C-wt.% = percentage in carbon basis

2.4 Experimental design and data analysis

The influence of reaction temperature (110-190°C), ethanol concentration in water (0-100 vol.%) and the amount of formic acid, used as a homogeneous catalyst, (0-5 vol.%) on bamboo fractionation was experimentally investigated. This ethanol/water system was very stable under the microwave conditions tested; i.e. the pressure achieved under the experiments was in good agreement with the saturation pressure of the solvent system at the temperature of the experiment, and neither runaway reactions nor explosions took place. 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. 9 axial experiments were performed to study non-linear effects and interactions according to the CCF design. In addition, 3 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. The ANOVA analysis helped for the selection of the operating variables and interactions that significantly influence the response variables under consideration. 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 (temperature, EtOH/H_2O and catalyst amount) were normalised from -1 to 1 (codec variables). This codification permits all operating variables (factors) to vary within the same interval and helps 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.

3. Results and discussion

3.1 Bamboo characterisation

Table 2 lists the characterisation results of bamboo. These include the proximate, ultimate and fibre analyses, the ash content and higher heating value (HHV). These results are fairly similar to those reported in the literature [37-39]. The presence of inorganics in bamboo is accounted for by the presence of powder derived from soil. Ca, Mg, K, Na, P and Si are the major inorganics detected. In addition, traces of other inorganic components, such as Fe, Al, S and Mn were also found in the feedstock.

Table 2. Proximate, fibre, elemental and calorific analyses of the bamboo used in this work.

Proximate analysis (wt.%)	
Moisture	3.46
Ash	8.15
Volatiles	63.52
Fixed carbon	20.31
Fibre analysis (wt.%)	
Cellulose	45.01±1.41
Hemicellulose	25.98±0.66
Lignin	20.80±0.17
Ash	8.15%
Elemental analysis (wt.%)	
C	47.58±0.34
H	5.83±0.03
N	0.48±0.04
O*	37.96±0.33
HHV (MJ/kg)	19.61±0.13
Ash composition (ppm)	
Ca	500
Mg	257.4
K	292
Na	170.41
P	108
Si	107

*Oxygen was calculated by difference

3.2 Effects of operating conditions on the overall conversion and products distribution

Table 3 summarises the operating conditions for the experiments and the experimental results. The overall bamboo conversion and the yields to gas, lignin-rich solid fraction, hemicellulose rich water fraction and cellulose rich solid fraction vary as follows: 6-49%, 1-9%, 2-23%, 2-32% and 51-94%, respectively. The relative influence of the operating variables on the global bamboo conversion and the yields of the three main fractions produced according to the ANOVA analysis, and the cause-effect Pareto principle is shown in Table 4. The gas yield is low and its not influenced by the operating conditions. This might suggest that formic acid decomposition did not take place due to the relative low temperature employed [40].

Table 3. Experimental conditions: temperature (ºC), solvent system (EtOH/H_2O, vol. %) and formic acid (vol. %) and results obtained in the experiments

Run	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9-12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
T (ºC)	110	190	110	190	110	190	110	190	150	110	190	150	150	150	150	190
EtOH/EtOH+H_2O (vol.%)	0	0	100	100	0	0	100	100	50	50	50	0	100	50	50	50
Formic acid (vol. %)	0	0	0	0	5	5	5	5	2.5	2.5	2.5	2.5	2.5	0	5	5
pH	4.16±0.39	4.16±0.39	5.42±0.02	5.42±0.02	1.53±0.04	1.53±0.04	1.37±0.02	1.37±0.02	2.12±0.03	2.12±0.03	2.12±0.03	1.83±0.03	1.83±0.03	3.46±0.21	1.45±0.03	1.45±0.03
Overall results																
Overall conversion (%)	6.55	34.74	10.27	12.62	14.12	48.75	5.59	16.45	20.27±1.71	13.43	46.91	29.79	10.80	20.63	19.04	49.00
Hemicellulose fraction yield (%)	4.17	23.22	6.77	8.12	10.98	32.33	2.33	10.94	12.35±1.46	9.04	24.04	23.60	7.44	12.22	11.73	25.82
Cellulose fraction yield (%)	93.45	65.26	89.73	87.38	85.88	51.25	94.41	83.55	79.73±1.71	86.57	53.09	70.21	89.20	79.37	80.96	51.00
Lignin fraction yield (%)	2.39	11.52	3.50	4.51	3.14	16.42	3.26	5.50	7.93±0.60	4.39	22.87	6.19	3.35	8.42	7.31	23.17
Gas yield (%)	1.36	3.23	2.94	2.31	4.85	8.67	3.76	2.39	2.79±0.64	2.45	2.47	2.25	2.58	1.47	2.44	3.74
Cellulose rich fraction fibre analysis																
Cellulose (wt.%)	43.94	57.62	49.33	51.85	55.82	61.92	49.33	46.78	52.86±2.87	49.65	61.55	49.32	56.79	54.61	45.79	83.00
Hemicellulose (wt.%)	14.88	4.22	19.57	15.76	17.82	0.00	18.95	19.10	14.09±0.79	19.85	2.46	7.20	20.86	15.10	12.99	1.99
Lignin (wt.%)	25.15	24.22	24.98	24.53	34.23	19.16	23.99	24.49	23.65±1.34	26.95	30.53	25.29	22.21	23.10	25.82	12.52
Lignin rich phase																
Mw (g/mol)	600	1528	3177	2758	2039	1053	3133	2540	2142±261	2262	1614	1567	2768	3060	2201	2323
Hemicellulose rich phase composition																
TOC (ppm)	0	353.05	174.37	34.31	206.44	157.76	89.69	56.06	148.75±90.27	34.52	172.94	126.11	56.58	177.44	93.28	397.27
Sugars (C- wt.%)	0	2.66	0	1.19	0.53	42.68	0.66	1.66	2.33±1.75	3.86	15.98	32.01	1.95	1.42	1.52	11.52
Acids (C- wt.%)	0	6.43	0	19.56	95.45	53.94	99.34	37.98	81.43±11.07	85.84	27.17	26.59	98.03	32.86	82.99	60.65
Oligomers (C- wt.%)	100.00	90.88	100.00	79.19	0	0	0	60.36	5.50±4.20	0	56.19	41.39	0.00	65.72	0	27.71

Table 4. Relative influence of the operating conditions according to the ANOVA analysis

