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Thermogravimetric Characteristic of *Spirulina platensis* for Production of Bio-oil by using Subcritical Hydrothermal Liquefaction

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Non-food type of feedstock to formed bio-oil through thermal reaction has attracted significant research attention. Spirulina Platensis can be converted into bio-oil via thermal process (pyrolysis, hydrothermal, hydrogenation) where the main challenges faces are to determine the potential product that will be upgraded to form biofuel. Microalgae become attractive feed for production of biofuel that give advantages in scale up for industrial purposed. The objective of this study is to determine the characteristic of bio-oil obtained by subcritical hydrothermal liquefaction including proximate, ultimate analysis, thermal degradation behaviour as well as analysis of liquid products by FTIR and GC-MS instrument. Raw Spirulina contain 45.32 % carbon and 8.08% hydrogen element with 93.10% of total solid and 83.62% of volatile matter considered potential to convert become bio-oil. Thermal gravimetric analysis exposed that the major mass change occurs at temperature between 200 °C to 350 °C. Hence subcritical hydrothermal liquefaction was conducted at temperature 350 °C in presence of air at the retention time 15 min by using 100 mL stainless steel reactor. Result of bio-oil shows that subcritical hydrothermal liquefaction able to hydrolyzes the glucose become small unit of sugar due to detection of functional group carboxylic acid, alkane and alcohols.

Keywords: Spirulina, bio-oil, thermal degradation, hydrothermal, glucose

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Finite stocks of fossil fuel worldwide become critical issue because this fuel is non-renewable and needed as main energy source [1]. Moreover, the fluctuation of fossil fuel price as well as high demand for this fuel (expected 34.90 billion barrels of fuel in 2030) caused decreasing of fossil fuel sources [2]. In addition, the fossil fuels that are taken out from under the earth's surface create many geothermal disturbances and negative impact on environment [3].

The use of alternative renewable energy Spirulina Platensis based is a brilliant idea to extract energy at low cost while avoiding the negative impact on environment [4,5]. Spirulina platensis is normally known as plankton and it is multicellular photosynthetic, with filamentous and spiral shaped cell character. This blue-green microalgae detected in alkaline saline water environment with pH 11 and survived in various types of habitats such as soils, seawaters, seabeds, thermal springs, including domestic and industrial water waste. Some advantages in utilizing Spirulina microalgae as feedstock: 1) mitigate CO2 which to an extent reduces global warming, 2) higher yields than other plant oil feedstock, 3) Sox and NOx trimmer, 4) biodiversity prospects, 5) easy growth and can be cultured during the year [6].

Basically, there are variety conversion technologies that have been used to convert microalgae into valuable bio-oils. The conversion technologies can be categorized into chemical conversion (condensation reaction and transesterification), thermochemical conversion (gasification, hydrothermal, pyrolysis and direct combustion) and biochemical conversion (anaerobic reaction, bio fermentation and interesterification) [7-8]. Previous study claimed that subcritical hydrothermal liquefaction (HTL) is environmentally friendly technology applied for the conversion of spirulina into bio-oil without prior drying [9-11], high conversion efficiency and low operation temperature compared to other thermal methods [2]. Previous study conducted HTL by using different categories of biomass as feedstock such as forestry and agricultures waste, animal manures, domestic and sewage waste [1]. Note that during HTL, degradation process occurred when biomass that consist of lipids, proteins and carbohydrates compounds involved in chemical mechanism starting with hydrolysis, repolymerization, dehydration, decarboxylation, and then deamination to form energy-dense bio-oil and biochar (an aqueous product rich in organic C (45% of initial feed)) [12]. The heterogeneity of biomass causes difficulties in energy recovery technologies by anaerobic digestion and combustion [2].

