

Evaluation of Major Fatty Acids Determination in Palm Oil by Gas Chromatography-Flame Ionization Detection

Moh. Taufik^{1,3}, Hanifah Nuryani Lioe², Nancy Dewi Yuliana²

¹Food Science Study Program, Bogor Agricultural University, IPB Darmaga Campus, Bogor 16680, Indonesia

²Department of Food Science and Technology, Faculty of Agricultural Technology and Engineering, Bogor Agricultural University, IPB Darmaga Campus, PO Box 220, Bogor, 16680, Indonesia

³Department of Food Science and Technology, Faculty of Bioindustry, Trilogi University, Jl. TMP. Kalibata No. 1, Jakarta Selatan, Daerah Khusus Ibukota Jakarta 12760, Indonesia

E-mail: hanilioe@hotmail.com

Submisi: 19 Mei 2015; Penerimaan: 8 September 2015

ABSTRACT

The fatty acid composition of palm oil is the major factor influencing its physical and chemical properties. The purpose of this research was to evaluate the analytical performance of major fatty acids (palmitic acid, stearic acid, oleic acid, and linoleic acid) analysis in palm oil. Triglycerides of palm oil were derivatized to fatty acid methyl esters (FAMES) by using boron trifluoride (BF₃) in methanol. FAMES were determined by gas chromatography-flame ionization detection (GC-FID) using DB-23 capillary column as stationary phase. The studied parameters were instrument performance analysis, the efficiency of fatty acid derivatization, stability of derivatized analytes, accuracy, repeatability, intra-lab reproducibility, ruggedness, and method uncertainty. The evaluation results showed the instrument linearity at a working range of 5 to 40 mg/mL marked by coefficient of determination (R^2) between 0.991-0.995. Instrument limits of detection (LOD) and instrument limits of quantification (LOQ) for 4 major fatty acids analysis were 26-35 µg/mL and 86-128 µg/mL, respectively. The increase of fatty acid concentration led to the decrease of derivatization efficiency in the fatty acids analysis. The result also showed that derivatized analytes were stable during 24 h storage at freeze temperature. The average recovery values by spiking method with the spiking concentration at 50 and 90 mg/g sample were at 75-94 % for stearic and linoleic acids analysis, however those for palmitic and oleic acids analysis were considered very low (<40 %), due to their low derivatization efficiency. Repeatability and intra-lab reproducibility of 4 major fatty acids analysis were at acceptable ranges, 0.45-1.38 % and 1.15-2.03 %, respectively. Determination by varying the volume of derivatizing agent showed the rugged method. Uncertainty of repeatability (U_r) and uncertainty of reproducibility (U_p) were ranged at 1.84-9.02 mg/g and 1.40-10.65 mg/g, respectively. This method was considerably reliable for the analysis of less abundance fatty acids in palm oil, stearic and linoleic acids.

Keywords: Analytical evaluation; fatty acid analysis; palm oil; gas chromatography-flame ionization detection; fatty acid derivatization

INTRODUCTION

Vegetable oils play an important role in the functional and sensorial properties of food and also have a function as carrier for fat soluble vitamins (Moreira *et al.*, 1999). The composition of fatty acids affects the physical and chemical characteristic of the oil (Knothe and Kenar, 2004). One of the important vegetable oils is palm oil. According to Gunstone

(2011), palm oil is consumed in many countries and also produced in the greatest amount. The major fatty acids in palm oil reported by Sundram *et al.* (2003), Gee (2007), Kumar and Krishna (2014) were palmitic acid (43.5 %, 44.1 % and 41.8 %), oleic acid (39.8 %, 39.0 % and 37.4 %), linoleic acid (10.2 %, 10.6 % and 14.1 %) and stearic acid (4.3 %, 4.4 % and 3.5 %).

Data of fatty acid composition is very important for food scientist and nutritionist to develop dietary formulation, food processing and new product (Muhamad and Mohamad, 2012). Furthermore, data of fatty acid composition can also be used to create nutrition facts of the product, especially to provide the facts of total saturated and unsaturated fat composition. Several methods have been introduced to determine the fatty acid composition of vegetable and animal oils. The most widely used technique to analyze the fatty acid composition is gas chromatography coupled with flame ionization detector (GC-FID) (Martinez *et al.*, 2009). The flame ionization detection is adequate for analytical applications of fatty acids (Petrovic *et al.*, 2010). This technique based on the AOAC Official Method 991.39 (AOAC, 2012). This official method basically provides clear guidelines for analyzing the fatty acid composition in fish oils.