Variable	R^2	I.Term	A (T)	B (EtOH%)	C (formic acid)	AB	AC	BC	ABC	A^2	B^2	C^2	A^2B	A^2C	AB^2	AC^2	A^2B^2
Overall results																	
Overall conversion (%)	0.99	20.17	16.21	-7.82	n.s	-6.2	1.74	-2.8	n.s.	9.48	n.s.	n.s.	n.s.	2.46	-6.71	n.s.	-11.01
			(28)	(17)		(12)	(7)	(5)		(3)				(8)	(8)		(11)
Hemicellulose fraction yield (%)	0.99	12.22	6.52	-8.08	n.s	-3.8	1.18	-2.19	n.s	4.25	3.3	n.s	2.76	1.77	n.s	n.s	-7.41
			(28)	(22)		(13)	(6)	(7)		(1)	(3)		(4)	(7)			(8)
Cellulose fraction yield (%)	0.99	79.83	-16.21	9.5	n.s	6.2	-1.74	2.8	n.s	-9.47	n.s	n.s	-2.09	-2.46	6.71	n.s	11.01
			(28)	(17)		(12)	(6)	(5)		(3)			(2)	(8)	(8)		(11)
Lignin fraction yield (%)	0.99	7.91	8.98	-1.95	n.s	-2.39	0.61	-0.61	n.s	5.46	-3.14	n.s	n.s	0.73	-5.77	n.s	-3.95
			(26)	(9)		(10)	(7)	(3)		(4)	(18)			(5)	(13)		(5)
Gas yield (%)	0.92	2.45	n.s	n.s	n.s	-0.96	n.s	-1	n.s	n.s	n.s	n.s	-0.84	1.19	n.s	0.42	1.24
						(17)		(18)					(18)	(23)		(7)	(18)
Solid residue fractionation composition																	
Cellulose (wt.%)	0.92	52.54	5.95	n.s	-4.41	-2.48	n.s	-2.66	n.s	n.s	n.s	n.s	-2.75	5.8	-26.6	23.12	n.s
			(20)		(9)	(7)		(8)					(9)	(10)	(17)	(20)	
Hemicellulose (wt.%)	0.99	14.06	-8.75	6.83	-1.05	3.1	-0.41	0.5	1.39	-2.96	n.s	n.s	-2.27	1.22	4.73	n.s	2.69
			(26)	(22)	(3)	(12)	(4)	(2)	(6)	(4)			(4)	(1)	(10)		(6)
Lignin (wt.%)	0.95	23.88	1.79	-0.79	n.s	2.01	-1.65	n.s	1.89	4.86	n.s	n.s	n.s	n.s	12.58	-16.36	-3.65
			(16)	(5)		(12)	(17)		(12)	(5)					(0)	(22)	(10)
Lignin phase																	
Mw (g/mol)	0.92	2097.5	-169.77	758.9	-429.5	n.s	-258.46	n.s	217.5	n.s	n.s	543.15	n.s	519.79	n.s	n.s	-537.15
			(8)	(40)	(1)		(10)		(11)			(5)		(13)			(13)
Water phase properties																	
TOC (ppm)	0.92	125.36	69.21	-43.23	n.s	-59.76	-36.91	n.s	63.52	n.s	n.s	n.s	n.s	n.s	-292.49	239.61	n.s
			(15)	(13)		(16)	(3)		(18)						(17)	(17)	
Sugars (C- wt. %)	0.93	13.5	n.s	27.83	n.s	-22.44	n.s	n.s	-22.15	n.s	57.79	n.s	-28.18	24.18	n.s	n.s	-44.9
				(5)		(18)			(18)		(12)		(10)	(21)			(15)
Acids (C- wt.%)	0.96	76.79	-29.33	35.72	31.54	n.s	-15.54	n.s	n.s	-14.52	-10.97	-13.09	-35.59	n.s	n.s	20.29	n.s
			(10)	(6)	(26)		(12)			(15)	(7)	(6)	(11)			(7)	
Oligomers (C- wt.%)	0.99	6.01	28.1	-20.7	-37.75	6.08	11.03	9.01	9.01	21.07	14.69	25.83	26.78	n.s	n.s	-24.55	-13.8
			(5)	(1)	(26)	(4)	(8)	(6)	(6)	(15)	(6)	(8)	(7)			(7)	(2)

n.s: Non significant with 95% confidence

Response = Indep. + Coefficient A·A + Coefficient B·B + Coefficient C·C + Coefficient AB·AB + Coefficient AC·AC Coefficient BC·BC Coefficient ABC·ABC + Coefficient $A^2 \cdot A^2$ + Coefficient $B^2 \cdot B^2$ + Coefficient $C^2 \cdot C^2$ + Coefficient $A^2B \cdot A^2B$ + Coefficient $A^2C \cdot A^2C$ + Coefficient $AB^2 \cdot AB^2$ + Coefficient $AC^2 \cdot AC^2$ + Coefficient $A^2B^2 \cdot A^2B^2$

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.

According to the cause-effect Pareto analysis, the temperature (with more than 26% of influence) is the operating variable exerting the greatest influence on the overall bamboo conversion and the yields to lignin, hemicellulose and cellulose rich solid fractions. In addition, the concentration of ethanol and the interactions (both linear and quadratic) between the temperature and the concentration of ethanol also have a significant influence on these response variables with more than 12% influence in all cases. The concentration of formic acid (catalyst) alone does not have a significant influence on these response variables. However, its interaction with the temperature is significant, thus suggesting a synergistic effect between these two variables; i.e. a certain temperature is needed for the acid to catalyse the process significantly. The gas yield is greatly influenced by the interaction between the temperature and the concentration of ethanol in water.

As regards the influence (positive or negative) of the operating variables on the process, the coefficients in the models indicate that an increase in the temperature leads to an increase in the overall conversion, the hemicellulose rich fraction and lignin rich fraction yields (positive terms), and a decrease in the cellulose rich solid fraction yield (negative term). The concentration of ethanol in water has the opposite effect; i.e. an increase in the proportion of ethanol results in a decrease in the overall bamboo conversion and the hemicellulose rich fraction and lignin rich fraction yields (negative terms) along with an increase in the cellulose rich solid fraction (positive terms). Moreover, as commented above, significant interactions between variables also take place, demonstrating that the other operating variables influence the effect that each variable has on the process. To gain a better understanding of the process, Figure 2 displays the effect of the operating variables and the most important interactions detected with the ANOVA analysis. Specifically, Figure 2 a and b illustrates the effect of the ethanol concentration and the temperature on the overall bamboo conversion in the absence of catalyst (formic acid = 0 vol.%) and for the highest amount of catalyst used in this work (formic acid = 5 vol.%), respectively. These effects are also shown for the lignin rich fraction, hemicellulose rich fraction and cellulose rich fraction yields in Figures 3 c-d, e-f and g-h, respectively.