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Reviews on liquid product obtained by hydrothermal by previous researchers as follow: 45-67% of bio-oil by lipids of Chlamydomonas reinhardtii [12]; 36 - 42 wt.% biocrude by Spirulina sp. and 34 - 58 wt.% biocrude by Tetraselmis sp. [13]; 62 wt % bio crude oil by Nannochloropsis gaditana and 60 wt.% biocrude oil by Scenedesmus almeriensis [4] ; 97.8% of the initial weight solid fuel by sewage sludge [11]; 28.4 wt.% bio-oil by macroalgae Enteromorpha prolifera [14]; 4.8 wt.% bio-oil by cellulose [15]; 2.4 % alkane (carbon mol % of input cellulose) [16]; 38.49 wt% of bio-oil by cattle manure [1]. The focus of previous study only on; amount of bio-oil [6,7, 17] produced, parameters that effect in bio-oil production [18-21], proximate and ultimate analysis [1, 22], types of catalyst used in the process [23-24], but not in thermal degradation behavior (mass change) and study on characteristic of bio-oil products by focus on chemical compound form by subcritical hydrothermal of Spirulina. Hence in this paper, the subcritical hydrothermal liquefaction of organic solid wastes is conducted where the bio-oil obtained was characterized in terms of elemental composition, proximate analysis, higher heating value, thermal degradation analysis, Fourier transform infrared spectroscopy (FTIR) and gas chromatography–mass spectrum (GC–MS) analysis. Current research results able to indicate the potential of microalgae to become feedstock for production of biofuel.

EXPERIMENTAL

Material

Chemicals and materials used in this study were Spirulina platensis powder (0.1–0.5 mm; Vigorous Technology, China), sodium hydroxide (NaOH) solution (0.5 mol), propanol, helium gas, distilled water, and Whatman No. 1 filter paper.

Method

Ultimate and Proximate Analysis

Spirulina Plantesis was obtained from Vigorous Technology, China in powder form (0.1 to 0.5 mm). About 5 g of *Spirulina* sample was heated at 70 °C for 1 hour before the ultimate and proximate analysis were conducted. Study on physical characteristics of *Spirulina* was conducted through the proximate analysis that covered analysis of moisture, total solid, volatile content, ash and fix carbon based on ASTM E949-88, E897-88, ASTM E830-87 and ASTM D5681-98a (2004). Meanwhile, ultimate analysis was conducted by instrument CHNS analyzer (LECO model CHNS-932) located in Faculty of Science Technology National University of Malaysia. Each elemental composition (carbon, hydrogen, nitrogen, sulphur and oxygen) of *Spirulina* determined to predict the high heating value (HHV) of the Spirulina sample. Value of HHV of bio-oil was calculated by equation 1[1]:

$$\text{HHV (KJ/kg)} = 3.55\,(C^2) - 232\,(C) - 2230\,(H) + 51.2\,(C) \times H + 131(N) + 20600 \qquad \text{[Eq. 1]}$$

in which C, H and O are the weight percentage of carbon, hydrogen, and oxygen in the bio-oil, respectively.

Thermal Gravimetric Analysis (TGA)

Instrument thermal gravimetric is used to investigate the weight loss of Spirulina under the temperature programmed condition in a controlled atmosphere. Study on thermal degradation was conducted via TGA instrument (Netzsch STA 449 F3 Jupiter) located in Department of Chemical Engineering National University of Malaysia. Pyrolysis of 1 g of Spirulina sample was conducted at room temperature up to 700 °C in presence of helium gas flowing at the rate of 250 ml/min. The effect of mass change as temperature increased was determined at the heating rate of 10 °C/min.

Subcritical Hydrothermal Liquefaction

Subcritical hydrothermal liquefaction of *Spirulina* was carried out in a 100 mL stainless steel reactor. Temperature and pressure limits of this reactor were 500 °C and 5000 psi, respectively. In each test, 5 g of sample mixed with 60 mL of 0.5 mol of NaOH solution was placed in the 100 mL reactor. Excess NaOH solution used so that the solid sample will fully submerge during the experiment followed subcritical water condition. After the sample was filled in the reactor was closed and placed inside furnace and operation condition was set for 350 °C at 15 minutes retention time. The reactor was cooled at room temperature for 3 hours. This experiment was conducted manually and the focus of this study is only on bio-oil products. The bio-oil was separated from the aqueous product by following the procedure as follow Yin et al. (2010) [1]: 1) solid residues in the aqueous product were removed by using Whatman No. 1 filter paper. 2) Then the liquid product was extracted by propanol with the volume ratio of 1 to 1 for 1 h then propanol extracted was removed together with water by evaporating in oven at 80 °C for 24 hours.

