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Full Length Article

Elucidation of reaction pathways of nitrogenous species by hydrothermal liquefaction process of model compounds

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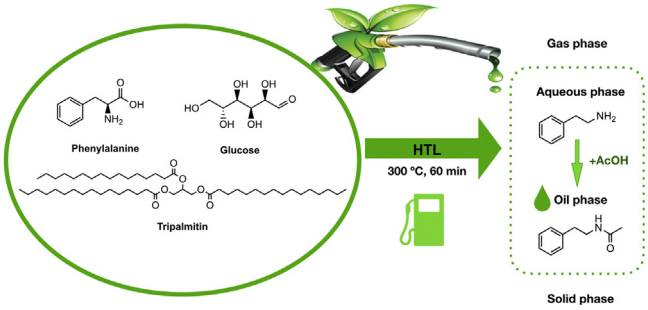
GRAPHICAL ABSTRACT



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ABSTRACT

The reaction pathway of nitrogen containing compounds under hydrothermal liquefaction (HTL) conditions was investigated by using amino acids as protein model compounds. The effect of organic acids and alkaline catalysts was also investigated by determining the structure and the partition of nitrogen containing species between the resulting solid, aqueous and bio-oil phases.

Representative results showed that operating in a water-acetic acid (95/5% v/v) binary solvent system resulted in a dramatic improvement in carbon recovery in the oil phase due to the transformation of water soluble hydrophilic products into oil soluble derivatives by acylation of the amino moiety.

The conclusions of this study provide a useful tool to improve the HTL process applied to nitrogen rich biomass, such as the organic fraction of urban waste, sewage sludge, and aquatic biomass (microalgae).

1. Introduction

The growing concerns over the availability of fossil fuel resources have increased an interest in searching new renewable alternatives. The waste organic biomass types, including sorted domestic organic waste and sewage sludge, are highly perishable wet materials with a typical moisture content ranging from 50 to 80% [1]. These feedstocks often cause serious environmental problems if not properly disposed. Due to the absence of the competition with the food and feed use, they are considered as sustainable feedstocks for energy production. They are also readily available: around 800 million tons of organic wastes are annually produced worldwide [2].

However, from a technological point of view, the high water content makes this feedstock not suitable for most thermochemical processes,

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such as fast pyrolysis and gasification, which require feedstock with lower moisture content, generally less than 30% [3]. Conversely, they are the typical feeds for the hydrothermal processes, such as hydrothermal liquefaction (HTL) [4] and hydrothermal carbonization (HTC) [5], since they do not need to dry the biomass before the treatment. Targeting the production of liquid biofuels, HTL has to be preferred to HTC that produces a solid hydrochar.

Generally, HTL process is carried out at 250–350 °C temperature range under 50–200 bar autogenous pressure. It is a non-selective process due to the complex composition of the waste biomass, containing polysaccharide, lipid and protein fractions.

The liquefaction mechanism comprises the following consecutive steps: (i) depolymerization of the biomass to form water soluble monomers, (ii) degradation of monomer by dehydration, deamination and decarboxylation reactions, (iii) recombination of the reactive fragments to form the bio-oil, (iv) further polymerization at prolonged reaction time to form char.

The produced bio-oil shows significantly higher oxygen and nitrogen contents, typically 10–20% and 1–8%, respectively, compared to the conventional crude oil (both elements < 1%) [6]. Therefore, the crude bio-oil is not directly suitable to fuel conventional engines and has to be upgraded by hydrotrating or cracking processes in order to produce liquid transportation fuels. The high heteroatom content, especially nitrogen, significantly reduces the efficiency of the typical refinery processes by poisoning the conventional catalysts, even in the case of a co-feeding of bio-oil with the current fossil fuels, hence creating a major technological challenge. For this reason the selection of an appropriate upgrading strategy needs a considerable effort to improve the bio-oil chemical composition and specifications that can be achieved only by a deep comprehension of the HTL reaction mechanism.

Although a number of studies [7–11] has been published on the decomposition of amino acids as representative model compounds of proteins, the liquefaction mechanism of the high-protein biomass is still unclear. Most of these studies are typically limited to the behavior of individual compounds and only a few works have explored binary and ternary mixtures with the carbohydrates [12,13] and lipid components [14,15]. Moreover, none of the studies on the model compounds has been focused on the role of catalysts, while it is known that alkali hydroxides, carbonates, bicarbonates and organic acids as homogeneous catalysts can affect the HTL product yields [16].

Therefore, the main goal of the present study is the investigation of the HTL reaction mechanism, focusing the attention on the nitrogen containing species pathways, with the goal to increase the energy yields and reduce the nitrogen content in the produced bio-oil. Due to the complex features of both waste feedstock and the reaction products, phenylalanine, leucine, glucose and tripalmitin were selected to simulate the interaction behavior between the main biomass components. Additionally, the effect of acid and alkaline homogeneous catalysts was also investigated.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma Aldrich and Alfa Aesar with the purity of 98–99% and used as received.

2.2. HTL experimental setup

The HTL experiments were performed in a Parr 2L batch reactor (4520 series) up to 300 °C at a heating rate of 2.5 °C/min under 80–85 bar of autogenous pressure and at residence times of 60 min. In a typical experiment, 300 g of water and 7 g of starting feedstock were loaded into the reactor. In case of binary mixtures, equal masses of each component (4 g) were used. After the reactor was sealed, pure nitrogen gas was used to purge the vessel. The reactor was then heated up to the designated experimental temperature and maintained for the given time. At the end of the reaction, the reactor was rapidly quenched at a cooling rate of 11 °C/min by flowing cold water through the cooling coil located inside the reactor. The residence time was defined as the elapsed time between the reaction temperature first achieving and the starting of cooling down procedure. In order to investigate the effect of alkali catalysts and organic acids on the bio-oil yields, the 0.02 M of alkali solutions and 95/5% v/v acid solutions were prepared using Na_2CO_3 and acetic or formic acids, respectively.

After reaction, the gas yield was calculated by the ideal gas law using the residual pressure (after cooling down the reactor) and the average molecular weight of the gases. The gas was then collected and analyzed by gas chromatography. The gas phase was found to consist mainly of CO_2 with the minor amount of CO. The recovery of the bio-oil phase was achieved by extraction with the diethyl ether. After the extraction of organic phase by the solvent, it was washed with water in order to remove the residual acetic acid partitioned into the oil phase when acetic acid-water binary solvent system was employed. After evaporation of the solvent, they were used for further analytical characterization.

2.3. Gas analysis

The GC–MS analysis of the gas phase was performed by gas chromatography (Agilent 7890A) equipped with a carboxen 1006 Plot column (30 m × 0.32 mm). The analysis was performed using the following method: 30 °C to 200 °C with 5 °C/min with splitless injection of 1 ml of gas. Source Temperature 230 °C, Inlet 250 °C. Scan from 25 to 200 Dalton.