Lipids must be converted into fatty acid methyl esters (FAMES) to achieve their volatility for GC analysis. There are many different derivatization methods in literature. The commonly used derivatization method in the analysis of fatty acids involves basic and acid catalysis. One of the basic catalysts commonly used in the derivatization of triacylglycerol is sodium methoxide (NaOCH_3) in methanol (Milinsk *et al.*, 2008). Derivatization by using NaOCH_3 in methanol can be implemented at room temperature in a short time (Macedo *et al.*, 2012), but the basic catalyst cannot transform free fatty acids into FAMES including free fatty acids used as internal standard (Ichihara and Fukubayashi, 2010). Boron trifluoride (BF_3) in methanol is one of the most commonly used acid catalysts as a derivatizing agent because this catalyst has high esterifying power (Liu, 1994). BF_3 can catalyze the methylation of fatty acids in both free and esterified forms in the presence of methanol (Wirasmita *et al.*, 2013). According to Knapp (1979), derivatization triglycerides to be FAMES by using acid catalyst can be divided in two steps i.e. saponification and esterification reaction. BF_3 can accelerate the esterification reaction between the salt of corresponding fatty acid and methanol.

Many researchers have studied about the evaluation of fatty acid analysis in some products. Omar and Salimon (2013) evaluated the fatty acid analysis in bakery products by using GC-FID and NaOCH_3 as catalyst. The results of Omar and Salimon (2013) showed that linearity for palmitic acid, oleic acid and linoleic acid analysis at concentration of 0.1 to 24 $\mu\text{g/mL}$ ranged from 0.993 to 0.998, while the recovery values and repeatability were ranged at 96.83-98.54 % and 0.56-0.71 %, respectively. Tong (2007) studied about palmitic acid, oleic acid and linoleic acid analysis in *Periploca sepium* using GC-FID and BF_3 in ether as catalyst. Tong *et al.* (2007) found that the recovery values were ranged at 99.2-100.2 %, while repeatability ranged from 0.5 % to 0.7 %.

According to AOAC Official Method 991.39 (AOAC, 2012), the amount of sample to be analyzed was 25 mg, while the volume of BF_3 methanolic (12% w/v) used was 2.0 mL. In fact, increasing the amount of sample will impact the improving of detectability of gas chromatography to the smallest concentration compound in sample (Orata, 2012). Parashar *et al.* (2009) used 200 mg of initial sample and 4.4 mL of BF_3 in ether to analyze the fatty acid composition of pomegranate oil. Wirasmita *et al.* (2013) used 350 mg of sample and 7 mL of BF_3 methanol (12%, w/v) to analyze the fatty acid composition of pumpkins oil. Tong *et al.* (2007) used 100 mg of initial sample and 2.0 ml of BF_3 in ether to analyze the fatty acid composition of *Periploca sepium*.

The objective of this research was to provide the analytical performance of major fatty acids analysis in palm oil using BF_3 in methanol for derivatization and GC-FID for determination. The analysis was performed at 100 mg sample weight. The parameters studied were chromatographic precision, instrument linearity, instrument limit of detection (LOD), instrument limit of quantification (LOQ), the efficiency of fatty acid derivatization, stability of derivatized analytes, accuracy, repeatability, intra-lab reproducibility, ruggedness, and method uncertainty.

MATERIALS AND METHODS

Materials

Palm oil was purchased from local market in Bogor, Indonesia. Palmitic acid (minimum purity 99 %), stearic acid (purity approximately 99 %), oleic acid (purity approximately 95 %), linoleic acid (purity approximately ≥ 99 %) and margaric acid (purity approximately 99 %) were purchased from Sigma-Aldrich (Steinheim, Germany). External standard (FAME Mix C8-C22) was purchased from Supelco (Bellefonte, US). Hexane, p.a., Na_2SO_4 anhydrous, p.a., NaOH, NaCl and methanol were purchased from Merck (Darmstadt, Germany). BF_3 in methanol (14 %, wt/vol) was purchased from Merck (Hohenbrunn, Germany).

Fatty Acids Analysis in Palm Oil

Derivatization

The derivatization was conducted following the procedure described by AOAC Official Method 991.39 (AOAC, 2012), with modification in the amount of initial sample, the kind of extracting solvent and the temperature of heating. Approximately 100 mg of palm oil was placed into a screw cap glass tube. As much as 0.1 mL of margaric acid solution (10 mg/mL) as internal standard and 1.5 mL of NaOH in methanol (0.5 N) were added into the tube and then nitrogen

was blown for 15 seconds to the tube. The tube was covered tightly, vortexed with a vortex 16700 Mixer Thermolyne (Maxi-Mix 1, Iowa, US), heated in a water bath W350 Memmert (Memmert GmbH and Co, Schwabach, Germany) for 5 minutes at 87 °C and then cooled. Furthermore, the tube was added by 2 mL of BF₃ in methanol (14 %, wt/vol), vortexed and then nitrogen was blown to the tube. The tube was covered tightly, heated for 30 minutes at 87 °C and then cooled. In this stage, fatty acids were converted to fatty acid methyl esters (FAMES). Extracting solvent, 1 mL of hexane, was added and then the mix was vortexed. Furthermore, 3 mL of saturated NaCl solution was added and then the mix solution was vortexed. Upper phase was transferred into the vial. Na₂SO₄ anhydrous was added into the vial to absorb moisture.

