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1 **Production of biocrude from organic waste: influence of feedstock composition on**
2 **hydrodenitrogenation reactivity in biocrude upgrading.**

3
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12
13 Hydrothermal liquefaction (HTL) process of protein-rich biomass produces biocrude with a
14 high nitrogen content that hinders its use as a biofuel intermediate. Nitrogen compounds in
15 the biocrudes are mainly present as fatty acid amides (FAAs) and nitrogen-containing
16 polycyclic aromatic compounds (NAs), having different hydrodenitrogenation reactivity
17 during the biocrude upgrading process. Therefore, it is crucial to shift reaction pathways
18 towards the formation of less recalcitrant compounds and reduce the nitrogen content of the
19 biocrudes. Herein, the impact of the chemical composition, in particular a lipid content, on
20 the biocrude yield, nitrogen content and types of nitrogen species has been studied using
21 macromolecular model compounds. By comparing the hydrodenitrogenation (HDN)
22 reactivity of FAAs and NAs, it was found that the latter are more recalcitrant compounds
23 compared to the FAAs and that the higher lipid content inhibits the generation of NAs. These
24 results suggest that lipids-rich waste can be supplemented with food waste to produce the
25 biocrudes highly suitable for conventional hydrotreatment processes.

26 *Keywords:* hydrothermal liquefaction, hydrotreatment, food waste, nitrogen, bio-oil, fatty acid
27 amides.

28

29 **1 Introduction**

30 About one-third of food produced for human consumption is wasted globally [1], while
31 approximately 88 million of food waste is accumulated only in the European Union per year [2].
32 Majority of the food waste are mainly ended up in landfills [3] and this has led to serious
33 environmental concerns, as different microorganisms cause serious bacterial contaminations and
34 infectious diseases due to their nutritional composition [4]. Moreover, the food waste causes the
35 greenhouse gas emissions of about 4.4 Gt CO₂ equivalent per year [5], accounting for 12.9% of
36 the global CO₂ emissions for energy production reported by the IEA World Energy Outlook 2021
37 (34.156 Gt CO₂ equivalent per year) [6].

38 Hydrothermal liquefaction (HTL) is a promising approach to minimize the generation of organic
39 food waste and recover energy [7]. Generally, HTL operates at the sub- and supercritical region
40 of water (250–400 °C; 200–300 bar) for a short reaction time [8]. The HTL process provides two
41 main advantages: (i) no requirement for drying the wet feedstock, which is an energy intensive
42 process; (ii) unique attributes of subcritical water, such as a high ionic product and lower dielectric
43 constant [9]. Under HTL conditions the biomass is depolymerized into four different products,
44 namely biocrude oil, aqueous, solid residue, and gas phases [9, 10]. Among these fractions, the
45 biocrude oil is of great interest, as it can be used as drop-in fuels after catalytic upgrading process.
46 Food waste consists of three main macromolecular fractions, namely carbohydrates, proteins and
47 lipids [11]. It has been reported that about 20-40 wt.% of nitrogen in the protein can migrate into
48 the biocrude fraction [12], causing a high nitrogen content (>10 wt.%). In comparison, the crude
49 fossil fuels are characterized by much lower one (>1 wt.%) [12]. Several studies on real petroleum
50 feeds showed that lower than 50 ppm of nitrogen in the feedstock can deactivate catalytic active
51 sites in conventional refinery units [13-15]. Consequently, the existing conventional refinery
52 infrastructures are not suitable for the upgrading of waste-derived oils, especially for
53 hydrodenitrogenation (HDN), while constructing new biorefinery units for the biocrudes is not

54 economically feasible due to the high capital investments, longer implementation period, higher
55 risks and uncertainty regarding the process efficiency [16]. In addition, little is known about the
56 HDN reactivity of nitrogen compounds of the biocrudes [17]. Optimization of the HTL reaction
57 parameters, such as reaction temperature, operating reaction time, heating rate, etc., is typically
58 not effective, as the low nitrogen content always accompanies with the lower bio-oil yield [18-
59 20]. On the other hand, engineering the feedstock composition for balancing the biocrude quantity
60 and quality (lower O and N contents) is highly relevant to meet the minimum specifications of
61 the upgrading process at the existing refinery units.

62 In this context, it is important to comprehend the behavior of proteins under hydrothermal
63 conditions and their contribution into the biocrude qualities. In our previous work [21] we
64 investigated the mechanism of the nitrogen moieties generation under HTL conditions employing
65 amino acids (leucine, phenylalanine) as protein model compounds in the presence of organic acids
66 and alkali catalysts. Our results showed that the addition of acetic acid into the system greatly
67 increases the biocrude yield as well as the carbon yield due to the transference of hydrophilic
68 amino moieties from the aqueous phase after acetylation reaction forming more hydrophobic
69 amides. Most importantly, we reported that with the addition of lipids the generation of fatty acid
70 amides (FAAs) competes with the formation of nitrogen- containing polycyclic aromatic
71 compounds (NAs) by Maillard reaction, observed in the presence of polysaccharides [21, 22].
72 Likewise, Fan, Y. et al. (2020) [23] confirmed the existence of the strong counteraction between
73 amidation and Maillard reaction products, using lactose, lysine and palmitic acid as model
74 compounds of carbohydrate, protein and lipid, respectively. As both studies have been performed
75 with only surrogate model mixtures, further investigations are still needed using more complex
76 mixtures in order to gain the exhaustive understanding about how the chemical composition of
77 biomass affect the specimens of the nitrogen in the final biocrude and their HDN reactivity during
78 the hydrotreatment process of the food waste-derived biocrudes.