Fourier Transform Infrared (FTIR) Spectrometry Analysis

Fourier transform infrared (FTIR) spectrometry analysis was performed to determine the atomic bonding on the products surface after subcritical hydrothermal was conducted over a range of 400–4000 cm^{-1}. The FTIR instrument used is Spectrum One model, Perkin Elmer from Faculty of Chemical University of Technology Mara Malaysia.

Gas Chromatography Mass Spectrometer (GC-MS) Analysis

Gas Chromatography Mass spectrometer instrument is used for qualitative analysis of bio-oils where the presence of specific chemical composition in the biomass is detected using Agilent 7890A gas chromatograph (GC) directly coupled to the mass spectrometer system (MS) of an Agilent 5975C inert MSD with triple-axis detector. Type of DB-5MS-UI column (30 m x 250 mm x 0.25) is used with 5% phenyl methylpolysiloxane stationary phase. The MSD Chemstation was used to find all the peaks in the raw GC chromatogram. A library search was carried out for all the peaks using the National Institute of Standards and Technology NIST/EPA/NIH version 2.0, and the results were combined in a single peak table. This instrument is located at Centre for Research and Instrumentation Management (CRIM), the National University of Malaysia.

RESULTS AND DISCUSSION

Proximate and Ultimate Analysis

Proximate analysis of raw data of Spirulina is presented in Table 1 where the total solid for all samples is high around 93.10% with low moisture content (6.89%) that typically <50% illustrated the suitable biomass feedstock for thermal conversion to bio-fuel [25-29]. The reason to measure the ash content because to determine the value of incombustible solid after hydrothermal process [10]. Spirulina contains high value of ash (7.80%) as the ash contains mineral and inorganic matter such as calcium, potassium, magnesium and phosphorus that can be used as nutrients for other processes [8, 10].

The percentage of fixed carbon (remaining mass of carbon) can be determined using the data moisture, ash and volatile matter obtained in the proximate analysis. The important of the VM and FC contents is that they provide a measure of microalgae ignition level subsequently gasified, or oxidised, related on microalgae to energy goal [29]. High values of volatile matter (83.62%) indicate the potential amount of solid transformed into other products either in liquid or gas phase. Approximately 1.69 % of fixed carbon is calculated from each TS, VS, ash and moisture content as listed in Table 1.

Ultimate analytical results for all the samples are shown in Table 1 important to make comparisons of element carbon, hydrogen, oxygen and nitrogen before (raw data) and after (bio-oil and biochar) hydrothermal process was conducted. Raw Spirulina contain high value of carbon (45.32%) with hydrogen value (8.08%). The high value of C and H contribute to high value of C:H indicates the high amount of both elements' presence in the biomass that possibly contain hydrocarbon compounds. HHV could be defined as the total energy content released when the fuel is burnt in air (together with latent heat contained in the water vapour) and hence gave the maximum amount of energy potentially recoverable from a given biomass source [29]. Raw Spirulina contains maximum HHV 19.45 KJ/kg. After hydrothermal process, value of C, H, N and O element for bio-oil were totally difference. High viscosity of bio-oil produced contain low value of carbon and hydrogen but high value of nitrogen, sulphur and oxygen than raw biomass required pre-treatment and upgrading of this bio-oil [2,23] in order to reduce the value of oxygen value so that this bio-oil qualified to be use as fuel.

Table 1. Proximate and Ultimate analysis.

Proximate Analysis	**Percentage (%)**	**Ultimate Analysis**	**Percentage (%)**	**Ultimate Analysis**	**Percentage (%)**
		Raw		**Bio-oil**	
Moisture	6.89	C	45.32	C	43.22
Total solid	93.10	H	8.08	H	7.32
Ash	7.80	N	10.22	N	9.84
Volatile matter	83.62	S	0.74	S	0.80
Fix carbon	1.69	O	35.64	O	38.83
		HHV (KJ/kg)	19.45	HHV (KJ/kg)	18.37

