Separation and Analysis of Microwave-assisted Liquefied Products of Corn Stover

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Corn stover was successfully liquefied by microwave heating at 160 °C with ethylene glycol (EG) used as the solvent and sulfuric acid as a catalyst. Gas chromatography and mass spectrometry (GC-MS) data indicated that methyl esters, including 3-(2-methyl-1,3-doxolane-2-yl) propionic acid methyl ester (PAME), levulinic acid isopropyl ester (LAE), methyl laurate, and methyl palmitate were the major degradation compounds, in addition to EG derivatives in the liquefied product of corn stover (LPCS). For high value-added utilization of LPCS, solvent extraction was applied to characterize the components and to separate it into useful fractions. After being dispersed in water, the water-soluble fraction of the LPCS was then extracted with organic solvents, including hexane, chloroform, diethyl ether, and ethyl acetate. Levulinic acid isopropyl ester showed the highest distribution in chloroform and ethyl acetate, while the lowest in hexane and ether. Levulinic acid isopropyl ester was selectively enriched to 28.76% and 43.65% by sequential extraction with chloroform and ethyl acetate, respectively, in accordance with the quantitative analysis.

Keywords: Corn stover; Liquefaction; Microwave; Separation

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INTRODUCTION

Corn stover is abundant in areas of large amounts of corn production throughout the world. It is by far the largest agricultural crop, in terms of the amount of biomass, both in the U.S. (Mullen *et al.* 2010) and China (Tan *et al.* 2013). It is, in many ways, an ideal feedstock for biomass utilization. Recently, thermo-chemical conversion of biomass to liquid biofuel has received considerable attention. The thermo-chemical processes applied in producing fuel from biomass include pyrolysis, solvolysis/liquefaction, gasification, and biological conversion (Liu *et al.* 2014). Liquefaction of corn stover may provide an alternative to fossil fuels, as well as reduce the contamination of the environment and increase traditional farmers' incomes.

Many reports have focused on the polyhydric liquefaction of biomass with external heating (Yamada and Ono 1999; Lee *et al.* 2000; Ge *et al.* 2003; Zhang *et al.* 2012, 2013, 2014). Microwave technology is one of the major achievements of modern science and has been developed rapidly in recent years. It has been widely used in chemistry since the 1980s because of its many good qualities. Kržan and Kunaver (2006) used microwave heating to liquefy wood with ethyl glycerol (EG) as the solvent and maleic anhydride as the catalyst, with yields as high as 90% when using 500 W microwave heating for 20 min. With microwave heating, the product yield improved and the product was characterized to be different from traditional liquefaction product, according to previous research by the authors (Xiao *et al.* 2011; Li *et al.* 2013). The characteristics of the liquefied products play a key role in their industrial utilization; however, little attention has been given to the separation and characterization of liquefaction products.

Fourier transform infrared (FTIR) analyses of corn stalk after liquefaction, including the oil-dissolved fraction, organic-dissolved fraction, and residues, indicated that cellulose, hemicellulose, and lignin were decomposed and depolymerized into small compounds (Zhang *et al.* 2007). Liu *et al*. (2006) also revealed that ethylene glycol (EG), diethylene glycol, EG derivatives, saccharides, alcohols, aldehydes, ketones, and phenols were found in the liquefied products from bagasse treatment with ethylene glycol. Zhang *et al.* (2012) reported that the liquefied products from acid hydrolysis residues (AHR) in EG/glycerol included alcohols, organic acids, esters, alkanes, phenols, and their derivatives, as characterized by gas chromatography and mass spectrometry (GC-MS).

In brief, the liquefaction reaction system is very complex and the product can vary according to the reaction conditions employed. It is necessary to separate and refine the bio-oil prior to its use in high-value added applications. Solvent extraction is a useful method for the isolation and fractionation of bio-oil (Wang *et al.* 2014).