GC Analysis

FAMES from external standard or FAMES resulted from sample derivatization were injected separately into Gas Chromatography instrument (GC). The GC analyses were performed on 7890A Gas Chromatography System (Agilent Technologies, California, US) equipped with flame ionization detector and splitless injector (1 µL). Injector and detector temperature were set at 270 °C and 280 °C, respectively. The utilized column was a DB-23 (60 m × 0.25 mm, with film thickness of 0.25 µm). This column was purchased from J and W Scientific (Folsom, CA). The GC oven program was as follows: 130 °C (hold 2 min), to 170 °C at 6.5 °C/min (hold 5 min), to 215 °C at 2.75 °C/min (hold 12 min), to 230 °C at 30 °C/min (hold 30 min). Helium and nitrogen of ultrahigh purity grade were used as carrier gases at flow rates of 11.07 and 31.24 mL/min.

Identification and Concentration of Fatty Acid

The fatty acid identification was determined by comparing retention time of the peaks with the respective external standards. The concentration of fatty acids was calculated with the following equation (AOAC, 2012).

$$[ALx] = \frac{Aalxs}{ASIs} \times \frac{[BSIs]}{BS} \times \frac{ASle}{Aalxe} \times \frac{[Balxe]}{[BSle]} \times 1000 \quad (1)$$

Where:

- [ALx] = Concentration of fatty acid X on sample (mg/g)
- Aalxs = Area of fatty acid X on chromatogram of sample
- ASIs = Area of margaric acid (internal standard) on chromatogram of sample
- [BSIs] = Concentration of margaric acid (internal standard) on sample
- BS = Weigh of sample (mg)

ASle = Area of margaric acid (internal standard) on chromatogram of external standard

Aalxe = Area of fatty acid X on chromatogram of external standard

[Balxe] = Concentration of fatty acid X on external standard

[BSle] = Concentration of margaric acid (internal standard) on external standard

Instrument Performance Analysis

Instrument performance analysis included chromatographic precision, instrument linearity, instrument limit of quantification (LOQ) and instrument limit of detection (LOD). The chromatographic precision was examined by following the method described by Ping (2013) i.e. using Coefficient of Variation (CV) of retention time and peak area from the injection multiple times. In this study, the chromatographic precision was determined by analysing seven replicates of 1 mL a fatty acid mix standard solution containing palmitic, stearic, oleic and linoleic acids at concentration of each fatty acid of 10 mg/mL in hexane solution. The chromatographic precision was expressed as CV (%) of retention time and peak area of every fatty acid in the mix solution as observed in their chromatogram. The CV of retention time and peak area was calculated with the following equation.

$$CV = \frac{SD}{\bar{x}} \times 100 \% \quad (2)$$

Where:

CV = Coefficient of Variation (%)

SD = Standard deviation of retention time or peak area

\bar{x} = Mean value of retention time or peak area

The instrument linearity was evaluated through a calibration curve by making a plot between peak area ratios of each fatty acid to internal standard versus the corresponding fatty acid concentration. The serial concentration of each fatty acid in fatty acids mix standard solutions were 5, 10, 20, 30 and 40 mg/mL. The fatty acid mix standard solutions, containing palmitic, stearic, oleic and linoleic acids, were analyzed as much as 1 mL in duplicate. The instrument linearity was observed by determining the coefficient of determination (R²).

LOD and LOQ were determined from the standard deviation (SD) of seven determinations at a low concentration of fatty acid mix standard solution at concentration of each fatty acid of 10 mg/mL. The fatty acid mix standard solution, containing palmitic, stearic, oleic and linoleic acids, was analyzed as much as 1 mL. LOD was calculated as 3 SD, while LOQ was calculated as 3.3 LOD (Squadrone *et al.*, 2010).

The Efficiency of Fatty Acid Derivatization

The efficiency of fatty acid derivatization was evaluated using fatty acids mix standard solutions at four concentrations of each fatty acid, i.e. 10, 20, 30 and 40 mg/mL. One mL of each fatty acids mix standard solution, containing palmitic, stearic, oleic and linoleic acids, was analyzed in duplicate. The efficiency of each fatty acid derivatization by BF₃-methanol was determined from its respective recovery value. Recovery values can be calculated by dividing the obtained concentrations to the true concentrations of fatty acid (Kazusaki *et al.*, 2012).

