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# Hollow fiber liquid-phase microextraction coupled with gas chromatography-flame ionization detection for the profiling of fatty acids in vegetable oils

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ABSTRACT

The development of a two phase hollow fiber liquid-phase microextraction technique, followed by gas-chromatography-flame ionization detection (GC-FID) for the profiling of the fatty acids (FAs) (lauric, myristic, palmitic, stearic, palmitoleic, oleic, linoleic, linolenic and arachidic) in vegetable oils is described. Heptadecanoic acid methyl ester was used as the internal standard. The FAs were transesterified to their corresponding methyl esters prior to the extraction. Extraction parameters such as type of extracting solvent, temperature, extraction time, stirring speed and salt addition were studied and optimized. Recommended conditions were extraction solvent, n-tridecane; extraction time, 35 min; extraction temperature, ambient; without addition of salt. Enrichment factors varying from 37 to 115 were achieved. Calibration curves for the nine FAs were well correlated ($r^2 > 0.994$) within the range of 10–5000 μg L$^{-1}$. The limit of detection (signal:noise, 3) was 4.73–13.21 ng L$^{-1}$. The method was successfully applied to the profiling of the FAs in palm oils (crude, olein, kernel, and carotino cooking oil) and other vegetable oils (soybean, olive, coconut, rice bran and pumpkin). The encouraging enrichments achieved offer an interesting option for the profiling of the minor and major FAs in palm and other vegetable oils.

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## 1. Introduction

Fatty acids (FAs) are the basic components of most naturally occurring lipids in both animals and plants. The diversity of the chain length, degree of unsaturation, geometry and position of double bonds determine the characteristic of these lipids and their origins [1]. The properties of oils are very much dependent on the FAs profile, which provide information on the chain length, percent saturation, monounsaturation and polyunsaturation. Based on these information, the recommended usage of each oil can be proposed. Profile determination of unsaturated FAs is useful in health care management (e.g., towards the prevention of diseases [2–7].

The most commonly used method for the analysis of FAs involves the determination of the corresponding methyl esters (FAMEs) using capillary gas chromatography (GC) with flame ionization detector (FID) [8], For lipids, fats and oils, often a transesterification procedure involving the direct conversion of FAs to alkyl esters (particularly methyl esters) by alcohol in the presence of a catalyst is often carried out. The derivatization procedure (especially for the longer chain FAs) is mandatory to increase the volatility and overcome adsorption of the polar functional groups to the GC column [9]. The thermally labile FAs can also be separated using high performance liquid chromatography (HPLC), capillary electrophoresis and supercritical fluid chromatography [10].

Prior to the analytical determination, the FAs or the FAMEs need to be isolated from the sample. This is commonly done using liquid–liquid extraction (LLE), and is used in the official methods recommended by regulatory bodies, e.g., the American Oil Chemists Society (AOCS) and the Malaysian Palm Oil Board (MPOB). Major problems of the LLE technique are the gross consumption of organic solvents, lack of selectivity, time consuming, labour intensive, and the extra evaporation step required prior to analysis to remove the excess solvent. This can lead to contamination problems and possible loss of analytes [11–13].

To overcome these problems and to meet the increasing demands for green approaches in analytical determinations, a considerable amount of effort has been directed to address these issues. In the early 1970s, the solid phase extraction (SPE) technique was introduced [11] and many reports utilized this technique for the analysis of FAs (e.g., determination of the composition of long chain FAs in transesterified palm oil [14], determination of methyl linolenate and methyl linoleate in soy-derived biodiesel [15], and free FAs in beer [16]). More recently, several microextraction and solvent minimized extraction techniques such as the solid phase microextraction (SPME) and liquid phase microextraction (LPME) were introduced. In particular, the hollow fiber LPME, originally

