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DETERMINATION OF TRIACYLGLYCEROL COMPOSITION AND ITS CONTENT IN REFINED AND FRACTIONATED PALM OIL BY USING LC-MS/MS WITH MULTIPLE NEUTRAL LOSS SCAN

SEONGEUNG LEE[1]*; SEUNGCHUL BAEK[1]; SIYOUNG YANG[1] and GYEONGHWEON LEE[1]

ABSTRACT

Palm oil is widely consumed as an edible vegetable oil in many countries. Triacylglycerol (TAG) is the main component of palm oil, and its composition and contents have been extensively linked with physical and chemical properties. However, the identification and quantitation of TAGs is a challenge due to the complexity of the TAG molecule. This study employed LC-MS/MS with multiple neutral loss scans (NLS) to analyse TAG composition and contents in refined and fractionated palm oil. Identifying and quantifying TAGs using LC-MS/MS in combination with multiple-NLS was an efficient way to improve accuracy and timeliness. For accurate quantification of TAGs, isotopic deconvolution and the adjustment factor (AF) were applied. A total of 31 TAGs were identified and quantified, and C50:1 (16:0/16:0/18:1) (20.5%-36.0%), C52:2 (16:0/18:1/18:1) (17.8%-25.0%), C52:3 (16:0/18:1/18:2) (7.8%-12.2%), C48:0 (16:0/16:0/16:0) (0.4%-17.4%), and C52:1 (16:0/18:0/18:1) (4.1%-9.3%) were the predominant TAGs depending on refined and fractionated palm oil. The obtained information has been elucidated concerning the fundamental properties of refined and fractioned palm oil, such as hardness and degree of oxidation.

Keywords: fatty acid, LC-MS/MS, neutral loss scan, palm oil, triacylglycerol.

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INTRODUCTION

Palm oil, extracted from palm fruit, is the major export of Southeast Asian countries such as Indonesia and Malaysia. Palm oil's widespread popularity stems from its lower cost when compared to other edible vegetable oils such as sunflower oil, olive oil, rapeseed oil and soybean oil and its various forms (Carter *et al*., 2007; Chandrasekharan *et al*., 2000; Murphy, 2009). Palm olein is the liquid fraction of refined palm oil, and palm stearin and palm mid-fraction is the solid fractions (Braipson-Danthine and Gibon, 2007). According to Gee (2007), the physical and chemical properties of fractionated palm oils were influenced by the composition of various lipid compound. The triacylglycerol (TAG) is the major compound of lipids in fractionated palm oil like other edible vegetable oils. The composition and the contents of TAGs have been most extensively linked with the structure and hardness of lipid and fats (Himawan *et al*., 2006; Noor Lida *et al*., 2002; Sato, 2001). Also, changes in TAG and fatty acid composition may impact lipid oxidative stability (Bates *et al*., 2012; Mateos *et al*., 2005). Therefore, analysing the composition of TAG species and its content is crucial to especially understanding more of the physical and chemical properties of refined and fractionated palm oil. However, the identification and quantification of TAG is a great challenge due to the complexity of the TAG molecule. TAGs are esters of a glycerol molecule linked to three fatty acid molecules. The fatty acid, in particular, has various characteristics depending on the number of carbons and double bonds (Li *et al*., 2014b).

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[1] Lotte R&D Center, 201,
Magokjungang-ro, Gangseo-gu, Seoul,
07594 Republic of Korea.

* *Corresponding author e-mail: sungoung.lee@lotte.net*

The composition of TAG species and its content has been determined by various analytical methods such as thin layer chromatography (TLC) (Khor *et al.*, 1980; Tan *et al.*, 1981), GC with flame ionisation detector (GC-FID) (Toschi *et al.*, 1993), HPLC (Buchgraber *et al.*, 2000), and liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Beccaria *et al.*, 2014; Cheong *et al.*, 2014). The TAG composition of various forms of palm oil including refined palm oil, fractionated palm oil and palm kernel oil have been especially determined by TLC, GC-FID and HPLC (Braipson-Danthine and Gibon, 2007; Chen *et al.*, 2007; Oboh, 2004). Recently, LC-MS/MS with multiple neutral loss scan (NLS) has been employed to determine TAG composition and its contents (Pizzo *et al.*, 2022; Sirbu *et al.*, 2018; Xie *et al.*, 2019; Xu *et al.*, 2018). The NLS continuously determined mass offset scanned data between the first and second analysers (Han *et al.*, 2012). In other words, the chemical components in the sample were scanned in the first analyser and then are fragmented in the collision cells, followed by scanning fragment ions at the second analyser. The mass offset was calculated using the difference of masses scanned in the first and second analysers. Notably, it is an effective method to identify TAGs since they have neutral loss parts like fatty acid chains. (Li *et al.*, 2014b). Multiple-NLS was used to determine the composition and contents of TAG in salmon muscle tissue. In this research, the author elaborated on TAG metabolism in Atlantic salmon where for example the TAG containing linoleic acid is metabolised in salmon muscle (Yeo and Parrish, 2020). However, this method has not been yet applied to study TAG contents and its composition in refined and fractionated palm oil.

Therefore, the objective of this study was to analyse the TAG composition and its contents in refined and fractionated palm oil using LC-MS/MS with multiple-NLS. Herein, the obtained multiple-NLS data were deconvoluted to reduce isotopic interference. These findings will be used to determine the chemical and physical properties of refined and fractionated palm oil.

