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Analysis of fatty acid methyl ester in bio-liquid by hollow fiber-liquid phase microextraction

Minseon Choi, Soyoung Lee and Sunyoung Bae★

Department of Chemistry, Seoul Women's University, 621 Hwarang-ro, Nowon-gu, Seoul 01797, Korea

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Abstract Bio-liquid is a liquid by-product of the hydrothermal carbonization (HTC) reaction, converting wet biomass into solid hydrochar, bio-liquid, and bio-gas. Since bio-liquid contains various compounds, it requires efficient sampling method to extract the target compounds from bio-liquid. In this research, fatty acid methyl ester (FAME) in bio-liquid was extracted based on hollow fiber supported liquid phase microextraction (HF-LPME) and determined by Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography/Mass Spectrometry (GC/MS). The well-known major components of biodiesel, including methyl myristate, palmitate, methyl palmitoleate, methyl stearate, methyl oleate, and methyl linoleate had been selected as standard materials for FAME analysis using HF-LPME. Physicochemical properties of bio-liquid was measured that the acidity was 3.30 (± 0.01) and the moisture content was 100.84 (± 3.02)%. The optimization of HF-LPME method had been investigated by varying the experimental parameters such as extraction solvent, extraction time, stirring speed, and the length of HF at the fixed concentration of NaCl salt. As a result, optimal conditions of HF-LPME for FAMEs were; n-octanol for extraction solvent, 30 min for extraction time, 1200 rpm for stirring speed, 20 mm for the HF length, and 0.5 w/v% for the concentration of NaCl. Validation of HF-LPME was performed with limit of detection (LOD), limit of quantitation (LOQ), dynamic range, reproducibility, and recovery. The results obtained from this study indicated that HF-LPME was suitable for the preconcentration method and the quantitative analysis to characterize FAMEs in bio-liquid generated from food waste via HTC reaction.

Key words: Bio-liquid, Hydrothermal carbonization, Fatty acid methyl ester, Hollow fiber-liquid phase microextraction, GC/MS

1. Introduction

Bio-liquid was designated as the liquid phase among products of the hydrothermal carbonization (HTC) reaction. HTC is a conversion process that converts wet biomass to following products; hydrochar in the solid phase, bio-liquid in the liquid phase, and bio-gas of the gas phase. The study of HTC reaction has been progressed by using lignocellulose, herbaceous biomass, microalgae, waste and so on.[1-3] The potential

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★ Corresponding author
Phone : +82-(0)2-970-5652 Fax : +82-(0)2-970-5972
E-mail : sbae@swu.ac.kr

of hydrochar used as an environmental sorbent, coal or soil amendment had been reported in the literature.[4-6] Bio-liquid is currently studied mainly for utilization as the fuel. The chemical properties of bio-liquid are known that it highly depends on the type of the biomass composition.[7] The main component that determines the availability of the fuel is known as fatty acid methyl esters (FAMEs). Among FAMEs, methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate, and methyl linoleate are considered as the major components of diesel.[8,9] Qualitative and quantitative analysis of these compounds play a significant role in utilization to fuel. Different biomass types yield the various compositions of bio-liquids.[10-12] However, a study on extraction method of the specific components has barely done. Since bio-liquid has a complex matrix from various components of biomass, the extraction method with selectivity and sensitivity for target compounds is required.

Previously, bio-liquid has been mainly analyzed using Liquid-Liquid Extraction (LLE) method.[13-14] This method extracts analytes using difference in density of each solvent. It requires a few milliliters of solvents and samples for pre-treatment and analysis procedure. This method is able to extract step-by-step in accordance with polarity, which is effective in whole screening of analytes. However, a large quantity of deleterious organic solvents are needed in this case and evaporation step on extraction process is time-consuming.

In order to overcome these draw backs, different extraction methods have been developed such as Solid Phase Extraction (SPE), Solid-Phase Microextraction (SPME) and In-Needle Microextraction (INME) even though these methods do not follow the same principle as LLE method.[15] Recently, researches tend to miniaturize traditional liquid-liquid extraction (LLE). In 1996, Liu and Dasgupta[16] and Jeannot and Cantwell[17] first published about liquid phase microextraction (LPME). Since then, new LPME techniques have been suggested such as single drop microextraction (SDME), dispersive liquid–liquid microextraction (DLLME) and hollow-fiber liquid-phase microextraction (HF-LPME), and so on.