In this study, we attempt to characterize the microwave liquefied products of corn stover (LPCS) treated with EG and to find a suitable way to separate the products for further utilization. The dissolution of LPCS in different solvents was investigated, and GC-MS was used for the analysis of liquefied products.

EXPERIMENTAL

Materials

Corn stover was collected from the Shangzhuang Experiment Station, China Agricultural University. The stover was ground and sieved through a 40-mesh screen. The reagents used in this study were of chemical grade and purchased from Beijing Chemical Plant (China). Butyl levulinate (Sigma-Aldrich, USA) was used as a standard for the calibration of levulinic acid isopropyl ester (LAE) in GC analysis. Chemical composition of corn stover was determined according to the NREL lab procedure of Sluiter *et al.* (2011). The chemical constituents of corn stover are shown in Table 1.

Table 1. Chemical Composition of Corn Stover

Components	Cellulose	Hemicelluloses	Lignin	Moisture	Ash
Content (%)	31.54 ± 0.18	17.54 ± 1.16	18.40 ± 0.62	2.81 ± 0.07	5.82 ± 0.06

Data expressed as the mean ± standard deviation

Methods

Liquefaction procedure

Corn stover (5.0 g) and EG liquefaction solvent (25.0 g) were added to a 100-mL Teflon reaction vessel in a Milestone Microwave Labstation (Ethos Touch Control, Italy). Sulfuric acid (98%, 0.875 g) was added as the liquefaction catalyst, and the contents mixed with an ASM-400 magnetic stirrer. The microwave frequency used was 2450 MHz, and the sample temperature maintained at 160 °C. The liquefied products from corn stover were designated as LPCS. The liquefaction yield was defined in Eq. 1 as,

$$X = (1 - \frac{M_1}{M_0}) \times 100\% \tag{1}$$

where X is the liquefaction yield (%), M_1 is the weight of residue (g), and M_0 is the weight of corn stover (g).

Separation of liquefaction products

The LPCS was filtered by a Büchner funnel using 1,4-dioxane. The solvent was evaporated with a rotary evaporator (RV 10, IKA; Germany) to obtain the evaporated product (M_2). Two folds of water were added to the concentrate after cooling, the mixture was centrifuged at 8,950 ×g for 20 min, and the precipitate (M_3) was weighed. The centrifugal sedimentation rate was defined in Eq. 2 as,

$$Y = \frac{M_3}{M_2} \times 100\% \tag{2}$$

where Y is the centrifugal sedimentation rate (%), M_2 is the evaporated liquefied product (g), and M_3 is the sediment after centrifugation (g).