As regard the effects of the temperature, Figure 2 shows that regardless of the other operating conditions, the temperature positively potentiates bamboo decomposition. Specifically, an increase in temperature from 110 to 190 ºC substantially increases the overall bamboo conversion as well as the yields of the lignin and hemicellulose fractions; which consequently results in a decrease in the cellulose fraction yield. The temperature positively enhances the kinetics of the process and greater microwave power is also needed to achieve higher temperatures, thus promoting the break of the intramolecular bonds between cellulose, hemicellulose and lignin in bamboo. Kassaye et al. [37] reported a similar trend during the hydrolysis of bamboo between 90 and 150 ºC with NaOH for 30 min.

The effect of the concentration of ethanol in water (solvent system) depends on the temperature due to the significant interaction detected between both variables, with two different developments occurring. At low temperature (110 ºC) the solvent system does not have a very important influence on the process, and similar conversions and yields to products are obtained regardless of the solvent composition, either using the pure solvents (ethanol and/or water) or any binary mixture. This suggests that a certain temperature is needed to both break the intermolecular bonds between the bamboo structural components and solubilise the released fractions into the solvent system. This is a consequence of the kinetics of the process and is in good agreement with the cause-effect Pareto results, which indicated that the temperature was the operating variable exerting the greatest influence on the overall conversion and the yields to different fractions. High temperatures are needed to achieve good conversion during the very short reaction (15 min) time employed in this work.

Conversely, as the temperature increases, the solvent system has a more important influence on the process, and an interaction between ethanol and water takes place. In particular, a synergetic interaction between EtOH and H_2O occurs for the overall bamboo conversion and the yields to lignin and cellulose fractions. Higher values are obtained for the bamboo conversion and the yield of lignin fraction and lower values for the yield of cellulose fraction with an EtOH/H_2O mixture than using the pure solvents (EtOH or H_2O alone), with a maximum occurring with a concentration of EtOH of around 40-50 vol.%. This maximum is in good accordance with other works reported in the literature [41-45] where maxima for lignin conversion occurred employing a concentration of 40-60 vol.% of EtOH in water. This solvent system-temperature interaction has been never detected in other works dealing with organosolv systems and can help to gain a deeper insight into this complex process.

This interaction is more obvious for the lignin yield than for the other variables, and it does not take place for the hemicellulose fraction yield, thus suggesting that the solvent system exerts the greatest influence on lignin fractionation. For lignin extraction, a nucleophilic solvent such as water is needed for extracting lignin from the biomass structure along with another solvent to dissolve the extracted lignin [28]. Water alone can extract lignin, however, the solubilisation of this lignin by water is the limiting step. Conversely, ethanol is not appropriate for lignin extraction, but it promotes lignin solubilisation. Therefore, the presence of both solvents in the system at the right temperature promotes both lignin extraction and solubilisation; which, therefore, significantly increases the lignin yield. This synergetic interaction does not take place for the hemicellulose fraction yield, for which the highest yield is produced with pure water; the addition of ethanol into the system progressively decreases the yield of this fraction. In addition, this effect is too weak for the rich cellulose solid fraction. These developments are a consequence of the different polarities of these two fractions compared to lignin as well as due to the lesser spread of hydrolysis reactions to extract hemicellulose in ethanol than in water [30].

The effect of the catalyst concentration on the overall bamboo conversion and the yields to the lignin, hemicellulose and cellulose rich fractions can be observed comparing Figure 2 a,c,e, and g with b, d,f and h, respectively. This comparison reveals that the effect of the catalyst for these response variables is relatively weak as predicted by the Pareto Analysis. In addition, the effect depends on the temperature. At low temperature (110ºC) the effect of the addition of up to 5 vol.% of formic acid in the process is almost negligible. However, at 190 ºC the catalyst has a more pronounced effect on bamboo decomposition as the kinetics of the process is enhanced. In addition, at this temperature, the specific effect of the catalyst depends on the solvent system. While an increase in the amount of catalyst promotes bamboo decomposition (increases bamboo conversion/decreases the cellulose solid yield and increases the yields to lignin and hemicellulose rich fractions) with pure water, the addition of ethanol progressively decreases the catalytic effect of formic acid in the process due to the lower solubilisation of formic acid protons in ethanol than in water [30].