79 In the present study we performed the HTL experiments by employing macromolecular model
80 compounds (albumin, starch, tripalmitin) to demonstrate the competition between the formation

81 FAAs and NAs. Moreover, we studied the influence of lipid content on the biocrude yield and
82 nature of nitrogen species in the biocrude by quantifying the relative contents of FAAs and NAs.
83 To the best of our knowledge, the impact of chemical composition of biomass waste on the nature
84 of nitrogen species (FAAs and NAs) has not been previously semi-quantified. Last but not least,
85 we also evaluated the HDN reactivity of the two main nitrogen compound classes, FAAs and
86 NAs, in the biocrude produced from the food waste. The results obtained in the present study are
87 important to build better fundamentals for formulating the feedstock composition to produce the
88 biocrude with the higher yield as well as quality, and, most importantly, make the biocrude
89 components less problematic for the existing refinery infrastructures, thus avoiding the additional
90 investment costs.

91

92 **2. Materials and methods**

93 **2.1 HTL experiments**

94 HTL experiments were carried out in a Parr 2L batch reactor (4520 series) at 300 °C for 60
95 minutes with a heating rate of 2.5 °C/min. The detailed experiment procedure has been described
96 elsewhere [21, 22]. The elemental composition of the albumin, starch and tripalmitin is given
97 Table S1. Shortly, 7 g of the feedstock was mixed with 300 g of water solution and placed into
98 the reactor. For the binary and ternary mixtures of albumin with starch and tripalmitin, equivalent
99 masses of each component (4 g) were employed with the same ratio of the feedstock to water.
100 Before running experiments, the reactor was purged with nitrogen to remove any residual air.
101 After 60 minutes of reaction time, the reactor was cooled with a cooling rate of 11 °C/min by
102 running cold water in an internal coil of the reactor.

103 The gas yield was determined by the ideal gas law taking into account the residual pressure. The
104 gas fraction was then collected in a gas sample bag and its composition was analyzed by Gas
105 Chromatograph (GC). The gas phase was found to consist mainly of CO₂. The biocrude extraction

106 has been done using ethyl acetate and the solvent was removed by rotary evaporation. The
107 biocrude and aqueous phases were stored at 5 °C until further analytical characterization.

108 **2.2 Characterization of HTL products**

109 The composition of the gas fraction was analyzed by Gas Chromatograph (Agilent 7890A)
110 equipped with a carboxen 1006 Plot column (30 m x 0.32 mm, average thickness 30 µm). The
111 method used for the gas analysis has been described elsewhere [21, 22].

112 The chemical composition of biocrudes were determined using a GC-MS with a Finnigan Trace
113 DSQ (Thermo) quadrupole mass spectrometry, interfaced to a Finnigan Trace GC Ultra. The
114 separation of biocrude components has been done using a DB-5 MS (Agilent J&W) fused silica,
115 non-polar capillary column (30 m x 0.25 mm ID x 0.25 m). Helium was used as a carrier gas (1
116 ml/min). The method was described well in previous studies [21, 22]. Shortly, about 1 mg/ml of
117 biocrude samples in diethyl ether were prepared. The oven temperature was set at the temperature
118 of 60 °C for 2 minutes, followed by a heating ramp of 10 °C/min to the final temperature of 320
119 °C. The acquisition of mass spectra was done in electronic ionization mode with a mass range of
120 50-650 Da.

121 The content of free fatty acids was quantified by GC-FID equipped with Supelco Petrocol
122 EX2887 (5m x 0.53 mm ID, thickness 0.1 µm). Helium was employed as a carrier gas (40 cm/sec).
123 BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) was used as a derivitization agent for
124 silylation reaction of fatty acids (palmitic, stearic, mono- and dioleic acids) present in the
125 biocrudes. After the addition of about 100 µl of BSTFA and 200 µl of pyridine to biocrude
126 samples, the solutions were heated at 70°C for 40 min. After silylation of fatty acids, the samples
127 were diluted with 1 ml of dichloromethane, followed by addition of 50 µl of tetradecane (4 mg/ml
128 in hexane) and 25 µl of tricaprins (8000 ppm in pyridine) as internal standards. The oven
129 temperature was set at the temperature of 50 °C for 2 minutes and heated up to the final
130 temperature of 350 °C with a heating rate of 10 °C/min. Elemental composition of biocrude and
131 solid fractions was analyzed using an elemental analyzer Flash 2000 Thermo Fisher.