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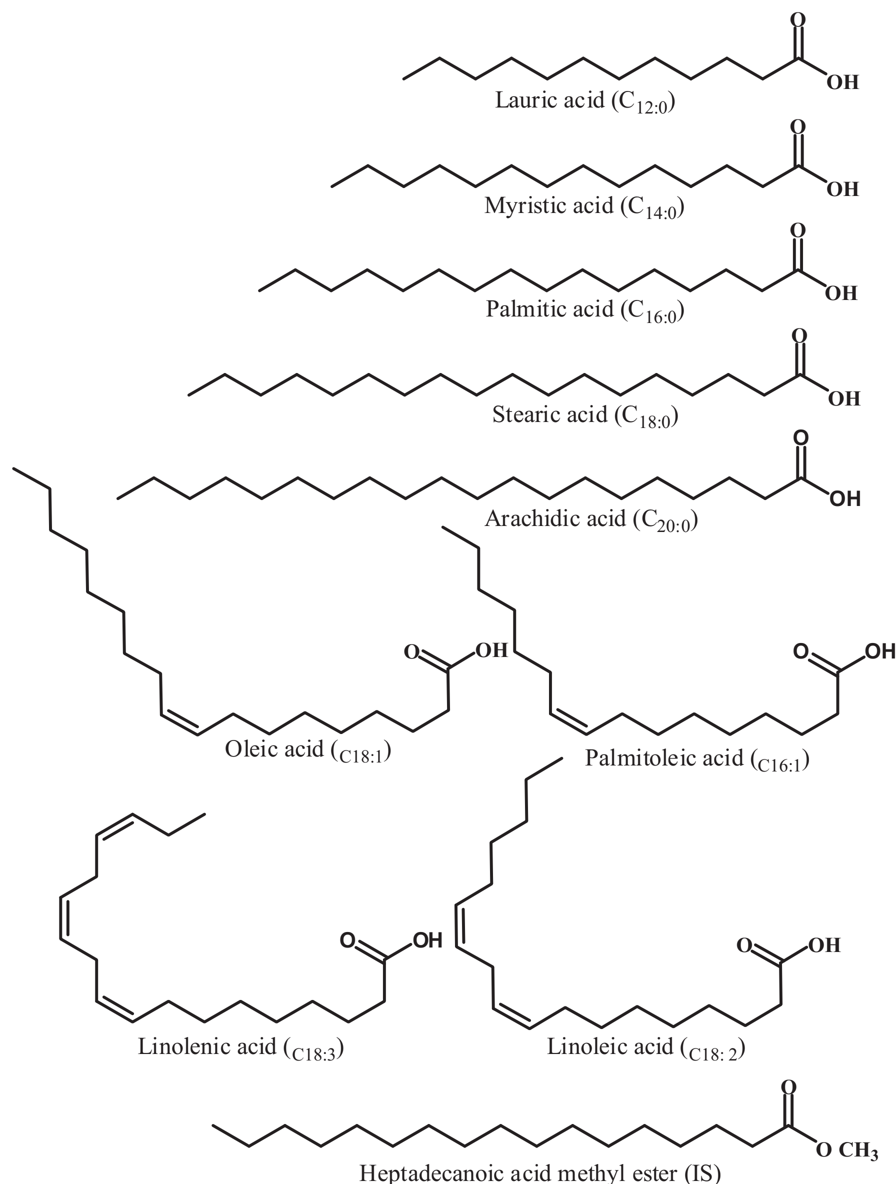


**Fig. 1.** Chemical structures of the FAs studied.

described by Bjergaard and Rasmussen [17] has attracted a lot of attention. SPME methods for the determination long chain FAs in several refined oils (avocado, camellia, pumpkin, sesame and soybean) [18], in lung tissues [12] have been reported. The SPME technique, however, is plagued with the fragile nature of the fiber, limited range of coating materials and the frequent sample carry-over between the runs [19,20].

Several LPME methods, especially for the short chain FAs have been reported [21–23]. Ion-pair dynamic fiber LPME combined with injection-port derivatization was used for the determination of three long chain FAs (myristic ($C_{14:0}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$)) in wastewater [13]. A dispersive liquid–liquid microextraction technique was applied to three FAs (azelaic, palmitic, and stearic acid) in water sample [24]. The sparce reports on FAs in general offer inspiration for us to explore the LPME technique in vegetable oil testing.

The LLE technique in combination with GC-FID only provides the profiling of the major FAs [8]. The minor FAs are not sensitive enough to be detected and are often reported as “others”. The use of more sensitive detectors such as the mass spectrometer is an option to increase sensitivity. Alternatively, enrichment techniques can also be deployed. As an example, the use of SPE has resulted in the identification of previously unidentified minor components in transesterified palm oil [14]. As mentioned earlier, the majority of the previous reported LPME methods described the extraction of FAs in water samples. The use of LPME for the analysis of FAs in vegetable oils is for the first time reported here. The oils were transesterified using standard procedures [8], and were subjected to the LPME treatment prior to the GC-FID determination. The chemical structures of the FAs studied are shown in Fig. 1.