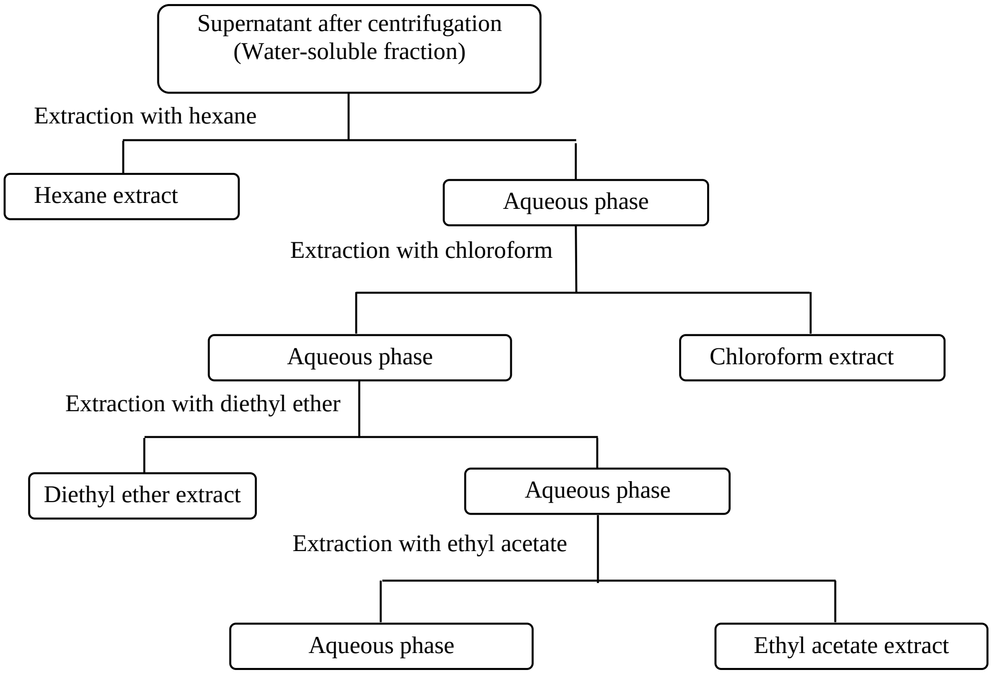

Fig. 1. Sequential extraction process for the supernatant of LPCS

The supernatant after centrifugal sedimentation was collected for further analysis. Organic solvents were used to extract the product to determine the properties of the liquefied product. Hexane, chloroform, diethyl ether, and ethyl acetate were used to extract different components. Two extraction routes were followed. One was a sequential extraction, as shown in Fig. 1, where the organic solvents (1:1, v/v) were used to extract the supernatant four times in the following order: hexane, chloroform, diethyl ether, and ethyl acetate. The other was to extract the supernatant with one organic solvent, designated as single solvent extraction. All non-aqueous phase extracts were evaporated to remove the organic solvents. The extraction rate is defined as,

$$Z = \frac{M_4}{M_2 \times (1 - Y)} \times 100\% \tag{3}$$

where Z is the extraction rate (%), M_4 is the extract after rotary evaporation (g), M_2 is the evaporated liquefied product (g), and Y is the centrifugal sedimentation rate (%).

Analysis of products using GC-MS

An Agilent 7890A/5975C GC-MS system (USA) was used for chemical identification in the liquefied products. Chromatographic resolution was achieved using a 30 m × 250 µm DB-5ms capillary column, which had a 0.25-µm film thickness (J & W Scientific; Folsom, CA). The inlet temperature was set at 280 °C. The oven was maintained at the initial temperature of 50 °C for 3 min, then heated to 180 °C at the rate of 6 °C/min, and finally ramped to 280 °C at 10 °C/min with a holding time of 3 min. Helium gas was used as the carrier gas at a flow rate of 1.0 mL/min. The injection volume was 1.0 µL, with a split ratio of 10:1. The ion source and quadrupole temperatures were 230 °C and 150 °C, respectively. Mass spectra were scanned from m/z 30 to 600 at a rate of 1.5 scans/s. The electron impact ionization energy was 70 eV.

RESULTS AND DISCUSSION

Liquefaction Yield of Corn Stover

Figure 2 shows that the microwave liquefaction yield of corn stover increased with reaction time. At 15 min, the liquefaction yield had reached 94%. Cellulose, hemicellulose, and lignin were decomposed and depolymerized into small compounds after liquefaction (Zhang *et al.* 2007).

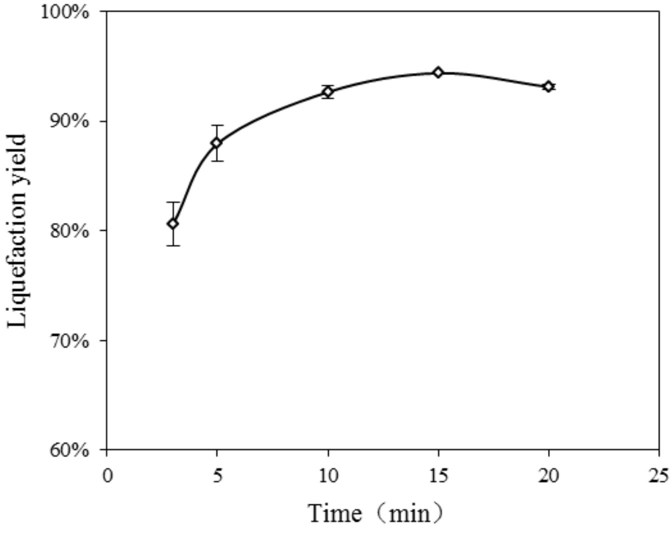

Fig. 2. Microwave liquefaction yield of corn stover in EG at 160 °C. Data provided as the mean ± SD