132 Fourier transform ion cyclotron resonance mass spectrometry was utilized to analyze the
133 composition of biocrude samples. The analysis was carried out using a 7 T FTICR MS (LTQ-FT
134 Ultra Thermo Scientific), equipped with ESI (Electrospray) ion source. The mass spectra were
135 mainly acquired in positive mode (ESI+). The typical conditions for the ESI+ analysis were
136 described in details elsewhere [21, 22, 24]. Generally, the spectra were collected simultaneously
137 with a low resolution linear ion trap (m/z 100-1000) and with a 7 T ultrahigh resolution FTICR
138 cell with a mass range of m/z 100-1000. The resolution was fixed at 400.000 (m/z 400) and the
139 ion accumulation time was defined by the automatic gain control, which was set to 10^6 . About
140 100 scans were collected and averaged for each analysis for the improvement of the signal-to-
141 noise ratio. Data acquired were processed by Xcalibur software (Thermo Scientific). Since a
142 single accurate mass could generate the great amount of potential different combinations of
143 elements, some restrictions are required to the element ranges. In the present study, the
144 restrictions were selected based on the elemental analysis of biocrude samples with the error range
145 of ± 2.5 ppm: 0-60 ^{12}C , 0-2 ^{13}C , 10-100 H, 0-6 N, 0-1 ^{31}S , 0-6 O. The assignment of molecular
146 formula was performed first below 400 Da due to the lower number of possible combinations for
147 a single mass and then the mass peaks above 400 Da were assigned through the Kendrick mass
148 [25]. The list of corresponding molecular formulas with the masses were categorized into groups
149 using a custom-built software ISOMASS [26] and the mass peaks related to isotopes were
150 determined and removed [24].

151 Then relevant peaks were grouped based on several parameters, in particular, the number of
152 heteroatoms (N, O, S) and the unsaturation degree, assigned as DBE (double bond equivalents)
153 [27, 28]. The DBE value for each molecular formula $\text{C}_c\text{H}_h\text{N}_n\text{O}_o\text{S}_s$ was defined as $\text{DBE} = c -$
154 $h/2 + n/2 + 1$. The molecular formulas were specified to about 90 % of the peaks with the relative
155 intensities higher than 0.1 %.

156

157 **2.3. Biocrude hydrocracking upgrade**

158 The biocrude samples were upgraded by a hydrocracking treatment carried out under the reaction
159 conditions typically used for the fossil heavy oil treatment [29]. The biocrude sample was added
160 into a stirred reactor together with an oleo-soluble catalyst precursor (Mo^{IV} 2-ethylhexanoate).
161 During the upgrading process, the catalyst precursor converts to highly dispersed molybdenum
162 sulfide (MoS_2) nanoparticles [29].
163 The reaction occurs at 430°C and 160 bar of hydrogen for 3 hours with 3000 wppm MoS_2 catalyst
164 concentration. At the end of the reaction, the mixture was extracted with tetrahydrofuran (THF),
165 filtered and directly analyzed.

166

167 **2.4. Product yield determination**

168 The yields of biocrude and solid fractions were determined according to Equation 1:

$$169 \quad X_{\text{fraction}} = \text{Mass}_{\text{fraction}} / \text{Mass}_{\text{feedstock}} * 100\% \quad (\text{Eq.1})$$

170 High heating values of biocrudes and upgraded oils were defined by Dulong's formula, where C,
171 H and O represent the weight percentage of the carbon, hydrogen and oxygen, respectively.

$$172 \quad \text{HHV} (\text{MJ/kg}) = 0.338 \cdot C + 1.428 \cdot (H - O/8) \quad (\text{Eq.2})$$

173 Energy Recovery (ER) in the biocrude samples was determined by the following formula:

$$174 \quad \text{ER} = X_{\text{biocrude}} \cdot \text{HHV}_{\text{biocrude}} / \text{HHV}_{\text{feedstock}} \quad (\text{Eq.3})$$

175 The nitrogen and oxygen removal are calculated by the following formula:

$$176 \quad \text{O}_{\text{removal}} = (\text{O}_{\text{initial}} - \text{O}_{\text{final}}) / \text{O}_{\text{initial}} * 100\% \quad (\text{Eq.4})$$

$$177 \quad \text{N}_{\text{removal}} = (\text{N}_{\text{initial}} - \text{N}_{\text{final}}) / \text{N}_{\text{initial}} * 100\% \quad (\text{Eq.5})$$

178

179 **3 Results and discussions**

180 **3.1 HTL of binary mixtures with starch and tripalmitin**

181

182 The nitrogen content in biocrudes is mainly originated from the protein source. Still, the presence
183 of lipids or carbohydrates can promote or inhibit the migration of nitrogen into the biocrude

184 fraction. To investigate the interaction mechanisms between biomass fractions, representative
 185 macromolecular model compounds, such as albumin, starch and tripalmitin, were tested in both
 186 water and water-acetic binary solvent system. Table 1 lists the biocrude yields, element analysis,
 187 HHV and ER values of biocrudes produced from binary mixtures HTL.

188

189 Table 1. Biocrude yields, elemental analysis, HHV and ER values of biocrudes from albumin
 190 and binary mixtures of albumin/starch and albumin/tripalmitin.

	Albumin	Albumin/Starch	Albumin/Tripalmitin
Biocrude yield, wt.%	7.4±1.5	6.3±0.21	70.5±3.6
Element analysis, wt.%			
C	72.6	71.0	74.3±0.6
N	6.6	6.2	2.2±0.07
H	9.1	7.5	11.4±0.3
S	3.5	2.6	<0.1
O*	7.9	12.7	12.1
Solid yield, wt.%	0.11±0.01	0.1±0.01	0.15±0.07
HHV, MJ/kg	35.6	32.7	38.2
ER, %	10.3	9.06	82.9

191 * *calculated by difference, %*

192

193

194 The biocrude yield produced from albumin and its nitrogen content was in agreement with the
 195 previous study performed with the egg albumin at 300°C [30]. The solid yield was negligible,
 196 thus implying a full conversion of albumin under hydrothermal conditions. It is worth noting that
 197 the yield of the biocrude fraction produced from protein fraction of different feedstocks vary
 198 widely based on its amino acid distribution. When the peptide bonds in the protein undergo

199 hydrolysis, some amino acids with hydrophobic side chains (phenylalanine, tryptophan, etc.) are
200 partitioned to the biocrude phase. On the other hand, hydrophilic amino acids (serine, asparagine,
201 etc.) contribute to the generation of water soluble compounds [22].