Hydrothermal of biomass to produce bio-oil occur by following steps: 1) hydrolysis of cellulose, hemicelluloses and lignin into glucose and fructose 2) degradation of cellulose and hemicelluloses become bio-oil 3) decomposition of bio-oil [10]. Yin et al. [1] state that minimum temperature 250 °C is required for hydrolysis to occur where 100% hemicellulose, 22% of cellulose and 60% of lignin were solubilized in hot water as monomeric sugar. The second step that formed bio-oil usually required temperature operation higher than 250 °C while at temperature 300 °C degradation of lignin occur [30]. It is suggested to choose temperature above than 300 °C to obtain maximum bio-oil yield due to lignin depolymerisation (bio-oil generation) usually followed by repolymerisation reactions (self-condensation) [4]. Temperature operating 350 °C allows the pressure inside autoclaves to rise to high levels, which enables specific structural transformations, such as converting crystalline cellulose into amorphous cellulose. Therefore, in this study, the operation temperature of 350 °C was chosen and able to obtain maximum 42.04 % of bio-oil considered high compared to previous study.

Thermal Degradation Analysis

It was observed from TGA results in Figure 1 that thermal decomposition of Spirulina occurred over the temperature range 150-450°C. The Spirulina was pyrolyzed as the temperature increased, which can be divided into three stages where the first stage at temperatures up to 150 °C corresponded to dehydration. Biomasses that contain high moisture content of biomass caused higher mass loss in this region [31-34]. Degradation of hemicelluloses and lignin occur at temperature 150 to 450°C called devolatilization stage followed with decomposition of carbohydrates [35-36]. Mass loss of all biomasses was increased drastically during this phase caused of liberation of volatile hydrocarbon from decomposition of hemicelluloses, cellulose and some part of lignin [36-37]. About 31.78% of mass loss was reported in Figure 1, which illustrated that most biomass was volatile into other compounds at this phase. Most lignin decomposition occurs at temperatures up to 450°C where proteins were decomposed over a wider temperature in between cellulose and hemicelluloses [38-39]. Previous study claimed that after operating temperature 650°C, the degradation rates are negligible because most volatiles had already been pyrolyzed [40-41]. Therefore, at this phase only 0.98 % to 8.40 % of the mass were considered less than mass loss during 150°C to 450°C. In general, biomass that contain high value of cellulose and hemicellulose will produce more volatiles compared to woody biomasses [42].