MATERIALS AND METHODS

Materials

Refined and fractionated palm oil, including palm olein, palm stearin and palm mid-fraction were obtained from Lotte Confectionery Co. (Seoul, Korea). Ammonium acetate, 14% boron-trifluoride in methanol solution, 37-component fatty acid methyl ester (FAME) standard mixture and TAG standards including glyceryl triundecanoate (C33:0, tri-11:0, internal standard for fatty acid analysis), glyceryl tridodecanoate (C36:0, tri-12:0), glyceryl trimyristate (C42:0, tri-14:0), glyceryl tripalmitoleate (C48:3, tri-16:1), glyceryl tripalmitate (C48:0, tri-16:0), glyceryl triheptadecanoate (C51:0, tri-17:0), glyceryl tristearate (C54:0, tri-18:0), glyceryl trioleate (C54:3, tri-18:1), glyceryl trilinoleate (C54:6, tri-18:2), glyceryl tri-linolenate (C54:9, tri-18:3), and glyceryl trinonadecanonate (C57:0, tri-19:0) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). TAG standard such as tripentadecanoin (C45:0, tri-15:0), triheptadecenoin (C51:3, tri-17:1, internal standard for TAG), trinonadecenoin (C57:3, tri-19:1), trinonadecadienoin (C57:6, tri-19:2), trieicosanoin (C60:0, tri-20:0), trieicosenoin (C60:3, tri-20:1), trieicosadienoin (C60:6, tri-20:2) and trieicosatrienoin (C60:9, tri-20:3) were obtained from Nu-check prep, Inc. (Elysian, MN, USA). Methanol, chloroform, isooctane and sodium hydroxide solution were purchased from J.T. Baker (Phillipsburg, NJ, USA).

Determination of Fatty Acid

The fatty acid analysis by GC-FID is an essential step since the fatty acid composition, and the molecular weight is the fundamental information for multiple-NLS. The analysis of fatty acids followed the modified method of Korean food code (KFDA, 2022). Refined and fractionated palm oil samples (50 mg) were placed in glass tubes. 1.5 mL of 0.5 N methanolic sodium hydroxide solution and 1.0 mL of internal standard (glyceryl triundecanolate) were added to the glass tube and heated for 5 min at 100°C. And then, 2.0 mL of 14% boron-trifluoride in methanol solution was added and incubated for 30 min at 100°C. During this step, fatty acid was converted into FAME. After heating, 1.0 mL of isooctane and 5.0 mL of saturated sodium chloride solution were added, mixed and centrifuged. The upper isooctane layer was determined by GC-FID (Model 7890a, Agilent, Santa Clara, CA, USA) with sp-2560 column (Supleco, Bellefonte, PA, USA). The sample (1.0 μL) was injected into the inlet of GC-FID using the split ratio of 200:1 at 250°C. The carrier gas was nitrogen with a flow rate of 1.0 mL min^{-1}. The initial temperature of the oven was 100°C for 3 min, then increased by 4°C min^{-1} to 190°C for 5 min, finally, ramped to 240°C at 3°C min^{-1} and kept at that temperature for 23 min. FAMEs were identified by comparing their retention time with the standard mixture (37 components of FAME standard mixture) and quantified by their peak area using internal standard and conversion coefficient.

Determination and Quantitation of TAG

First, refined and fractionated palm oil samples (50 mg) were placed in the glass tube, and diluted using 20.0 mL solvent (Chloroform:Methanol = 2:1). The mixture was thoroughly vortexed for 10 min and the upper layer mixture (0.5 mL) was mixed with 0.5 mL internal standard (C51:3, tri-17:1, 0.5 nmol) and 4.0 mL ammonium acetate mixture solution (Chloroform:Methanol:300 mM ammonium acetate = 300:665:35) and then vortexed for 2 min. In this step, TAG reacted with ammonium acetate to form ammonium-adducted TAG. The prepared samples were directly infused into the LC-MS/MS (Model Xevo TQ-S, Waters, Milford, MA, USA) with electrospray ionisation (ESI) to identify and quantify TAGs. The sample (300 µL) was direct infused at a flow rate of 20 µL min^{-1}. The TAG was identified and quantified in the positive mode with 4.0 kV capillary voltage, 60 V cone voltage, and 450°C desolvation temperature. The gas flow of desolvation and cone were 600 and 150 L hr^{-1}, respectively. The collision gas was nitrogen, and the collision energy was 25 V. TAG analysis was carried out as ammonium-adducted ion using multiple-NLS in positive mode. The content and molecular weight of fatty acid was used for multiple-NLS. The obtained data were processed using MassLynx software version 4.1 (Waters, Milford, MA, USA).

The acquired spectrum intensity was corrected by deconvolution to reduce the effect of isotopic interference and improve quantification accuracy. The calculation of isotopic deconvolution was followed, as shown by Li *et al.* (2014a), and the detailed process is explained below. The corrected spectrum intensity was applied to quantify the TAG by applying it to the statistically estimated equations using adjustment factors (AF). The AF was calculated using the correlation between commercial TAG and internal standard (Li *et al.*, 2014b). And then, the factor, which was unavailable to calculate, was estimated by a regressive curve generated by statistical software SPSS version 25 (IBM, Armonk, NY, USA).

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using SPSS version 25 (IBM, Armonk, NY, USA). Means were compared using Duncan's test with a significance level of 0.05.

RESULTS AND DISCUSSION

Fatty Acid Composition of Refined and Fractionated Palm Oil

The fatty acid composition of refined and fractionated palm oil was analysed using GC-FID. The fatty acids, including myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), arachidic acid (C20:0) and linolenic acid (C18:3) were determined (*Figure 1*). The quantification data of fatty acids are shown in *Table 1*. In this study,