202 The Albumin/Starch binary mixture produced lower biocrude yield than that from the albumin
203 alone. However, the nitrogen content of the biocrude was not reduced correspondingly even if the
204 albumin content in the binary mixture was twice less compared to the individual experiment with
205 the albumin. This indicates the occurrence of the interaction reactions between starch and
206 albumin decomposition products, causing the generation of more nitrogen compounds in the
207 biocrude fraction.

208 It should be pointed out that the experiments with albumin and albumin/starch binary mixture
209 have been also performed in an acetic acid-water solution (5/95 % wt.%) (Table S2). As
210 previously reported for model amino acids [21], also in the present study a significant
211 improvement of the biocrude yield was observed due to the selective acetylation of water soluble
212 amines forming more hydrophobic amides.

213 The binary mixture of albumin/tripalmitin resulted in the highest biocrude yield due to the
214 contribution of fatty acids produced from the tripalmitin under HTL conditions and their
215 derivatives into the biocrude phase. In this case, the decreased nitrogen content can be attributed
216 to two main factors: the dilution of protein derived fragments by long carbon chains of fatty acids
217 that are quantitatively partitioned into the biocrude phase and the reaction between the acidic
218 moiety of palmitic acid and water soluble amines to give hydrophobic amides [32].

219 Energy recovery is an important indicator for the efficiency of the HTL process. In this context,
220 the greatest ER was observed for the albumin/tripalmitin binary mixture due to the highest
221 biocrude yield and HHV values. In contrast, co-feeding albumin with starch resulted in the
222 slightly reduced ER compared to that from albumin only owing to its lower biocrude yield and
223 HHV, which is consistent with previous literature [34, 35]. The presence of starch also negatively
224 affected both oxygen and nitrogen contents of the biocrude. In general, the biocrude with higher
225 heteroatom contents is more difficult to upgrade into transport fuels.

226

227 3.2 Characterization of main chemical species in biocrudes produced from albumin and 228 binary mixtures.

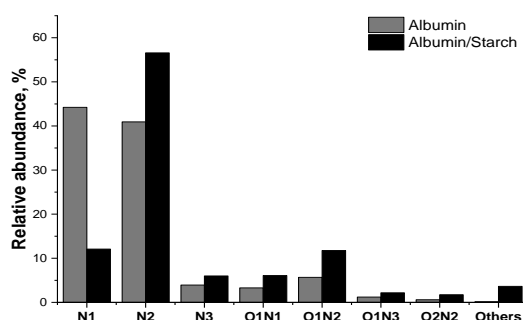
229

230 The biocrude samples produced from albumin and albumin/starch mixture were analyzed by
231 FTICR MS ESI+, as basic nitrogen species are more efficiently ionized in this ionization
232 condition. The mass spectra of biocrudes contained thousands of different peaks and each of them
233 is related to a specific molecular formula. The spectra were then elaborated and the molecular
234 formulas were categorized into groups based on their heteroatoms using a petroleomic approach.

235 Fig.1 depicts the main class components of biocrude products produced from the albumin and
236 albumin/starch mixture. The abundance of one or two nitrogen atom containing compounds (N1
237 and N2, respectively) was observed for the biocrude produced from the albumin HTL.

238 For the albumin and starch mixture HTL the N2 class was clearly predominant in the biocrude
239 followed by N1, O1N2 and O1N1 class components.

240



241

242 Fig. 1. Heteroatom class components of biocrudes from albumin and albumin/starch mixture.

243

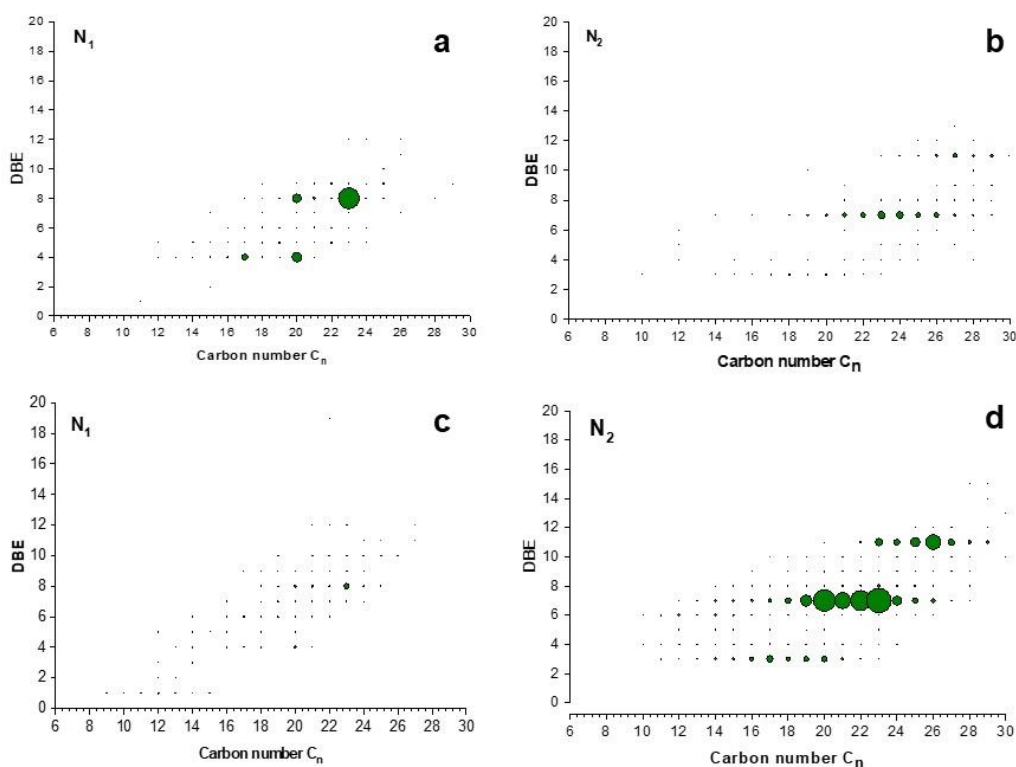
244 In order to determine the possible structures of the biocrude components the abundance plots of
245 double bonds equivalents DBE (the sum of rings and double bonds) versus carbon number (Cn)
246 for the main heteroatom classes are depicted in Fig. 2. In the case of the biocrude obtained from