2.4. GC–MS of oil samples

Bio-oil samples have been first diluted in diethyl ether to a final concentration of 1 mg/ml. About 1 µl of these diluted samples were analyzed by GC–MS with a Finnigan Trace DSQ (Thermo) quadrupole mass spectrometry interfaced to a Finnigan Trace GC Ultra equipped with a DB-5 MS (Agilent J&W) fused silica, non-polar capillary column (30 m × 0.25 mm inner diameter and 0.25 m film thickness) using helium as carrier gas (1 ml·min^{-1}), in splitless mode. Elution was performed using the following protocol: an initial temperature of 60 °C for 2 min, and a temperature ramp to 320 °C at a rate of 10 °C min^{-1}. Mass spectra were acquired in electronic ionization (EI) mode with a mass range 50–650 Da.

Quantitative determination of phenylethylacetamide, cinnamic acid, and styrene was performed using external standard calibration in the concentration range between 0.02 and 0.1 mg/ml in ethyl acetate or diethyl ether. The amount of dimers and trimers of styrene, 2-hydroxy-N-phenylethyl-3-phenylpropanamide and N-phenylethyl-3-phenylpropanamide were determined by the response factor of styrene and phenylethylacetamide.

2.5. GC-FID analysis

Quantitative analysis of free fatty acids was performed by GC-FID equipped with Supelco Petrocol EX2887 (5 m × 0.53 mm ID) using helium as a carrier gas (40 cm/sec constant flow). The analysis was performed using the following method: an initial temperature of 50 °C for 2 min, and a temperature to 350 °C with a rate of 10 °C/min. The silylation of oil components was performed using BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide). About 100 µl of BSTFA and 20 µl of pyridine were added into oil samples and the solutions were heated at 70 °C for 40 min. After the dilution of the solutions with 1 ml of dichloromethane, about 50 µl of IS 1 (40.77 mg of tetradecane in 10 ml hexane) was added.

2.6. Elemental analysis

Elemental analysis of bio-oil products and solid residues was performed by an elemental analyzer Flash 2000 Thermo Fisher. Carbon, hydrogen, nitrogen and sulfur were determined simultaneously by quantitative analysis of their relative combustion gas products (carbon dioxide, water vapor, nitrogen, and sulfur dioxide). About 2–3 mg of each sample was directly weighed in a tin cup. Once inserted into the instrument, a complete combustion at 950 °C was performed. Helium was used as carrier gas and oxygen as combustion gas. The analysis of oxygen was done by EA1100 Thermo Fisher. The samples were weighed about 1–2 mg in silver cups and underwent to pyrolysis at 1060 °C.

2.7. Total organic carbon (TOC) and total nitrogen (TN) analysis

TOC analysis of aqueous samples was performed by SHIMADZU - TOC-V instrument and TN analysis was done using TNM.

2.8. ESI-FTICR-MS direct flow injection analysis

Mass spectrometry analysis was performed on a 7 T FTICR MS (LTQ-FT Ultra Thermo Scientific), equipped with ESI (Electrospray) ion source. The mass spectra were collected in positive and negative mode. The sample was infused at a flow rate of 10 L min^{-1}; typical ESI (+) conditions were as follows: source voltage 3.5 kV, capillary voltage 43 V, tube lens voltage 130 V, capillary temperature at 275 °C, sheath gas 10 arbitrary units, auxiliary gas 5 arbitrary units. The spectra were acquired both with a low resolution linear ion trap (m/z 100–1000) and with a 7 T ultrahigh resolution FTICR cell with a mass range of m/z 100–1000. The resolution was set to 400.000 (at m/z 400). The ion accumulation time was defined by the automatic gain control (AGC), which was set to 10^6. A minimum of 100 scans was collected and averaged for each analysis to improve the signal to noise ratio. The data were processed by the software Xcalibur (Thermo Scientific), after setting the following restrictions to the element ranges: 0–60 ^{12}C, 0–2 ^{13}C, 10–100H, 0–6 N, 0–1 S, 0–6 O taking into account the elemental analysis, while the error range was set at ± 2.5 ppm. These restrictions are required because of the great number of possible different combinations of elements that can be generated from a single accurate mass. The first step of the molecular formula assignment was done below 400 Da, since in this range the assignments are more reliable due to the lower number of possible combinations for a single mass. Secondly, the higher mass peaks (above 400 Da) were assigned through the Kendrick mass [17]. The lists of the masses and the corresponding molecular formulas were then grouped with a custom designed software (ISO-MASS) [18] and the mass peaks relevant to isotopic distributions were identified and deleted. The relevant signals were categorized according to different parameters, such as the number of heteroatoms (N, O and S) and the number of unsaturations expressed as DBE (Double Bond Equivalents) [19]. For each molecular formula the DBE was calculated according to the following equation (for $C_cH_hN_nO_oS_s$): DBE = c − h/2 + n/2 + 1. The molecular formulas were assigned to approximately 90% of the peaks presenting relative intensities higher than 0.1%.

2.9. LC–MS analysis

Aqueous phase products were quantitatively determined on a YMC Triart C18 column, using the following LC gradient method: from 60 to 100% Acetonitrile in 12 min, followed by isocratic conditions for 4 min at a constant flow rate of 0.4 ml/min. The flow was then split with a T union before entering the heated H-ESI ion source operated in positive ion mode with the following conditions: Source voltage 5 kV, ESI temperature 100 °C, Capillary voltage 43 V, Tube lens Voltage 130 V, Capillary temperature 280 °C, Sheath gas 50, Auxiliary gas 10. The mass spectra were acquired in high resolution in profile mode with the ICR Cell analyzer (200000 RP) with a mass range from m/z 80 to 450. Quantitative determination of the phenylethylamine was done using external standard calibration in the concentration range between 0.02 and 0.1 mg/ml in acetonitrile.

2.10. Ion exchange chromatography

For the quantification of acetic acid, the samples were filtered with 0.45 µm membranes and injected on an ionic chromatograph (ICS3000 DIONEX instrument) with an AS11-HC column. The elution was achieved by a gradient from 1 mM of NaOH to 60 mM of NaOH in 35 min. The detection was conductimetric. Quantification was made with external standard.

2.11. ^{13}C solid state NMR spectroscopy

Solid residue was analyzed by ^{13}C solid state NMR spectroscopy, with a Bruker Avance 400 NMR WB for solid state. Samples were put in 4 mm. zirconia rotors and then analyzed with the following experimental conditions: spectral width: 30 kHz, rotor speed: 12 kHz, Delay: 4.5 s, mixing time (for cross-polarization) contact time t_C = 3 ms, number of scans: 20000.