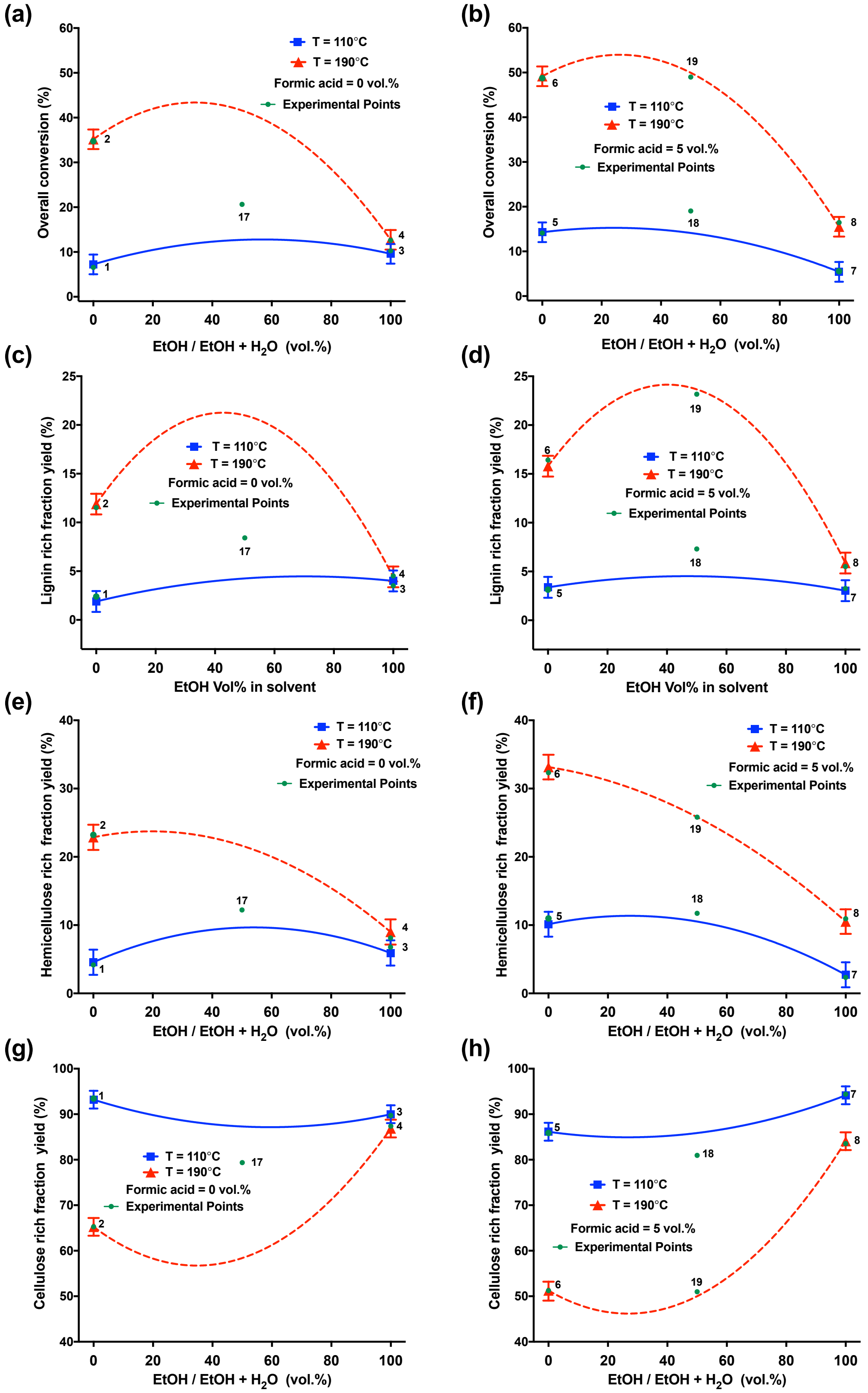

Figure 2. Interaction plots between the temperature and the solvent system (EtOH/H_2O) without/with catalyst for the overall bamboo conversion (a/b), lignin rich solid fraction yield (c/d), hemicellulose rich liquid fraction yield (e/f), and cellulose rich solid fraction yield (g/h). Bars are LSD intervals with 95% confidence.

3.3 Effects of operating conditions on the properties of each fraction

This section examines the effects of the operating conditions on several of the most important properties of the three products produced during the fractionation of bamboo: i) the cellulose-rich spent solid fraction, ii) the hemicellulose rich water sample fraction and iii) the lignin rich fraction.

3.3.1 Cellulose-rich solid fraction

This cellulose-rich solid fraction consists of the solid residue obtained by filtration after the microwave treatment of bamboo. The characterisation of this solid by chemical titration revealed that this fraction contains cellulose and, in less proportion, unreacted hemicellulose and lignin with varying compositions depending on the operating conditions. The amounts of cellulose, hemicellulose and lignin in the spent solid vary by: 44-83 wt.%, 0-21 wt.% and 12-34 wt.%. The effect of the operating conditions on the composition of this fraction according to the ANOVA analysis, and the cause-effect Pareto principle is shown in Table 4. The temperature and its interaction with the catalyst amount and in lesser extent with the solvent system are the factors exerting the greatest influence on the proportions of cellulose and lignin. The temperature, the solvent system and the interaction between these two variables primarily influence the proportion of hemicellulose. The effects of the operating conditions and interactions between them on the relative amounts of cellulose, hemicellulose and lignin are plotted in Figure 3. In particular, Figure 3 a, b and c shows the effects of the solvent system and the temperature in the absence of catalyst. Figures 3 d, e and f show these effects when using the highest concentration of catalyst (5 vol.%) employed in this work.

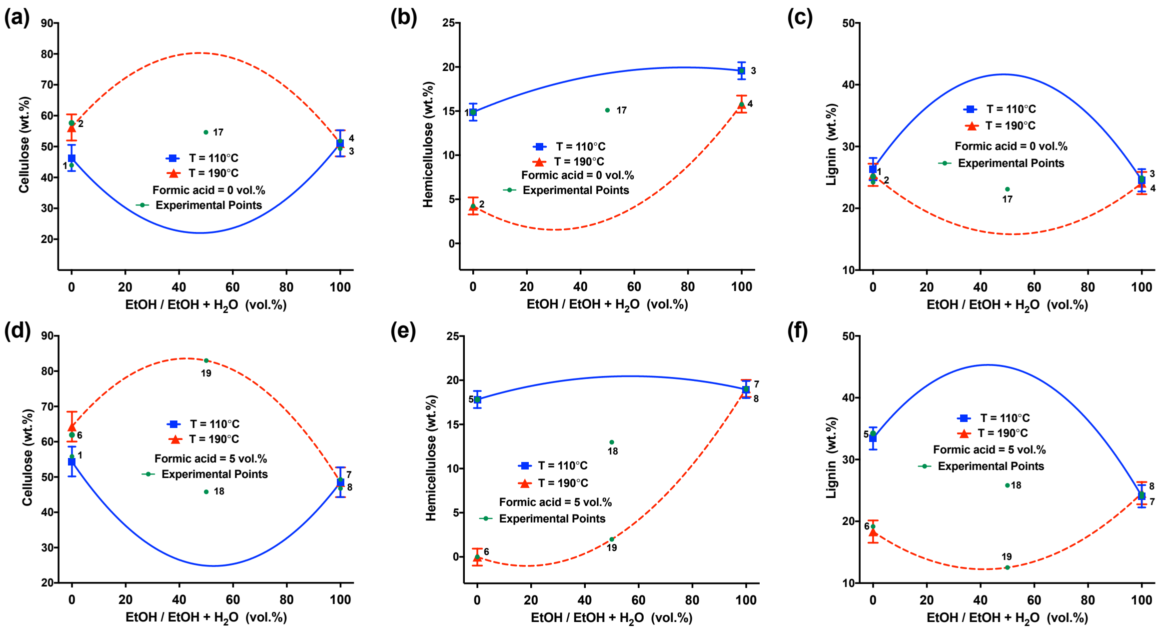

Figure 3. Interaction plots between the temperature and the solvent system (EtOH/H_2O) without/with catalyst for the relative amounts of cellulose (a/d), hemicellulose (b/e) and lignin (c/f) of the cellulose rich solid fraction. Bars are LSD intervals with 95% confidence.