After 15 min, the liquefaction yield decreased slightly due to the condensation reactions of the liquefied products into insoluble substances. This trend suggests that the liquefaction process is a combination of the degradation of polymers and condensation reactions of the liquefaction products (Tan *et al.* 2012; Liu *et al.* 2014).

Separation of LPCS with Water

Solvent extraction was used to separate the constituents of the LPCS. Among the different extraction solvents, water is economical and efficient (Wang *et al*. 2014). Zhang *et al.* (2012) separated the liquefied products of acid hydrolysis residue (AHR) from glycerol into three fractions: a water-soluble part, an acetone-soluble part, and a residue. Fourier transform infrared (FTIR) analyses showed that the water-soluble fraction mostly contained EG, EG derivatives, and cellulose degradation products; the residue mostly contained lignin derivatives. Cellulose is a major constituent of corn stover (Table 1). Water was utilized to separate the chemical components of the liquefied product; however, the solubility of the various chemical components of LPCS varied as the products changed with time. As shown in Fig. 3, the centrifugal sedimentation rate increased as the liquefaction reaction time increased; that is, the amount of water-soluble substances decreased as the reaction time increased. At 15 min, the centrifugal sedimentation rate was almost up to 45% and remained constant up to 20 min. This could indicate the repolymerization of the degraded products *via* condensation reactions as the liquefaction time increased (Niu *et al*. 2011).

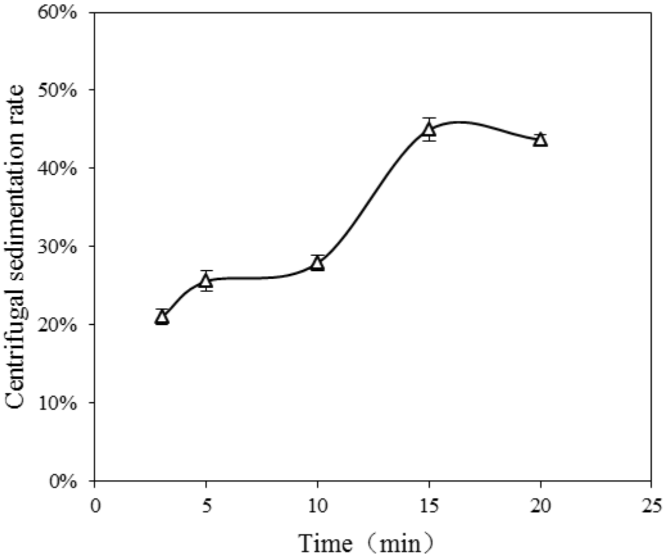

Fig. 3. Centrifugal sedimentation rates of LPCS

Extraction of Water-soluble Fraction with Organic Solvents

Sun *et al.* (2008) reported that bio-oil consists of organic acids, aldehydes, ketones, alcohols, phenols, ethers, and esters, and that bio-oil needs to be refined before it can qualify as a substitute for petroleum. For the better utilization of the microwave-liquefied products, solvent extraction was attempted in our study to separate the complex product mixture.

The water-soluble fraction of LPCS after liquefaction of 15 min at 160 °C was extracted with organic solvents in two different ways. The results in Fig. 4 show that as much as 53.19% of the liquefied product in the supernatant cannot be extracted by sequential organic solvents. Hexane, chloroform, diethyl ether, and ethyl acetate extracted, respectively, 1.78%, 23.69%, 6.81%, and 14.52% of the supernatant by sequential extraction, while as much as 2.92%, 29.97%, 11.58%, and 27.90%, respectively, of the mixture can be extracted by single-solvent extraction. The data indicate the highest solubility in organic solvents using single-solvent extraction when compared to sequential extraction.

