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The storage stability of biocrude obtained by the hydrothermal liquefaction of microalgae

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ABSTRACT

Hydrothermal liquefaction (HTL) is a promising technology that can produce biocrude oil from wet biomass. The biocrudes, while generally acknowledged to be more stable than pyrolysis oils, are still thought to degrade relatively quickly, which limits their applicability. In this investigation, the storage stability of biocrude produced from hydrothermal liquefaction of microalgae was systematically studied over 60 days, and the effect of the storage material, feedstock species, liquefaction temperature and storage temperature were assessed. Biocrudes obtained at 300 °C and 350 °C from the microalgae Spirulina and *Chlorella vulgaris* were stored at three temperatures: cold (4 °C), ambient (20 °C) and elevated temperatures (35 °C), over the two-month period. The dynamic viscosity, higher heating value, thermogravimetric analysis and elemental and chemical composition were assessed. The viscosity of the biocrudes only increased considerably at 35 °C. The reaction temperature and biomass type were also strong determining factors of the impact on biocrude stability. Biocrudes produced from *C. vulgaris* were more stable than the Spirulina, and the crudes formed at 350 °C were considerably less reactive than those produced at 300 °C. This demonstrates that biocrudes can be stored without substantial degradation, allowing a more flexible approach to upgrading to value products.

1. Introduction

Advanced drop-in biofuels are an alternative to fossil-derived fuels, giving similar performance while reducing net emissions of CO_2 and other greenhouse gases (GHGs) [1]. Microalgae are a viable feedstock for biofuel production – some species have shown up to 50 times higher photosynthetic efficiencies compared to terrestrial plants, and do not require fertile soil for cultivation, posing no competition to other agricultural activities. However, the use of microalgae as a feedstock for "traditional" biofuels, such as biodiesel or bioethanol, is limited by poor economics and the extremely dilute nature of cultivated algae (0.1–2.5 g L^{-1}), requiring energy-intensive dewatering [2,3].

One promising conversion technology is hydrothermal liquefaction (HTL), which can convert wet microalgal biomass into a petroleum-like liquid (biocrude) with a heating value higher than the original raw material. The biocrude can then be converted into hydrocarbon fuels separately or alternatively co-refined with fossil fuels [4,5]. Yields vary depending on feedstock but are typically between 30 and 40% with respect to the dry microalgal biomass. The HTL reaction occurs at conditions close to the critical point of water, with loadings between 5 and 20% solids and reaction temperatures and pressures between 200 and 400 °C and 10–20 MPa, respectively. The physicochemical properties, yield and elemental and chemical composition of the biocrude are strongly dependent on the reaction conditions (HTL temperature and time) and the characteristics of the raw material [6,7].

The biocrude is a complex mixture of linear and branched hydrocarbons, nitrogenated and oxygenated cyclic compounds, fatty acids, esters, phenolic derivatives and alternative oxygenates [8]. The biocrude is similar to heavy oil, though has a higher nitrogen and oxygen content, up to 7% and 19%, respectively [6–8]. The dynamic viscosity typically falls between 35 and 6,200 mPa s at 40 °C, whilst the densities generally range between 0.9 and 1.3 g mL^{-1}. HHV values of around 31–39 MJ kg^{-1} are typical,

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depending on the carbon content [9–14].

In the large-scale production of microalgal biocrude, it is also necessary to consider its stability during transport and long-term storage, since thermal and storage stability studies of similar fuels report an increase in their viscosity, density and molecular weight, among other properties [15–18]. The change in the physicochemical properties of the biocrude during storage is known as aging, and is attributed to the polymerization, esterification and condensation reactions between the volatile and oxygenated compounds found in biocrude [19–21]. One of the key requirements for HTL biocrudes is the potential to co-refine with fossil resources, which would potentially require longer-term storage and transport.

A number of studies on the stability of bio-oil obtained from pyrolysis of lignocellulosic biomass have been published to date, in which two main evaluation methods are used: prolonged storage under controlled conditions, and accelerated aging at 80 or 90 °C. The main metrics for bio-oil stability are viscosity, water content, pH and total acid number (TAN) [15–18,22–27]. Whilst it has been noted that due to the lower levels of reactive oxygenated species, HTL biocrudes tend to be more stable than the corresponding pyrolysis bio-oils [12], no investigations have yet examined the long term storage of biocrude from the HTL of microalgae, or the effect of the various parameters on the physical and chemical properties during storage. This is key to determining how biocrudes will fit into the existing transportation infrastructure. In this investigation, the stability of biocrude obtained by HTL of two separate phototrophic species (Spirulina and *C. vulgaris*) at 300 and 350 °C were examined. The biocrudes were stored at three temperatures: cold (4 °C), ambient (20 °C) and hot (35 °C), over 60 days. The dynamic viscosity, higher heating value (HHV) and thermogravimetric analysis (TGA) were measured as indicators of biocrude aging. The chemical and elemental composition of each biocrude was also analyzed at the beginning and at the end of the test period.

2. Material and methods

2.1. Materials

The cyanobacteria *Arthrospira platensis* (Spirulina) and microalgae *Chlorella vulgaris* were obtained from Naturya (Bath, United Kingdom). Feedstock chemical compositions were provided by the supplier. The ash and moisture content of the biomass was quantified using TGA; the mass loss between 20 and 105 °C was used to determine the sample moisture content and the percentage mass residual at the end of the TGA was taken to be the ash (Fig. S1). Quantification of the elemental analysis and HHV is described in section 2.4. The characterization of the two microalgae is shown in Table 1. All other reagents were purchased from Sigma Aldrich and used without further purification.

Table 1
Characterization of biomass in percent by mass on dry basis (%).