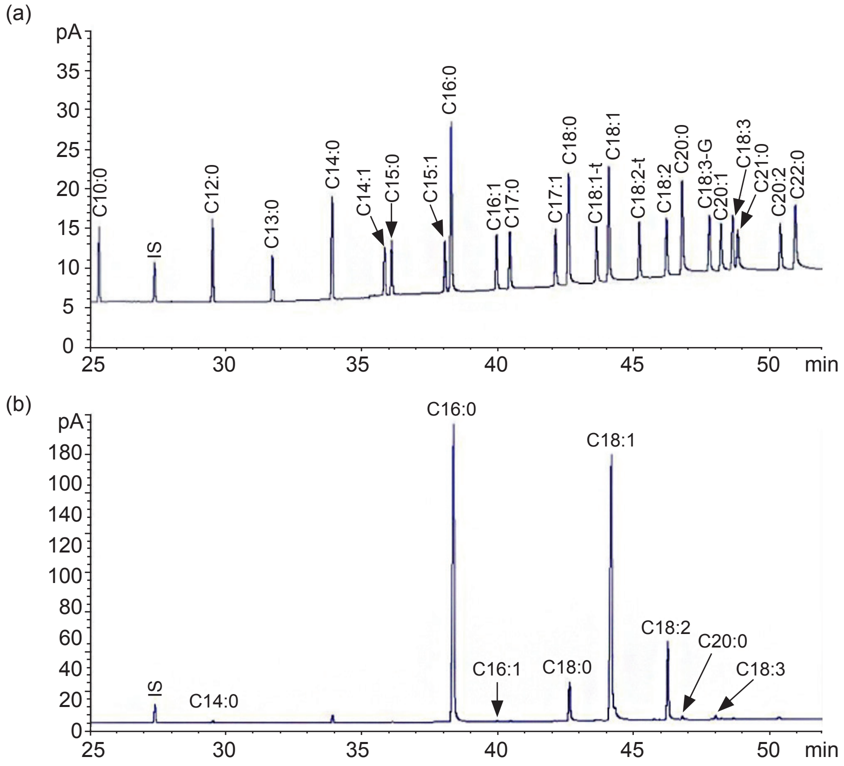

Figure 1. Chromatogram of FAME determined by GC-FID. (a) Standard (FAME 37 mixture) and (b) refined palm oil.

palmitic acid and oleic acid showed higher contents other than fatty acids. Palm stearin showed 63.38 g/ 100.00 g of palmitic acid, which could influence the hardness characteristics. On the other hand, palm olein showed 44.92 g/100.00 g of palmitic acid, which could impact their soft characteristics. Also, palm olein showed the highest oleic acid content (37.84 g/100.00 g). Myristic acid, palmitoleic acid, arachidic acid and linolenic acid were less abundant at level under 1.00 g/100.00 g. Gee (2007) and Koushki *et al.* (2015) showed similar results in fatty acid composition and its contents of refined and fractionated palm oil. Especially, according to Gee (2007), iodine value and melting point which could explain the properties of oils and fats including oxidative stress were different between palm olein and palm stearin. These differences were influenced by the composition and contents of fatty acids. Moreover, the composition and contents of TAGs would specifically explain the differences in the physical and chemical properties of fractionated palm oils. The fatty acid composition from this study and molecular weight of ammoniated ion was utilised based on the condition of multiple-NLS.

Identification of TAG using Multiple Neutral Loss Scan (NLS)

The multiple NLS is an effective scan method for the identification of lipids that possess neutral loss moieties such as fatty acyl chains in the TAG structure. The condition of multiple NLS was established using the fatty acids profile obtained by GC-FID, including NLS 245 (C14:0), NLS 273 (C16:0), NLS 271 (C16:1), NLS 301 (C18:0), NLS 299 (C18:1), NLS 297 (C18:2), NLS 329 (C20:0), NLS 295 (C18:3) and NLS 285 (C17:1, IS). The range of multiple NLS was at m/z 600-1050. The spectrum obtained from multiple NLS were utilised to confirm combinations of fatty acid chain and identify each TAG using their molecular masses of ammoniated ion form (*Figure 2*). For example, the spectrum at 875 was found in NLS 273, 297 and 299 means the loss of C16:0, C18:2 and C18:1 fatty acid from its TAG compounds. Besides the confirmation of the three fatty acids determined using multiple-NLS, the masses of ammoniated ion of TAG matched with 875, leading to confirmation of C54:3 (16:0/18:1/18:2). This process was repeated using different NLS conditions of fatty acid to identify TAG in refined and fractionated palm oil. A total of 32 TAGs were detected depending on the sample type and the range of m/z 797-993 in neutral loss scan with positive mode. Depending on the sample, various numbers of carbon (46-56) and double bonds (0-6) were identified. Most TAGs identified from the multiple-NLS were composed of palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2), similar to fatty acid analysis data.

Isotopic Deconvolution

The spectrums of identified TAGs were corrected using isotopic deconvolution. The main atoms of TAG are carbon, oxygen and hydrogen which have their own isotope, causing the interference in the mass spectrometry analysis. Particularly, this interference is influenced by carbon variants, including ^{13}C and ^{12}C. In other words, one TAG molecule (M) may have isotopes such as M+1, M+2, M+3, *etc.*, and some of them may interfere with different mass (Li *et al.*, 2014b). For example, C50:1 has a monoisotopic spectrum (M) at 851. On the other hand, its second isotopic spectrum (M+2) with two ^{13}C isotopic carbon is at m/z 853. The M+2 spectrum of C50:1 interferes with the monoisotopic spectrum of C50:0, which has m/z 853 (*Figure 3a*). C50:1 has one more double bond than C50:0, which means the loss of two hydrogen atoms.

Thus, the interference of isotope affects the accurate quantification of TAGs in mass spectrometry analysis, causing the requirement of isotopic deconvolution, which means removing the effect of isotope. This study conducted isotopic deconvolution for a spectrum determined using LC-MS/MS with multiple-NLS.