HF-LPME is a method for extraction of analytes by using several microliter solvents exist inside the porous fiber. This method is able to extract analytes using very small amount of solvent for a short time without pretreatment. The sample and organic solvent are separated by hollow fiber, where small amount of solvent can be immiscible and withstand during extraction. This is an effective method to extract non-polar compounds in aqueous sample.[18-20]

This study evaluated the effectiveness of HF-LPME as an effective extraction method for FAMEs in aqueous bio-liquid. Extraction solvents, extraction time, amount of extraction solvent, and agitation speed at the fixed concentration of salt were investigated in this study to optimize HF-LPME method. Validation and comparison of extraction efficiency of HF-LPME were suggested under the optimized condition. FAMEs present in bio-liquids generated from various biomass were qualitatively analyzed by HF-LPME, Gas chromatography-Flame Ionization detector (GC-FID), and Gas Chromatography/ Mass Spectrometry (GC/MS).

2. Experimental

2.1. Reagents and materials

Methyl myristate (≥ 99.5 %), methyl palmitate (≥ 99.5 %), methyl stearate (≥ 99.5 %), and methyl linoleate (≥ 99.9 %) were purchased from TCI (Japan). Methyl palmitoleate (analytical standard) was supplied from Sigma-aldrich (USA). Methyl oleate (analytical standard) was purchased from Fluka (Italy). The solvents for stnadard solution preparation was methanol (HPLC grade, Samchun, Korea) and for extraction of the analyte was n-octanol (98 %, Junsei, Japan), hexyl ether (98 %, TCI, Japan), and n-tridecane (99 %, TCI, Japan).

Bio-liquid was generated from various food wastes through HTC reaction at 220 °C for 3 hours in our laboratory. The food wastes that used in this experiment were taken from a cafeteria and a Chinese restaurant in Seoul. The bio-liquid was stored in an amber bottle at room temperature to keep out of direct sunlight. The accurel Q3/2 (polypropylene, 200 μm thickness, 600 μm I.D.) was chosen as hollow fiber membrane to be used for HF-LPME. Acidity and water contents of bio-liquid produced in this study were pH 3.30 (± 0.01) and 100.84 (± 3.02)%, respectively.

2.2. Hollow fiber-liquid phase microextraction (HF-LPME)

2.2.1. Installation of HF-LPME

For the installation of the equipment shown in *Fig.* 1, a 10 μL autopipette tip, a 12 mL screw vial, and a septum were used. The end of the hollow fiber was pressured by heated pate to seal it. Then, it was washed out with acetone for 30 min to remove the impurities in the fiber and dried at 60 °C for 30 min. HF in extraction solvent for 20 s in order to fill the pores with the extraction solvent. To remove the extraction solvent on the surface and the inner wall of the HF, sonication was applied for 3 s. Then, HF was filled with the fresh extraction solvent using a 10 μL GC syringe.

Then, it was assembled to the 10 μL autopipette tip attached to the screw vial cap. The septum was inserted into the screw vial cap to be tighten. Finally, HF was immersed into the 12 mL screw vial which a 10 mL of bio-liquid was transferred into. In order to enhance the extraction efficiency, sample solution was stirred constantly at various speeds using a cross type stirrer bar (1 cm). After extraction reaction reached at equilibrium, 10 μL syringe was used for transferring the extraction solvent from the HF and for GC injection simultaneously. The HF was discarded after each extraction.

2.2.2. Optimization of HF-LPME analysis conditions

To investigate various parameters effect on extraction efficiency, the optimization of HF-LPME was performed through various reaction parameters, including extraction solvent, extraction time, stirring speed, and the length of HF. Extraction amount was quantitated by peak area obtained from GC-FID. Each reaction parameter was varied while other reaction parameters were fixed. From the previous study, the NaCl salt concentration was optimized at 0.5 w/v%.