Properties	Spirulina	*C. vulgaris*
Moisture (%)	5.2	3.4
Ash (%)	7.7	8.7
HHV ($MJ \cdot kg^{-1}$)	23.0	24.0
Elemental composition (%)		
C	49.7	51.4
H	7.2	7.4
O[a]	24.3	22.1
N	11.3	10.2
Chemical composition[b] (%)		
Lipids	1.1	2.3
Protein	70.2	61.8
Carbohydrates	19.3	26.7
Others	0.7	0.4

[a] By difference.
[b] From supplier.

2.2. HTL reactions

Biocrude for the stability study was obtained at two different reaction temperatures (300 and 350 °C), using two different biomass types (Spirulina and *Chlorella vulgaris*). Liquefaction was carried out in a 50 mL batch reactor. The reactor consisted of a section of 1" stainless-steel tubing fitted with a pressure gauge, a thermocouple, a needle valve for venting gaseous products at the end of the reaction, and a pressure relief valve. The design of the reactor and reaction procedures have been previously reported [28].

In a typical reaction, the reactor was charged with 5 g (dry weight) of microalgae and 20 mL of deionized water (20% total initial solids). The reactor was then introduced into a vertical tubular furnace pre-heated to 550 °C and left until it reached the desired reaction temperature. HTL was performed at two temperatures 300 °C (10 MPa) and 350 °C (17 MPa), with an average heating speed of 13 and 10 $°C \cdot min^{-1}$, respectively. Once the reaction temperature was reached, the reactor was immediately removed from the oven and allowed to cool to ambient temperature. A typical HTL thermal profile can be found in Fig. S2. After the reaction, gaseous products were released *via* the needle valve; aqueous phase products were subsequently decanted and filtered to separate them from the biocrude and solid products. The biocrude was recovered by washing the reactor and filter paper several times with chloroform. The chloroform-biocrude solution was filtered and the solvent was removed *in vacuo* at 35 °C.

2.3. Storage stability test

The storage stability test was performed on four types of biocrude: *C. vulgaris* biocrude obtained at 300 °C (Ch300) and 350 °C (Ch350), and Spirulina biocrude obtained at 300 °C (Sp300) and 350 °C (Sp350). Each sample of biocrude was stored for 60 days in a sealed glass vial, in the dark and at controlled temperatures: cold (4 °C), ambient (20 °C) and hot (35 °C). A further stability test was performed on the *Chlorella* biocrude obtained at 300 °C (Ch300m) and 350 °C (Ch350m): a stainless-steel strip (2.0 cm × 1.6 cm x 0.025 cm thickness) was added to the vial containing biocrude and stored for the same time interval at 20 °C. This was to establish whether biocrude aging was influenced by corrosion effects (which would affect the type of storage container used industrially). The stainless-steel plate was weighed before and after the test.

The dynamic viscosity was primarily used to determine the stability of the biocrude. Elemental and chemical composition and HHV of the biocrude were recorded at the start (0 days) and end of the aging study (60 days). All measurements were made in duplicate. TGA was carried out during the first 15 and 20 days of the test.

2.4. Analytical methods

The dynamic viscosity, TGA, HHV and chemical and elemental composition of the biocrude were analyzed during the storage stability test. The dynamic viscosity of the biocrude was measured using a Bohlin C-VOR rheometer, using a CP 1°/20 mm spindle, and a gap width of 0.07 mm. The value of shear stress was chosen empirically, depending on the expected viscosity of the biocrude, generally between 8 and 800 Pa. The viscosity measurements were made at a temperature of 40 °C and the viscosity index is defined in Eq. (1), where V_1 and V_2 are the dynamic viscosities of fresh sample and aged sample, respectively.

Table 2
Yield of biocrude and biochar of microalgae Spirulina and *C. vulgaris* in percent by mass on dry basis (%).

Microalgae	HTL Temperature (°C)	Biocrude (%)	Solid (%)
Spirulina	300	31.0 ± 1.6	10.0 ± 0.8
	350	36.2 ± 1.3	6.8 ± 1.2
C. vulgaris	300	39.6 ± 1.9	8.1 ± 0.8
	350	42.1 ± 1.5	5.5 ± 0.7

$$\Delta\text{Viscosity} = V_2 - V_1 / V_1 \quad (1)$$

TGA of the biocrude was performed on a Setaram TG-92 thermogravimetric analyzer. The sample was heated from ambient temperature to 800 °C at a heating rate of 10 °C·min^{-1}, with 1.5 bar nitrogen as carrier gas and 0.5 bar argon supplied as furnace gas.

Elemental analysis of the biomass and biocrude were carried out externally in OEA Laboratories Limited (Cornwall, United Kingdom). HHV was calculated from elemental composition using Eq. (2) proposed by Channiwala & Parikh [29].

$$\text{HHV}\ (\text{MJ}\cdot\text{kg}^{-1}) = 0.3491\text{C} + 1.1783\text{H} - 0.1034\text{O} - 0.1034\text{N} + 0.1005\text{S} - 0.0211\text{A} \quad (2)$$

where C, H, O, N, S, and A represents carbon, hydrogen, oxygen, nitrogen, sulfur and ash, respectively, as weight % on a dry basis.

Biocrude samples were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) (Agilent Technologies 7890A-5975C). An HP-5MS capillary column (30 m × 250 μm × 0.25 μm) was used to separate the compounds. The biocrude samples were diluted in tetrahydrofuran (THF) to a concentration of 1 μg mL^{-1}. The injection volume was 1 μL, with a split ratio of 1:10 and an inlet temperature of 250 °C. The initial temperature of the column was held at 50 °C for 1 min, then ramped at 7.5 °C·min^{-1} to 290 °C, and held at 290 °C for 3 min. The major compounds were identified according to the National Institute of Standards and Technology (NIST11) spectral database.

3. Results and discussion

The positive energy balance for the hydrothermal liquefaction of microalgae is well established, irrespective of the temperature of production, as long as the aqueous fraction can be recycled and the nutrients recovered [30]. In this study we selected the two highest industrially produced photosynthetic micro-organisms for the liquefaction; the eukaryotic *Chlorella vulgaris* and prokaryotic Spirulina.