2.12. Product yield determination

After all analyses were done, the product yields of bio-oil and solid residue phases were calculated by the following equations

$$X_{bio-oil} = Mass_{bio-oil}/Mass_{feedstock}* 100\% \quad (1)$$

$$X_{solid} = Mass_{solid}/Mass_{feedstock} * 100\% \quad (2)$$

The carbon and nitrogen recovery in the bio-oil and solid residue were determined by following equations, where C and N are the mass percentages of carbon and nitrogen, respectively:

$$C_{bio-oil} = C\ (\%)_{bio-oil}/C\ (\%)_{feedstock}* X_{bio-oil} \quad (3)$$

$$N_{bio-oil} = N\ (\%)_{bio-oil}/N\ (\%)_{feedstock}* X_{bio-oil} \quad (4)$$

$$C_{solid} = C(\%)_{solid}/C(\%)_{feedstock} * X_{solid} \quad (5)$$

$$N_{solid} = N\ (\%)_{solid}/N(\%)_{feedstock}* X_{solid} \quad (6)$$

The carbon and nitrogen recovery in aqueous phase were determined by TN and TOC analyses. In case of experiments with acetic acid-water solvent system, the carbon fraction which is contributed from acetic acid is subtracted from the total carbon of the system in order to consider amino acid only as a carbon source.

$$TOC_{phenylalanine} = TOC_{total} - TOC_{acidic\ acid} \quad (7)$$

The High Heating Value (HHV) was calculated by Dulong's formula as below, where C, H and O are the mass percentages of the carbon, hydrogen and oxygen, respectively.

$$HHV\ (MJ/kg) = 0.338\ C + 1.428\ (H - O/8) \quad (8)$$

Energy Recovery in the bio-oil is calculated by the following formula:

$$ER = X_{bio-oil}* HHV_{bio-oil}/HHV_{feedstock} \quad (9)$$

3. Results

3.1. HTL of phenylalanine and leucine

The effect of alkali and acid catalysts on the bio-oil yield and nitrogen content is presented in Table 1. The bio-oil yields produced from phenylalanine and leucine HTL in the reference water medium were 7.9 and 6%, respectively. A significant increase in bio-oil yield up to 30.2 and 28.6%, was observed for both the model substrates of

Table 1
Bio-oils from model amino acids HTL.

	Phenylalanine HTL				Leucine HTL			
	Ref.[a]	AcOH[b]	HCOOH[c]	Na_2CO_3[d]	Ref.	AcOH	HCOOH	Na_2CO_3
Bio-oil yield, %	7.9	30.2	8.7	5.9	6.0	28.6	4.7	3.7
Element analysis								
C, %	80.4	67.9	75.6	83.4	72.3	63.9	60.1	62.9
N, %	4.4	6.5	5.3	3.8	6.3	8.9	5.9	6.7
H, %	7.5	9.0	7.4	6.3	11.3	10.8	10.2	10.6
O, %	6.5	14.1	8.5	6.8	10.0	15.6	12.0	9.0
Solid residue, %	8.6	5.7	16	–	3.3	4.8	0.4	0.5
HHV, Mj/kg	36.5	33.2	34.6	36.1	38.8	34.2	32.7	34.7
ER, %	9.6	33.6	10.1	5.9	8.0	36.3	5.3	4.4

[a] Water solvent.
[b] 0.8 M acetic acid.
[c] 1 M formic acid.
[d] 0.2 M Na_2CO_3.

phenylalanine and leucine, respectively, when water-acetic acid medium was employed. The bio-oil composition was also affected by an increase in oxygen and nitrogen content. Similar phenomena were not observed in the presence of formic acid, probably due to its fast decomposition to CO_2 and H_2, whereas acetic acid was reported to be more stable under hydrothermal conditions [18]. Apart from acetic acid, all the other tested reaction conditions did not significantly affect the bio-oil yield and elemental composition with respect to the reference reaction.

As shown in Table 1, bio-oils produced from Phenylalanine and Leucine HTL in the presence of acetic acid showed a lower HHV value compared to those obtained in the reference test, due to the higher oxygen content. However, a significant increase in energy recovery was observed due to the higher bio-oil yield.

3.2. The role of acetic acid in the improvement of energy recovery

The fate of carbon and nitrogen and their distribution in the product streams are crucial points as they determine the bio-oil specifications to meet the requirement of refinery upgrading processes. In order to understand the role of acetic acid in the observed yield increase, the standard experiment in water cannot be directly compared to that carried out in water-acetic acid solvent, since the additional mass contribution of acetic acid has to be considered. Therefore, the carbon and nitrogen mass balances related to amino acid only were calculated taking into account of the unreacted acetic acid (mainly in the aqueous phase) and the acetyl moieties (introduced in the bio-oil species).

The elemental balance of carbon and nitrogen were determined by combining the total organic carbon content (TOC) and total nitrogen (TN) analyses of the aqueous phase along with the elemental analysis of the bio-oil products and solid residues taking into account the product yields.

As reported in Fig. 1, in the reference test with the water solvent only 8.5% of the carbon and 4.2% of nitrogen coming from phenylalanine were distributed into the bio-oil phase. The carbon and nitrogen contents in the solid residue phase were 12.5% and 12.6% respectively. Both carbon (69.5%) and nitrogen (76.7%) were preferentially partitioned into the aqueous phase. A fraction of nitrogen (not quantified) produced via the deamination of phenylalanine and phenylethylamine was known to transfer into the water phase as ammonium salt [10], while a fraction of the carbon (9.5%) coming from the decarboxylation of phenylalanine was detected in the gas phase as CO_2. In the presence of acetic acid a threefold increase in the carbon partitioned into the bio-oil (28.8%) was observed and a significant amount of nitrogen (21.0%) also moved into the oil phase (Fig. 1).

This phenomenon is not simply related to a pH effect promoting the formation of more hydrophobic species, since the increase in the bio-oil yield was not observed in the control experiment that was carried out at the same pH = 5, but using sulfuric acid instead of acetic acid. This suggests an active role of acetic acid in a reactive extraction mechanism with the migration of water soluble molecules from the aqueous phase into the oil phase after acetylation. In order to confirm this hypothesis the reaction products were characterized in detail and quantified in all phases of the final reaction mixture. Fig. 2 reports the complete reaction pathway for phenylalanine HTL.

According to Fig. 2, decarboxylation and deamination are competitive reactions in the first step of phenylalanine hydrothermal degradation, forming cinnamic acid (1a) and phenylethylamine (2a) respectively. Cinnamic acid further reacts with water to form 2-hydroxy-3-phenylpropanoic acid (3a). Both 2-hydroxy-3-phenylpropanoic and cinnamic acids react with phenylethylamine to form the corresponding 2-hydroxy-N-phenylethyl-3-phenylpropanamide (4a) and N-phenylethylcinnamamide (5a), respectively.