The extraction solvents were varied as n-tridecane, dihexyl ether, and n-octanol while extraction time for 30 min, stirring speed at 1200 rpm, HF length of 20 mm, and salt concentration of 0.5 w/v% were fixed. The extraction times were varied for 10 min, 20 min, 30 min, and 40 min while extraction solvent of n-octanol, stirring speed at 1200 rpm, HF length of 20 mm, and salt concentration of 0.5 w/v% were fixed. The stirring speeds were varied at 600 rpm, 900 rpm, 1200 rpm, and 1500 rpm while extraction solvent of n-octanol, extraction time for 30 min, HF length of 20 mm, and salt concentration of 0.5 w/v% were fixed. The HF lengths were varied at 15 mm, 20 mm, and 25 mm while extraction solvent of n-octanol, extraction time for 30 min, stirring speed at 1200 rpm, and salt concentration of 0.5 w/v% were fixed. Calibration curve, validation of the anlysis method, and comparison of extraction efficiency were performed at the optimized conditions.

2.2.3. Gas chromatography/mass spectrometry (GC/MS) and gas chromatography-flame ionization detector (GC-FID)

Optimization of HF-LPME, validation of analytical method, comparison of extraction efficiency, and

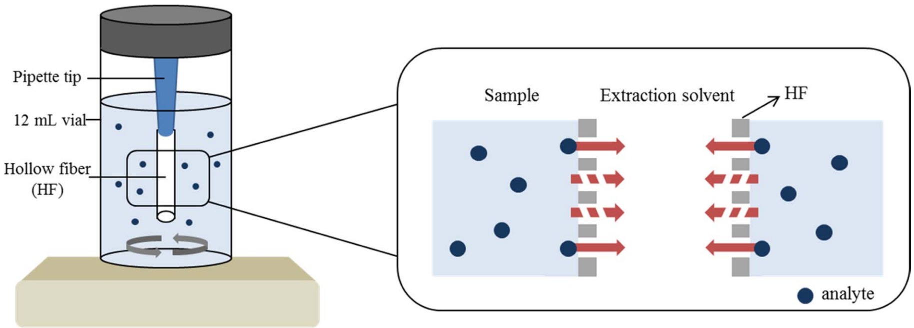

Fig. 1. A schematic illustration of mechanism for hollow fiber–liquid phase microextraction. HF represents hollow fiber.

Table 1. The operating conditions of gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC/MS) used in this study

	GC-FID	GC/MS	
	GC (HP 5890)	GC (7820A , Agilent)	
Column	SPB1® (60 m × 0.25 mm × 0.25 μm, 100 % dimethyl polysiloxane, Supelco)	SPB1® (60 m × 0.25 mm × 0.25 μm, 100 % dimethyl polysiloxane, Supelco)	
Oven Temp. program	150 °C (for 3 min)→ 20 °C/min → 190 °C (for 1 min)→ 20 °C/min → 280 °C (for 15 min)	150 °C (for 3 min)→ 20 °C/min → 190 °C (for 1 min) → 20 °C/min → 280 °C (for 15 min)	
Injector Temp.	300 °C	300 °C	
Split ratio	1:10	1:30	
Carrier gas	N_2 (99.99 %)	He (99.999 %)	
Flow rate	1 mL/min	1 mL/min	
Injection volume	1 μL	0.4 μL	
Detector			
	FID	MS (5977E, Agilent)	
Detector temp.	300 °C	Mass transfer line	300 °C
FID gas	H_2 (99.99 %), Air (99.99 %)	Ionization voltage	70 eV
		Ion source	230 °C
		Quadruple	150 °C
		Mass range	35 ~ 500 m/z

bio-liquid quantitative analysis were determined by Gas Chromatography-Flame Ionization Detector (GC-FID) (Hewlett-Packard, HP 5890, Palo Alto, CA). Bio-liquid qualitative analysis was determined by Gas Chromatography/Mass Spectrometry (GC/MS) (Agilent 7820A/Agilent 5977E MSD, Santa Clara, CA, USA). SPB1® (60 m × 0.25 mm × 0.25 μm, 100 % dimethyl polysiloxane, Supelco, Bellefonte, PA, USA) was used. Detailed analysis conditions are shown in *Table* 1.