## 2. Experimental

### 2.1. Chemicals and reagents

Lauric ($C_{12:0}$, 99%), myristic ($C_{14:0}$, 99–100%), palmitic ($C_{16:0}$, 99%), palmitoleic ($C_{16:1}$, 99%), stearic ($C_{18:0}$, ≥99%), oleic ($C_{18:1}$, 99%), linoleic ($C_{18:2}$, 99%), linolenic ($C_{18:3}$, ≥99%) and arachidic ($C_{20:0}$, ≥99%) acids, sodium hydroxide, iso-octane, n-octane, n-decane, n-tridecane and n-hexadecane were purchased from

Sigma–Aldrich (St. Louis, MO, USA). Heptadecanoic acid methyl ester (IS, ≥99.5%) was purchased from Fluka (Buchs, Switzerland). Methanol (HPLC grade), boron trifluoride and n-hexane were purchased from Merck (Darmstadt, Germany). Vegetable oil samples were purchased from local supermarkets and crude palm oil samples were kindly donated by Carotino Sdn. Bhd. (Johor Bahru, Malaysia).

### 2.2. Materials

Q3/2 Accurel polypropylene hollow fiber membrane (600 μm inner diameter, 200 μm wall thickness and 0.2 μm pore size) was purchased from Membrana GmbH (Wuppertal, Germany). 4 cm of the hollow fiber was used. The hollow fiber was discarded after single use. A 25 μL Hamilton microsyringe (model 702SNR) with a blunt needle tip was used to introduce the acceptor phase, support the hollow fiber and acts as an injector for the analysis after the extraction. The syringe with the attached hollow fiber was clamped to a retort stand during the extraction. A hot plate stirrer (model GLHPS-G) purchased from Global Lab (Penang, Malaysia) was used for stirring during the extraction.

### 2.3. Chromatographic conditions

GC analysis was performed using a Clarus 500 GC unit that was purchased from Perkin Elmer (Shelton, CT, USA). A Supelcowax 10 fused silica capillary column of 30 m × 0.32 mm I.D., film thickness 0.25 μm from Supelco (Bellefonte, PA, USA) was used. The chromatographic conditions were performed according to the AOCS procedure (Ce 1e-91) [8]. The oven temperature was programmed as follows: 80 °C (hold for 2 min) at 20 °C $min^{-1}$ to 125 °C (hold for 1 min) then at 3 °C $min^{-1}$ to 220 °C (hold for 5 min). The injector and the FID were operated at 240 °C. Nitrogen was used as carrier gas at a flow rate of 1 mL $min^{-1}$. A split ratio of 1:10 was applied. Chromatographic data were processed using Total Chrom Workstation version 6.3.1 software. Quantification was done using an eight-points external standard calibration assay.

### 2.4. Preparation of standards

A stock solution (1000 mg $L^{-1}$) was prepared by dissolving the nine FAs (lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic and arachidic) in boron trifluoride in methanol (14%). The mixture was heated at 80 °C with continuous stirring for 60 min. The transesterified mixture was stored at 4 °C. Working solutions were prepared fresh every day in methanol after spiking with IS (1000 μg $L^{-1}$ for LPME method and 100 mg $L^{-1}$ for LLE method, prepared by direct dilution in methanol).

### 2.5. Transesterification of samples

Prior to the LPME-GC-FID analysis, a transesterification process was carried out by mixing oil (15 mg) with 0.5 M sodium methanoate (1.25 mL) in a vial (16 mL). The vial was tightly capped and was heated for 5 min at 60 °C. The vial was then cooled and 14% boron trifluoride in methanol (2 mL) was added, and heated (80 °C) with continuous stirring for 60 min. The vial was allowed to cool and the mixture was than diluted to the 100 mL mark with methanol after spiking with IS (1000 μg $L^{-1}$). For the LLE, 30 g of oil was mixed with 2.5 mL of 0.5 M sodium methanoate. Similar procedure was used except that 4 mL of 14% boron trifluoride was used. The mixture was spiked with IS (100 mg $L^{-1}$).

### 2.6. Extraction procedures

For the LPME method, transesterified standard or sample (10 mL) were transferred to a sample vial (16 mL). A magnetic stirrer (15 mm × 5 mm) was placed in the solution. Next, n-tridecane (5 μL) was withdrawn using a syringe. The syringe needle was then inserted into the hollow fiber segment and the assembly was immersed in n-tridecane for 20 s to impregnate the pores of the hollow fiber. Subsequently, the acceptor phase (n-tridecane) was injected into the lumen of the hollow fiber. The hollow fiber was than placed immediately in the sample solution and the sample vial was agitated at 1700 rpm. After a prescribed time of extraction, the magnetic stirrer was switched off and the acceptor phase was carefully withdrawn into the syringe and the hollow fiber was discarded. Finally, the acceptor phase was injected into the GC system. For the LLE method, 2.5 mL of water and 2.5 mL of n-hexane were added to the transesterified cooled mixture, vortexed for 15 min and the upper layer was injected into the GC system.