TABLE 1. FATTY ACID COMPOSITION AND CONTENTS OF REFINED AND FRACTIONATED PALM OIL

Fatty acid	Abbreviation	Fatty acid contents (g/100 g)			
		Refined palm oil	Palm olein	Palm stearin	Mid-fraction
Myristic acid	C14:0	1.07 ± 0.08^{ab}	0.93 ± 0.10^{b}	1.19 ± 0.07^{a}	0.95 ± 0.10^{b}
Palmitic acid	C16:0	46.08 ± 0.49^{bc}	44.92 ± 0.65^{c}	63.38 ± 0.57^{a}	51.59 ± 0.58^{b}
Palmitoleic acid	C16:1	0.14 ± 0.01^{a}	0.15 ± 0.02^{a}	0.10 ± 0.00^{a}	0.09 ± 0.08^{a}
Stearic acid	C18:0	4.60 ± 0.10^{bc}	4.33 ± 0.19^{c}	5.05 ± 0.10^{a}	4.63 ± 0.17^{b}
Oleic acid	C18:1	37.51 ± 0.35^{a}	37.84 ± 3.03^{a}	24.38 ± 0.55^{b}	35.36 ± 0.98^{a}
Linoleic acid	C18:2	9.32 ± 0.08^{a}	9.09 ± 2.63^{a}	5.04 ± 0.11^{b}	6.35 ± 0.26^{b}
Arachidic acid	C20:0	0.14 ± 0.25^{a}	0.35 ± 0.06^{a}	0.35 ± 0.06^{a}	0.20 ± 0.17^{a}
Linolenic acid	C18:3	0.17 ± 0.01^{a}	0.15 ± 0.03^{a}	0.07 ± 0.01^{b}	0.11 ± 0.01^{b}

Note: All values are means ± SD of triplicate analysis. Mean values in a row followed by different superscript letters are significantly (p<0.05) different (Duncan's multiple range test).

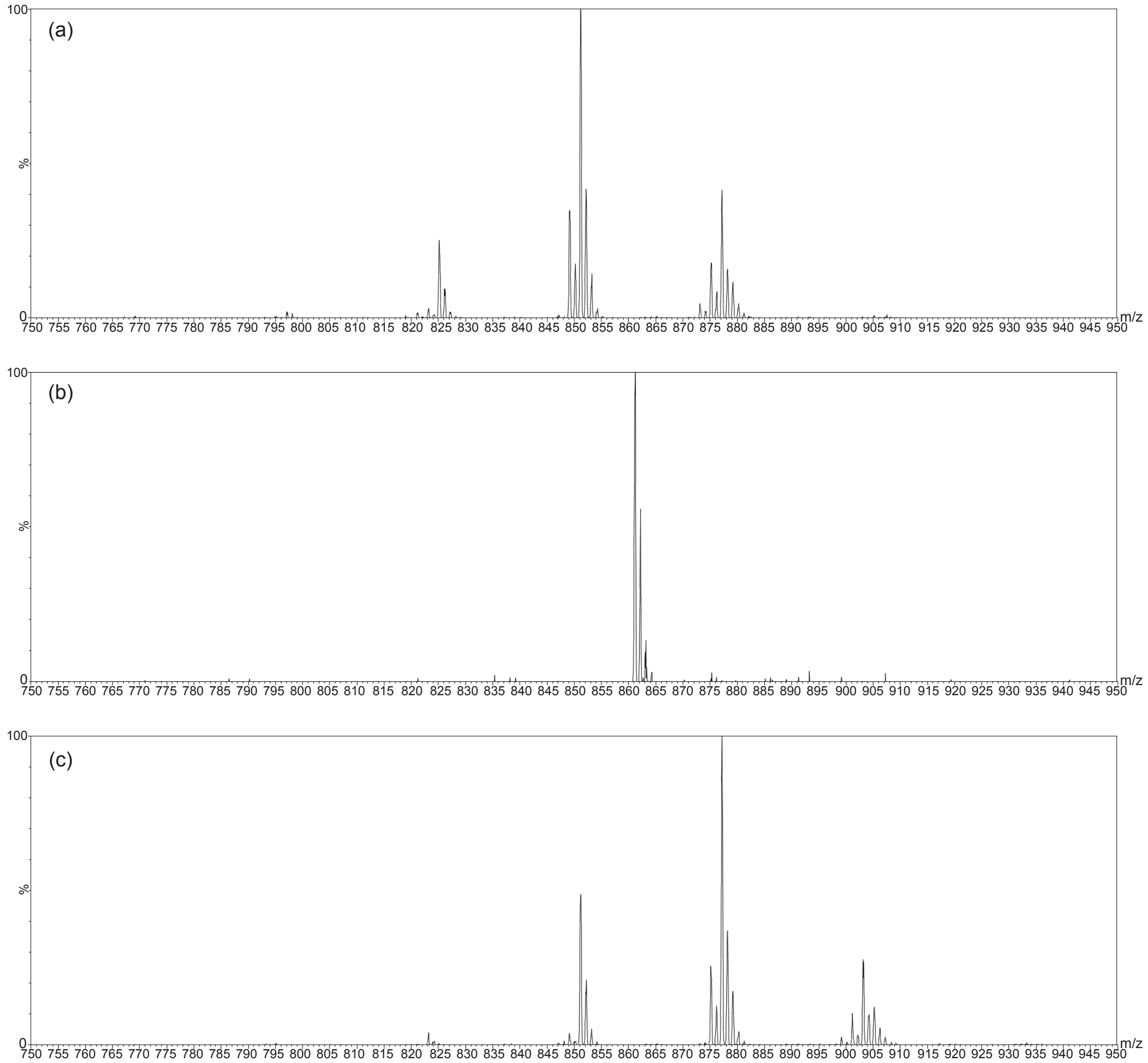

Figure 2. Partial spectrum of multiple NLS of ammoniated TAG ion in refined palm oil. (a) NLS 273 (Loss of C16:0), (b) NLS 285 (Loss of C17:1, internal standard), and (c) NLS 299 (Loss of C18:1).