With respect to the effects of the temperature on the composition of the cellulose rich fraction, Figure 3 shows how regardless of amount of catalyst employed (0-5 vol.% formic acid), an increase in the temperature between 110 and 190 ºC results in a decrease in the proportions of hemicellulose and lignin, and consequently, the proportion of cellulose increases. An increase in the temperature helps to solubilise the hemicellulose and lignin fractions of bamboo into the solvent system, and therefore a cellulose-rich solid is obtained due to the positive kinetic effect of the temperature in the process. A solid with a high proportion of hemicellulose (80-85%) can be obtained at 190 ºC employing a concentration of EtOH of around 40-50 vol.%. This is in good agreement with other works where the EtOH/H_2O solvent system was used for the fractionation of biomass [41-45].

In addition, an interaction between the temperature and the solvent system was detected in the ANOVA analysis and can be observed from the results plotted in Figure 3. This indicates that the effect of the temperature depends on the solvent system and vice versa. The temperature has a significant influence on the process for the majority of the binary mixtures considered (between 15 and 85 wt.% of ethanol in water). However, for the pure solvents or high concentrated in one compound (>85 wt.%) binary mixtures, the effect of the temperature decreases dramatically, being insignificant in some cases. This development might be a consequence of the positive influence of the temperature on the solvent system in this work.

At 190 ºC, the positive kinetic effect of the temperature can mask the effect on the solvent system on lignin solubilisation, resulting in a cellulose solid fraction with similar lignin content regardless of the solvent system. Conversely, the effect of the solvent can be observed at low temperature. Pure water helps lignin depolymerisation [28], while ethanol can react with α-hydroxyl groups in bamboo to increase the solubility of lignin, preventing its condensation; thus decreasing the lignin content of this fraction at 110 ºC [28]. For these reactions to take place at low temperature, high biomass/ethanol and biomass/water ratios might be needed, therefore pure solvents are required, and the synergetic effect between both solvents does not take place a low temperature. The opposite effect takes places for the relative amount of hemicellulose in the solvent system; i.e. the proportion of hemicellulose is not strongly influenced by the solvent system at low temperature, and this fraction has a high proportion of hemicellulose due to the low solubilisation of this structural component at low temperature. Conversely, the solvent system significantly influences the relative amount of hemicellulose at high temperature (190ºC). Using pure water the proportion of hemicellulose in the solid is very low, and the addition up to a 40 vol.% of ethanol in the system does not influence the relative amount of hemicellulose in the solid. However, a further increase in the proportion of ethanol from 40 to 100 vol.% results in a sharp increase in the relative amount of hemicellulose in the spent solid due to the lesser spread of hydrolysis reactions to extract hemicellulose in ethanol than in water [30]. The variations observed for the relative amount of cellulose in this fraction are related to the variations observed for hemicellulose and lignin, as this fraction is not very reactive under the operating conditions tested in this work.

The catalyst has a weak effect on the proportions of cellulose, hemicellulose and lignin of this fraction, with two different effects occurring depending on the temperature. At low temperature an increase in the amount of formic acid between 0 and 5 vol.% leads to a decrease in the proportion of cellulose along with an increase in the relative amount of hemicellulose and lignin, the opposite is observed at high temperature (190 ºC); i.e. the proportion cellulose increases and the relative amounts of hemicellulose and lignin decrease.

3.3.2 Lignin-rich solid fraction

This rich lignin fraction consists of the solid residue produced after removing the ethanol from the liquid collected after the microwave experiments. The lignin content of this solid fraction was greater than 95 wt.%, which is in good agreement with other works reported in the literature that regard this solid as high purity organosolv lignin and suggests that the formation of humins does not substantially take place under the operating conditions tested in this work. This highlights the good controllability and selectivity of this microwave-assisted organosolv process for the fractionation of bamboo. The molecular weigh of this fraction, determined by GPC, shifts between 600 and 3177 g/mol. The effects of the operating conditions on the molecular weight of the organosolv lignin produced according to the ANOVA analysis, and the cause-effect Pareto principle is shown in Table 4. This analysis shows that the solvent system (ethanol concentration in water) is the factor exerting the greatest importance on the molecular weigh of this fraction, the temperature and the catalyst amount having a lower influence. Figure 4 a and b show the effect of the solvent system on the molecular weight of the organosolv lignin produced at low and high (110 and 190 ºC) temperatures for two catalyst loadings: 0 vol.% and 5 vol.%, respectively. Regardless of the temperature and the catalyst loading, this fraction has the lower molecular weight when only water is used in the process. The addition of up to 45-50 vol.% of ethanol in the solvent mixture increases the molecular weight of this fraction, while a further increase of up to 100 vol.% does not modify the molecular of the lignin produced. The presence of water in the solvent system helps to depolymerise lignin, thus decreasing the molecular weight of the lignin produced [20].

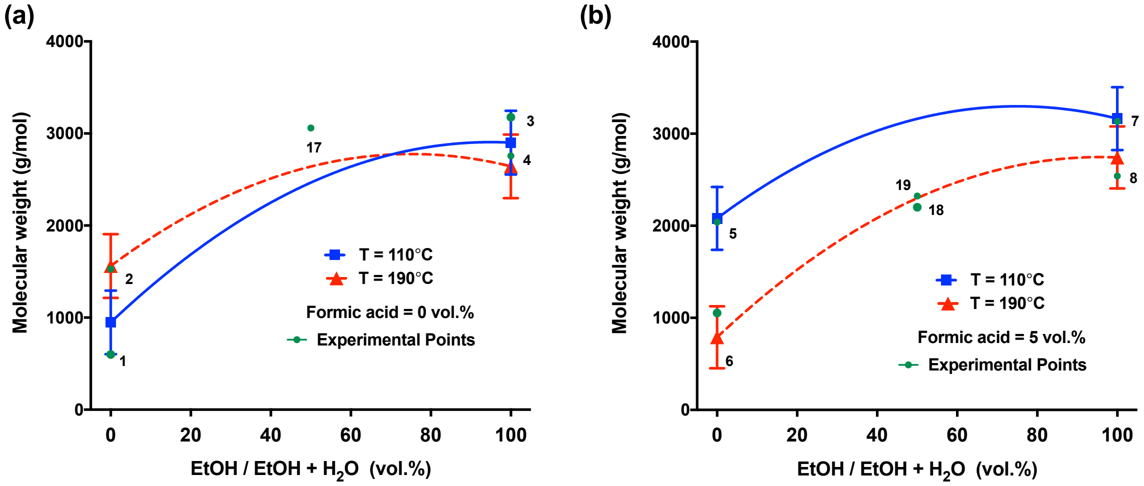

Figure 4. Interaction plots between the temperature and the solvent system (EtOH/H_2O) without/with catalyst (a/b) for the molecular weight of the lignin produced. Bars are LSD intervals with 95% confidence.