### 2.7. Validation of the LPME procedure

Linearity of the calibration curve was established after the extraction of the FAMEs standard solutions at eight different concentrations and three replicates were prepared at each level. The limits of detection (LOD) were calculated as the minimum concentration providing chromatographic signals three times higher than the background noise. The repeatability of the procedures was evaluated by carrying out six replicates of a mixture of the FAMEs that was prepared in methanol within the same day at three concentrations (50, 500, 5000 μg $L^{-1}$). The mixtures were subjected to the LPME procedure and analysed using GC-FID.

## 3. Results and discussion

Generally, the chemical analysis of vegetable oils is difficult due to the complexity of the sample as they contain a diverse range of major and minor components. The intense colour of some oils (e.g., crude palm and carotino cooking oils) and its viscosity adds to the complication.

### 3.1. LPME conditions

Several parameters (e.g., type of organic solvent, temperature, extraction time, stirring speed and salt addition) that influence the extraction efficiency were studied and optimized. To evaluate the effects of these parameters, a mixture that contained 500 μg $L^{-1}$ of each FAME and spiked with IS (1000 μg $L^{-1}$) was used.

#### 3.1.1. Selection of organic solvent

The choice of extracting organic solvent is generally important in extraction work. For the hollow fiber LPME technique, the selected solvent should be effectively impregnated in the pores of the fiber. In addition, the solvent must be of low volatility, immiscible with methanol (used in the transesterification step) and suitable for the GC analysis. Some common solvents such as n-hexane, cyclohexane, n-heptane, iso-octane and n-octane were not considered due to their volatility and difficulty to sample from the hollow fiber lumen. The use of n-decane resulted in low enrichments for all the FAMEs studied. However, significant improvements in the extraction were found when n-tridecane and n-hexadecane were used, but between these two solvents, n-tridecane is preferred as it offered higher enrichments (Fig. 2). The use of n-tridecane and n-hexadecane resulted in better extraction efficiencies due to their low polarity ($\log P = 7.5$ and 8.8, respectively) when compared to n-decane ($\log P = 5.6$) [25,26]. The low affinity for n-hexadecane to the

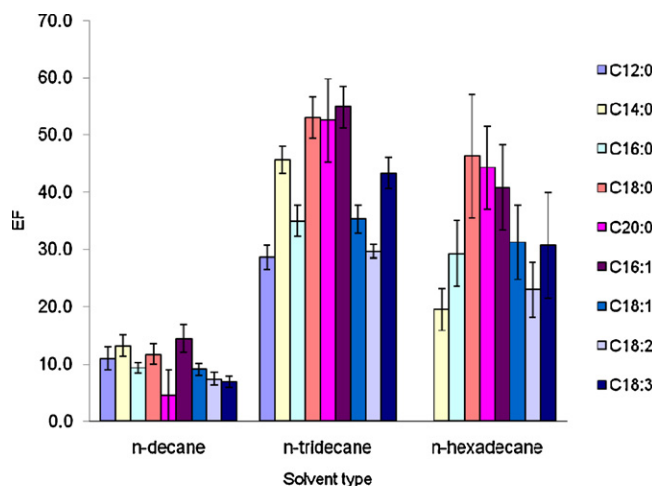


**Fig. 2.** Effect of extraction solvent on the LPME efficiency. FAs concentration, 500 μg L$^{-1}$; stirring speed, 1700 rpm; extraction time 25 min; ambient temperature.

target analytes resulted in a reduction of the extraction efficiency due to its longer chain length compared to n-tridecane.

### 3.1.2. Effect of extraction temperature

Higher enrichment factors can be anticipated by increasing the temperature. However, it was noticed that increasing of temperature above ambient can cause losses in the acceptor phase due to the increase in solubility of the selected acceptor phase in the donor phase (methanol). The increase in temperature will decrease the partition coefficient. This dominating effect causes an increase in the mass transfer, and resulted in a decrease in efficiency [27]. Furthermore, high temperature leads to solvent losses due to evaporation, thus decreasing the extraction yield. Therefore, the experiments were carried out at ambient temperature.