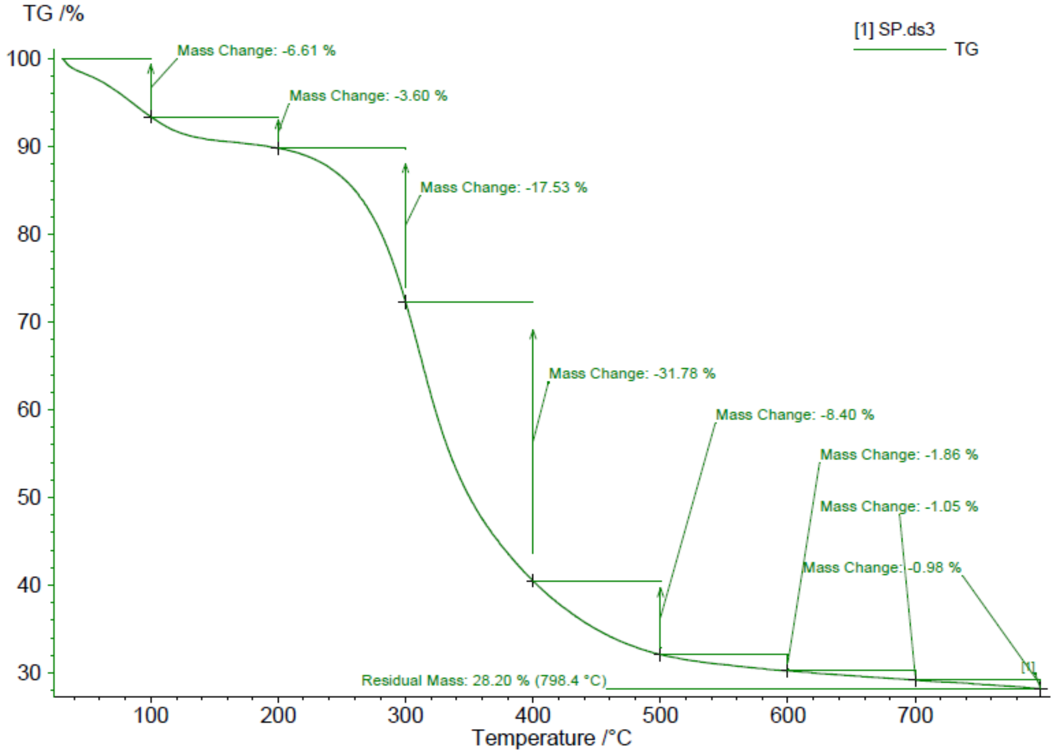

Figure 1. Percentage of mass change by Spirulina.

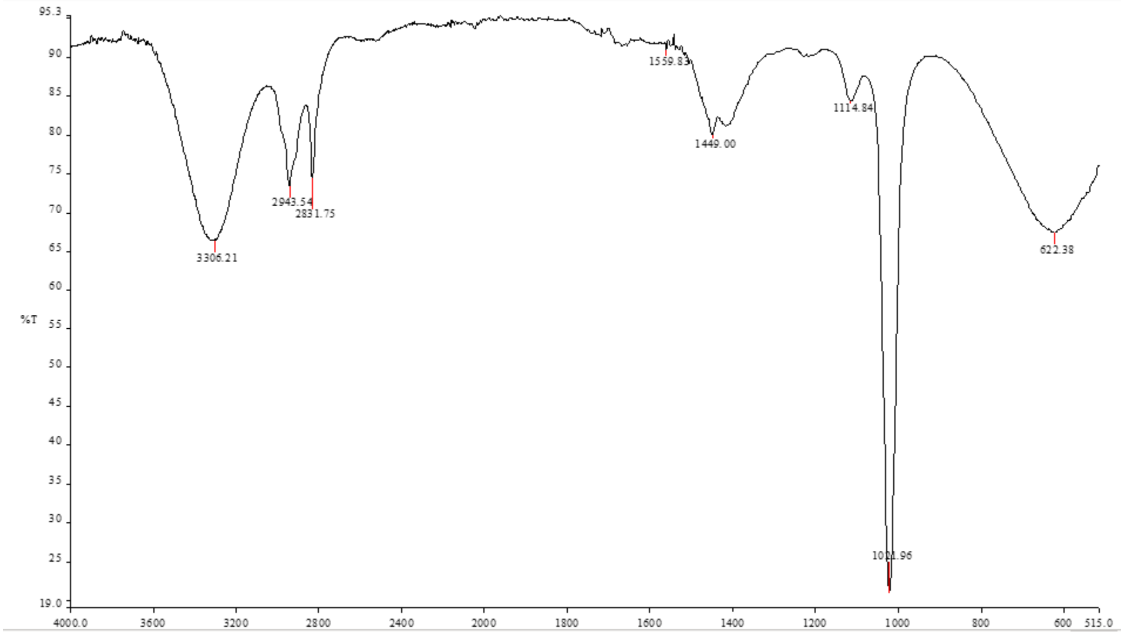

Figure 2. FTIR result of Spirulina.

Table 2. FTIR peak position for solid wastes.

Peak position (wave number) cm $^{-1}$ [33]	Functional group	Peak position (wave number) cm $^{-1}$
3300-3600	Alcohol OH	3306.21
2870-2960	Alkane C–H	2943.54 2831.75
1500 - 1600	Aromatic C=C	1559.83
1440-1305	Carboxylic acid	1449.00
1100	Secondary alcohol	1114.84

The present result of biomass volatile matter loss obtained are different compared to other previous study; Yang et al. [43] give range 46.1% of mass loss by empty fruit bunch palm (EFBP) while in this study about 26.95% of EFBP mass loss. Authors Alias et al. [41] obtained 60.71% of mass loss by rice husk and 80.43 % mass loss by coconut pulp. About 60-75% of mass loss by switchgrass, wheat straw and red cedar studied by authors Pasangulapati et al. 2012 [37]. The varieties of mass loss obtained may be due to difference size and heating rate of biomass. All the biomass thermal degradation study indicates that the maximum volatile hydrocarbon product can be obtained when the temperature condition for thermal process at between 250°C to 350°C. However, for present study the temperature 350°C suggested chosen for subcritical hydrothermal liquefaction process as degradation of lignin occurred from temperature more than 300°C.