Stability of Derivatized Analytes

The stability of derivatized analytes stored at freeze temperature (-10 ± 2 °C) prior to GC injection was examined by following the method described by Petrovic *et al.* (2010) i.e. analyzing derivatized analytes during a certain period. The determination of derivatized analytes resulted from palm oil derivatization was performed at 0 and 24 h after storage. This analysis was done in duplicate. Statistical analysis by t-test was performed to determine the stability of derivatized analytes stored at the freeze temperature.

Evaluation of Analytical Method

Accuracy and repeatability

The accuracy was determined by recovery test. The spiking method using palm oil sample (100 mg) at spiking concentrations of 50 and 90 mg fatty acid/g palm oil was performed. The samples were analyzed in five replicates for spiked sample and duplicate for non-spiked sample. Repeatability was expressed as relative CV (%) from spiked sample, while accuracy was calculated as recovery (AOAC 2012).

Intra-lab reproducibility

Intra-lab reproducibility was evaluated by analyzing palm oil with the same instrument, same operator but at three

different months. The replication of each analysis was done in duplicate. Intralab reproducibility was expressed as CV (%).

Ruggedness

According to AOAC (2012), the volume of BF₃ in methanol used in fatty acid derivatization was 2.0 mL. In this study, ruggedness of the method was evaluated by varying the volume of BF₃ in methanol (14 %, wt/vol) i.e 1.8, 2.0 and 2.2 mL. Palm oil was used for this analysis. Each analysis was done in duplicate. Statistical analysis by one-way ANOVA was performed to determine the ruggedness of this method.

Uncertainty of Repeatability (U_r) and Uncertainty of Reproducibility (U_R)

The calculation of U_r and U_R followed Česnik and Gregorčič (2006). U_r and U_R were determined with the following equation

$$U_r = t_{95;9} \times SD_r; U_R = t_{95;9} \times SD_R \quad (3)$$

Where:

SD_r = standard deviation of repeatability,

SD_R = standard deviation of intra-lab reproducibility and

t_{95;9} = student's t factor for 9 degrees of freedom and 95% confidence level (t_{95;9} = 2.262).

RESULTS AND DISCUSSION

Instrument Performance Analysis

Chromatographic precision shows the stability of gas chromatography-flame ionization detector (GC-FID) with certain conditions and parameters in detecting fatty acids. The chromatographic precision of retention time and peak area for palmitic acid, stearic acid, oleic acid and linoleic acid are presented in Table 1. CV of retention time and peak area for palmitic acid and stearic acid of this result is comparable to that performed by Ping (2013) i.e. 0.03-0.09 % for retention time and 0.21-1.55 % for peak area. CV of retention time

Table 1. Precision of retention time and peak area in fatty acids analysis by GC-FID with BF₃-methanol derivatization, evaluated from 7 determinations of fatty acids at low concentration

Fatty acid	Concentration (mg/mL)	Average		CV (%)	
		Retention time (min)	Peak area	Retention time	Peak area
Margaric acid (C17:0)	10	39.251 ± 0.030	437.08 ± 7.12	0.08	1.63
Palmitic acid (C16:0)	10	35.777 ± 0.024	3940.91 ± 65.11	0.07	1.65
Stearic acid (C18:0)	10	43.114 ± 0.051	4040.24 ± 66.64	0.12	1.65
Oleic acid (C18:1)	10	44.413 ± 0.039	4245.42 ± 69.72	0.09	1.64
Linoleic acid (C18:2)	10	46.831 ± 0.040	4355.91 ± 72.84	0.08	1.67

and peak area was also lower than 2.0 %. Maximum CV for chromatographic precision is 2.0% (JECFA, 2006). This result indicated that the GC-FID with conditions and parameters used was stable in detecting fatty acids.

Instrument linearity is the ability of analytical methods to show a proportional response to the concentration of analyte at a given range (Kazusaki *et al.*, 2012). Instrument linearity was verified at range of fatty acid concentration from 5 to 40 mg/mL. The results of instrument linearity for palmitic acid, stearic acid, oleic acid and palmitic acid are shown in Table 2. Obtained results showed that the determination coefficient (R^2) values for all fatty acids were acceptable. A linear regression with an R^2 value > 0.990 is a very good fit (AOAC, 2012). Instrument limit of detection (LOD) is the lowest concentration of the analyte in standard solution that can be detected but not necessarily quantified, while instrument limit of quantification (LOQ) is the lowest concentration of the analyte in standard solution that can be quantified with acceptable precision. LOD and LOQ of this research can be seen in Table 3. LOQ of this result is comparable to that performed by Tong *et al.* (2007) i.e. LOQ at a range of 19-87 $\mu\text{g/mL}$. Tong *et al.* (2007) validated a method to determine fatty acid composition in vegetable oil by GC-FID with BF_3 -methanol derivatization. In derivatization step, Tong *et al.* (2007) used the larger NaOH-methanol volume and the shorter reaction time i.e. 5 mL and 5 min, respectively. According to Wang (2008), LOD and LOQ values are affected by physicochemical properties of the analytes, methods used and instrumental parameter.