Among the four kinds of organic solvents investigated, the water-soluble fraction of LPCS had the highest solubility in chloroform and ethyl acetate and the lowest solubility in hexane and diethyl ether (*via* both extraction methods). According to the similarity-intermiscibility theory, there was a considerable amount of polar components in the liquefaction products. Liu *et al.* (2006) also used organic solvents to extract liquefaction products with ethanol and water as liquefaction reagent in supercritical/subcritical conditions and reported that the oil-dissolved fraction had the highest solubility in chloroform. In this study, chloroform and ethyl acetate are shown to be efficient extraction solvents for the separation of the liquefied products.

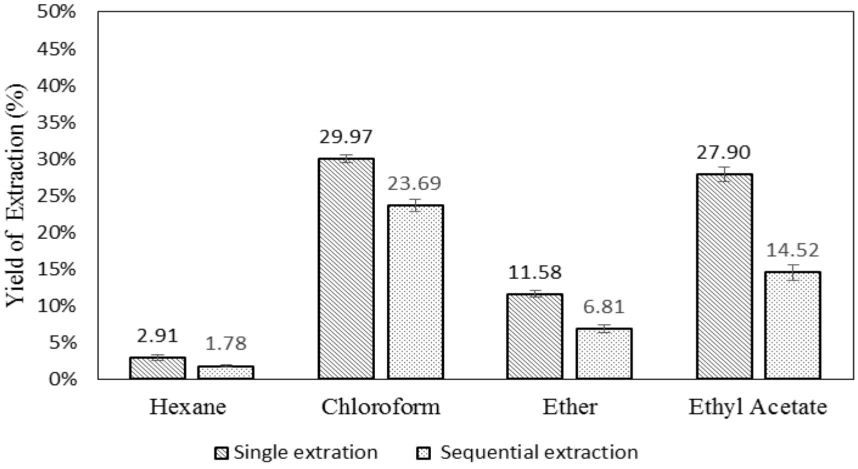

Fig. 4. Extraction yields by sequential extraction and single extraction. Data provided as the mean ± SD

Analysis of the Liquefaction Products by GC-MS

The GC-MS analysis was used to identify the chemical composition of different solvent extraction fractions from LPCS. The chromatograms of each sample are shown in Figs. 5a through 5f. The area under the curve percentages of each compound (defined by the percentage area for each peak in the chromatogram in relation to the total area) are shown in Table 2, listing the major compounds detected by GC-MS. The relative peak area % may be used to show the relative concentrations of the compounds in the sample.

As shown in Fig. 5a and Table 2, there are two types of major compounds in LPCS. One is EG and its derivatives, including diethylene glycol (DEG) and triethylene glycol (TEG). The other is methyl esters, including 3-(2-methyl-1,3-doxolane-2-yl) propionic acid methyl ester (PAME), levulinic acid isopropyl ester (LAE), methyl laurate, and methyl palmitate. Ethylene glycol and its derivatives dominated the liquefied products, as reported by Zhang *et al.* (2007) and Zhang *et al.* (2012). Compounds in the latter category were assumed to be the degradation products from cellulose, in which PAME was the dominant component. 3-(2-Methyl-1,3-dioxolan-2-yl) propanoic acid and methyl palmitate were also reported in the liquefied compounds from acid hydrolysis residue of corncob in polyhydric alcohols (Zhang *et al*. 2012).