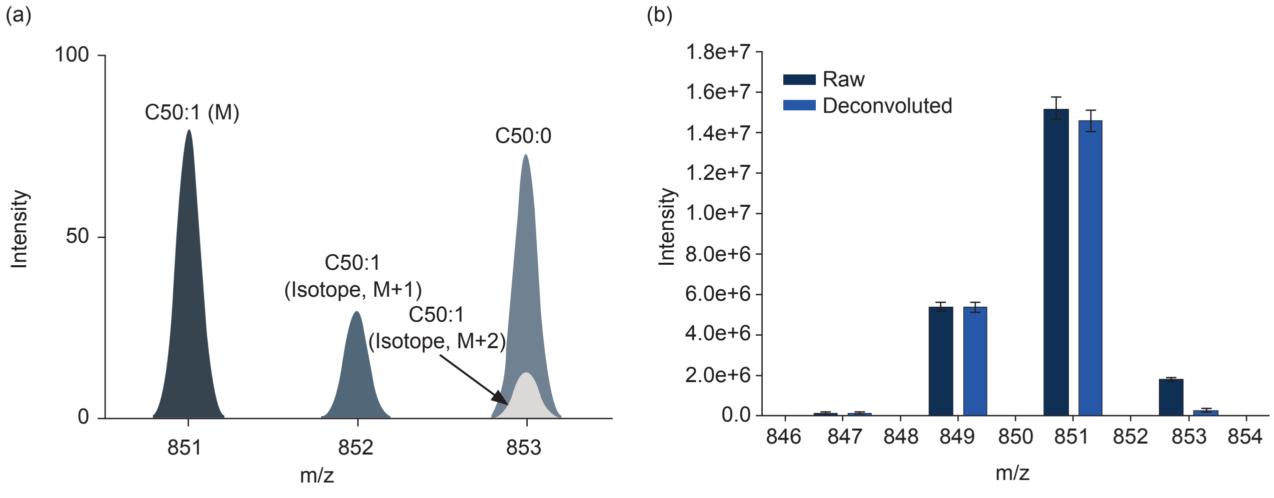

Figure 3. (a) Example of deconvolution to reduce effect of isotopic interference, and (b) partial result of isotopic deconvolution in NLS 217.

The calculation for deconvolution was followed as given by Li *et al.* (2014a; 2014b). First, the Z_M was calculated using Equation (1):

$$Z_M = [(N_C \times 1.12) \times (N_C \times 1.12) \; / \; 200 + (N_O \times 0.204)] \; / \; 100 \qquad (1)$$

where N_C and N_O means the numbers of carbon and oxygen atoms of the diacylglycerol (DAG) product ion from fragmented TAGs. And then, the Z_M was applied to the following Equation (2) to compute the corrected peak signal:

$$^{C}I_{M+2} = I_{M+2} - {}^{C}I_M \times Z_M \qquad (2)$$

where I_{M+2} means the gained raw spectrum intensity, and $^{C}I_M$ means the corrected spectrum intensity of molecule M. In this process, 32 TAG spectrum were deconvoluted depending on the sample, and the spectrum of C50:0 (16:0/16:0/18:0) TAG was removed because of isotopic interference. The partial results of isotopic deconvolution in NLS 217 are shown in *Figure 3b*. In this figure, the spectrum at m/z 847 showed the same intensity after isotopic deconvolution. On the other hand, the spectrum at m/z 853 showed the most decreasing intensity after isotopic deconvolution.

Calculation and Estimation of AF

After performing isotopic deconvolution on the spectrum signal obtained at individual NLS, the adjustment factors were used to correct the variable NLS spectrum of acquired TAGs that varied in the number of carbon atoms and double bonds in the fatty acid chain. The AF is defined as a coefficient where the NLS spectrum intensity of the internal standard (C51:3) is divided by the NLS spectrum intensity of each TAG, when the TAG and internal standard are equimolar (Li *et al.*, 2014a). The AF was calculated by utilising commercially purchased TAG standards, and then statistical estimation for regressive curve was conducted by using calculated factors. First, the regressive curve for saturated TAG was obtained using the calculated AF of saturated TAGs (*Figure 4a*). After that, the regressive curve was obtained for unsaturated TAG, including three double bond (*Figure 4b*) and six double bonds (*Figure 4c* and *4d*). Lastly, the final regressive curve was deduced about each carbon of TAGs (48-60) using acquired AF from calculation and estimation (*Figure 4e*). All deduced and computed AF are shown at *Table 2*.

These factors were applied to the isotopically deconvoluted spectrum intensity of NLS. For instance, the AF for m/z at 903 (C54:3) was 1.81, which means that 1.85 moles of C54:3 and 1.00 mole of internal standard (C51:3) would show the same spectrum intensity. Thus, the amount at m/z 903 (C54:3) from NLS 299 was corrected by multiplying 1.85. The increase in the AF indicated a decrease in the intensity of the NLS spectrum, which was found to increase with the number of carbons in the range of C46-C60 in a fatty acid chain. The results are consistent with the findings of Li *et al.* (2014a) in which the TAGs with short fatty acid chains were shown to have higher sensitivity than that of long fatty acid chain. The AF was increased depending on the increase in unsaturation of TAGs in the range of 3-6 double bonds. On the other hand, it was decreased in the range of 0-3 double bonds in the TAGs, which means the highest intensity of NLS spectrum at 3 double bonds. The similar results were reported in the study by Han and Gross (2001) and Li *et al.* (2014a).

Quantitation of TAG in Refined and Fractionated Palm Oil

The contents of individual TAG species in refined and fractionated palm oil were quantified

TABLE 2. CALCULATED AND DEDUCED ADJUSTMENT FACTOR (AF) FOR QUANTIFICATION OF TAG DETECTED IN REFINED AND FRACTIONATED PALM OIL (TAG 51:3, INTERNAL STANDARD = 1.0)

TAG	AF[1]	TAG	AF	TAG	AF	TAG	AF	TAG	AF
46:0	0.51	50:2	0.80	52:5	1.89	56:1	3.47	58:4	3.81
48:0	0.78	50:3	0.88	52:6	2.51	56:2	2.85	58:5	4.51
48:1	0.55	50:4	1.14	54:0	2.89	56:3	2.58	58:6	5.81
48:2	0.49	50:5	1.59	54:1	2.28	56:4	2.67	60:0	10.65
48:3	0.62	50:6	2.23	54:2	1.92	56:5	3.11	60:1	7.69
48:4	0.92	52:0	1.87	54:3	1.81	56:6	3.92	60:2	5.90
48:5	1.39	52:1	1.46	54:4	1.96	58:0	6.89	60:3	5.28
48:6	2.05	52:2	1.26	54:5	2.36	58:1	5.23	60:4	5.83
50:0	1.21	52:3	1.26	54:6	3.01	58:2	4.17	60:5	7.55
50:1	0.91	52:4	1.47	56:0	4.46	58:3	3.69	60:6	10.44

Note: [1] The adjustment factor is defined as NLS intensity of internal standard divided by NLS intensity of commercially purchased TAG standard for each.