The yields of biocrude and solid residue for HTL reactions of microalgae at different temperatures are shown in Table 2. The yields obtained are in line with those obtained in similar studies [31–34].

3.1. Dynamic viscosity

Biocrude yields were higher for *C. vulgaris* than Spirulina, increasing slightly with increasing temperature for both species. To

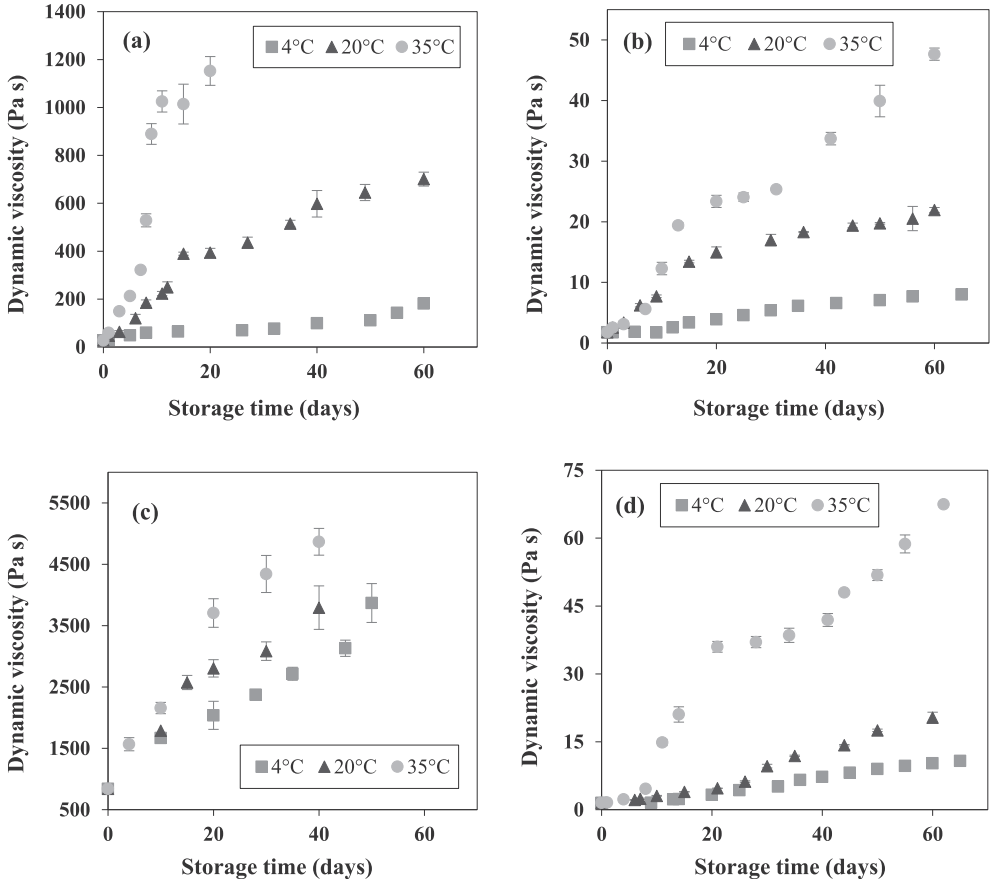

Fig. 1. Change of dynamic viscosity of biocrude of microalgae: (a) Ch300, (b) Ch350, (c) Sp300, and (d) Sp350. The biocrude was stored at 4 °C (square), 20 °C (triangle) and 35 °C (circle). Error bars indicate standard deviation.