247 albumin, the main N1 class distribution corresponded at DBE= 4 and Cn=17-20; DBE=8 and
248 Cn=20-23. Concerning N2 class, a broad distribution of species ranging from Cn=20-26 and
249 DBE=7 was observed. The N1 species could be tentatively assigned to aniline and pyridine
250 derivatives with a single ring (DBE=4) or functionalized with a benzene moiety (DBE=8). The
251 N2 class could be assigned to condensed pyrazine or pyrimidine derivatives (DBE=7).

252 Generally, NAs are generated through Maillard reaction; however, their formation was also
253 observed when amino acids were treated solely [39, 40]. For instance, the degradation of ¹³C-
254 labelled serine in the absence of any sugars led to the generation of different α-aminocarbonyl
255 intermediates that are common precursors to pyrrols, pyrazines and pyridines via Knorr pyrrole
256 synthesis [39]. The other mechanism for the formation of pyrazines can also occur through the
257 intra- and intermolecular cyclization of amino acids followed by dehydration reactions [12, 34,
258 38].

259 The biocrude from the albumin/starch mixture contained mainly N2 homologues series of
260 compounds with the same DBE values of 7 and 11 with Cn=16-26, indicating a higher degree of
261 unsaturation and functionalization. These species could be indicative for quinazoline and
262 naphthyridine derivatives. Based on the FTICR MS analysis, it was shown that NAs, mainly N1
263 and N2 containing compounds, can be produced from the albumin without any carbohydrate
264 source; however, in the presence of starch, the formation of N2 class components with higher
265 DBE values is enhanced via reactions of amines with oxygenated derivatives (aldehydes, ketones)
266 generated by sugars. Some hypothesized structures based on the elemental analysis and DBE
267 values are illustrated in Fig. S1.

268



269

270

271 Fig. 2. C_n vs DBE plots for main classes found in biocrudes from a) N1 classes for albumin; b)

272 N2 classes for albumin; c) N1 class for albumin/ starch; d) N2 classes for albumin/starch.

273

274 The predominant nitrogen species in the biocrude produced from albumin/tripalmitin mixture
 275 were mainly in the form of fatty acid amides (FAAs). Therefore, GC-FID was employed for the
 276 quantification of FAAs. Under hydrothermal conditions, tripalmitin underwent hydrolytic
 277 cleavage to form palmitic acid, corresponding to 72 wt.% of the biocrude. The amidation reaction
 278 took place between palmitic acid and ammonia and amines, resulting in the formation of about 4
 279 wt.% FAAs (Fig. S2).

280 These results confirm that Maillard reaction is the main reaction pathway that takes place in the
 281 binary mixture of protein and carbohydrate, thus generating NAs, while the presence of lipids
 282 shifts the reaction pathway towards the formation of FAs and FAAs, which are expected to be
 283 less resistant to upgrading process compared to the NAs.

284

285 3.3 HTL of ternary mixture

286

287 In order to verify the influence of the lipid content on the nitrogen distribution in the biocrudes,
288 comparative experiments were carried out with the ternary mixture containing two different lipid
289 contents of 20 wt.% and 33 wt.%. Two ternary mixtures of albumin/starch/triolein with the weight
290 ratios of 1:1:0.5 and 1:1:1 were processed under identical HTL conditions. Triolein was selected
291 herein in replace of tripalmitin, as oleic acid is the main component of waste cooking oils [41].
292 Table 2 represents the yields, HHV and ER values of biocrudes.