### 3.1.3. Effect of extraction time

The extraction was conducted for different times (15, 20, 25, 30, 35 and 40 min). An increase in the extraction efficiency was observed as the extraction time was increased up to 35 min (Fig. 3). After 35 min, it became increasingly difficulty to withdraw the solvent from the lumen, due to the increasing dissolution of the acceptor phase to the donor phase with time [28]. Thus, 35 min was selected for the subsequent experiments.

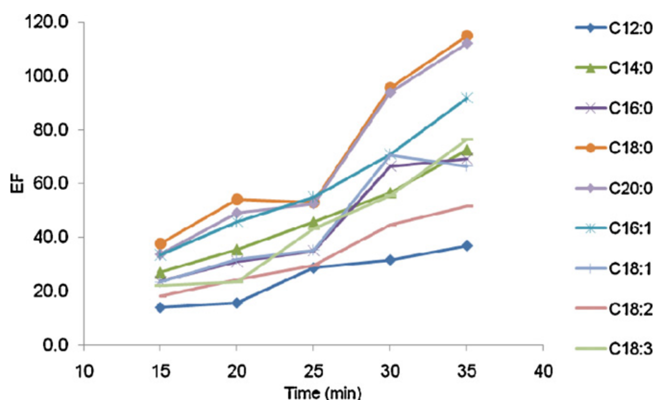


**Fig. 3.** Effect of extraction time on the LPME efficiency. FAs concentration, 500 μg L$^{-1}$; extraction solvent, n-tridecane; stirring speed, 1700 rpm; ambient temperature.

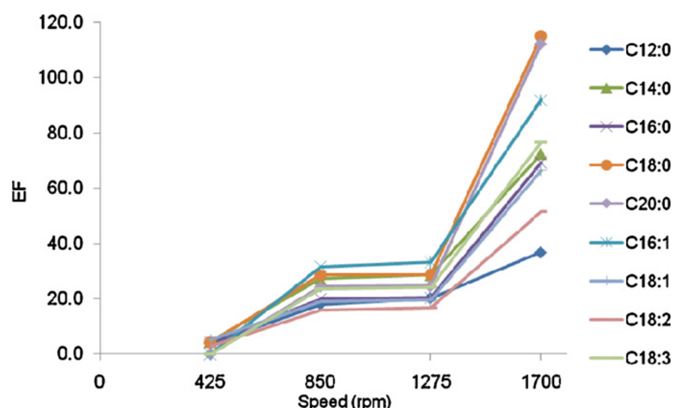


**Fig. 4.** Effect of stirring speed on the LPME efficiency. FAs concentration: 500 μg L$^{-1}$; extraction solvent: n-tridecane; extraction time 35 min; ambient temperature.

### 3.1.4. Effect of stirring speed

The effect of stirring rate (425–1700 rpm) on the extraction efficiency was also investigated. It was found that the extraction efficiency increases with the increase in stirring rate (Fig. 4). The high stirring rate enables greater exposure of the extraction surface to the sample. Therefore, stirring at 1700 rpm was selected for the rest of the studies.

### 3.1.5. Effect of salt addition

The addition of salt may increase the extraction efficiency because it can cause a decrease in the solubility of analytes in the sample solution (salting out effect) and thus enhancing their partitioning into the organic phase. Thus, different amounts of NaCl (0–20% w/v) were added to the sample solution and the best extraction was obtained when no NaCl was added (Fig. 5). This is due to the increase in the viscosity for the bulk solution which affects the kinetics of the partitioning of analytes between the organic and extraction solvents [29] that results in a decrease in the diffusion rate of the analytes from the bulk solution to the extraction solvent. It was also reported earlier that the addition of salt did not improve the extraction efficiency significantly for certain LPME work [29,30]. Therefore, further experiments were performed without the addition of NaCl.