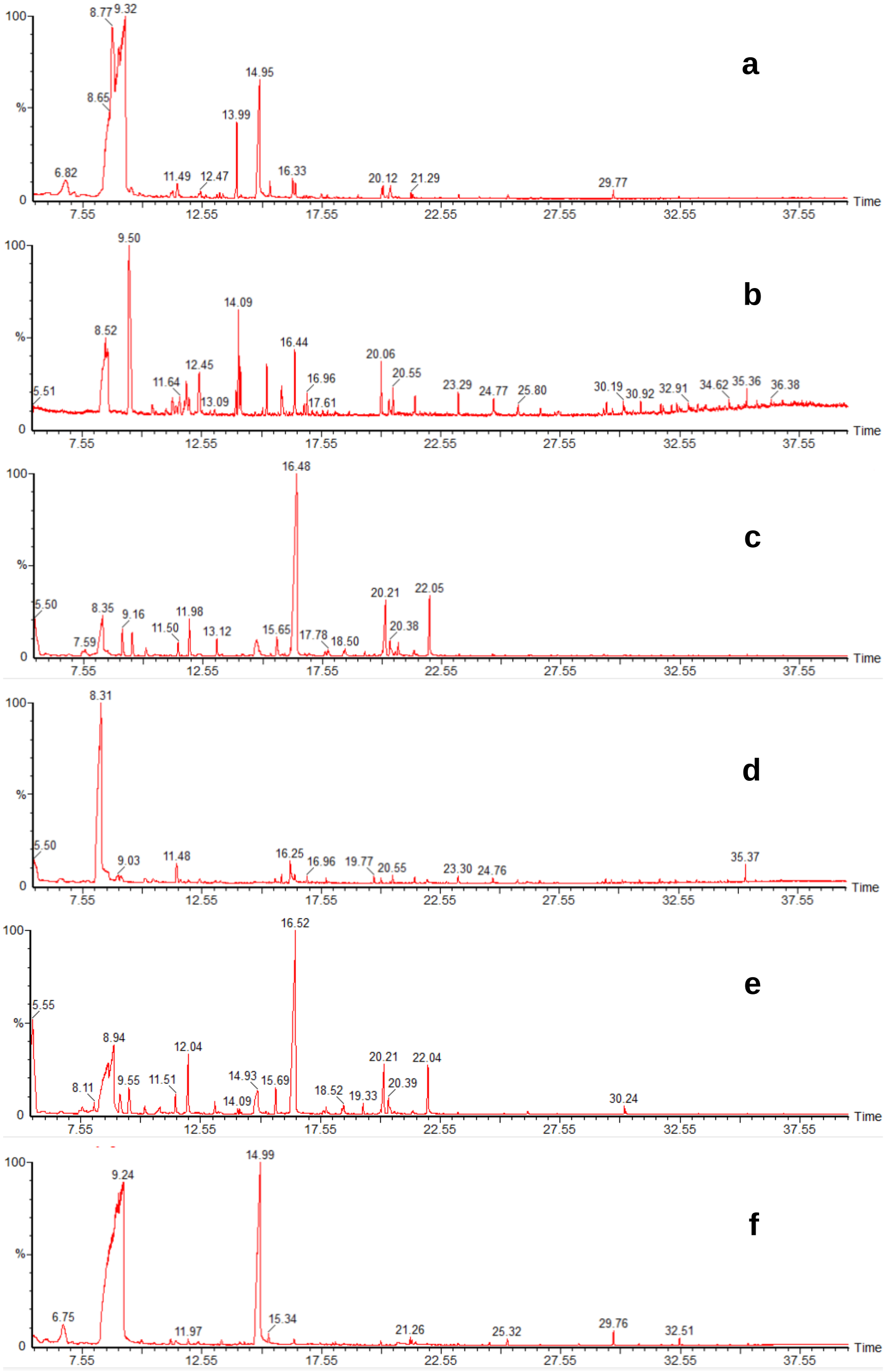

Fig. 5. GC-MS chromatograms of LPCS and its extraction fractions. (a) LPCS after sequential extraction with (b) hexane, (c) chloroform, (d) diethyl ether, and (e) ethyl acetate; (f) water phase after extraction