The effect of the temperature depends on the catalyst amount. On the one hand, in the absence of a catalyst, the temperature does not exert a significant influence regardless of the solvent system. On the other, when the highest amount of catalyst is used, the effect of the temperature depends on the solvent system with two developments observed. In particular, an increase in the temperature decreases the molecular weight of lignin when pure water is used. The addition of ethanol progressively reduces the effect of the temperature and as a consequence, the effect of this variable stops having a significant influence on the molecular weight of the organosolv lignin produced for concentrations of ethanol higher than 75 vol.% due to the lower effectiveness of ethanol than water in lignin depolymerisation [20].

3.3.3 Hemicellulose-rich liquid fraction

This fraction comprises water-soluble hemicellulose decomposition products. It is made up of a mixture of oligomers, sugars (cellobiose, glucose and xylose), carboxylic acids (acetic acid), ketones (γ-valerolactone, GVL) and furans (furfural and 5-hydroxymethylfurfural, HMF). The relative amounts (in C basis) of these families of compounds vary by 0-100 wt.%, 0-100 wt.%, 0-100 wt.%, 0-13 wt.% and 0-4 wt.%, respectively. Table 4 lists the effects of the operating conditions on the composition of this fraction according to the ANOVA analysis and the cause-effect Pareto principle. This analysis indicates that the operating conditions do not significantly influence on the relative amounts of ketones and furans of this fraction, probably due to their low concentration in the liquid phase under the operating conditions used in this work. In addition, methyl acetate was not found in the chemical analysis of the water-soluble hemicellulose rich fraction; thus suggesting that the possible esterification reaction between ethanol and formic acid did not significantly take place under the operating conditions tested in this work. The statistical analysis shows that the interaction between the temperature and the solvent system is the factor with the highest influence on the proportion of sugars, while the amount of catalyst is the operating variable with the highest influence on the relative amounts of carboxylic acids and oligomers. In addition, significant interactions between the operating variables also take place.

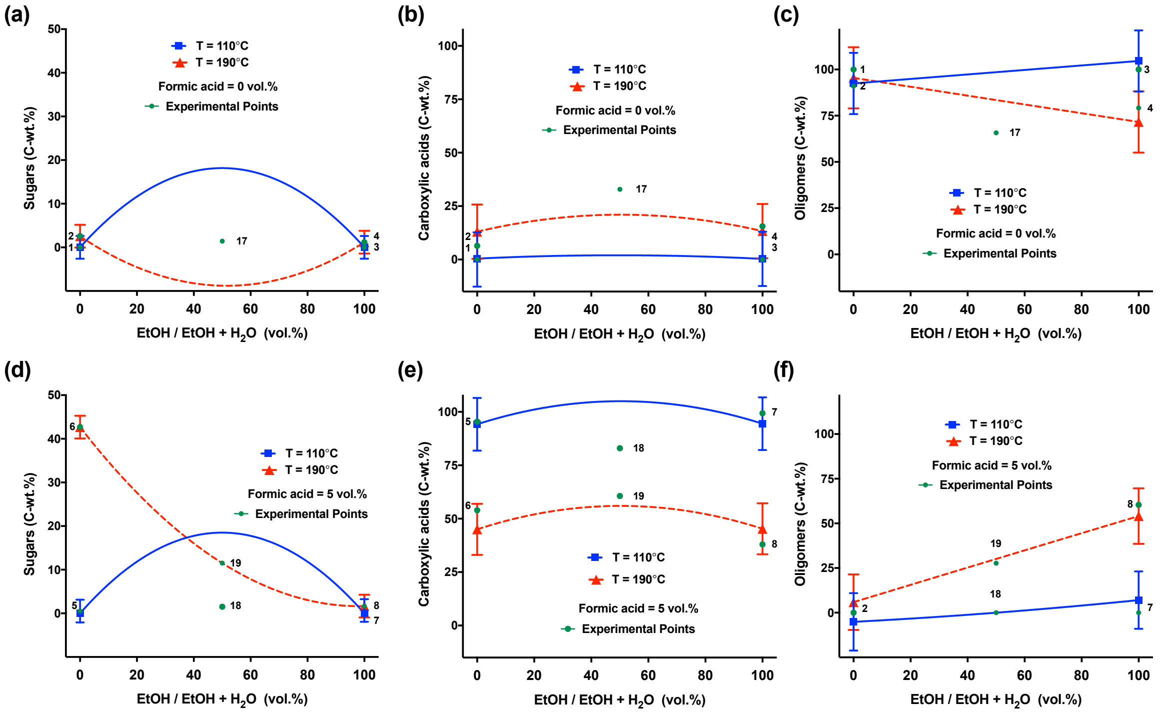

Figure 5. Interaction plots between the temperature and the solvent system ($EtOH/H_2O$) without/with catalyst for the relative amounts of sugars (a/d), carboxylic acids (b/e) and oligomers (c/f) present in the water-soluble hemicellulose rich fraction. Bars are LSD intervals with 95% confidence.

The effect of the operating conditions and the most important interactions for the proportions of sugars, carboxylic acids and oligomers are shown in Figure 5. In the absence of a catalyst, this hemicellulose rich water fraction is primarily made up of oligomers, the proportions of sugars and carboxylic acids being quite low regardless of the solvent system. Conversely, the addition of a catalyst (formic acid) decreases the proportion of oligomers and increases the concentration of sugars, and carboxylic acids in this fraction. At low temperature (110 ºC) carboxylic acids are the most abundant compounds of this fraction, while an increase in the temperature increases the relative amounts of sugars and oligomers and decreases the proportion of carboxylic acids in the liquid due to greater conversion of hemicellulose and lignin. At 190 ºC the proportion of carboxylic acids is not influenced by the solvent system, while this operating variable exerts a significant influence on the relative amounts of sugars and oligomers. With pure water, this fraction has a high concentration of sugars, and a negligible quantity of oligomers is observed. The increase of the proportion of ethanol in the solvent system decreases the proportion of sugars and increases the proportion of oligomers. The presence of water in the solvent system promotes hydrolysis reactions, thus decreasing the proportion of oligomers and, therefore, increasing the amount of sugars [28].