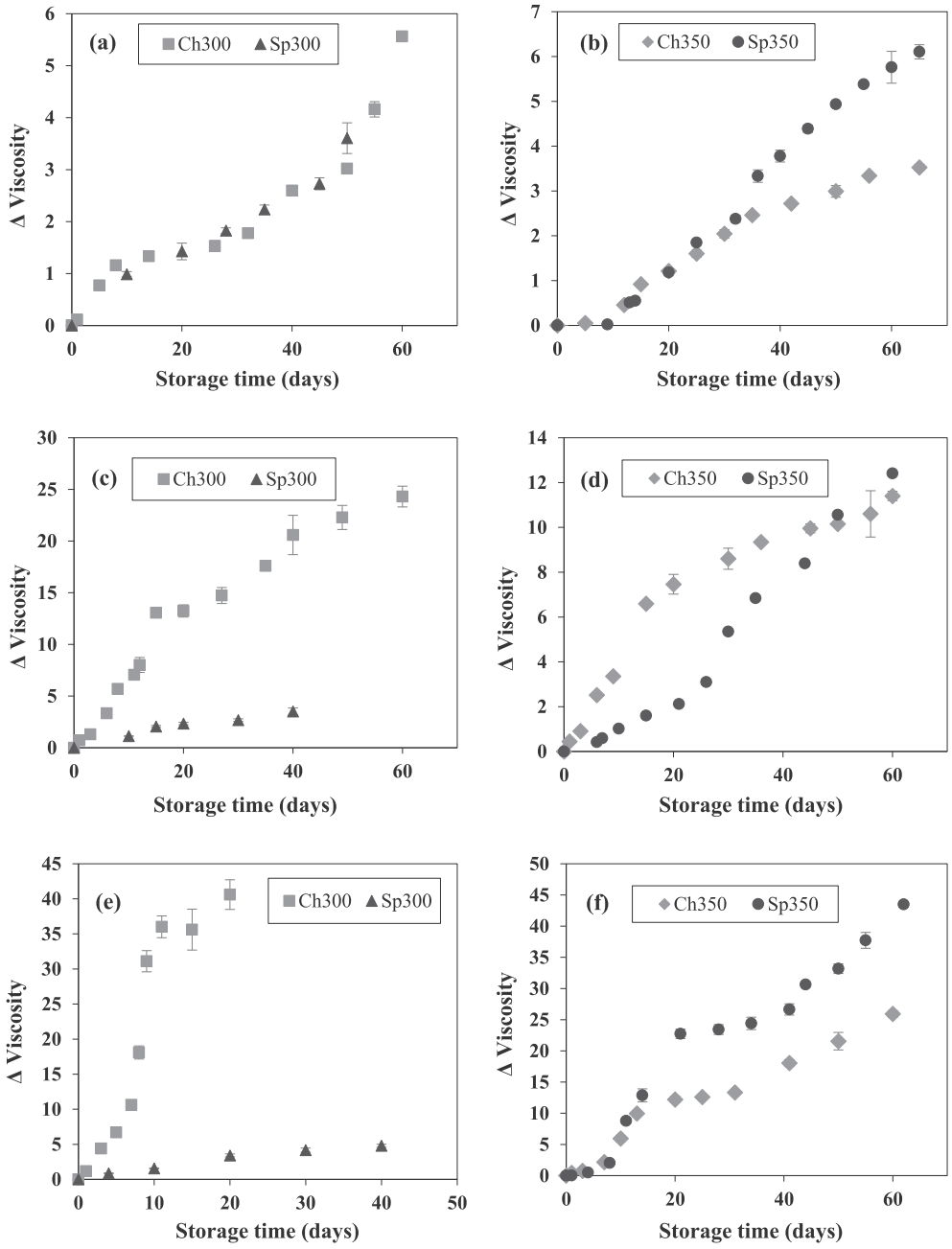

Fig. 2. Viscosity index of biocrude of microalgae: (a) and (b) – biocrude stored at 4 °C, (c) and (d) – biocrude stored at 20 °C, (e) and (f) – biocrude stored at 35 °C. Error bars indicate standard deviation.

assess the stability, the samples were stored at three different temperatures, 4 °C, chosen as the coldest reasonable refrigeration temperature, 20 °C, room temperature and 35 °C, realistically the highest temperature the crude would be exposed to over long periods of time if stored poorly in hot climates. The biocrude viscosity and behavior, however, were significantly different for the two species, and changed significantly during storage (Fig. 1). All samples showed Newtonian behavior in the shear stress range 8–800 Pa. The initial viscosity of the biocrude decreased considerably with increasing reaction temperature for both microalgae. This behavior is similar to previous observations [31,35–37] and is presumably due to more extensive depolymerization of larger macromolecules into smaller, less viscous fragments at higher temperatures. In general, the viscosity of the biocrude increased with time and storage temperature, which suggests that condensation and polymerization reactions were occurring [19,21,38]. For both species, the biocrudes generated at lower temperatures polymerized to a much greater extent than the crudes formed at 350 °C. Microalgal biocrude obtained at 350 °C remained liquid throughout the entire 60 days storage period.

The Sp300 sample was considerably more viscous than the Ch300 sample. However, at 4 °C, both samples changed, on average, at a rate of 7% day^{-1} (Fig. 2a). The rate of repolymerization was heavily influenced by storage temperature. At 20 °C and 35 °C, the change in dynamic viscosity in Ch300 was 5 and 20 times higher than in the Sp300 sample, respectively (Fig. 2c and e). Sp300 solidified before the end of the test period, independent of the storage temperature.

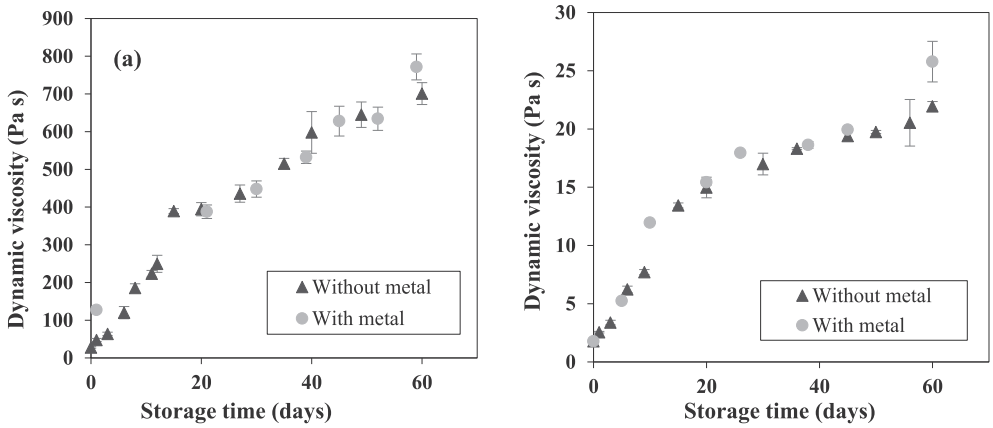

Fig. 3. Change of dynamic viscosity of biocrude of *C. vulgaris* obtained to different HTL temperature (a) 300 °C, and (b) 350 °C with (circle) and without (triangle) contact with a stainless-steel plate. The biocrude was stored at 20 °C. Error bars indicate standard deviation.

At 4 °C, the dynamic viscosity of the Sp350 and Ch350 biocrudes remained unchanged during the first 10 days of storage. This demonstrates that biocrudes could potentially be stored and transported under temperature-controlled conditions if transportation times were relatively short. However, after 10 days, the viscosity of the samples increased linearly at a rate of approximately 10%·day^{-1}. After 35 days of storage, the viscosity increase rate of the Sp350 was 2.6 times higher compared to the change in viscosity of Ch350 (Fig. 2b). At 20 °C, the viscosity of samples Ch350 and Sp350 do not show this lag time, and rather, start reacting immediately. The viscosity of Ch350 increased 3.7 times faster than the viscosity of Sp350 during the first 20 days. After this point, the viscosity increase rate of the Ch350 stabilized (9%·day^{-1}), while the viscosity growth rate of the Sp350 sample increased from 11 to 26%·day^{-1}. At the end of the storage period, both biocrudes had a similar viscosity (Fig. 2d).