293

294 Table 2. Biocrude yields, elemental analysis, HHV and ER of biocrudes from HTL of ternary mixtures

	Albumin/Starch/Triolein 20	Albumin/Starch/Triolein 33
	wt.%	wt.%
Biocrude yield, %	25.0±2.9	36.1±1.5
Biocrude yield without triolein contribution, %	7.1	6.4
Solid yield, %	2.1	1.0
<i>Element analysis, %</i>		
C%	74.0±0.7	74.5±0.6
N %	3.8±0.1	3.0±0.05
H, %	10.5±0.4	11.1±0.2
O, %	11.4	11.4
S, %	0.3±0.04	0.1
HHV, MJ/kg	36.7	38.0

ER, %	37.17	51.2
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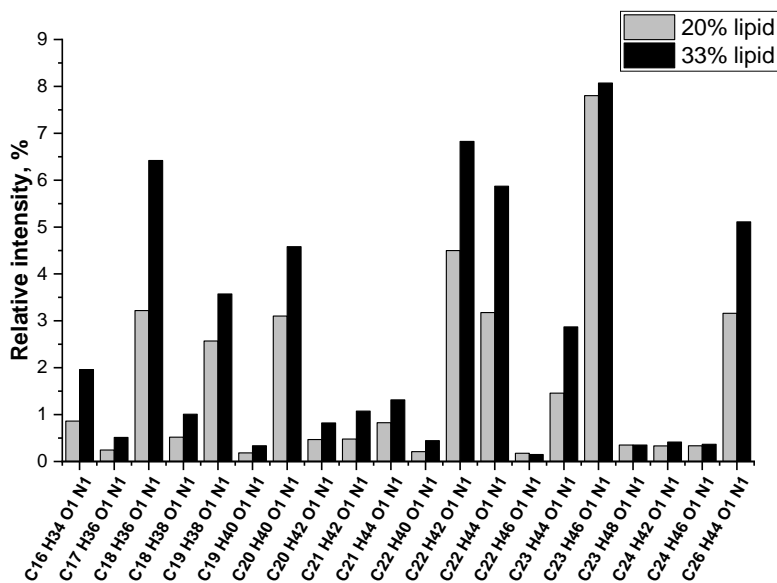
295

296 As expected, the higher biocrude yield and lower nitrogen content were obtained with the higher
297 lipid content mainly due to the direct contribution of fatty acids to the biocrude. As result of this,
298 the ER was substantially improved as well. In this case, the synergistic effect of the addition of
299 lipid to albumin on the biocrude yield, previously reported for the binary mixture, was not
300 prominent. In fact, most of the biocrude is derived from the hydrolysis of the lipid fraction with
301 formation of oleic acid. If it is subtracted, the yields decrease to 6-7%, which are similar to the
302 cases of the binary albumin/starch or albumin alone tests. This can be explained by the occurrence
303 of hydrolysis of three macromolecular fractions, albumin, starch and triolein, at different
304 temperatures. In fact, starch is rapidly hydrolyzed to produce oligosaccharides and
305 monosaccharides at 180–250 °C [42], while the hydrolysis of lipids, for instance, soybean,
306 linseed, and coconut oils proceeds at 260–280 °C [43]. Thus, starch-derived products are present
307 in the reaction mixture since the first stages of the process and they limit their effect on the
308 formation of amides, that would have increased the biocrude yield due to their solubility in the
309 oil phase.

310 Deep characterization of the products was thus carried out. Given the poor resolution of the GC-
311 MS analysis for polar nitrogen compounds, FTICR ESI+ was utilized to characterize the FAAs
312 and NAs species of the biocrudes. It should be pointed out that by ESI+ the presence of FAs was
313 not detected, as they are selectively ionized in the ESI- mode.

314 In order to determine the effect of the lipid content on the formation of the NAs and FAAs in the
315 biocrudes produced from the ternary mixtures, the relative contents of FAAs and NAs were
316 determined by FTICR-MS, taking into account their relative intensities in the mass spectra
317 normalized with respect to that of an internal standard (caffeine) and also considering dilution
318 factors. The distribution of FAAs in the biocrude products from ternary mixtures is illustrated in
319 Fig.3. As it can be seen from Fig. 3, the biocrude produced from the 33% lipid containing ternary

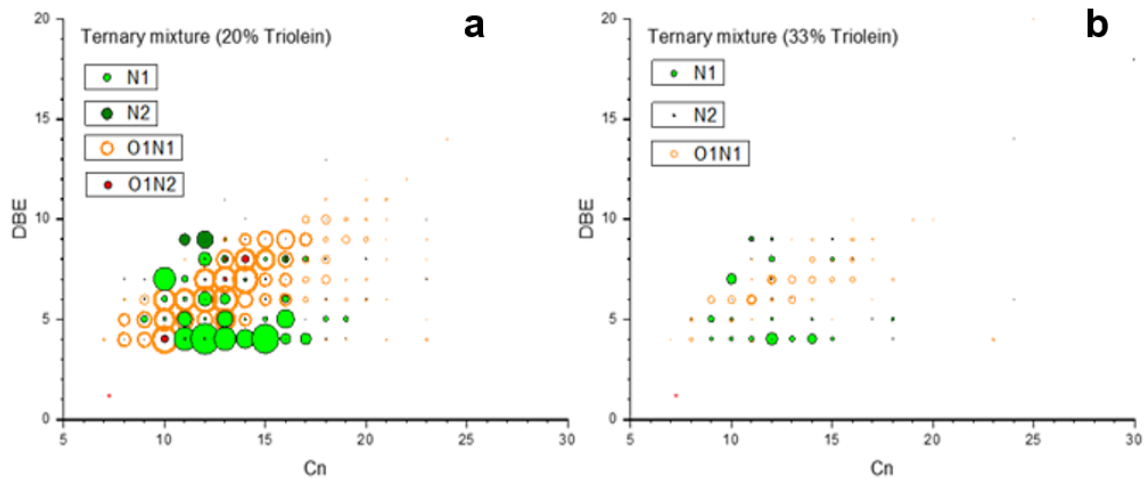
320 mixture contained more FAAs compared to that from the mixture with the 20% lipid content. The
 321 ratio of the total relative peak intensity of FAAs in the biocrudes from the mixtures with 33 %
 322 and 20 % lipid contents was estimated as 1.51, thus indicating that the high-lipid mixture produced
 323 a higher amount of FAAs.
 324



325
 326 Fig. 3. FTICR ESI+ based distribution of main FAAs species as protonated ions for ternary
 327 mixture with 20 % and 33 % triolein contents.