Characterization of Bio-oil

Fourier Transforms Infrared (FTIR) Spectrometry Analysis

FTIR is a technique to determine functional groups in the raw material and bio-oil [33]. The representative FTIR spectrums for bio-oils obtained from the hydrothermal liquefaction of Spirulina is shown in Figure 2. The values of peak position for functional group are estimate according to reference in Table 4. According to FTIR analysis, bio-oil from hydrothermal of Spirulina contains alcohol, alkane, carboxylic acids and aromatic functional group. Note by Yin et al. [1], mostly absorbance brands of bio-oil were referred to poly alcohols (range 3600–3500 cm^{-1}), carboxylic acids (range 3000–2500, 1780 and 1440–1305 cm^{-1}), phenol derivatives (range 3610 and 1200 cm^{-1}), alpha, beta-unsaturated ketones (range1650–1600 cm^{-1}), and secondary alcohols (1100 cm^{-1}). Author Pereira et al. [44] summarized the band characteristic; 1) OH group present in lignin 2) C-H group (CH_2 and CH_3) are from cellulose, lignin and hemicellulose. 3) C=O present in hemicelluloses 4) C=C skeletal aromatics generally found in the lignin structure. In addition, the present of poly alcohol and phenol derivatives gave high viscosity of HTL bio-oil yield meanwhile the present of carboxylic acids cause corrosive HTL bio-oil formation [1].

Gas Chromatography Mass Spectrometry

Formation of all compounds detected by GC-MS can be explained as in Figure 3 and Table 3 where products such ester, furan and its derivatives formed illustrated this process able to degrade the glucose become small units of sugar. Through hydrothermal liquefaction hemicelluloses, cellulose and lignin are decomposed at temperature range 150 to 350°C [35-39]. The product (bio-oil) composition through subcritical hydrothermal liquefaction was affected by increasing reaction temperature and self-generated pressure caused in degradation of cellulose, hemicellulose and lignin.

Cellulose mainly hydrolyzes to glucose, then forms fructose via isomerization, which then undergoes dehydration and fragmentation reactions leading to the formation of various soluble products, such as hydroxymethylfurfural-related (5-HMF), 1,2,4-benzanetriol and aldehydes [45]. However, in this study 5-HMF was not detected may be due to unstable characteristic of 5-HMF compound that easily converted become acidic compounds [46]. Hemicellulose mainly hydrolyzes pentose and some oligomers of xylan and mannan, followed by dehydration to furfural compounds [47]. Small-molecules products, mostly carboxylic acid were obtained. After polymerization or condensation reactions induced by intermolecular dehydration or aldol condensation reactions between those intermediate products (furan and benzene derivatives) occurred, C=O and C=C bonds appear due to dehydration and the keto-enol tautomerization. The final products were condensed polyaromatic, and furfural structure [48] connected through condensation, polymerization, linear linking, decarbonylation with alkanes, hydroxyl and carboxyl groups at the end of side chains. Degradation of glucose formed alcohol and carboxylic acid proved that further transformation occurred with the solubilized compounds during the hydrothermal process [49]. Present result illustrated that the bio-oil mostly contain alcohol and carboxylic acids such as Hexadecanoic acid, Palmitoleic acid and Octadecanoic acid detected in Spirulina bio-oil. Acid compounds formed when the acetyl group that present in xylose was removed due to degradation of cellulose, hemicelluloce and lignin during hydrothermal of Spirulina [50].