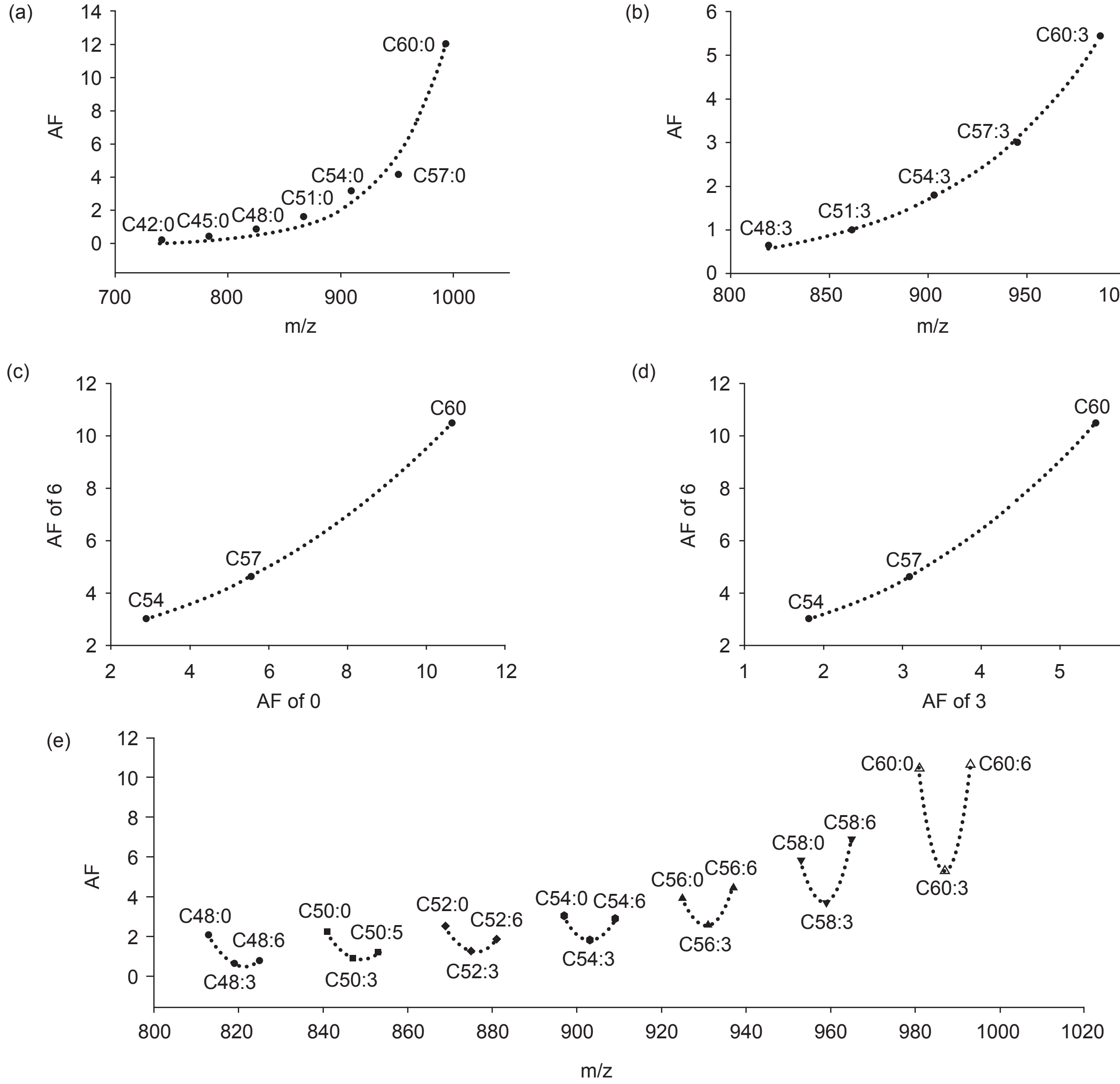

Figure 4. The process of estimation of AF using regression curve to determine TAG in refined and fractionated palm oil. (a) Regression curve of saturated TAG, (b) regression curve of TAG with 3 double bonds, (c) estimation of TAG with 6 double bonds (TAG:0 → TAG:6), (d) estimation of TAG with 6 double bonds (TAG:3 → TAG:6), and (e) combination of regression curve to estimate AF along with carbon number.

using a spectrum acquired from multiple-NLS with isotopic deconvolution and adjustment factor. The calculation for TAG quantification was followed as given by Li *et al.* (2014a). First, each fatty acid in TAGs (Am) at a particular m/z was calculated (in nmol) following the Equation (3):

$$A_m = \frac{\text{(Observed TAG intersity} \times \text{Internal standard amount)} \times \text{Adjustment factor}}{\text{Observed internal standard intensity}} \qquad (3)$$