328
 329 For the semi-quantitative analysis of NAs, the mass spectrum peaks that are relevant to the
 330 nitrogen-containing heterocyclic and aromatic compounds were considered by elaborating the
 331 compounds only with a DBE value higher than 4. Fig.4 illustrates the abundance plots of DBE
 332 versus Cn for the NAs. It can be seen that the NAs in the biocrudes from the ternary mixtures
 333 were present in forms of N1, N2 and O1N1 class components at DBE=4-9 and Cn=8-20 and their
 334 relative abundances were more significant for the mixture with the 20% of lipid content. In fact,
 335 the ratio of the total peak intensity of NAs between 20% and 33% triolein containing mixtures
 336 was 0.47 (Total Peak area of NAs for 20% triolein/Total Peak area for 33% triolein), thereby
 337 confirming the lower content of the NAs in the biocrude obtained from the mixture with the higher

338 lipid content. These results confirm the positive impact of the lipids on the promotion of amidation
339 reactions, forming FAAs and inhibiting the formation of NAs, the latter being more recalcitrant
340 for the hydrotreatment process.
341



342
343 Fig. 4. Cn vs DBE plots for aromatic nitrogen classes (DBE >4) for biocrudes from ternary
344 mixtures: a) 20% and b) 33% triolein content.

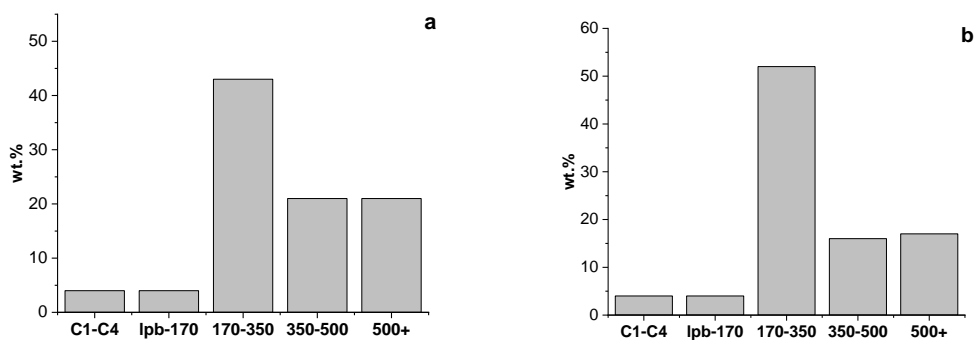
345

346 3.4 Hydrotreatment of biocrude produced from organic food waste

347

348 In order to validate the hypotheses elaborated using model compounds, the composition of the
349 biocrudes produced from the ternary mixture of albumin/cellulose/tripalmitin and from a real
350 municipal organic food waste were characterized by FTICR MS with APPI and compared
351 considering the number of common products and their intensities. According to the results, only
352 about 44% common products were present in both model and biomass waste biocrudes [44].
353 Even though the employment of the ternary mixtures can help to understand the interaction
354 mechanisms that occur under HTL conditions and optimize the process, they may not be fully
355 reliable for the next upgrading stage via hydrodeoxygenation and hydrodenitrogenation.

356 Therefore, in order to accurately evaluate the hydrodenitrogenation reactivity under relevant
 357 hydrotreatment conditions, and particular the effect of FAAs and NAs content, the two biocrude
 358 samples were produced starting from real food wastes with the different lipid contents. A standard
 359 sorted household organic food waste was selected with the dry matter of 35%, carbon content of
 360 42% and lipid content of 20%. The comparison was made with a supermarket food packaging and
 361 expiring waste with the dry matter of 50%, 55% of carbon content, and 31% of lipid content (due
 362 to the presence of meat, cured meats and dairy products packaging).
 363 The HTL of sorted household and supermarket wastes resulted in the biocrude yields of 35% and
 364 58%, respectively. Afterwards, the HTL oils were upgraded by conventional hydrocracking
 365 process that is typically employed for the refinery upgrading of fossil heavy oil, such as the
 366 vacuum distillation residue, also referred to as the bottom-of-the-barrel [29]. The yields of
 367 upgraded oils were 92 and 93 % for the biocrudes obtained from organic waste with 20 and 33 %
 368 lipid contents, respectively. As shown in Fig. 5, the upgrading of the biocrude from the 33% lipid
 369 containing food waste resulted in the increased yields of diesel (170-350 °C) fraction as expense
 370 of vacuum gas oil VGO (350-500 °C) and heavy oil (+500 °C) fractions compared to those from
 371 the food waste with the 20% lipid content.