## 3.2. Adopted extraction conditions

Based on the above experiments, the conditions that lead to the optimum extraction of the FAMEs were: n-tridecane as organic extracting solvent; stirring speed, 1700 rpm; extraction

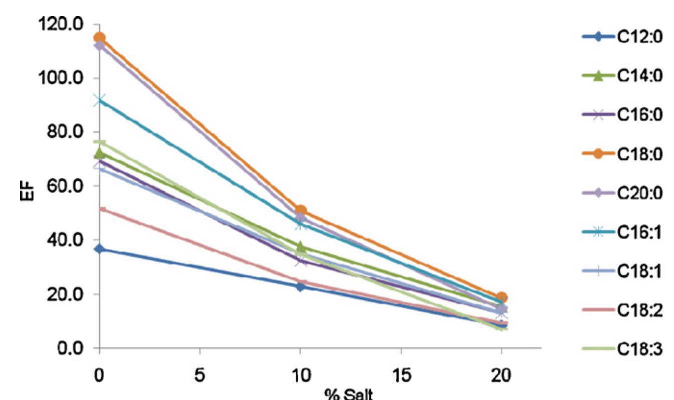


**Fig. 5.** Effect of addition of salt on the LPME efficiency. FAs concentration: 500 μg L$^{-1}$; extraction solvent: n-tridecane; stirring speed 1700 rpm; extraction time 35 min; ambient temperature.

**Table 1**
Analytical characteristics of the proposed LPME-GC-FID method.[a]

| FA | $r^2$ | LOD (ng $L^{-1}$) | Enrichment factor |
|---|---|---|---|
| $C_{12:0}$ | 0.9945 | 13.2 | 36.9 |
| $C_{14:0}$ | 0.9999 | 7.92 | 72.5 |
| $C_{16:0}$ | 0.9994 | 6.31 | 69.2 |
| $C_{18:0}$ | 0.9984 | 5.78 | 115.0 |
| $C_{20:0}$ | 0.9983 | 5.53 | 112.2 |
| $C_{16:1}$ | 0.9997 | 6.50 | 91.9 |
| $C_{18:1}$ | 0.9987 | 4.73 | 66.5 |
| $C_{18:2}$ | 0.9993 | 6.01 | 51.6 |
| $C_{18:3}$ | 0.9998 | 9.47 | 76.5 |

[a] Linearity range of FAs studied, 10–5000 μg $L^{-1}$.

time, 35 min at ambient temperature without the addition of salt.

The overall extraction efficiency was evaluated by the enrichment factor (EF). As it is impossible to detect the target analytes in the donor phase at very low concentrations, a reference standard (50 mg $L^{-1}$ in methanol) was directly injected into the GC system. EF was calculated according to the formula:

$$EF = \frac{C_a}{C_d}$$

where $C_a$ is the concentration of analyte in the reference standard (50 mg $L^{-1}$) and $C_d$ is the concentration of analyte obtained from the calibration curve of the analyte after the LPME extraction. Under these extraction conditions, respectable enrichment factors ranging from 37 to 115 were achieved (Table 1).

### 3.3. Method validation

#### 3.3.1. Linearity

Eight different concentrations (10–5000 μg $L^{-1}$ for LPME and 10–150 mg $L^{-1}$ for LLE) of standard solutions, each containing a mixture of nine FAMEs spiked with IS were injected into the GC column. Each mixture was injected thrice. The ratio of peak area of FAME to that of the IS was plotted against concentration of the FAME. The LPME method shows good linearity with correlation coefficient ($r^2 > 0.994$) (Table 1).

**Table 2**
Repeatability (%RSD) in the determination of the nine FAs using the proposed LPME-GC-FID method.

| FA (μg $L^{-1}$) | %RSD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $C_{12:0}$ | $C_{14:0}$ | $C_{16:0}$ | $C_{18:0}$ | $C_{20:0}$ | $C_{16:1}$ | $C_{18:1}$ | $C_{18:2}$ | $C_{18:3}$ |
| 50 | 6.84 | 9.17 | 10.0 | 10.2 | 10.4 | 10.8 | 12.5 | 10.2 | 5.37 |
| 500 | 6.66 | 2.45 | 1.76 | 2.37 | 3.43 | 2.31 | 2.07 | 1.51 | 2.91 |
| 5000 | 3.72 | 1.97 | 1.67 | 1.48 | 1.78 | 1.43 | 1.12 | 1.01 | 1.89 |

$n = 6$.

#### 3.3.2. Limit of detection (LOD)

The proposed LPME method gave comparable LODs for the nine FAMEs (4.73–13.2 ng $L^{-1}$) compared to the ion-pair dynamic fiber LPME-GC–MS (9.3–15 ng $L^{-1}$) [13], but is significantly lower compared to the reported methods using LLE-GC-FID (700–800 μg $L^{-1}$) [31], the dispersive liquid–liquid microextraction-GC-FID (0.67–1.06 μg $L^{-1}$) [24] and the SPME-GC–MS (0.51–170 μg $L^{-1}$) [12].

#### 3.3.3. Repeatability

A satisfactory relative standard deviation (RSD) of the peak areas was obtained (1.01–12.5%) for the tested FAs (Table 2).