The calculated amount of fatty acid in TAG at each m/z was used to calculate the amount of each TAG molecule. For instance, TAG mass m/z at 877.0 (C52:2) has various fatty acid chains. The most amount of fatty acid chain at C52:2 and they are C16:0, C18:1, C18:0 and C18:2, in this order. Based on the number of carbons and double bonds on the three fatty acid chains, two kinds of fatty acid combinations were deduced for C52:2, such as 16:0/18:1/18:1 and 16:0/18:0/18:2. The amount of 16:0/18:1/18:1 was represented by the amount of 18:1, $A_{18:1}$. The 16:0/18:1/18:1 have two C18:1 acyl chains. Thus, the amount of this TAG was calculated using the formula, *i.e.*, $A_{18:1}/2$. Also, the level of 16:0/18:0/18:2 was represented by the amount of C18:0, $A_{18:0}$ and the amount of C18:2, $A_{18:2}$. The formula to calculate the level of 16:0/18:0/18:2 was $(A_{18:0} + A_{18:2})/2$. Individual TAGs (31) identified by their fatty acyl chain were calculated this way, depending on samples. The final calculated results are shown in *Table 3*, and the results are presented as nmol/g and % portion separately. The two most abundant TAGs, C50:1 (16:0/16:0/18:1) (1415.8-1943.9 nmol/g, 20.5%-36.0%) and C52:2 (16:0/18:1/18:1) (962.0-1717.8 nmol/g, 17.8%-25.0%) accounted for nearly 40% of the TAGs found in refined and fractionated palm oil. In contrast, the TAG, which exhibited the third highest content, varied greatly depending on the samples.

The third highest TAG of refined palm oil and palm olein was C52:3 (16:0/18:1/18:2) (814.5-911.9 nmol/g, 12.2%-13.3%), however, palm stearin did not contain this TAG. Whereas, palm stearin showed the third highest contents of C48:0 (16:0/16:0/16:0) (1057.7 nmol/g, 17.4%). On the other hand, palm olein and mid-fraction showed relatively lower contents at 29.1 and 53.8 nmol/g, respectively. Braipson-Danthine and Gibon (2007) determined the TAG composition in fractionated palm oil by HPLC and reported similar results with our study. In their study, palm olein showed relatively higher contents of C52:2 (16:0/18:1/18:1) at 25.41%-43.03%. Whereas, palm stearin showed relatively higher contents of C50:1 (16:0/16:0/18:1) at 30.01%-40.67%. Palm stearin especially showed the TAG composition of C48:0 (16:0/16:0/16:0), however, palm olein did not contain this TAG. Chen *et al.* (2007) and Oboh (2004) also determined the TAG composition in refined palm oil by HPLC and TLC with GC-FID, respectively. In their studies, the refined palm oil mainly contained the TAG of C52:2 (16:0/18:1/18:1) and C50:1 (16:0/16:0/18:1). The other analysis methods such as HPLC, TLC and GC-FID had been used to determine the TAG compositions and contents in refined and fractionated palm oil due to its relatively effective values. However, a quantitative literature of TAG species in absolute amount, *e.g.*, nmol/g, is still lacking for various forms of palm oil.