At 35 °C, the viscosity of Sp350 and Ch350 increased at an average rate of 28%·day^{-1} during the first 15 days. During the period between 20 and 35 days, the viscosity for both samples stabilized to a degree. After this point the dynamic viscosity increased dramatically for both Sp350 and Ch350 samples, at an average rate of 70% and 43%·day^{-1}, respectively (Fig. 2f). Although it is difficult to make a direct comparison with literature due to the lack of information available on the stability of microalgal biocrude, Spirulina biocrude produced by Jena et al. showed a similarly rapid and linear increase in viscosity during the first 75 days of storage [12].

The effect of the storage material on the viscosity of the biocrude is shown in Fig. 3. In general, the presence of metals did not have a significant effect on the viscosity of the *C. vulgaris* biocrude during storage at 20 °C. Likewise, there was no corrosion damage to the steel plate and its weight remained unchanged. A slight increase in viscosity for the samples stored with metal was observed at the start of the test, and once again at the end of the 60 days storage period. This behavior can be attributed to the fact that metals can act as a catalyst, accelerating biocrude aging. The biocrudes showed a similar behavior to those observed in a study by Garcìa-Pèrez et al. examining the effect of stainless steel and copper on the rheological characteristics of biocrude obtained by pyrolysis of wood residues, during accelerated aging [39].

3.2. Thermogravimetric analysis

To assess the impact of storage on the biocrude properties, the crudes were also examined by TGA. The thermogravimetric curves (TG) of the biocrudes Ch300 and Ch350 stored at 35 °C for 15 days are shown in Fig. 4a and Fig. 4b. Thermogravimetric weight loss in biocrudes is commonly divided into three stages: (1) evaporation of volatile compounds between 20 and 200 °C, (2) decomposition of intermediate compounds between 200 and 480 °C, and (3) formation of solid residues at 535 °C [27,40,41].

For the crude Ch300, over 10% of the weight was lost prior to 200 °C for samples at the early stages of storage. These more volatile fractions were depleted slightly within the first 7 days of storage but disappeared entirely by day 9. This corresponds with the sharp increases in the viscosity. Over the entire storage period, the volatility of the sample increased slightly, suggesting that the volatiles react with the alternative components and the molecular weight of the crude increases over the storage time. In the Ch350 sample, the loss of volatile compounds was lower, and as such, the viscosity increased substantially less. The residual mass fraction increased slightly for both biocrudes from 20% to 24%. The weight loss of the volatile fraction and the increase in the generation of solid residue in the biocrude during storage show the degree of aging of the biocrude, which can be attributed the volatile compounds, such as ketones, alcohols and aldehydes, phenol, furfural and their derivatives reacting to obtain higher molecular weight compounds [19,21,38].

Samples Ch300 and Ch350 stored at 4 °C and 20 °C showed similar behavior to that described above. At 20 °C, the solid residue increased by 3% and 2% for Ch300 and Ch350 samples, respectively. The level of volatile compounds decreased by 51% in the Ch300 sample during 12 days of storage, whereas the volatile fraction remained unchanged in Ch350 (Fig. 4c and d). At 4 °C, the volatile fraction and the solid residue in the Ch350 sample remained unchanged during 15 days of storage. The solid residue content of the Ch300 sample remained constant, and the volatile fraction only decreased by 25% (Fig. 4e and f). The TG curves for Sp350 stored at 4, 20 and 35 °C are given in the supporting information (Fig. S3); where the volatile and solid fractions had changes like those shown in Ch350 during the first 15 days of storage.

3.3. Elemental composition and HHV

Generally, heteroatoms such as bound oxygen and nitrogen are responsible for the instability of biocrudes during storage and transport, since most nitrogenated and oxygenated compounds are comparatively more reactive [21]. Some studies have shown that a reduction in oxygen content makes the biocrude more stable during storage, in addition to improving other properties, such as viscosity and HHV [42,43].