3. Results and Discussion

3.1. Optimization of analysis conditions

The enrichment factor of HF-LPME at the various conditions was calculated by the following Eq. (1) and used to determine the optimized conditions. The enrichment factor (EF) was defined as the ratio of the peak area of analyte by HF-LPME (A_{HF}) to that of analyte without pretreatment (A_0) at the same concentration.

$$EF = \frac{A_{HF}}{A_0} \qquad (1)$$

3.2. Effect of extraction solvent and extraction time

To evaluate the extraction solvents, three solvents were selected on the basis of durability against extraction period, less volatility, and immiscibility with bio-liquid. In this study, a preliminary experiment on appropriate extraction solvents for FAMEs was referenced.[18] As a result, n-tridecane, dihexyl ether, and n-octanol were selected as tentative solvents for this extraction. As shown in *Fig.* 2, n-octanol showed the greatest EF among extraction solvents tested. The largest EF represented the excellence of sensitivity and effective extraction. According to other studies, for various nonpolar compounds, n-octanol was selected as optimum extraction solvent.[21] It is considered that n-octanol was proven to be less loss due to the features of larger viscosity and low volatility.

HF-LPME is a process that requires equilibrium to be reached. Therefore, equilibrium time can be significant factor on extraction efficiency.[22] As shown in *Fig.* 2, the EFs of FAME compounds were increased by increasing of extraction time. After 30 min extraction time, there is no dramatic increase of *EF*

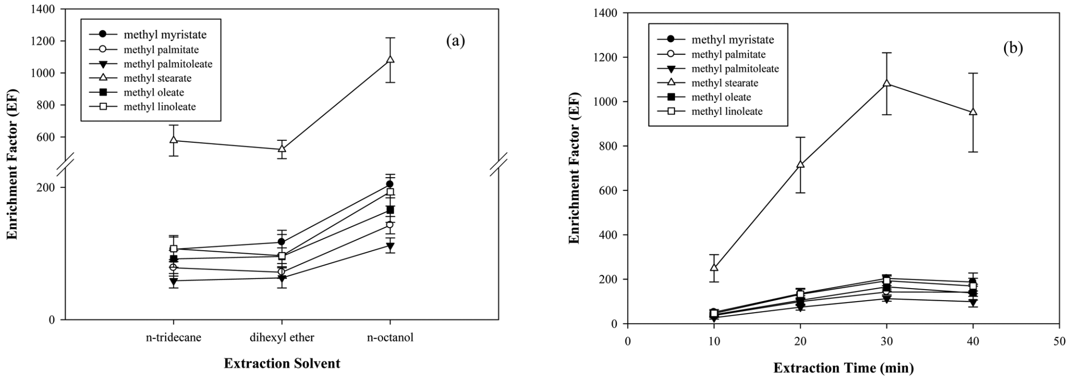

Fig. 2. Optimization of (a) extraction solvent and (b) extraction time determined by enrichment factor of standard FAME compounds. HF-LPME conditions : extraction time for 30 min, HF length of 20 mm (7 μL), stirring speed at 1200 rpm, and NaCl salt of 0.5 w/v%

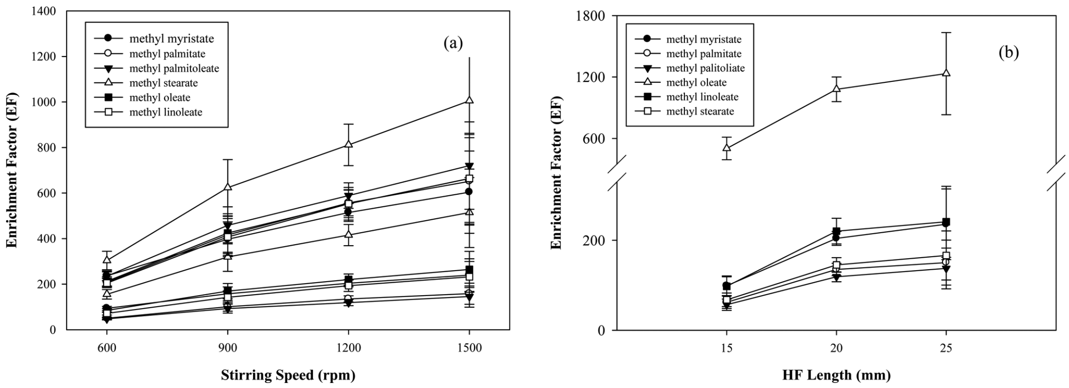

Fig. 3. Optimization of (a) stirring speed determined and (b) HF lengh by enrichment factor of standard FAME compounds. HF-LPME conditions : extraction solvent of n-octanol, extraction time for 30 min, HF length of 20 mm (7 μL), and NaCl salt of 0.5 w/v%.

values. At 40 min extraction time, *EF* values of most standard FAME compounds showed the large relative standard deviation (RSD) of 18.6~25.1 %. For this reason, the optimal extraction time was selected as 30 min.