Table 2. Result of instrument linearity of fatty acids analysis by GC-FID with BF_3 -methanol derivatization (N=2)

Fatty acid	Regression equation	R^2
Palmitic acid (C16:0)	$y = 4.109x + 0.070$	0.994
Stearic acid (C18:0)	$y = 4.072x - 0.123$	0.995
Oleic acid (C18:1)	$y = 3.827x + 0.130$	0.991
Linoleic acid (C18:2)	$y = 5.186x - 0.010$	0.993

Table 3. LOD and LOQ of fatty acids analysis by GC-FID with BF_3 - methanol derivatization from determined by 7 determinations of fatty acids at low concentration

Fatty acid	Instrument limit of detection (LOD) ($\mu\text{g/mL}$)	Instrument limit of quantification (LOQ) ($\mu\text{g/mL}$)
Palmitic acid (C16:0)	26	86
Stearic acid (C18:0)	35	116
Oleic acid (C18:1)	27	89
Linoleic acid (C18:2)	38	128

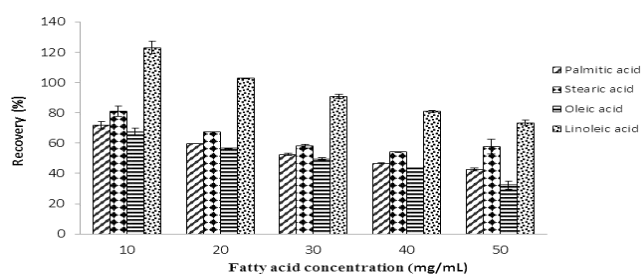


Figure 1. The efficiency of fatty acid derivatization by BF_3 -methanol in fatty acids analysis by GC-FID at different concentrations of fatty acids in the mix standard solution.

The Efficiency of Fatty Acid Derivatization

The efficiency of fatty acid derivatization indicates the percentage of fatty acid in sample which can be derivatized by derivatizing agent and detected by GC-FID instrument. The efficiency of derivatization can be predicted by the recovery value of fatty acid standard analysis. The efficiency of derivatization for palmitic acid, stearic acid, oleic acid and linoleic acid analyses at four concentrations are shown in Figure 1. In this study, it is revealed that at the same concentration of 4 fatty acids, the efficiency of derivatization was different to each other.

The derivatization efficiency showed by recovery values was higher for stearic and linoleic acids (averaged 65.2-99.5 %), however for palmitic and oleic acids gave lower efficiency (averaged 54.3-57.7 %). The low recovery might be affected by the shorter reaction time, lower reaction temperature and methanol volume used in derivatization step in this study. Hallmann *et al.* (2008) analyzed the efficiency of BF_3 to derivatize fatty acid into fatty acid butyl ester (FABEs). The research result of Hallmann *et al.* (2008) showed that the efficiency of fatty acid derivatization can be improved by the increase of reaction time, reaction temperature and methanol volume.

The efficiency of derivatization decreased with the increase of fatty acids concentration to be analyzed. The low recovery for higher concentration of fatty acids might be caused by the insufficient amount of BF_3 -methanol used in this study. The efficiency of derivatization can be increased by using high volume of catalyst or small amount of sample. Liu (1994) recommended to use small amount of sample in fatty acid derivatization.

Stability of Derivatized Analytes

According to Paligova *et al.* (2008), derivatized analytes (FAMES) have low storage stability due to their high potential to be hydrolyzed and oxidized. However, the stability of the FAMES during freeze storage 24 h has been proved to be acceptable as presented in Table 4.

Table 4. Stability of fatty acid methyl esters (FAMES) with and without freeze storage 24 h

Freeze storage (h)	Fatty acid (mg/g)			
	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)
0	241.6 ± 0.4	28.1 ± 0.1	283.0 ± 0.7	104.0 ± 0.2
24	240.5 ± 2.2	28.0 ± 0.1	281.0 ± 0.2	102.0 ± 0.1

No significant difference in fatty acid concentrations was observed in the analysis of fatty acids in palm oil with and without freeze storage 24 h of analytes solution after derivatization. This result indicated that FAMES was stable during 24 h storage at freeze temperature. According to Ashraful *et al.* (2014), the main factor affecting FAMES oxidation is storage temperature. Freeze storage temperature (-6.8 ± 0.3 °C) was predicted causing the stability of FAMES during storage. In this study, FAMES resulted from palm oil derivatization was stored in dark vial. FAME samples stored in dark glass vessels are more stable (Paligova' *et al.*, 2008).