In addition, phenylethylamine undergoes dimerization and deamination reactions to form diphenylethylamine (6a) and styrene (7a), both partitioned into the bio-oil phase. Styrene further reacts to form minor amounts of hydration (8a) and oligomerization (9a) products. All these products were identified and quantified with an overall molar balance up to 70% on the basis of GC–MS and LC–MS analyses.

The missing fraction of products in bio-oil that were not detectable by GC were identified by FTICR-MS ESI analysis (Supp. Info. A). Considering the mass yields of the solid residue and reaction products, the amount of higher molecular weight material was calculated by difference and it reached 40.3 and 34.5 wt% in the reactions performed in water and acetic acid-water solvent systems, respectively.

According to the results, in the reference water solvent the presence of 6a and 4a as the main products of the bio-oil was confirmed. In addition, minor amounts of higher molecular weight N1- and N2-containing species were detected with carbon number and DBE ranging from C25 to C40 and 15 to 25, respectively. The high carbon content and unsaturation degree (DBE) of these species suggests that they can be formed by further reactions of styrene intermediates. In the solid residue mainly phenylalanine oligomerization and polymerization products (10a) were detected by ^{13}C solid state NMR spectroscopy (Supp. Info. B).

In contrast, a different pathway was observed in water/acetic acid binary solvent, since phenylethylamine (2a) reacts with acetic acid to form phenylethylacetamide (11a) which is predominantly partitioned in the oil phase, as shown in Table 2, reporting the main reaction product yields and phase partitions.

Acetyl moiety (C_n = 2) is also incorporated into the oil phase, however the additional carbon recovery because of phenylethylamine (C_n = 8) transferring is the main reason for the increased yields as well as the energy recovery in the bio-oil.

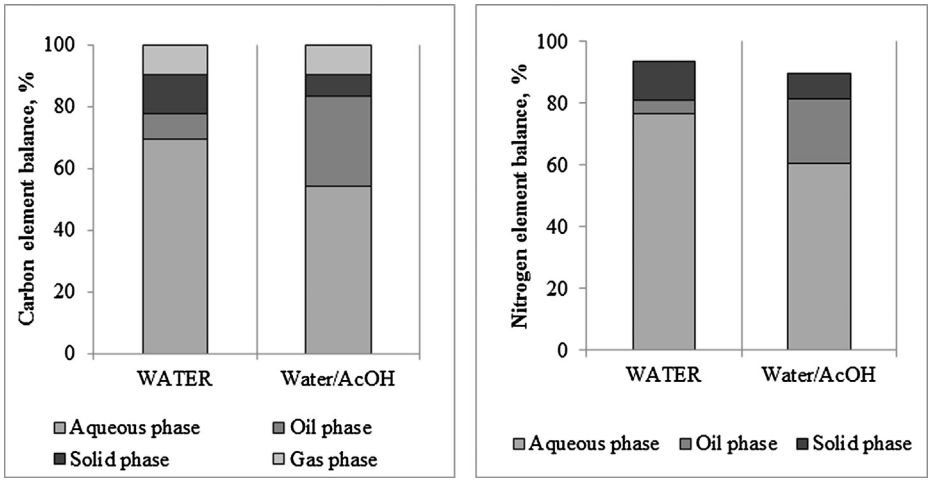

Fig. 1. Carbon and Nitrogen balance in Phenylalanine HTL product streams.

According to Table 2, in both reaction conditions more than 95% of species are related to the decarboxylation pathway through the formation of phenylethylamine (2a) with a minor contribution of the deamination pathways leading to acid derivatives. The reduced molar yield of phenylethylamine (from 61.4 mol. % to 46.2 mol. %) in the aqueous phase well correlates with phenylethylacetamide formation in the oil phase (15.6 mol. %).

This explains the effect of acetic acid on the relocation of carbons from the aqueous phase into the oil phase and the consequent improvement in mass and energy recovery from the substrate. Also in this case a minor amount of high molecular weight N1 and N2-containing species with carbon number and DBE up to 52 and 25, respectively were detected by FTICR-MS ESI analysis.

As shown in Fig. 3, the similar reaction pathway was also observed in Leucine HTL tests. According to GC–MS and LC–MS analyses, the decarboxylation pathway was confirmed to be predominant over the deamination route, being isopentylamine (2b) as the most abundant specie, as already determined for Phenylalanine HTL. The amine (2b) also undergoes dimerization reaction to form the secondary amine (6b), while minor amounts of 4-methylpentanoic acid derivatives (1b and 3b) produced by the deamination of leucine reacted with 2b to form the corresponding amides (4b and 5b). The main components of solid residues are confirmed to be leucine oligomers (7b) by ^{13}C solid state NMR spectroscopy (Supp. Info. App. C).

Also in this case the addition of acetic acid resulted in improved bio-oil yields from 6.0%, measured in the reference water solvent, up to 28.6%, as reported in Table 1. The main difference in the reaction pathways (Fig. 3) is the transformation of the hydrophilic