TABLE 3. IDENTIFICATION AND QUANTIFICATION DATA OF TAG IN REFINED AND FRACTIONATED PALM OIL

m/z$^{1)}$	TAG	Composition	Refined palm oil		Palm olein		Palm stearin		Mid-fraction	
			nmol/g	Portion	nmol/g	Portion	nmol/g	Portion	nmol/g	Portion
797	46:0	14:0-16:0-16:0	17.0 ± 0.2^{b}	0.3	5.2 ± 0.1^{d}	0.1	50.6 ± 1.6^{a}	0.8	7.9 ± 0.9^{c}	0.2
821	48:2	14:0-16:0-18:2	19.4 ± 1.5^{a}	0.3	18.9 ± 0.2^{a}	0.3	0.0	0.0	18.4 ± 0.7^{a}	0.3
823	48:1	14:0-16:0-18:1	39.4 ± 5.4^{bc}	0.6	47.9 ± 1.5^{a}	0.7	43.2 ± 1.1^{ab}	0.7	34.7 ± 1.3^{c}	0.7
825	48:0	14:0-16:0-18:0	9.7 ± 0.4	0.1	N.D	0.0	N.D	0.0	N.D	0.0
825	48:0	16:0-16:0-16:0	222.9 ± 6.3^{b}	3.3	29.1 ± 2.3^{d}	0.4	1066.7 ± 18.4^{a}	17.4	53.8 ± 0.8^{c}	1.0
847	50:3	14:0-18:1-18:2	20.8 ± 1^{a}	0.3	17.2 ± 0.8^{b}	0.3	4.1 ± 1.3^{c}	0.1	15.1 ± 3.2^{b}	0.3
847	50:3	16:0-16:1-18:2	27.2 ± 0.4^{a}	0.4	14.9 ± 2.2^{b}	0.2	10.8 ± 1^{c}	0.2	N.D	0.0
847	50:3	16:0-16:0-18:3	N.D	0.0	15.3 ± 0.8^{a}	0.2	15.1 ± 0.9^{a}	0.3	10.2 ± 0.8^{b}	0.2
847	50:3	16:1-16:1-18:1	4.3 ± 1.3^{b}	0.1	3.8 ± 1.5^{b}	0.1	10.1 ± 1.7^{a}	0.2	N.D	0.0
849	50:2	14:0-18:1-18:1	28.7 ± 0.7^{b}	0.4	33.8 ± 2.9^{a}	0.5	17.7 ± 1.5^{c}	0.3	6.7 ± 1.7^{d}	0.1
849	50:2	16:0-16:1-18:1	21.5 ± 1.7^{c}	0.3	25.9 ± 1.5^{b}	0.4	17 ± 2.4^{d}	0.3	32.5 ± 2.5^{a}	0.6
849	50:2	16:0-16:0-18:2	403.4 ± 8.9^{b}	6.0	476.4 ± 2.0^{a}	6.9	304.6 ± 4.3^{d}	5.0	347 ± 1.2^{c}	6.5
851	50:1	14:0-18:0-18:1	N.D	0.0	N.D	0.0	N.D	0.0	11.4 ± 0.8	0.2
851	50:1	16:0-16:0-18:1	1437.5 ± 11.1^{c}	21.4	1410.6 ± 11.5^{c}	20.5	1686.1 ± 5.2^{b}	27.4	1934.3 ± 30^{a}	36.0
853	50:0	16:0-16:0-18:0	112.6 ± 1.9^{b}	1.7	N.D	0.0	302.7 ± 3.2^{a}	4.9	N.D	0.0
873	52:4	16:0-18:1-18:3	42.3 ± 1.4^{b}	0.6	48.2 ± 1.4^{a}	0.7	N.D	0.0	25.7 ± 0.7^{c}	0.5
873	52:4	16:0-18:2-18:2	216 ± 2.6^{b}	3.2	248.7 ± 4.0^{a}	3.6	129.5 ± 4.8^{c}	2.1	91.6 ± 2.2^{d}	1.7
875	52:3	16:0-18:1-18:2	815.8 ± 19.7^{b}	12.2	911.0 ± 7.1^{a}	13.3	N.D	0.0	416.6 ± 7.1^{c}	7.8
877	52:2	16:0-18:0-18:2	218.2 ± 100.8^{a}	3.3	133.7 ± 2.0^{ab}	2.0	102.9 ± 4.2^{c}	1.7	156.4 ± 43.1^{ab}	2.9
877	52:2	16:0-18:1-18:1	1487.9 ± 27.8^{b}	22.2	1718.9 ± 24.7^{a}	25.0	1109.9 ± 39.6^{c}	18.1	956.5 ± 20.3^{d}	17.8
879	52:1	16:0-18:0-18:1	277.4 ± 9.1^{d}	4.1	335.8 ± 19.4^{c}	4.9	424.3 ± 8.7^{b}	6.9	501.7 ± 16.8^{a}	9.3
897	54:6	18:2-18:2-18:2	20.7 ± 1.4^{c}	0.3	33.0 ± 2.1^{a}	0.5	8.9 ± 1.7^{d}	0.1	24.6 ± 0.9^{b}	0.5
899	54:5	18:1-18:2-18:2	186.2 ± 7^{a}	2.8	113.9 ± 1.9^{b}	1.7	51.6 ± 1.6^{c}	0.8	49.5 ± 0.8^{c}	0.9
901	54:4	18:1-18:1-18:2	219.7 ± 7.9^{b}	3.3	251.1 ± 3.3^{a}	3.7	158.7 ± 3.1^{c}	2.6	123.7 ± 6.5^{d}	2.3
901	54:4	18:0-18:2-18:2	51.7 ± 3.5^{a}	0.8	50.2 ± 1.7^{a}	0.7	34.6 ± 1.8^{a}	0.6	66.9 ± 40.4^{a}	1.3
903	54:3	18:0-18:1-18:2	118.1 ± 2^{b}	1.8	144.7 ± 4.7^{a}	2.1	77.5 ± 6.1^{c}	1.3	63.6 ± 3.3^{d}	1.2
903	54:3	18:1-18:1-18:1	325.3 ± 13^{b}	4.8	410.2 ± 5.5^{a}	6.0	249.3 ± 1.8^{c}	4.1	199.6 ± 2.6^{d}	3.7
905	54:2	16:0-18:2-20:0	30.8 ± 1.3^{b}	0.5	37.3 ± 1.0^{a}	0.5	31.7 ± 1.6^{b}	0.5	N.D	0.0
905	54:2	18:0-18:1-18:1	230.2 ± 5.5^{b}	3.4	259.4 ± 12.2^{a}	3.8	137.2 ± 21.8^{c}	2.2	131.9 ± 0.9^{c}	2.5
907	54:1	16:0-18:1-20:0	79.1 ± 3.1^{a}	1.2	44.8 ± 1.0^{c}	0.7	71.9 ± 3.4^{b}	1.2	68.5 ± 4.1^{b}	1.3
933	54:2	18:1-18:1-20:0	31.6 ± 1.3^{a}	0.2	29.9 ± 1.5^{a}	0.4	32.2 ± 1.5^{a}	0.5	23.6 ± 0.7^{b}	0.4

Note: All values are means ± SD of triplicate analysis. Mean values in a row followed by different superscript letters are significantly (p<0.05) different (Duncan's multiple range test). $^{1)}$ - m/z is the molecular weight of ammoniated ion of TAG. N.D - not detected.

A quantitative TAG profiling method for the refined and fractionated palm oil is described in the present study based on multiple-NLS.

The differences in composition and content suggest that the TAGs have been closely related to the physical and chemical properties of palm oils which are refined and fractionated. For example, palm stearin is semi liquid form like butter and shortening. According to Gee (2007), palm stearin showed a higher melting point than palm olein. Palm stearin is thought to have more palmitic acid than other fractionated palm oil, including palm olein. Its predominant type of palmitic acid is C48:0 (16:0/16:0/16:0), making palm stearin harder than other fractionated palm oil. Because saturated TAGs strongly linked with each other. Also according to Almeida *et al.* (2018), palm stearin showed relatively higher oxidative stability than palm olein. Although both fractionated palm oil contained palmitic acid, but also their predominant type of palmitic acid is different.

CONCLUSION

In conclusion, LC-MS/MS with multiple-NLS was effectively used to determine the TAG composition and its contents in refined and fractionated palm oil. To obtain precise quantification, isotopic deconvolution and the AF of various TAG were used. A total of 31 TAGs were identified and quantified, and C50:1 (16:0/16:0/18:1) (20.5%-36.0%), C52:2 (16:0/18:1/18:1) (17.8%-25.0%), C52:3 (16:0/18:1/18:2) (7.8%-12.2%), C48:0 (16:0/16:0/16:0) (0.4%-17.4%) and C52:1 (16:0/18:0/18:1) (4.1%-9.3%) being the most abundant TAGs, depending on refined and fractionated palm oils. Using direct infusion LC-MS/MS with repeated neutral loss scans to identify and quantify TAG was an efficient approach that improved the detection accuracy. This approach effectively revealed the composition and content of TAG in refined and fractionated palm oil. This has ramifications for the physical and chemical properties and could be positively used to understand the phenomenon, like hardness, oxidation levels and other properties.

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