Evaluation of Analytical Method

Accuracy and repeatability

Accuracy describes the closeness of the analytical results obtained to the true values of the analyte (Kazusaki *et al.*, 2012). Accuracy was evaluated by recovery experiment at two spiking concentrations. The recovery values of palmitic acid, stearic acid, oleic acid and linoleic acid analysis at two spiking concentrations are summarized in Table 5. These four major fatty acid were totally present at concentration of 687-690 mg/g in non-spiked palm oil (N=4).

Table 5. Recovery and repeatability of four fatty acids in palm oil at two spiking concentrations analyzed by GC-FID with BF₃-methanol derivatization (N=5)

Fatty acid	Concentration (mg/g)			Recovery (%)	Repeatability	
	Initial	Spiking	Found		CV analysis (%)	2/3 CV Horwitz (%)
Palmitic acid (C16:0)	256.3 ± 1.5	50.0 ± 3.2	273.4 ± 2.2	34.34 ± 5.60	0.79	1.62
Stearic acid (C18:0)	31.2 ± 0.1	54.1 ± 3.4	72.8 ± 0.8	77.06 ± 3.91	1.12	1.98
Oleic acid (C18:1)	302.6 ± 2.1	48.8 ± 3.1	313.8 ± 2.9	23.20 ± 6.60	0.91	1.59
Linoleic acid (C18:2)	110.3 ± 1.4	49.4 ± 3.1	155.5 ± 0.9	93.65 ± 5.69	0.56	1.76
Palmitic acid (C16:0)	255.6 ± 1.7	90.0 ± 3.6	284.8 ± 1.3	32.47 ± 2.46	0.45	1.61
Stearic acid (C18:0)	30.34 ± 0.1	97.4 ± 3.9	103.0 ± 1.4	74.68 ± 1.66	1.38	1.88
Oleic acid (C18:1)	301.6 ± 2.2	87.9 ± 3.5	322.8 ± 3.9	24.24 ± 4.89	1.24	1.58
Linoleic acid (C18:2)	110.1 ± 0.6	87.2 ± 3.5	188.5 ± 1.9	89.98 ± 2.00	1.02	1.71

The averaged recovery values by spiking method at 50 and 90 mg/g spiking concentration for palmitic acid and oleic acid were very low because the efficiency of BF₃-methanol derivatization for these fatty acids at given concentration was also very low (derivatization efficiency: 53 % for palmitic acid and 49 % for oleic acid). The averaged recovery values for stearic acid and linoleic acid analysis were higher. The higher recovery might be resulted from the high efficiency of BF₃ in methanol derivatization for stearic acid and linoleic acid at found concentration (81 % for stearic acid and 104 % for linoleic acid).

Repeatability is the variation from multiple analyses of a single homogeneous sample (Kazusaki, 2012). Repeatability was calculated as CV (%) of fatty acids from spiked sample. CV resulted from analysis and CV calculated by Horwitz formula according to AOAC PVM (as an acceptable CV value, 2/3 CV Horwitz) at two spiking concentrations are presented in Table 5. CV analysis from this research was lower than 2/3 CV Horwitz. This result suggested that repeatability of this method was acceptable. The CV values obtained were also lower than the result of Tong *et al.* (2007). Tong *et al.* (2007) reported that CV for palmitic acid, oleic acid and linoleic acid analysis was 2.1, 4.4 and 2.9 %, respectively.

Intra-lab Reproducibility

Intra-laboratory reproducibility indicates the degree of variation from analyses at different time. CV of intra-lab reproducibility from the results of the major fatty acids analysis in palm oil in three different months can be seen in Table 6.

Table 6. Intra-lab reproducibility of fatty acids analysis by GC-FID with BF₃-methanol derivatization (N=2)

Months	Fatty acid (mg/g)			
	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)
I	248.6 ± 0.6	30.0 ± 0.1	294.0 ± 0.7	108.0 ± 0.4
II	256.3 ± 1.5	31.2 ± 0.1	302.6 ± 2.1	110.3 ± 1.4
III	255.6 ± 1.7	30.3 ± 0.1	301.6 ± 2.2	110.1 ± 0.6
CV analysis (%)	1.68	2.03	1.57	1.15
CV Horwitz (%)	2.46	3.38	2.40	2.79

CV analysis for palmitic acid, stearic acid, oleic acid and linoleic acid analysis ranged from 1.15 % to 2.03 %. CV analysis from this research was lower than CV Horwitz. This result indicated that reproducibility of this method was acceptable at three mounts analysis. The CV values obtained were comparable to other research result as reported by Omar and Salimon (2013) who validated a gas chromatographic method for determining fatty acids in bakery products. They reported that CV for palmitic acid, oleic acid and linoleic acid analysis ranged from 1.83 to 4.09 %.