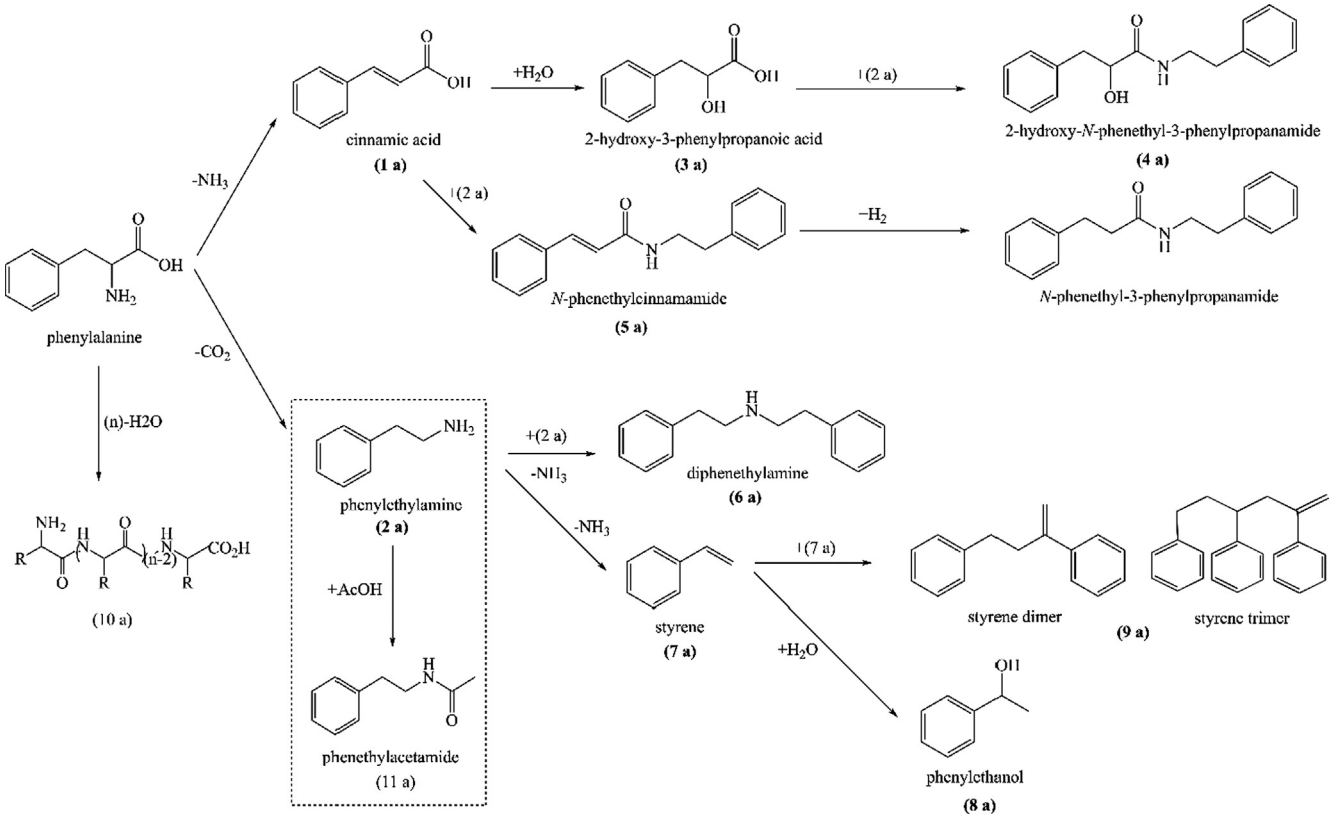

Fig. 2. Chemical HTL pathway of Phenylalanine.

Table 2
Phenylalanine HTL main product yield and partition.

Products, (mol, %)	Water		Water/AcOH	
	Aqueous phase	Oil phase	Aqueous phase	Oil phase
1a		0.20		0.23
2a	61.4		46.2	
3a		0.61		2.06
4a		0.89		1.43
5a		0.25		0.24
6a		1.72		2.27
7a		0.70		0.82
8a		0.15		0.4
9a		0.07		0.05
11a			1.42	15.6

Table 3
Bio-oil yields, elemental analysis, HHV and ER of bio-oil products.

	Phenylalanine/Tripalmitin		Phenylalanine/Glucose	
	Water[a]	Water/AcOH[b]	Water	Water/AcOH
Bio-oil yield, %	68.1	62	7.3	23.5
Element analysis				
C,%	77.0	75.0	76.3	69.1
N,%	2.1	2.5	5.2	6.9
H,%	11.8	11.2	8.5	8.7
O,%	9.1	11.2	10.0	15.3
Solid residue yield,%	1.6	8.2	6.9	10.5
HHV, Mj/kg	39.8	38.3	35.9	33.1
ER in bio-oil, %	91	79.7	8.8	26.1

[a] Water solvent.
[b] 0.8 M acetic acid.

isopentylamine (2b) into the lipophilic acylated amide derivatives (8b) with a significant relocation of the carbon from the water phase into the bio-oil phase (up to 25% with respect to 7.4% measured in the water solvent).

As a consequence, the bio-oil produced in water/AcOH solvent showed an increased nitrogen content (8.9% by weight) with respect to that measured in the reference test (6.3%) (Table 1).

The relocation phenomenon observed with acetic acid could be a useful tool to understand and predict the behavior of more complex real organic waste feedstock or oleaginous microalgae biomass containing different acyl donor species, such as the lipid component from oil and fat fractions.

3.3. HTL of Phenylalanine/Tripalmitin binary mixture

As reported in the literature [20], the bio-oil yield is related to the feedstock composition and the contribution tendency of biomass macromolecules to the oil yield in HTL is in the following order: carbohydrate < protein < lipid. The typical composition of sorted domestic organic waste includes a consistent fraction of lipids as mono-, di-, tri-glycerides and free fatty acids ranging from 10 to 35% [21]. Microalgae also can have lipid contents as high as 80%, although this is usually in the range of 15–35% and is dependent on the growth conditions [22].

In order to investigate the effect of fatty acid derivatives on nitrogen species pathway, the binary mixture of phenylalanine and tripalmitin was tested as the model substrate HTL. As expected, the higher bio-oil yield of 68% with Energy recovery of 91% (Table 3), compared to those obtained from phenylalanine alone HTL, was due to the almost quantitative partition of hydrophobic fatty acids derived products into the bio-oil phase. The simplified reaction pathway is shown in Fig. 4.