3.4 Optimisation of the fractionation process and energetic assessment

Optimum conditions were sought for the selective fractionation of bamboo making use of the experimental models developed (Table 4). The predicted R^2 of all the models are greater than 0.90, allowing their use for prediction purposes within the range of study. The optimisation process comprises the minimisation of the cellulose conversion (maximisation of the cellulose solid rich fraction and proportion of cellulose of this fraction) and the maximisation of the yields of hemicellulose rich and lignin rich fractions. To meet this objective, a solution that strikes a compromise between the optimum values for all the response variables was sought. To do so, 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 5 lists the relative importance assigned to each variable as well as the criteria used in the whole optimisation. A relative importance of 3 was given to the overall conversion and the cellulose, hemicellulose and lignin yields, while a relative importance of 5 was given to proportions of cellulose, hemicellulose and lignin in the cellulose rich fraction to increase the purity of this fraction and therefore the purity of the lignin and hemicellulose fractions produced. This strategy allows striking a compromise between yield and purity to maximise the process by using the desirability objective function D(X) shown in Eq.1.

$$D(X) = (d_1^{r1} \cdot\ d_1^{r2} \cdot \ldots \cdot d_1^{rn})^{\frac{1}{\Sigma rj}} = (\textstyle\prod_{j=1}^{n} d_j^{rn})^{\frac{1}{\Sigma r_j}} \qquad \text{(Eq. 1)}$$

Where n is the number of responses considered in the optimisation and r_j is the relative importance of each response (1-5)

Table 5. Theoretical optimisation: operating conditions and response variables: objectives, the interval of variations, relative importance, theoretical and experimental optimum values.

Variables	**Objective**	**Interval of variation**	**Relative importance (1-5)**	**Optimum Theoretical**	**Optimum Experimental**
Temperature (ºC)	none	110-190		190	
EtOH/EtOH+H_2O (vol.%)	none	0 - 100		45	
HCOOH (vol.%)	none	0 - 5		5	
Overall Conversion (%)	maximise	0-100	3	52	51.23
Cellulose rich faction					
Yield (%)	maximise	0-100	3	49	50.46
Cellulose content (wt.%)	maximise	0-100	5	84	84.87
Hemicellulose content (%)	minimise	0-100	5	1	1.85
Lignin Content (%)	minimise	0-100	5	12	11.55
Lignin rich fraction					
Yield (%)	maximise	0-100	3	24	23.87
Molecular weight (DA)	none			2203	2201
Hemicellulose rich fraction					
Yield (%)	maximise	0-100	3	27	26.82
Oligomers (C-wt.%)	none	0-100		27	27.80
Sugars (C-wt.%)	none	0-100		56	60.11
Carboxylic acids (C-wt.%)	none	0-100		14	12.65

Taking these conditions into account, the optimisation predicts an optimum at 190 ºC using a solvent system consisting of 45% EtOH/50% H_2O (vol/vol) with a concentration of formic acid of 5 vol.%. Under these conditions, it is possible to selectively fractionate bamboo into cellulose, hemicellulose and lignin. The cellulose rich fraction contains 84 wt.% of cellulose and 12 wt.% of lignin. The yield to organosolv lignin is very high (24%), which corresponds to a recovery of 90 wt.% of the lignin present in the original bamboo. In addition, hemicellulose is completely removed from the original feedstock, and a water solution consisting of a mixture of oligomers, sugars and carboxylic acids can be produced. This optimum was checked experimentally (Table 5) and non-statistically significant differences were found between the theoretical prediction and the experimental results with 95% confidence.

To gain a better insight into this fractionation process, the original bamboo and the spent solid produced at the optimum condition were characterised by Scanning Electron Microscopy (SEM) and X-ray Diffraction (XRD), and a comparison was established between both materials. The SEM images (Figure 6) show how after the microwave-assisted organosolv treatment the original micro-fibrous structure of bamboo disappears and the solid residue surface becomes significantly rougher. In addition, some small spheroidal particles appeared on the cellulose fibrillar structure after the treatment (under 1500 magnification), which was possibly caused by lignin precipitation onto the fibres [46, 47]. These differences suggest that most of the lignin and hemicellulose are removed during the organosolv process. A small quantity of lignin still remains on the surface of the cellulose rich fraction due to the lignin condensation. These developments are in a good agreement with the experimental results listed in Table 5.

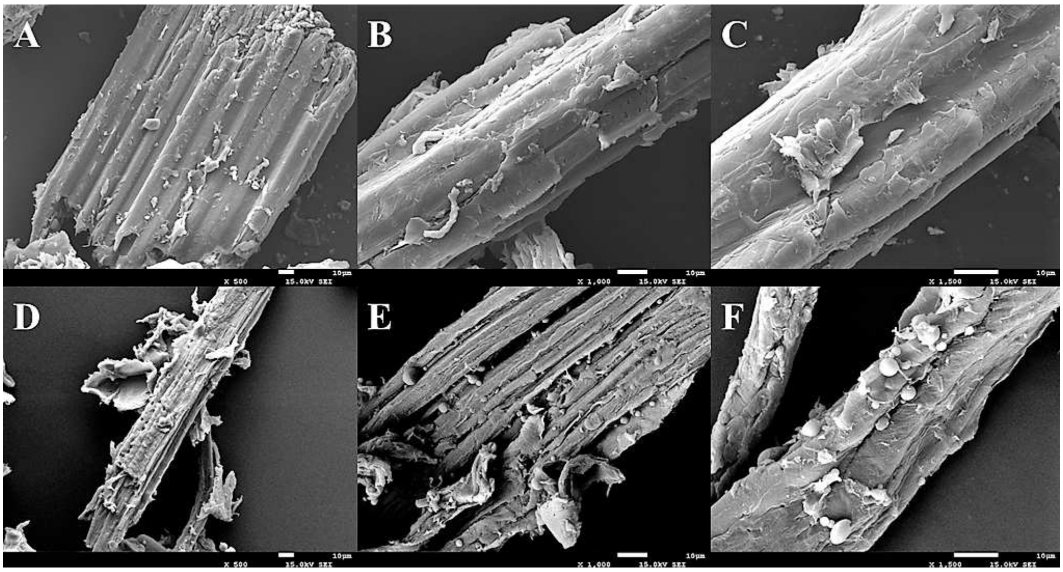

Figure 6. SEM images of bamboo powder (A: ×500, B: ×1000, C: ×1500) and optimum experimental run solid residue (D: ×500, E: ×1000, F: ×1500).