Ruggedness

Ruggedness is the ability of a method to not be affected by variation in reagent or procedure analysis. Ruggedness of this method was evaluated by the variation of BF₃ volume i.e. 1.8, 2.0 and 2.2 mL. BF₃-methanol is a catalyst in this fatty acid analysis.

The analysis results of major fatty acids analysis in palm oil with variation of BF₃-methanol volume are given in Table 7. The result of one-way ANOVA showed that the concentration of fatty acids at every treatment was no significant difference. It suggested that derivatization method was rugged to the variation of BF₃-methanol volume in the given range (1.8 – 2.2 mL, 14 % w/v).

Table 7. Ruggedness of fatty acids analysis by GC-FID with different volumes of BF₃-methanol solution for fatty acids derivatization (N=2)

BF ₃ -methanol volume (mL)	Fatty acid (mg/g)			
	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)
1.8	230.8 ± 7.5	26.9 ± 0.7	271.6 ± 9.1	99.6 ± 3.2
2.0	241.6 ± 0.4	28.1 ± 0.1	283.0 ± 0.7	104.0 ± 0.2
2.2	243.8 ± 0.1	28.4 ± 0.2	290.0 ± 6.3	106.8 ± 2.3

Table 8. Uncertainty of repeatability (U_r) and uncertainty of reproducibility (U_r) of fatty acids determination in palm oil by GC-FID with BF₃-methanol derivatization

Fatty acid	U _r (mg/g)		U _R (mg/g)
	Spiking concentration		
	50 mg/g	90 mg/g	
Palmitic acid (C16:0)	4.91	2.91	9.65
Stearic acid (C18:0)	1.84	3.22	1.40
Oleic acid (C18:1)	6.49	9.02	10.65
Linoleic acid (C18:2)	1.99	4.35	2.84

Uncertainty of Repeatability and Uncertainty of Reproducibility

There are many sources of uncertainty in the analysis, two of which are uncertainty of repeatability (U_r) and reproducibility (U_R). U_r and U_R from the analysis of major fatty acids analysis in palm oil by GC-FID are presented in Table 8.

CONCLUSIONS

The major fatty acids analysis in palm oil by using gas chromatography-flame ionization detection (GC-FID) has been evaluated and shown the acceptable results for stearic and linoleic acid analysis in terms of chromatographic precision, accuracy by recovery test, repeatability, intra-lab reproducibility and ruggedness. Due to the lower efficiency of fatty acid derivatization by BF₃-methanol, the recovery values of palmitic and oleic acids were very low, less than 40 %. This method was considerably reliable for the analysis of less abundance fatty acids in palm oil, stearic and linoleic acids.

ACKNOWLEDGEMENTS

We thank Yane Regiyana for her technical help to use GC-FID at Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Indonesia.

REFERENCES

- AOAC. (2012). *Official Methods of Analysis of AOAC International* (19th ed.). AOAC International Press, Maryland. Chapter 41, p 27-29 and Appendix K, p 8-10.
- Ashraful, A.M., Masjuki, H.H., Kalam, M.A., Rahman, S.M.A., Habibullah, M. and Syazwan, M. (2014). Study of the effect of storage time on the oxidation and