According to the GC–MS and GC-FID analyses, in the first step

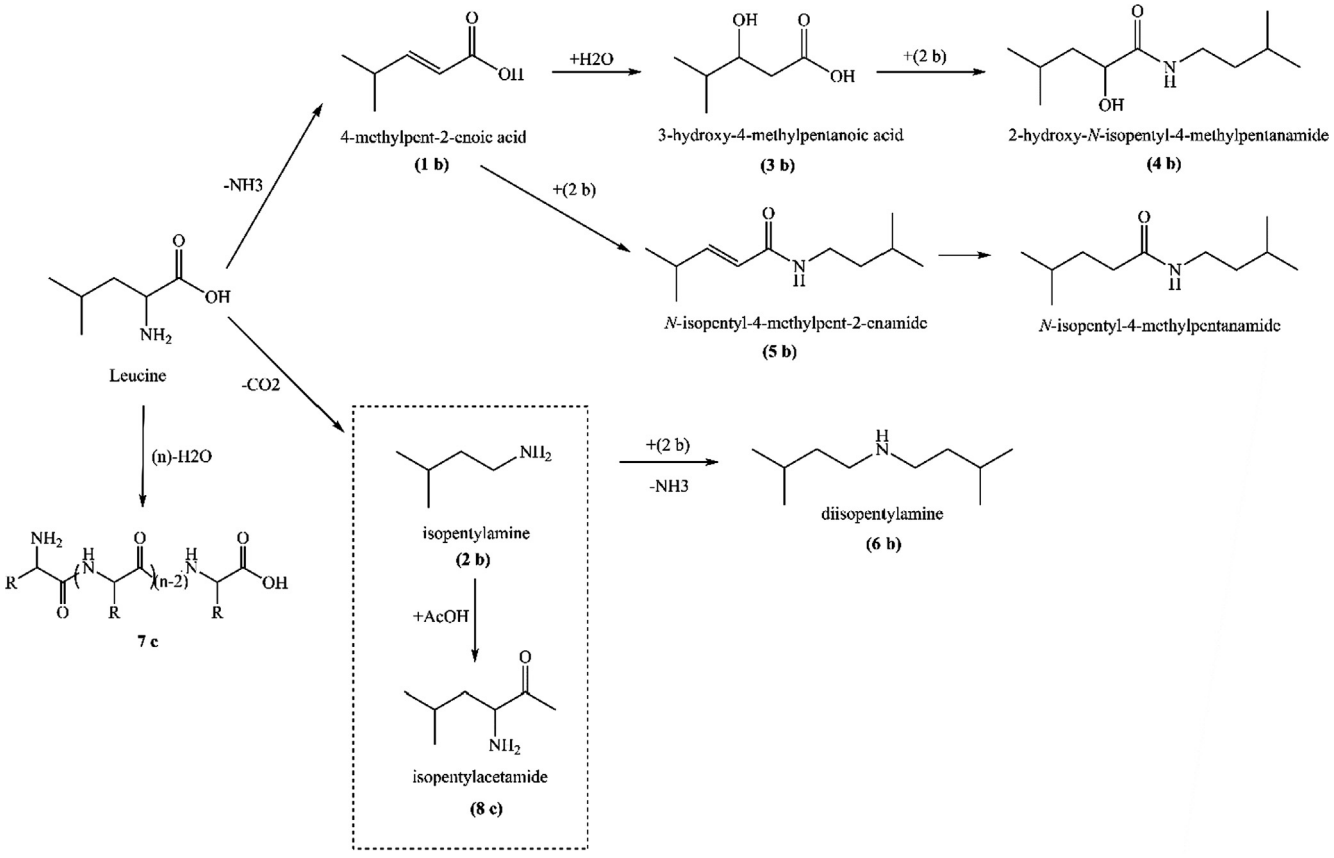

Fig. 3. Chemical HTL pathway of Leucine.

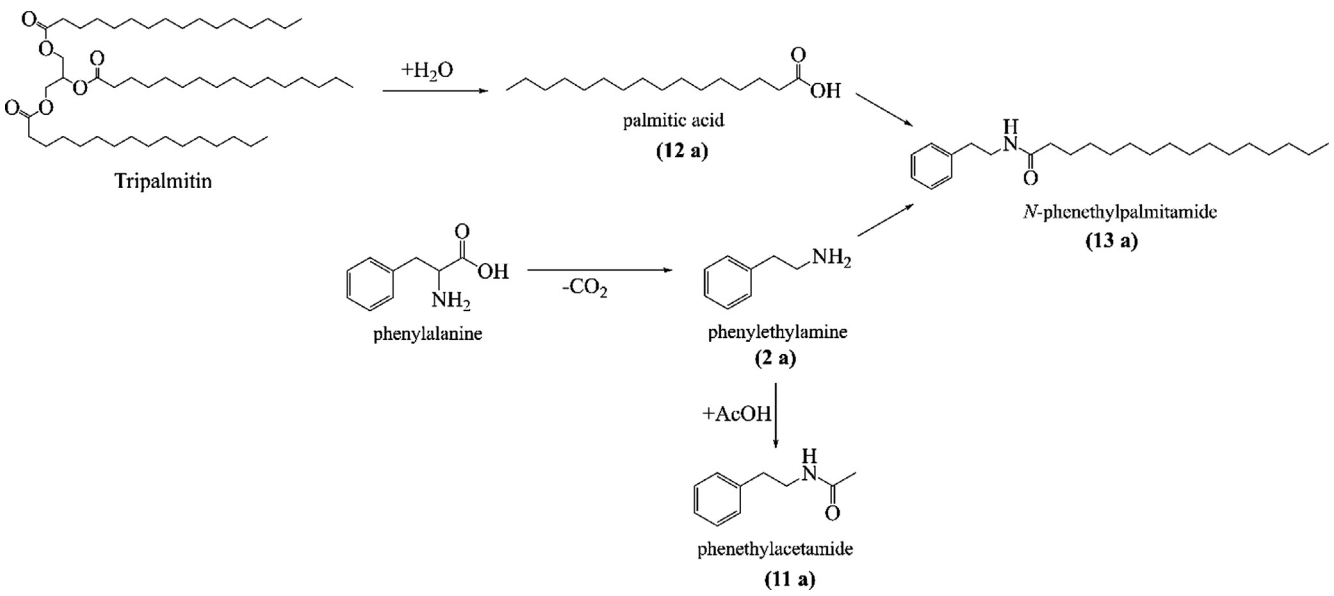

Fig. 4. Chemical pathway of Phenylalanine/Tripalmitin binary mixture.

tripalmitin was fully hydrolyzed to palmitic acid (12a) and only traces of glycerol monopalmitate were detected. Phenylethylamine (2a) formed by decarboxylation of phenylalanine was then acylated by palmitic acid to form phenylethylpalmitamide (13a). Palmitic acid (47.0 wt%) and phenylethylpalmitamide (30.7 wt%) were the main products in the bio-oil.

After the subtraction of the total contribution of palmitic acid (3.8 g of free acid and its amides), the fraction of bio-oil deriving from phenylalanine component alone is 1.6 g, thus corresponding to 40% of the initial mass of amino acid. This value is considerably higher than that obtained with phenylalanine without tripalmitin in water (8%) and in water/AcOH solvent (30%), as reported in Table 1, hence suggesting a synergic effect exerted by the lipid fraction on the bio-oil formation chemistry.

In the presence of acetic acid a competition with palmitic acid was observed to form amides 11a and 13a, resulting in no beneficial effect on bio-oil yield (Fig. 4). On the basis of the bio-oil composition: palmitic acid (12a) (70.5 wt%), phenylethylacetamide (11a) (21.5 wt%), N-phenylethylpalmitamide (13a) (5.2 wt%), acetic acid turned out to be more effective in the acylation of phenyleltylamine. A negligible amount of nitrogen containing high molecular weight species was detected by FTICR-MS ESI analysis. As shown in Table 3, the nitrogen content in the bio-oil from the binary mixture (2.1 wt%) was remarkably lower than that measured for Phenylalanine alone (4.4%) due to the dilution of nitrogen containing species the by fatty acid derivatives. The similar dilution effect was observed in the presence of AcOH with the nitrogen content decreasing from 6.5 to 2.5%.

3.4. Characterization of bio-oil products from phenylalanine/glucose binary mixture

As shown in Table 3, the bio-oil yield obtained from the binary mixture of Phenylalanine/Glucose (1:1 by weight) was similar to that measured for Phenylalanine alone, suggesting an equivalent contribution from the two components.