The GC-MS analysis of different extracts showed that the primary components after sequential extraction by hexane (Fig. 5b) and diethyl ether (Fig. 5d) were EG and its derivatives. Levulinic acid isopropyl ester was detected as the major extracted compound with a retention time of 16.29 min after sequential extraction of chloroform (Fig. 5c) and ethyl acetate (Fig. 5e). Significant amounts of the liquefied products remained in the water phase after solvent extraction (Fig. 5f). Chloroform and ethyl ester showed advantages in the enrichment of LAE from the liquefied products. The PAME was not detected in the different solvent extract fractions, which was supposed to be unstable in the succeeding separation processing.

Table 2. Major Compounds in Liquefied Product of Corn Stover and Its Extraction Fractions

RT (min)	Component	Formula	Relative area (%)					
			a	b	c	d	e	f
8.65	Ethanol	$C_4H_{10}O_3$	5.95	22.78	12.79	79.4	19.42	-
9.32	Diethylene glycol (DEG)	$C_4H_{10}O_3$	63.66	32.68	3.31	2.29	2.96	75.85
11.49	2-isopropoxyethyl proponate	$C_8H_{16}O_3$	1.76	2.41	1.56	3.22	-	0.25
13.99	3-(2-methyl-1,3-doxolane-2-yl) propionic acid methyl ester (PAME)	$C_8H_{14}O_4$	4.65	0.58	0.29	0.35	1.01	0.23
14.95	Triethylene glycol (TEG)	$C_6H_{14}O_4$	19.05	3.84	2.60	0.48	1.90	19.11
16.33	Levulinic acid isopropyl ester (LAE)	$C_8H_{14}O_3$	1.32	5.62	52.23	4.25	35.16	-
20.12	1,6-dioxaspiro[4,4]nonane-2,7-dione	$C_7H_8O_4$	0.81	4.01	9.08	0.67	5.10	0.11
21.29	Methyl laurate	$C_{13}H_{26}O_2$	0.29	1.80	2.19	0.68	1.14	0.14
29.77	Methyl hexadecanoate	$C_{17}H_{34}O_2$	0.51	0.80	0.16	0.36	0.43	0.36

Table 3. LAE Concentration in Different Extraction Fractions

Sample	Concentration % (m/m)
Untreated liquefied product	0.67
Chloroform single extract	20.51
Ethyl acetate single extract	23.48
Chloroform sequential extract	28.76
Ethyl acetate sequential extract	43.65

To determine the LAE enrichment effect of the solvents chloroform and ethyl acetate, the concentration of the LAE in the different solvent extraction fractions was quantified (Table 3). The original concentration of LAE in the LPCS was determined to be 0.67% (m/m). It was enriched to 20.51% in the chloroform single extract and to 23.48% in the ethyl ester single extract. The concentrations in the chloroform sequential extract and the ethyl acetate sequential extract were as high as 28.76% and 43.65%, respectively.

The data show that chloroform and ethyl acetate can selectively separate LAE from LPCS while hexane and diethyl ether work for removal of the liquefaction EG and its derivatives.

CONCLUSIONS

1. The microwave liquefaction yields increased during 5 to 15 min, while the water-soluble substances in the LPCS decreased.
2. Among the four organic solvents selected for extraction, the water-soluble fraction of LPCS had the highest solubility in chloroform and ethyl acetate and the lowest solubility in hexane and diethyl ether.
3. Gas chromatography-mass spectroscopy was used to identify the components in the liquefied product and extracts with solvent; the LPCS consisted of EG derivatives and methyl esters, including PAME, LAE, methyl laurate, and methyl palmitate.
4. After extraction with water and organic solvents, the concentration of LAE increased from 0.67% in the LPCS to 28.76% in the sequential extract of chloroform and 43.65% in the sequential extract of ethyl acetate.
5. Chloroform and ethyl acetate were observed to be efficient at selectively extracting chemical constituents of the microwave-liquefied products and enriching LAE.

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