- thermal stability of various biodiesels and their blends. *Energy Fuels* **28**(2): 1081-1089.
- Česnik, H.B. and Gregorčič, A. (2006). Validation of the method for the determination of dithiocarbamates and thiuram disulphide on apple, lettuce, potato, strawberry and tomato matrix. *Acta Chimica Slovenica* **53**: 100-104.
- Gee, P.T. (2007). Analytical characteristics of crude and refined palm oil and fractions. *European Journal of Lipid Science and Technology* **109**(4): 373-379.
- Gunstone, F.D. (2011). *Vegetable Oils in Food Technology: Composition, Properties and Uses, Second Edition*. Blackwell Publishing, Oxford. p 8.
- Hallmann, C., Van aarssen, B.G.K. and Grice, K. (2008). Relative efficiency of free fatty acid butyl esterification, choice of catalyst and derivatisation Procedure. *Journal of Chromatography A* **1198-1199**: 14-20.
- Ichihara, K. and Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal of Lipid Research* **51**(3): 635-640.
- JECFA. (2006). *Analytical methods, Test Procedures and Laboratory Solutions Used by and Referenced in the Food Additive Specifications*. Food and Agriculture Organization of The United Nations, Rome. p 23.
- Kazusaki, M., Ueda, S., Takeuchi, N. and Ohgami, Y. (2012). Validation of analytical procedures by high-performance liquid chromatography for pharmaceutical analysis. *Chromatography* **33**(2): 65-73.
- Knapp, D.R. (1979). *Handbook of Analytical Derivatization Reactions*. Wiley-Interscience, Canada. p 148.
- Knothe, G. and Kenar, J.A. (2004). Determination of the fatty acid profile by ¹H-NMR Spectroscopy. *European Journal of Lipid Science and Technology* **106**: 88-96.
- Kumar, P.K.P. and Krishna, A.G.G. (2014). Physico-chemical characteristics and nutraceutical distribution of crude palm oil and its fractions. *Grasas y Aceites* **65**(2): 1-12.
- Liu, K.S. (1994). Preparation of fatty acid methyl esters for gas chromatographic analysis of lipids in biological materials. *Journal of the American Oil Chemists' Society* **71**: 1179-1187.
- Macedo, L.F.A., Lacerda, E.L.Q., Silva, R.D., Simionato, J.I., Pedrao, M.R., Coro, F.A.G. and De Souza, N.E. (2012). Implications of method chosen for analysis of fatty acids in meat: a review. *American Journal of Agricultural and Biological Sciences* **7**(3): 278-284.
- Martínez, M.V., De Quirós, A.R.B., Hernández, J.L. and Yusty, M. (2009). Fatty acid profile and total lipid content of chionoecetes opilio shells. *The Open Food Science Journal* **3**: 93-97.
- Milinsk, M.C., Matsushita, M., Visentainer, J.V., De Oliveira, C.C. and De Souza, N.E. (2008). Comparative analysis of eight esterification methods in the quantitative determination of vegetable oil fatty acid methyl esters (FAME). *Journal of the Brazilian Chemical Society* **19**(8): 1475-1483.
- Moreira, R.G., Castell-Perez, M.E. and Barrufet, M.A. (1999). *Deep-Fat Frying: Fundamentals and Applications*. Aspen Publication, Maryland.
- Muhamad, N.A. and Mohamad, J. (2012). Fatty acids composition of selected Malaysian fishes. *Sains Malaysiana* **4**(1): 81-94.
- Omar, T.A. and Salimon, J. (2013). Validation and application of a gas chromatographic method for determining fatty acids and trans fats in some bakery products. *Journal of Taibah University for Science* **7**: 56-63.
- Orata, F. (2012). Derivatization Reactions and Reagents for Gas Chromatography Analysis. In: Mohd, M.A. (ed.). *Advanced Gas Chromatography-Progress in Agricultural, Biomedical and Industrial Applications*. Rijeka: InTech. p 83-108.
- Paligova', J., Jori'Kova', L. and Cvengros, J. (2008). Study of FAME stability. *Energy and Fuels* **22**: 1991-1996.
- Parashar, A., Sinha, N. and Singh, P. (2010). Lipid contents and fatty acids composition of seed oil from twenty five pomegranates varieties grown in india. *Advance Journal of Food Science and Technology* **2**(1): 12-15.
- Petrovic', M., Kezic', N. and Bolanc', V. (2010). Optimization of the GC method for routine analysis of the fatty acid profile in several food samples. *Food Chemistry* **122**: 285-291.
- Ping, B.T.Y. (2013). Gas chromatography-flame ionization detector method for determination of carbon chain length distribution of palm-based fatty alcohol. *Journal of Oil Palm Research* **20**: 36-43.
- Squadrone, S., Ferro, G.L., Marchis, D., Mauro, C., Palmegiano, P., Amato, G., Poma Genin, E. and Abete, M.C. (2010) Determination of melamine in feed: Validation of a gas chromatography-mass spectrometry method according to 2004/882/CE regulation. *Food Control* **21**(5): 714-718.
- Sundram, K., Sambanthamurthi, R. and Tan, Y. (2003). Palm fruit chemistry and nutrition. *Asia Pacific Journal of Clinical Nutrition* **12**(3): 355-362.

- Tong, L., Zhang, L., Yu, S., Chen, X. and Bi, X. (2007). Analysis of the fatty acids from *Periploca sepium* by GC-MS and GC-FID. *Asian Journal of Traditional Medicines* **2**(3): 110-114.
- Wang, P.G. (2008). *High-Throughput Analysis in the Pharmaceutical Industry*. CRC Press, Taylor and Francis Group, Boca Raton. p 174.
- Wirasnita, R., Hadibarata, T., Novelina, Y.M., Yusoff, A.R.M. and Yusop, Z.A. (2013). Modified methylation method to determine fatty acid content by gas chromatography. *Bulletin of the Korean Chemical Society* **34**(11): 3239-3242.