Since HTL of glucose leads to highly oxygenated polar molecules [23], its contribution to the bio-oil yield is supposedly related to the formation of more hydrophobic species by reaction with the phenylalanine degradation water soluble products. This phenomenon was indirectly confirmed by the increased nitrogen content in the bio-oil from 4.4% measured for phenylalanine alone, up to 5.2% for the binary mixture, thus indicating a partial relocation of water soluble nitrogenous species from the aqueous into bio-oil phase.

Even if starting from the simple binary mixture of monomeric model compounds, a very broad spectrum of products, each with low relative concentration was displayed by GC–MS analysis of bio-oil. The polar species directly generated by glucose through dehydration and retro-aldol condensation reactions, such as 5-hydroxymethyl furfural, glyceraldehyde, pyruvaldehyde and glycolaldehyde were not detected in the bio-oil, since they are quantitatively partitioned into the aqueous phase. Additional carbonyl species could be formed by further transformation of the above mentioned products (e.g. 1, 4-dicarbonyl compounds from furan ring opening) [24].

Conversely, the products of phenylalanine decomposition (Fig. 3) were also identified in the binary mixture, namely diphenylethylamine (6a) and the amides 4a and 5a, but only as minor components.

According to the EI mass spectrum fragmentation patterns, the main compounds were attributed to the nitrogen containing species with the aromatic heterocyclic structures. In particular, the more intense GC–MS peaks showed the ions 108/122 as base peaks in their EI Mass spectra, which are likely related to the alkylated pyrazine cores (ethylpyrazine and propylpyrazine). In minor extent alkylated pyridines and other N, O- containing heteroaromatic compounds were detected.

Aromatic compounds are typically formed by the condensation and cyclization of unstable low molecular weight aldehyde and ketones intermediate fragments generated by the degradation of glucose. Instead, nitrogen containing heterocyclic species are formed by the combination of amino acid derived water-soluble materials, such as ammonia, primary and secondary amines with the glucose derived carbonyl compounds, through imines, enamines and enols intermediates [25].

In contrast, a completely different product distribution was obtained in the presence of acetic acid, where the main species were the same as identified in the case of the reaction with Phenylalanine alone (Fig. 2). Phenylethylacetamide (11a) was confirmed to be the most abundant peak, while the formation of a smaller amount of nitrogen containing heterocyclic species was also detected in this case with a broad structural differentiation. This considerably different behavior can be explained by the greater reactivity of the amines towards the acylation rather than the condensation with carbonyl species leading to amides instead of nitrogen containing heterocyclic products.

This is a key point in the rationalization of HTL mechanism, since the reactivity of amides and nitrogen containing heterocyclic compounds in the hydrodenitrogenation (HDN) processes is dramatically

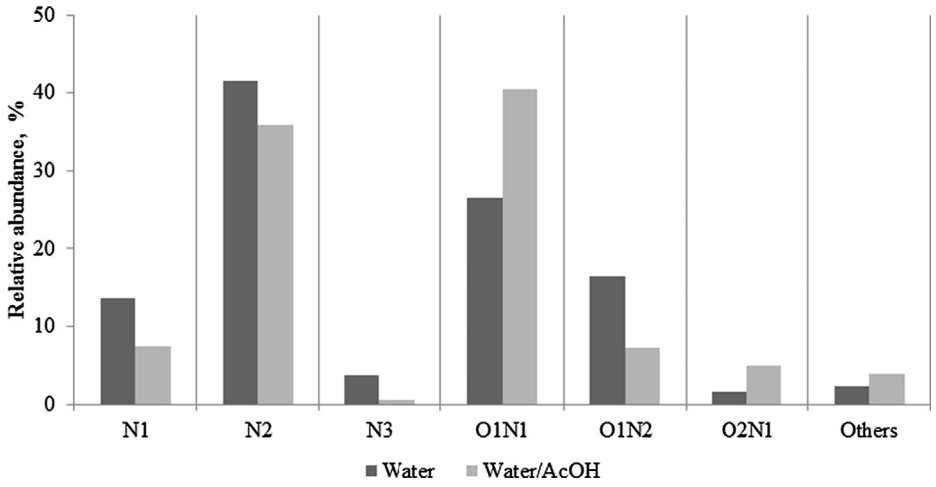

Fig. 5. Distribution of the main heteroatoms classes in Phenylalanine/Glucose binary mixture bio-oil.

different, being the latter more recalcitrant and making the upgrading of bio-oils to fuels problematic [26]. In addition to the products identified via GC–MS, other heavier and more polar compounds have been detected by (ESI+) FTICR MS analysis. The molecular formulas determined through the accurate mass were grouped into specific classes according to their content of heteroatoms, and according to their double bond equivalents (DBE) and carbon number (C_n) (Fig. 5).

On the basis of these results, N2 is the most abundant heteroatom class, followed by O1N1, N1, and N2 classes, while in the presence of acetic acid the reduction of N2, N1 and N2O1 components was observed and N1O1 class becomes predominant. This is in agreement with the formation of acetylated nitrogenous species, such as phenylethylacetamide (11).

Only negligible amounts of products were detected for the fully oxygenated O, O1 and O2 classes, confirming that these polar species are predominantly partitioned into the water phase and contribute to the bio-oil formation only after the reaction with the nitrogen containing intermediates. The compounds that belong to the most abundant classes (N2, O1N1) were then plotted in DBE versus C_n plots, according to their C_n, DBE value and relative abundance in the mass spectrum (size of the spots in the plot) (Fig. 6).

Concerning N2 class, a broad distribution of species ranging from C_n = 15–50 and DBE 10–30 was determined in the bio-oil. The main N2 class compounds in the bio-oil are related to C_n = 15–17 and DBE = 10, C_n = 21–23 and DBE = 14, C_n = 31–33 and DBE 19, C_n = 39–41 and DBE = 23, C_n = 46–47 and DBE = 27. Remarkably, this distribution shows discreet differences of benzene or alkylated benzene rings (DBE ≥ 4) between the more abundant species. Each of these species could be tentatively assigned to the pyrazine derivatives formed by the successive addition of aromatic rings (likely related to styrene) to the pyrazinic core (DBE = 3). The same distribution was observed in the presence of acetic acid, however, the regions of highest abundance are located to higher values of carbon number and DBE (C_n = 39 and DBE = 23, C_n = 47 and DBE = 27), indicating the higher abundance of the more unsaturated species.