### 3.4. Analysis of vegetable oils

The proposed method was applied for the profiling of FAs in palm oils (crude, olein, kernel and carotino cooking oils) and other vegetable oils (soybean, olive, coconut, rice bran and pumpkin oils). Quantification was done using an eight points external standard calibration assay of the ratio of peak area to that of the IS versus concentration of analyte. Qualitative analysis was done by comparing the retention times to those of the standards. Using the LLE in conjunction with GC-FID as suggested in the standard AOCS and MPOB methods, only the major FAs ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$) can be determined in crude and olein palm oils (Table 3). The minor FAs ($C_{12:0}$, $C_{20:0}$, $C_{16:1}$, $C_{18:3}$) cannot be determined using the standard methods. Reasonable agreement of the major FAs was found between the LPME results and the standard method for the samples tested. The enormous complexity of the matrix especially the crude palm and carotino cooking oils do affect the extraction process. The

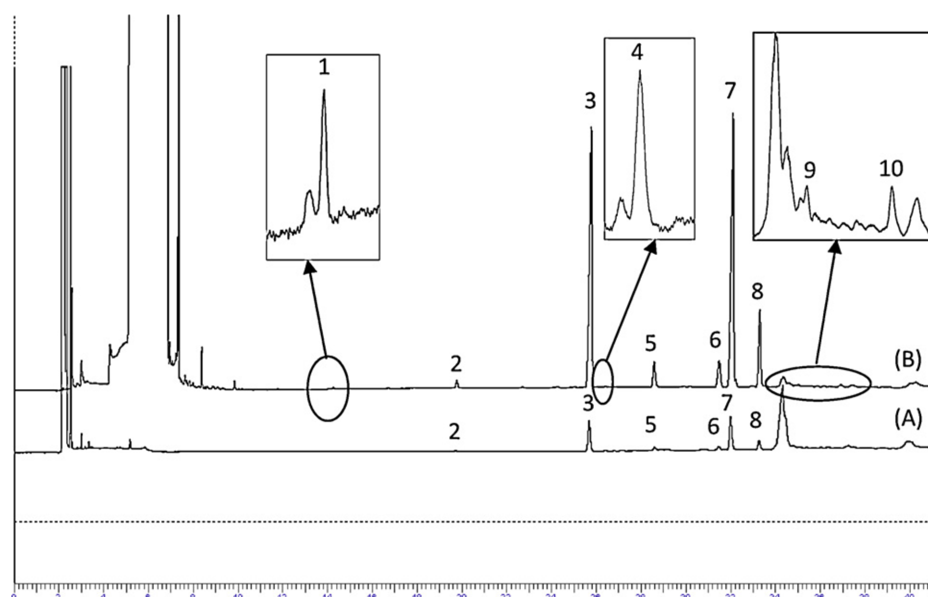


**Fig. 6.** Profiling of FAs of palm olein sample after (A) LLE, and (B) LPME. Peak assignments: (1) lauric, (2) myristic, (3) palmitic, (4) palmitoleic, (5) IS, (6) stearic, (7) oleic, (8) linoleic, (9) linolenic, and (10) arachidic acids.