372
 373 Fig. 5. Distillation cuts for upgraded oils from: a) household food waste; b) supermarket food
 374 waste.
 375

376 The characterization of the biocrudes before and after the upgrading process is given in Table 3
 377 [45]. It can be seen that the upgrading of the household waste HTL oil resulted in the removal of

378 around 75% of oxygen, 64% of sulphur and 60% of nitrogen and, as a result of the removal of
 379 heteroatoms, the HHV increased from 34.8 to 43. MJ/kg, similar to that of fossil fuels. In the case
 380 of the household waste HTL oil, the main difference was the denitrification efficiency that
 381 increased up to 71%.

382

383 Table 3. Element analysis of biocrudes produced from the organic food waste [45].

	HTL oil from sorted household organic waste	Upgraded HTL oil	HTL oil from supermarket food waste	Upgraded HTL oil
<i>Element analysis, %</i>				
C	72.0	82.0	72.5	83.0
H	9.0	12.3	9.3	12.2
O	12.5	3.1	12.3	3.1
N	5.8	2.3	5.2	1.5
S	0.75	0.27	0.68	0.27
HHV, MJ/kg	34.8	43.3	35.6	44.9

384

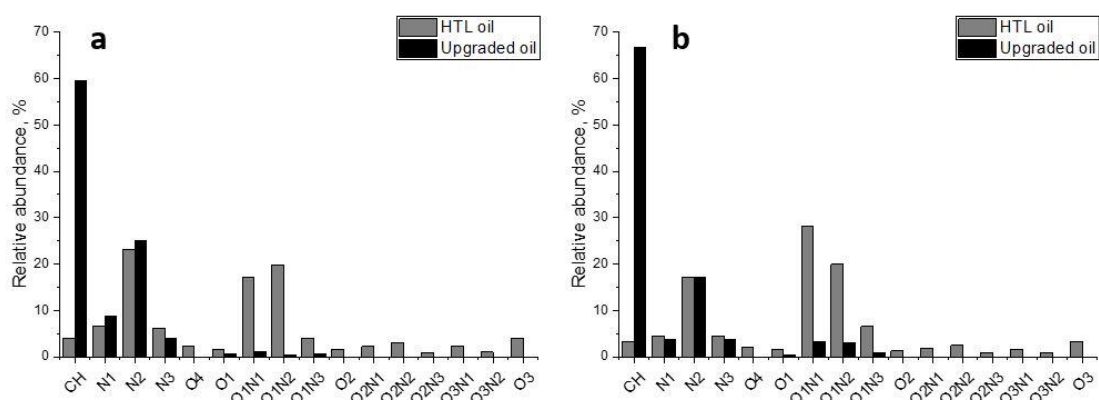
385 The upgraded oil produced from household waste was analyzed by GC-MS to determine the type
 386 of residual nitrogen containing species (Fig.S4). The native biocrude was found to contain FAs,
 387 FAAs and some NAs. After the hydrotreatment, the main peaks were related to the linear saturated
 388 hydrocarbons with the carbon numbers ranging from C14 to C30 were detected; however, the
 389 elemental analysis clearly showed that nitrogen was still present in the upgraded oil.

390 The nitrogen compounds that are not detectable by GC-MS due to the low volatility and high
 391 polarity, were additionally characterized by FTICR MS with the APPI (Atmospheric pressure
 392 photoionization) in positive ion mode (Fig. 6). The classes were determined and compared for the
 393 biocrudes and the upgraded oils, normalizing the abundances according to the elemental analysis

394 results. The class distribution confirmed the efficient oxygen removal, as only trace amounts of
 395 compounds related to the O1 and O1Nx classes (FAAs) were detected. On the other hand, the
 396 amount of NAs belonging to N1, N2, N3 classes, decreased slightly, thereby confirming that the
 397 NAs are more difficult to remove by the hydrotreatment process. Similar results were obtained
 398 with the supermarket food waste upgraded oil, confirming the NAs belonging to N1, N2, N3
 399 classes are the most recalcitrant, under the given hydrocracking conditions. In this case, the
 400 relative abundance of the Nx species was lower than that from the oil with the lower lipid content
 401 (even if by a semi-quantitative comparison, due to complexity of the mixture), thus confirming
 402 the effect of lipids on the distribution of nitrogenous products, which are shifted more towards
 403 the FAAs components. This is in fully agreement with the lower nitrogen content measured with
 404 the elemental analysis.

405 Based on these results, blending of the protein-rich biomass with acyl donor species, such as
 406 oleaginous biomass, microalgae, waste cooking oils and fat fractions from animal waste, can be
 407 one of the most optimal solutions to inhibit the Maillard reactions and promote the formation of
 408 nitrogen compounds with the higher HDN reactivity, thereby producing the biocrudes with
 409 specifications that can meet the requirements of the existing refinery facilities.

410



411

412

Fig. 6. Main molecular classes distribution of HTL oil and upgraded oil.

413

The relative abundances of the classes have been normalized according to the elemental analysis

414

results for a) household food waste; b) supermarket food waste [45].

415

416 **4. Conclusion**

417 HTL tests carried out on model molecules have given general indications that interactions
418 between proteins and lipids lead to an improved biocrude yield and a lower content of nitrogen-
419 containing aromatic compounds, compared to that observed with protein and carbohydrate
420 mixtures. The presence of lipids in the HTL feedstock favors the formation of fatty acid amides,
421 which can be converted into hydrocarbons, thereby resulting in the more efficient removal of
422 nitrogen in the biocrude upgrading process. These results suggest that HTL feedstock should be
423 supplemented with a lipids-rich waste biomass to produce a biocrude more suitable for upgrading
424 by conventional hydrotreatment processes.

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432

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