For the N1O1 class the main peaks correspond to C_n = 22 and DBE = 14, C_n = 28–30 and DBE = 18, C_n = 35–36 and DBE = 22. Here again discreet differences (DBE ≥ 4) are found between the more abundant species supposedly formed by the successive addition of the aromatic rings (likely related to styrene). The N1O1 class compounds in the bio-oil obtained from binary mixture HTL in the presence of acetic acid show similar DBE regions shifted by one or two units of Cn, suggesting a higher degree of alkylation. In both bio-oil samples (with and without acetic acid) N1, N2, O1N1 compounds show high values of DBE, indicating the presence of high unsaturated nitrogen containing compounds. These results prove that the phenylalanine pathway to oligomerized products is significant in the bio-oil from the binary mixture of phenylalanine and glucose HTL. At higher temperatures (100 °C) styrene is reported to auto-polymerize up to 300 °C [27], so oligomerization products containing a vinyl group (9a, in Fig. 3) can undergo addition reactions with phenylalanine derivatives to form nitrogenated polycyclic aromatic hydrocarbons.

4. Conclusion

This paper has presented the modeling study on HTL focusing on the mechanism of nitrogen species formation. The choice of monomeric substrates, representative of protein, polysaccharide and lipid biomass components, has made possible to simplify the spectrum of the products, thus allowing the identification of the main reaction pathways. In particular, the key role of the acetylation reaction of nitrogen containing compounds was highlighted in the case of biomass feedstock containing protein and acyl donor components, such as acetylated sugar in the hemicellulose and fatty acid chains in the lipid fraction.

It is confirmed that lipids are the main contributors to the bio oil yield. They also display the synergistic effect on the protein contribution into bio-oil yield by the reactive extraction of hydrophilic species (amines). In the case of phenylalanine, the relocation of the produced phenylethylamine from the aqueous phase to the oil phase after amide formation results in the recovery of valuable ethylbenzene after hydrotreating of the bio-oil, while, in the case of short chain amino acids a fraction of light hydrocarbons is produced, with an overall enhancing of the carbon and energy yield to renewable fuels.

Lipids also exert the reduction of nitrogen content in the bio-oil by mass effect, thus making the resulting product less critical for the co-feeding in the refinery upgrading processes. More importantly, in the presence of lipids, amides formation is in the competition with the generation of nitrogen containing heterocyclic species, observed in the presence of carbohydrates.

This is a crucial point that determines the bio-oil specifications for the subsequent upgrading by HDN, being conventional catalysts poorly efficient in removing heterocyclic nitrogen.

The result of this model-based study represents a useful tool to design proper waste biomass blends, tuning its composition to produce bio-oils with the standard quality suitable for co-feeding in refinery processes.

However, further investigations are still needed for the exhaustive understanding of the reactivity of the real feedstock, focusing on molecular structures and distribution of nitrogen containing heterocyclic and aliphatic products in order to promote the formation bio-oils with higher HDN reactivity and eventually the development of new specific hydrotreatment catalysts.

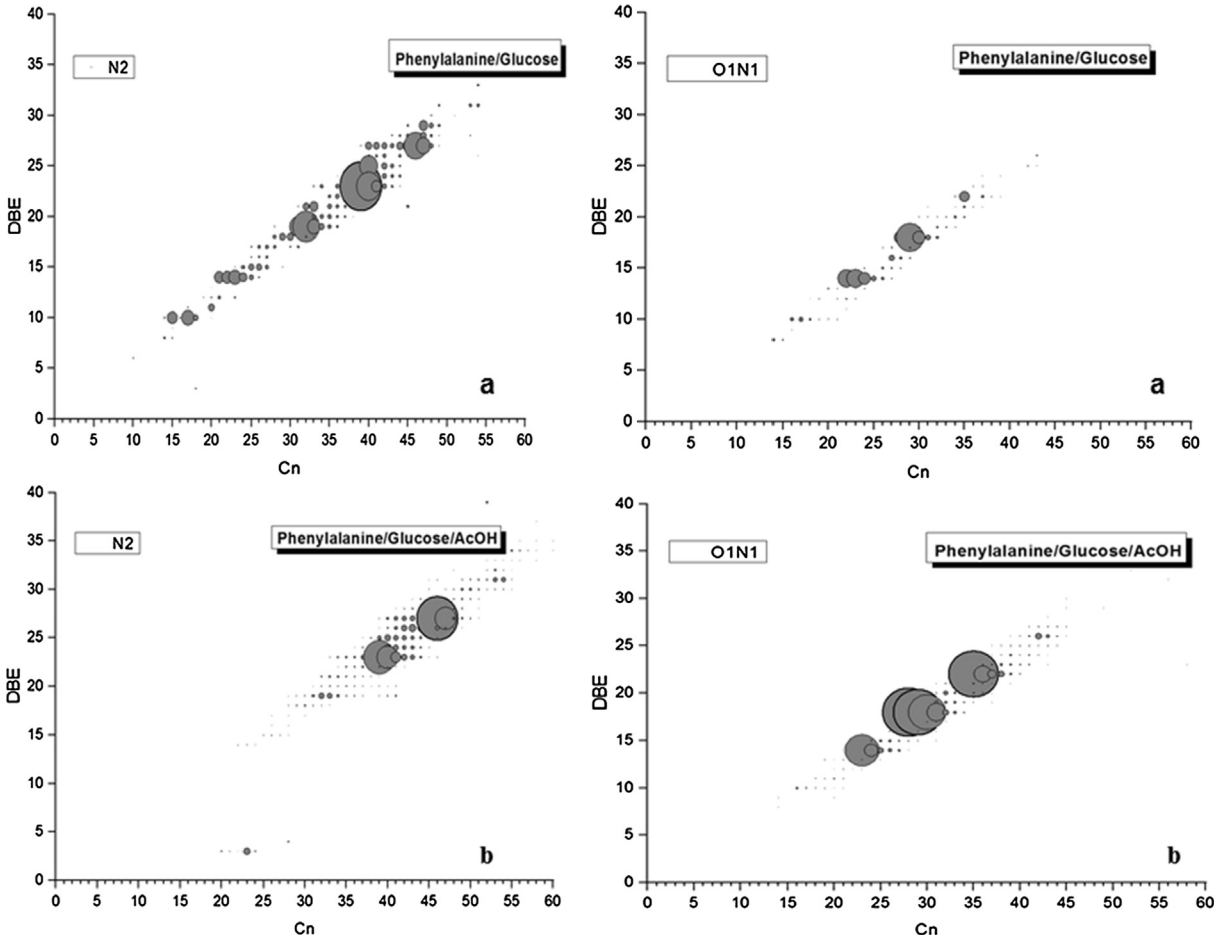

Fig. 6. DBE versus carbon number distributions for N2 and O1N1 class compounds in Phenylalanine/Glucose binary mixture bio-oil.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fuel.2018.11.136.

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