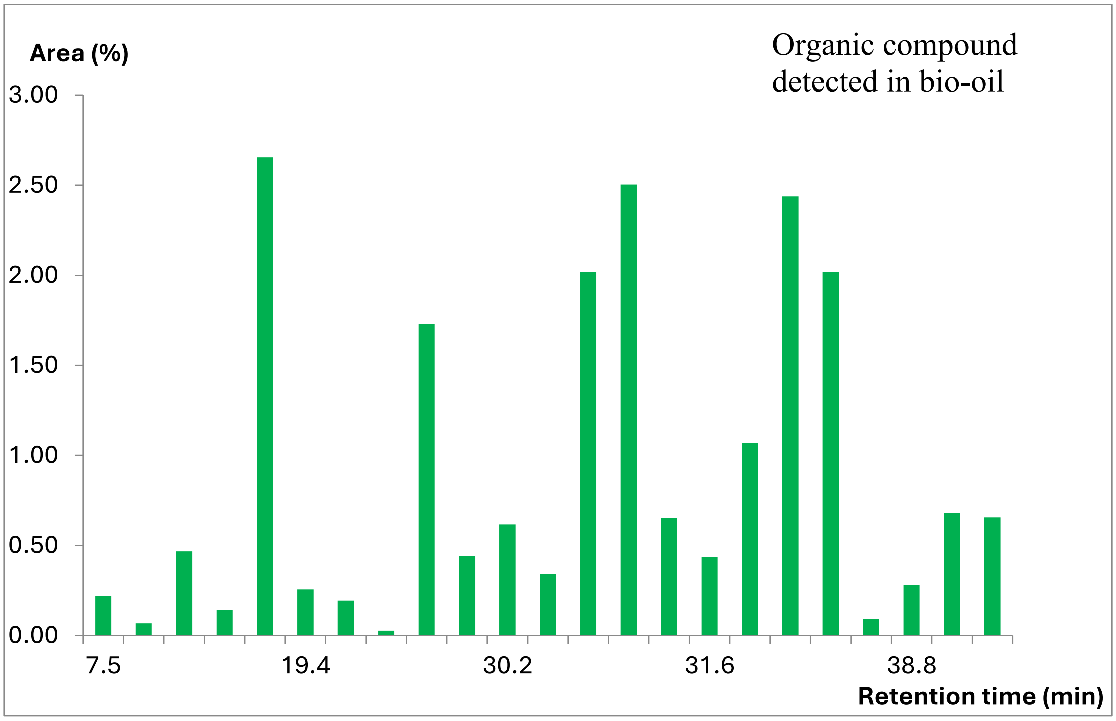

Figure 3. Chemical compounds detected in bio-oil by GCMS.

Table 3. Major component of bio-oils determined by GC-MS analysis.

Retention time (min)	Area (%)	Name of chemical compound
7.52	0.22	Dimethyl trisulfide
11.93	0.07	1-Piperidinecarboxaldehyde
13.58	0.47	2-Piperidinone
15.07	0.14	Caprolactam
17.83	2.65	Methyl ester
19.42	0.26	1-Dodecanol
20.64	0.19	Benzofuranone
21.80	0.03	2H-Pyran-2-one
27.77	1.73	Hexadecanoic acid
28.24	0.44	Palmitoleic acid
30.17	0.62	Linolenic acid
30.68	0.34	Phytol
31.02	2.02	Gamolenic Acid
31.22	2.50	Octadecadienoic acid
31.28	0.65	6-Octadecenoic acid
31.60	0.44	Octadecanoic acid
31.84	1.07	Hexadecanamide
32.86	2.44	2,5-Piperazinedione
34.30	2.02	Pyrrolo
37.22	0.09	Undecanoic acid
38.82	0.28	Ethanone
39.55	0.68	2,5-Piperazinedione
47.02	0.66	Propanoic acid

CONCLUSION

According to proximate, ultimate, thermal degradation, FTIR and GC-MS analysis proved that the subcritical hydrothermal liquefaction of Spirulina can be applied commercially to produce bio-oil and biofuel. Subcritical hydrothermal liquefaction of Spirulina decomposes lignin, hemicelluloses and cellulose to form valuable other organic compounds such as carboxylic acid alkane and alcohols. The experiment condition chooses (350 °C and 15 minutes) by study on thermal degradation of Spirulina (TGA) that achieved 31.78% of mass change. However, Spirulina based-bio-oil should be upgraded into biofuel for specific application such as for biodiesel and bio-gasoline. Besides further work is required (upgrading technology) to increase the product of bio-oil.

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