3.3. Effect of stirring speed and the volume of extraction solvents

The variation of stirring speed was taken a role of constant movement between organic solvent and analyte.[23,24] The effect of stirring speed on extraction efficiency was investigated by changing stirring speed from 600 rpm to 1500 rpm. Extraction efficiency increased with stirring speed (*Fig.* 3). The higher stirring speed may make the exposure of the extraction solvent to sample effectively. However, the *EF* value at 1500 rpm showed a large variation in RSD. High speed would produce many air bubbles, hindering the transport of analytes.[25] It might also cause solvent loss due to severe turbulence around the HF. Therefore, the optimum condition of stirring speed was selected at the 1200 rpm.

The volume of extraction solvents was varied by the length of HF. The length of HF about 15 mm was equivalent to 5 μL, 20 mm to 7 μL, and 25 mm to 9 μL of extraction solvent. The *EF* values were increased as solvent amount increase and standard deviations of *EF* values were also increased in HF length (*Fig.* 3). As a result, the extraction efficiency was increased up to a certain amount of extraction solvent. In this study, the optimum HF length was determined to be 20 mm HF. Similar results have been reported for some other LPME systems.[26]

3.2. Validation of analysis method

To validate HF-LPME method, limit of detection (LOD), limit of quantitation (LOQ), recovery, and reproducibility were obtained through the calibration curve. Total ion chromatogram of bio-liquid compounds obtained from HF-LPME followed by GC/MS was shown in *Fig*. 4. LOD and LOQ were defined and calculated following ISO 11843-2. From the calibration curve of FAMEs, all of FAME standard compounds have great r^2 (coefficient of determination) value higher than 0.996. The ranges of LOD for all FAME standard compounds were calculated for 51.7 ng/mL ~ 292 ng/mL, LOQ for 172 ng/mL ~ 975 ng/mL, and dynamic range for 5.2×10^{-2} μg/mL ~ 1.8×10^{2} μg/mL.

Accuracy of HF-LPME was evaluated through the recovery test. It was investigated by adding 10 μg, 50 μg, and 100 μg of FAME standard compounds to a 10 times diluted sample of bio-liquid. The rate of recovery was 112.50 % ~ 121.49 % at low level. Recovery values of 50 μg and 100 μg were ranged between 78.55 % and 99.66 %. The reproducibility of HF-LPME method was represented as relative standard deviation. Inter assay (n=3) was performed at the same concentration of FAME standard compounds using three different HFs. All values showed lower than 10 % which meant good precision.

The EF of HF-LPME was explained in section 3.1, which was able to be calculated by the ratio of the slope from each calibration curve (Eq. (2)).

$$EF = \frac{A_{HF}}{A_0} = \frac{k_{HF}C_{HF}}{k_0 C_0} = \frac{k_{HF}}{k_0} \tag{2}$$

where k_{HF} and k_0 are the experimental coefficients, and C_{HF} and C_0 are the same concentration of FAME standard compounds used for HF-LPME analysis and without pretreatment. From the computation obtained from Eq. (2), HF-LPME methods with