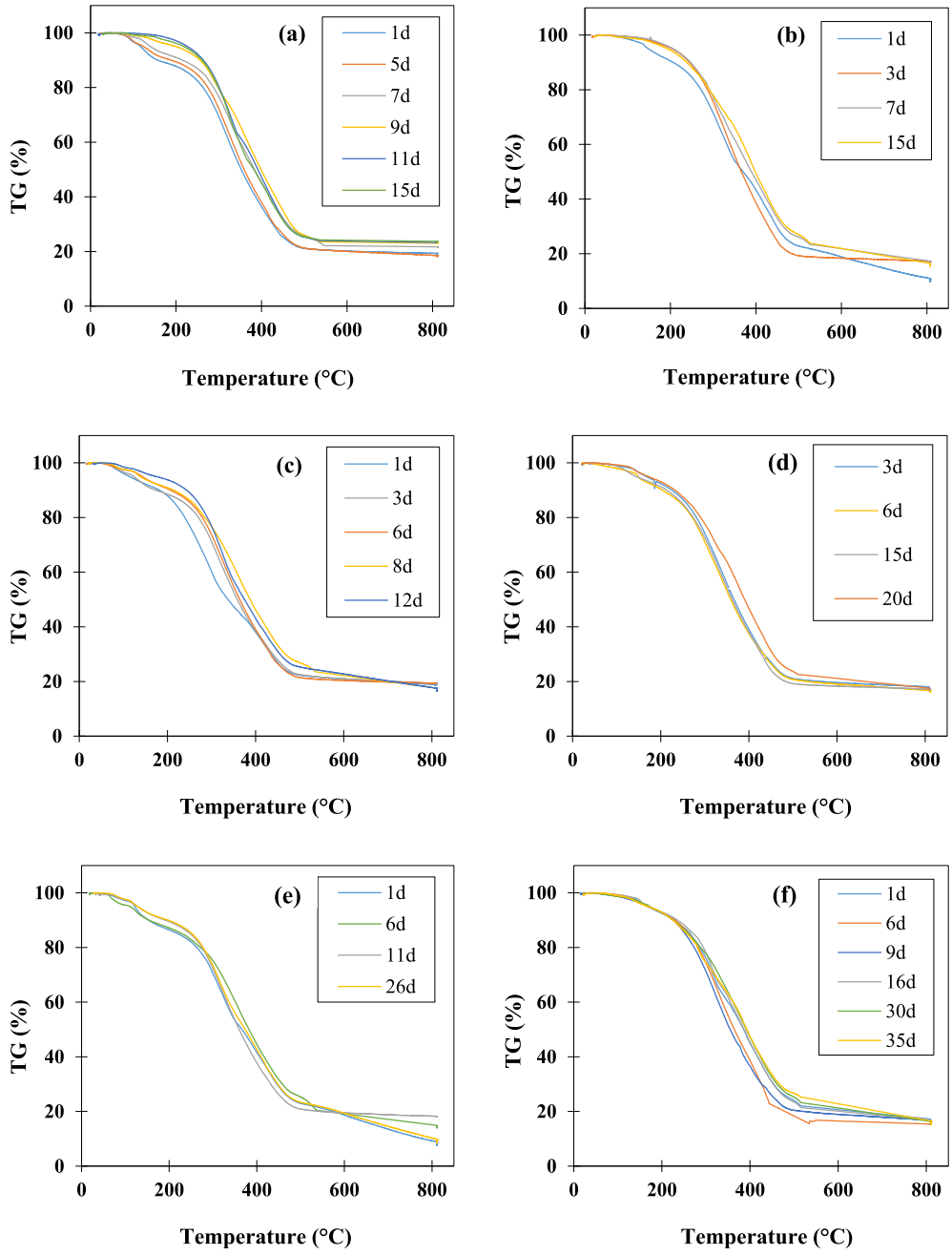

Fig. 4. Thermogravimetric analysis (TGA) of *C. vulgaris* biocrude stored for several days. (a) Ch300 stored at 35 °C, (b) Ch350 stored at 35 °C, (c) Ch300 stored at 20 °C, (d) Ch350 stored at 20 °C, (e) Ch300 stored at 4 °C, and (f) Ch350 stored at 4 °C.

Changes in elemental composition during storage of the microalgal biocrudes are shown in Table 3. In general, there was a slight increase in carbon content with storage time and temperature, as well as a small reduction in oxygen content in the biocrude. This suggests that condensation reactions are occurring and removing oxygen. The nitrogen and hydrogen content in the biocrude remained relatively unchanged during storage. Although the elemental composition remained relatively stable with time and storage temperature, the structure of the compounds changed, evidenced by the increase in the viscosity of the biocrude (Section 3.4).

These results are comparable with lignocellulosic bio-oils, where the elemental composition of the bio-oils remained approximately unchanged during the first 2 months of storage [18,20,44]. However, Jo et al. reported that the oxygen content of biocrudes decreased significantly from 42% to 27%, and the carbon content increased from 42% to 63%, when the storage time was greater than 6 months [43]. This reduction of the oxygen content in biocrude can be attributed to condensation and esterification reactions that eliminate oxygen in the form of water, and the loss of volatile compounds by evaporation [21].

HHV of the microalgal biocrudes before and after storage is shown in Fig. 5. The HHV of the fresh biocrude samples before commencing the stability test was (in descending order) Ch350 > Ch300 > Sp350 > Sp300; increasing the HTL processing temperature favored the deoxygenation and denitrogenation of

Table 3
Change in elemental composition during storage (0 and 60 days) of microalgae biocrude.

Biomass	HTL temperature (°C)	Element	Composition (%)			
			Before storage	After storage		
				4 °C	20 °C	35 °C
Spirulina	300	C	67.2 ± 0.1	67.7 ± 0.1	68.6 ± 0.03	69.2 ± 0.03
		H	8.7 ± 0.03	8.8 ± 0.04	8.7 ± 0.03	8.6 ± 0.05
		O[a]	15.6 ± 0.03	15.0 ± 0.03	14.0 ± 0.03	14.0 ± 0.1
		N	8.4 ± 0.03	8.5 ± 0.03	8.7 ± 0.03	8.1 ± 0.03
	350	C	68.2 ± 0.05	69.4 ± 0.1	69.0 ± 0.2	70.0 ± 0.1
		H	8.7 ± 0.05	8.9 ± 0.04	8.4 ± 0.1	8.9 ± 0.04
		O[a]	16.1 ± 0.03	14.4 ± 0.04	15.5 ± 0.2	14.0 ± 0.03
		N	7.0 ± 0.04	7.4 ± 0.03	7.0 ± 0.03	7.2 ± 0.03
C. vulgaris	300	C	68.8 ± 0.1	66.9 ± 0.2	67.8 ± 0.1	67.5 ± 0.03
		H	8.9 ± 0.03	8.6 ± 0.1	8.7 ± 0.03	8.7 ± 0.1
		O[a]	15.1 ± 0.1	17.2 ± 0.3	15.7 ± 0.1	16.6 ± 0.03
		N	7.2 ± 0.1	7.4 ± 0.03	7.9 ± 0.03	7.3 ± 0.03
	350	C	70.8 ± 0.1	72.3 ± 0.2	72.8 ± 0.03	73.3 ± 0.03
		H	9.0 ± 0.03	9.3 ± 0.03	9.1 ± 0.03	9.1 ± 0.2
		O[a]	13.6 ± 0.03	11.7 ± 0.2	11.5 ± 0.03	10.8 ± 0.03
		N	6.6 ± 0.05	6.6 ± 0.03	6.7 ± 0.03	6.8 ± 0.03

[a] By difference.