**Table 3**
Composition of FAs in vegetable oils analysed using the proposed LPME-GC-FID and the LLE-GC-FID methods.[a]

| FAs | % Fatty acid | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Palm oil | | | | | | | | Other oils | | | | | |
| | CPO1 | CPO2 | PO1 | PO2 | PO3 | PO4 | CPKO | Carotino | Soybean oil 1 | Soybean oil 2 | Olive oil | Coconut oil | Pumpkin oil | Rice bran oil |
| $C_{12:0}$ | 0.21 | 0.61 | 0.28 (ND) | 0.33 | 0.28 | 0.32 | 35.8 | ND | 0.02 | 0.44 (ND) | 0.02 | 38.4 | 0.02 (ND) | 0.02 |
| $C_{14:0}$ | 4.48 | 4.90 | 1.50 (1.50) | 4.08 | 4.36 | 4.46 | 38.0 | 1.56 | 1.81 | 0.85 (0.41) | ND | 43.4 | 1.94 (0.43) | 2.26 |
| $C_{16:0}$ | 45.9 | 45.9 | 43.0 (39.3) | 43.9 | 40.3 | 40.9 | 5.76 | 15.2 | 12.3 | 12.8 (13.5) | 13.5 | 5.48 | 13.7 (13.7) | 22.5 |
| $C_{18:0}$ | 4.87 | 6.63 | 4.30 (4.70) | 4.97 | 4.64 | 4.46 | 1.67 | 3.82 | 5.12 | 5.30 (7.7) | 4.44 | 2.11 | 7.72 (7.06) | 2.79 |
| $C_{20:0}$ | 0.42 | 0.31 | 0.25 (ND) | 0.36 | 0.39 | 0.44 | 0.07 | 0.42 | 0.45 | 0.34 (ND) | 0.50 | 0.06 | 0.43 (ND) | 0.92 |
| $C_{16:1}$ | 0.20 | 0.25 | 0.26 (ND) | 0.27 | 0.27 | 0.28 | ND | 0.27 | ND | ND (ND) | 0.79 | ND | ND (ND) | 0.29 |
| $C_{18:1}$ | 32.4 | 31.4 | 38.0 (43.9) | 35.0 | 37.0 | 37.5 | 8.19 | 54.4 | 17.9 | 20.4 (22.5) | 72.7 | 2.64 | 28.8 (28.0) | 35.2 |
| $C_{18:2}$ | 10.5 | 10.1 | 12.5 (9.5) | 10.8 | 12.5 | 11.8 | 1.67 | 20.3 | 54.3 | 53.2 (49.5) | 8.00 | 0.63 | 47.1 (47.0) | 34.9 |
| $C_{18:3}$ | 0.62 | 0.02 | 0.24 (ND) | 0.17 | 0.18 | 0.30 | 0.02 | 5.90 | 7.16 | 5.93 (4.62) | 0.48 | 0.01 | 0.17 (ND) | 1.21 |
| Total sat. | 55.9 | 58.4 | 49.3 (45.5) | 50.0 | 50.0 | 50.6 | 81.3 | 21.0 | 19.7 | 19.8 (21.6) | 18.5 | 89.4 | 23.8 (21.2) | 28.5 |
| Total unsat. | 43.7 | 41.7 | 51.0 (53.4) | 46.2 | 50.0 | 49.9 | 9.88 | 80.9 | 79.3 | 79.5 (76.6) | 82.0 | 3.28 | 76.0 (75.0) | 71.6 |
| Total | 99.6 | 100.0 | 100.3 (98.9) | 99.9 | 100.0 | 100.5 | 91.2 | 101.9 | 99.0 | 99.3 (98.2) | 100.5 | 92.6 | 99.8 (96.2) | 100.1 |

CPO—crude palm oil; PO—palm olein oil; CPKO—crude palm kernel oil.

[a] Figures in brackets refer to LLE-GC-FID results.

good enrichments of the FAMEs from the proposed LPME to that of the LLE techniques are evident from the chromatograms (Fig. 6).

The compositions of the FAs in crude palm oil and palm olein are in agreement to the previous reports based on GC–MS analysis [32]. The palm kernel oil is dominated by the shorter chain length FAs ($C_{8:0}$, $C_{12:0}$, $C_{14:0}$), its total saturation is high, making it suitable for frying and baking purposes [2]. Carotino cooking oil is a unique blend of palm and canola oils which is produced from a process that involves a deacidification and deodourisation of red palm oil, retaining as much as 80% of the original carotenoids [33]. The high carotenoids impart a distinct red colour to this oil. This highly coloured oil was found to be rich in unsaturated FAs (80.9%), comparable to soy bean, olive, pumpkin and rice bran oils (Table 3). In particular, it is rich in linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$). The amount of all FAs in the other vegetable oils is consistent to those of the reported GC–MS [32] work. The amount of sample and organic solvent consumed are significantly less compared to the SPE or the LLE methods [16].

## 4. Conclusions

A two phase hollow fiber LPME method for the extraction of FAMEs in vegetable oils has been successfully developed. Under the optimized conditions, enrichments of 37–115, depending on the FAME, were achieved. In conjunction with the GC-FID, the major ($C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$) and minor FAMEs ($C_{12:0}$, $C_{20:0}$, $C_{16:1}$, $C_{18:3}$) in palm oils can be simultaneously determined, a feature that is not possible using the standard methods. The official method involving LLE and GC-FID is not sensitive enough to determine these minor FAMEs. Enrichments and speed of extraction can be further improved by using the dynamic [34] and electromigration [35] approaches, respectively. The good enrichments that allows the profiling of the minor FAMEs opens up many exciting possibilities for further studies, e.g., oil adulteration, shelf life, medical diagnostics, etc.

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