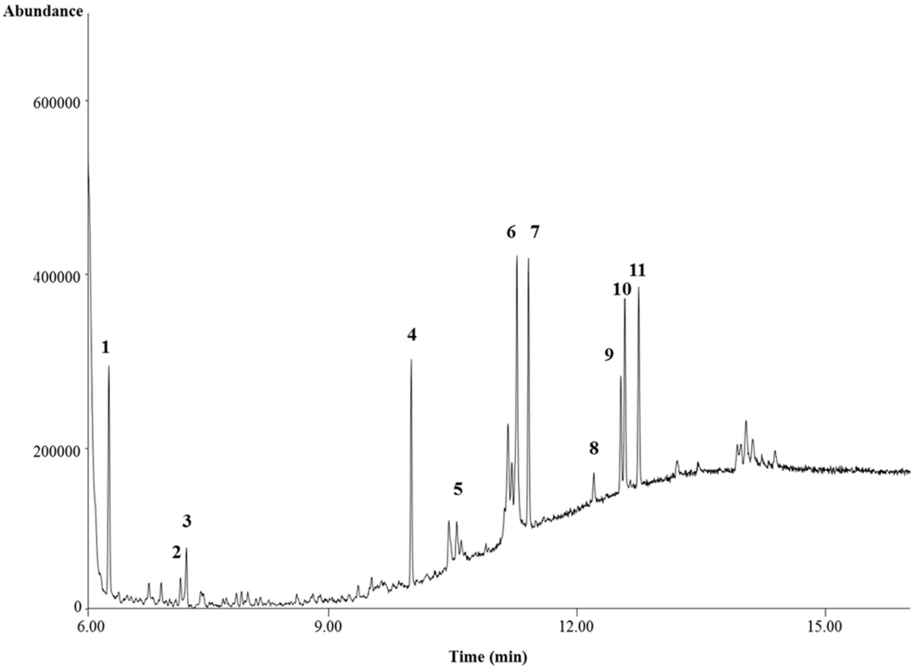

Fig. 4. Total ion chromatogram of bio-liquid compounds obtained from HF-LPME and analyzed by GC/MS. Peak notation: 1, tridecane; 2, 2-methyl-5-hydroxybenzofuran; 3, tetradecane; 4, methyl myristate; 5, 5,8-Dimethoxy-4-phenyl-2(1H)-quinolinone; 6, methyl palmitoleate; 7, methyl palmiate; 8, 5,8-Dimethoxy-4-phenyl-2(1H)-quinolinone; 9, methyl oleate; 10, methyl linoleate; 11, methyl stearate.

enrichment factor was 30.88 ~ 41.90.

3.3. Comparison of extraction efficiency

The concentration factor (CF) was calculated to compare extraction efficiency obtained from the two different extraction methods. In Eq. (3), A_{HF} means the peak area obtained from HF-LPME method while A_{LLE} means the peak area obtained from LLE method. Bio-liquid was extracted with diethyl ether: dichloromethane (1:1), evaporated, and re-concentrated in dichloromethane before GC injection. Single LLE was applied in this study. Both methods were performed by adding the same amount of FAME standard compounds to the sample solution.

$$CF = \frac{A_{HF}}{A_{LLE}} \tag{3}$$

As a summary, the *CF* values were 20.81(± 3.92) of methyl myristate, 3.77(± 1.80) of methyl palmitoleate, 26.23(± 6.49) of methyl stearate, and 51.84(± 14.86) of methyl linoleate, respectively. The *CF* values of methyl palmitate and methyl oleate were not able to be calculated because they were not extracted by single LLE extraction. If the number of extraction was increased in LLE, the *CF* values would be varied. Consequently, it was concluded that HF-LPME showed advantages such as high extraction efficiency in short extraction time and less solvent usage.

4. Conclusions

Bio-liquid was generated from food waste via hydrothermal carbonization (HTC) reaction. Among various compounds presented in bio-liquid, FAMEs were analyzed using hollow fiber-liquid phase microextraction (HF-LPME) followed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC/MS). The optimization, validation and application of HF-LPME method were performed.

The optimum conditions of HF-LPME were determined for FAMEs including methyl myristate, methyl palmitate, methyl palmitoleate, methyl stearate, methyl oleate, and methyl linoleate. The optimum conditions were n-octanol of extraction solvent, 30 min of extraction time, 1200 rpm of stirring speed, 20 mm of the length of hollow fiber (equivalent amount of extraction solvent : 7 μL), and 0.5 w/v% of the concentration of NaCl salt. Calibration curve, recovery, reproducibility, enrichment factor and concentration factor were determined to validate HF-LPME at the optimum conditions. Qualitative analysis results of bio-liquid obtained by GC/MS after HF-LPME were alcohol, aldehyde, ethanol, fatty acid, FAME, and pyrazine. Quantitative analysis of FAMEs presented in bio-liquid from food waste was performed by GC-FID using a calibration curve.

In conclusion, HF-LPME method suggested in this study was successfully applied and validated to extract and concentrate the FAMEs in extraction solvent. It has been proven to be a prompt extraction method using less solvent usage, and simple method with satisfactory accuracy and reproducibility.

Acknowledgements

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