XRD was used to evaluate the effectiveness of the optimised microwave assisted organosolv process by comparing the degree of crystallinity (DoC) of both materials (before and after the treatment) [48, 49]. Figure 7 shows the XRD patterns for the original material and the spent solid (high purity cellulose fraction) produced after the microwave treatment. The comparison of both spectra does not show great differences between both solids, which is in good agreement with the experimental results, as only crystal cellulose can be detected by XRD. In addition, the crystallinity degree increases from 0.54 (original bamboo) to 0.64 (cellulose rich fraction). This is in good agreement with the experimental results described above, as hemicellulose and lignin (amorphous) are removed during the treatment and, therefore, the proportion of cellulose in this fraction increases; thus augmenting the degree of crystallinity of this fraction.

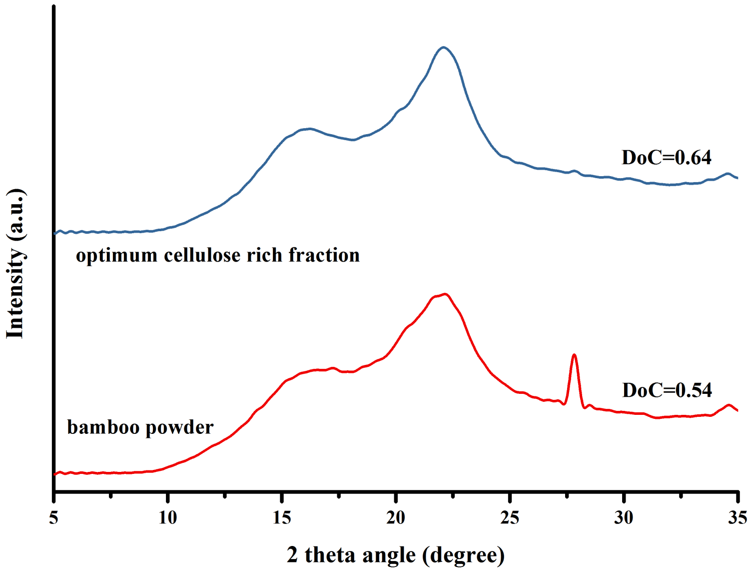

Figure. 7 XRD patterns for the original bamboo powder and spent solid produced the optimum conditions.

Finally, a basic energetic assessment was conducted to analyse the energy feasibility of this organosolv process. This process relies on thermal energy, i.e., for heating of the feedstock to reaction conditions, for product separation by drying and for thermal solvent recovery. The physicochemical properties used for this calculation are listed in Table 6. At the optimum operating conditions (reaction temperature and solvent system), the energy required to heat up the solvent system and bamboo from 25 to 190 ºC is 10.89 MJ/kg bamboo and 0.25 MJ/kg bamboo, respectively. In addition, the energy required for ethanol evaporation is 9.4 MJ/kg bamboo. Therefore, the input energy, calculated as the sum of energy needed to heat the feed up to the reaction temperature and the energy needed for solvent evaporation is 20.5 MJ/kg bamboo. In a real case, it would be necessary to include heat losses as well as the energy demand for all the operation units required in the process. Viell et al. [50] and Garcia et al. [51] reported energy requirements of 28.8 MJ/kg biomass and 11.84 MJ/kg biomass for wood biomass, respectively. Very interestingly, it was also reported that the energy requirement of organosolv processes could be reduced up to around 6 MJ/kg biomass using process unit heat integration [50], which makes this organosolv process more energetically feasible.

Table 6. Physicochemical properties of biomass and solvents used for energy calculations

Chemical property	Energy
Water heat capacity	0.00418 $MJ\cdot Kg\text{-}^{1}\cdot K^{-1}$
Water enthalpy vaporisation at normal boiling point	2.26 $MJ\cdot Kg\text{-}^{1}$
Ethanol heat capacity	0.00242 $MJ\cdot Kg\text{-}^{1}\cdot K^{-1}$
Ethanol enthalpy vaporisation at normal boiling point	0.841 $MJ\cdot Kg\text{-}^{1}$
Biomass heat capacity (estimated) [52]	0.0015 $MJ\cdot Kg\text{-}^{1}\cdot K^{-1}$

4. Conclusions

This work addresses a novel microwave-assisted, acid catalysed, organosolv process for the selective fractionation of bamboo analysing how and to what extent the reaction temperature (110-190 ºC), solvent system (EtOH/H_2O) and catalyst amount (0-5 vol.% formic acid) affect the yields and the most important properties of each fraction. The most important conclusions obtained from this work are summarised as follows.

1. The operating variables have a significant influence on bamboo fractionation, with three main fractions being obtained: i) a cellulose-rich solid fraction, ii) a hemicellulose rich water-soluble fraction and iii) a lignin rich fraction. The yields to each one of these fractions varied by 51-94%, 2-23% and 2-32%, respectively.

2. Increasing temperature positively enhances bamboo decomposition, increasing the overall bamboo conversion. The effect of the concentration of ethanol in water (solvent system) depends on the temperature. At low temperature (110 ºC) the solvent system does not exert a very important influence, while a synergetic interaction between EtOH and H_2O takes place at high temperature, and better results are obtained with EtOH/H_2O mixtures than with pure solvents alone. The effect of the catalyst is relatively weak, however it does demonstrate a noticeable effect when using a high temperature and high proportions of water in the solvent system.

3. The cellulose rich solid fraction consists of the solid residue obtained after the microwave treatment of bamboo, and it is made up of un-reacted cellulose (44-83 wt.%), hemicellulose (0-21 wt.%) and lignin (12-34 wt.%). The water-soluble hemicellulose rich fraction consists of a mixture of oligomers, sugars, carboxylic acids, ketones and furans. The relative amounts (in C basis) of these families of compounds vary by 0-100 wt.%, 0-100 wt.%, 0-100 wt.%, 0-

13 wt.% and 0-4 wt.%, respectively. The solid rich lignin fraction comprises high pure (>95%) organosolv lignin, with a molecular weight range of 600 to 3177 g/mol.

4. An optimum for this bamboo fractionation process was found at 190 ºC using a solvent system consisting of 45% EtOH/50% H_2O (vol/vol) with a concentration of formic acid of 5 vol.%, employing a reaction time as short as 15 min; thus converting this process in a very selective and efficient process for the valorisation not only of bamboo but also of many other types of biomass. These conditions maximise the solubilisation of lignin and hemicellulose in the organosolv system and minimise cellulose conversion; thus allowing the selective fractionation of bamboo into a high purity (84 wt.%) cellulose solid fraction, pure (>95%) organosolv lignin and a rich hemicellulose fraction consisting of a mixture of oligomers (27 C-wt.%), sugars (56 C-wt.%) and carboxylic acids (14 C-wt.%) in water.

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