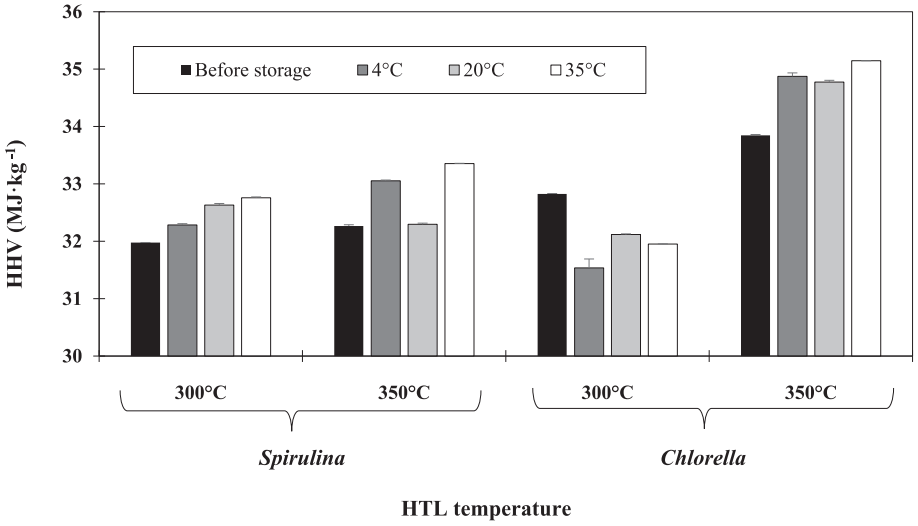

Fig. 5. Change in HHV of microalgae biocrude before and after 60 days of storage.

Table 4
Elemental composition (%) and HHV (MJ kg^{-1}) of biocrude from *C. vulgaris* stored with and without metal.

HTL temperature (°C)	Characteristic	Before storage	After storage at 20 °C	
			Without metal	With metal
300	C	68.8 ± 0.1	67.8 ± 0.1	69.5 ± 0.1
	H	8.9 ± 0.03	8.7 ± 0.03	8.7 ± 0.04
	O*	15.1 ± 0.1	15.7 ± 0.1	14.7 ± 0.04
	N	7.2 ± 0.1	7.9 ± 0.03	7.1 ± 0.03
	HHV	32.8 ± 0.03	32.1 ± 0.05	32.9 ± 0.03
350	C	70.8 ± 0.1	72.8 ± 0.03	69.2 ± 0.2
	H	9.0 ± 0.03	9.1 ± 0.03	8.5 ± 0.03
	O*	13.6 ± 0.03	11.5 ± 0.03	15.3 ± 0.03
	N	6.6 ± 0.05	6.7 ± 0.03	6.9 ± 0.03
	HHV	33.8 ± 0.03	34.8 ± 0.05	32.5 ± 0.1

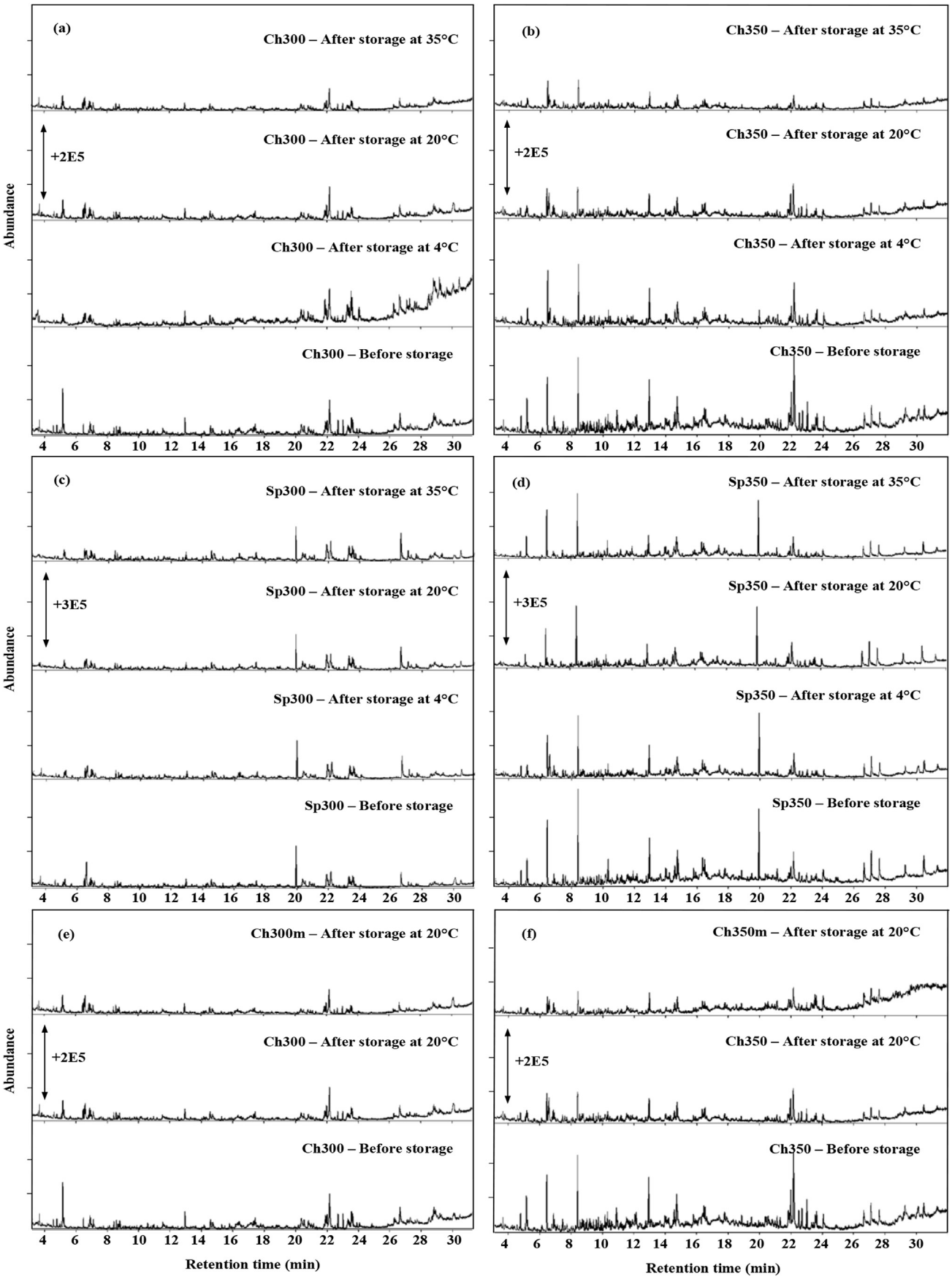

Fig. 6. Chromatograms from GC–MS of biocrude of microalgae before and after 60 days of storage. (a) Ch300. (b) Ch350. (c) Sp300. (d) Sp350. (e) Ch300 and Ch300m. (f) Ch350 and Ch350m.

biocrudes (Table 3). Comparing the HHV values before and after storage, HHV of Sp300 increased by 1%, 2% and 2.4% when the sample was stored at 4 °C, 20 °C and 35 °C, respectively. Similarly, for Sp350 and Ch350, small but positive changes in HHV (0.1–4%) were observed when storage temperature was increased. However, the opposite behavior was observed in the sample Ch300: HHV decreased with increasing storage temperature.

These small changes in the energy content of the biocrudes during storage are congruent with the changes in elemental composition, particularly the oxygen content. In general, HHV fluctuated by ±4% with respect to samples at the beginning of the aging period, demonstrating that HHV remains relatively stable over 2 months of storage. Since, the storage of the biocrude in the industry will occur in tanks built mainly of steel, it was possible to demonstrate that the presence of metals did not significantly affect the elemental composition and HHV of the Ch300m sample stored at 20 °C. Only a small decrease (4%) in the HHV of the Ch350m sample was observed (Table 4). This suggests that storage in glass lined vessels will potentially not be necessary.

3.4. Chemical composition

The results of the GC-MS analysis for the samples Ch300 and Ch350 before and after storage are shown in Fig. 6a and Fig. 6b. GC-MS was used as a qualitative tool to demonstrate the changes in the main volatile components of the biocrude during storage. It is evident from the chromatograms of the two samples that HTL temperature had an impact on the chemical composition of the biocrude. The Ch350 sample contained a greater variety of volatile compounds than the Ch300 sample (Fig. 6a and b). This could be the reason for the significant difference between the viscosity of the two samples.

In both samples, a decrease in the overall number and area of the main peaks is observed with increasing storage temperature, which could be attributed to the formation of other compounds of higher molecular weight (polymerization) [19,20]. The peaks corresponding to oxygenated and nitrogenated compounds such as phenol, pyrazine, pyrrole, pyrimidine and indole and their derivatives (5–15 min), were significantly reduced with increasing storage temperature, especially in the Ch350 sample stored at 35 °C. (Fig. 6b). Likewise, a reduction was observed in the peaks detected between 21 and 23 min corresponding to fatty acid derivatives, such as esters, as well as some unsaturated hydrocarbons and amides. In general, increasing storage temperature caused the loss of many light components, corresponding to retention times between 5 and 30 min.

In the Ch350 biocrude, a significant reduction in volatile compounds was observed when the sample was stored at 20 °C and 35 °C, compared to sample stored at 4 °C, where only a slight variation of these compounds is observed, suggesting that cooling the biocrude can delay the polymerization reactions. The decrease in the level of compounds such as phenol (6.5 min), 4-methylphenol (8.4 min) and indole (13 min) indicate that they may be involved in polymerization reactions that can occur at temperatures as low as 20 °C [45].

These results suggest that aging of the microalgal biocrude could be due to the presence of phenolic, nitrogenated and unsaturated compounds, which continue to react during storage until they reach equilibrium [43,44]. Unlike biocrude obtained from lignocellulosic biomass, the biocrude produced from microalgae contains up to 8 times more nitrogen, so that other reactions involving nitrogenous species could occur, such as the polymerization of indoles [46]. These reactions involving nitrogenous species could be the reason that the increase in viscosity was 5 times faster, on average, in biocrude obtained from microalgae than bio-oil obtained by pyrolysis of lignocellulosic biomass [17,44,47].

Results obtained by GC-MS were consistent with the changes evidenced by macroscopic properties, such as viscosity. However, although the larger molecules generated by polymerization reactions in the biocrude were expected to appear in the chromatograms at higher retention times, these expected new peaks were not observed, as the compounds were probability too large or insufficiently volatile to be detected by GC-MS. Similar results were observed for the Sp300 and Sp350 samples (Fig. 6c and d). The presence of steel during biocrude storage did not seem to affect the chemical composition of the biocrude. (Fig. 6e and f).

4. Conclusions

HTL processing temperature and biomass type were determining factors in the initial physicochemical characteristics of the biocrude. According to the type of biomass, HTL temperature and storage temperature, the stability of the biocrude was *Chlorella* > *Spirulina*, 350 °C > 300 °C and 4 °C > 20 °C > 35 °C, respectively. The viscosity of the Ch350 and Sp350 biocrudes stored at 4 °C did not show any significant change throughout the 60 day storage period. However, biocrude viscosity increased considerably when stored at 35 °C, especially for the Ch300 biocrude. The increase in the viscosity of the biocrude with storage time and temperature can be attributed to the loss of volatile compounds and the increase of the residual fraction, as demonstrated by TGA, suggesting that polymerization and esterification reactions were occurring between the biocrude compounds. The presence of stainless-steel strips did not affect the aging of the biocrude during storage at 20 °C for two months, suggesting that bio-crudes could be stored in steel vessels. The elemental composition and HHV of the biocrude changed slightly over the storage period for all storage temperatures. GC-MS analysis revealed that storage temperature had a significant influence on the reduction of volatile compounds in the biocrudes. Utimately, biocrude upgrading is necessary to improve its long-term stability and displace traditional fossil-based fuels. However, this work demonstrates that, with careful handling, algal biocrudes are stable enough for storage and transportation and could be shipped from point of production to a traditional refinery or biorefinery for further upgrading.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.renene.2019.07.084.

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