Comparison of Peak-area Ratios and Percentage Peak Area Derived from HPLC-evaporative Light Scattering and Refractive Index Detectors for Palm Oil and its Fractions

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Abstract: High-Performance Liquid Chromatography (HPLC) methods via evaporative light scattering (ELS) and refractive index (RI) detectors are used by the local palm oil industry to monitor the TAG profiles of palm oil and its fractions. The quantitation method used is based on area normalization of the TAG components and expressed as percentage area. Although not frequently used, peak-area ratios based on TAG profiles are a possible qualitative method for characterizing the TAG of palm oil and its fractions. This paper aims to compare these two detectors in terms of peak-area ratio, percentage peak area composition, and TAG elution profiles. The triacylglycerol (TAG) composition for palm oil and its fractions were analysed under similar HPLC conditions i.e. mobile phase and column. However, different sample concentrations were used for the detectors while remaining within the linearity limits of the detectors. These concentrations also gave a good baseline resolved separation for all the TAGs components. The results of the ELS method’s percentage area composition for the TAGs of palm oil and its fractions differed from those of RID. This indicates an unequal response of TAGs for palm oil and its fractions using the ELSD, also affecting the peak area ratios. They were found not to be equivalent to those obtained using the HPLC-RID. The ELSD method showed a better baseline separation for the TAGs components, with a more stable baseline as compared with the corresponding HPLC-RID. In conclusion, the percentage area compositions and peak-area ratios for palm oil and its fractions as derived from HPLC-ELSD and RID were not equivalent due to different responses of TAG components to the ELSD detector. The HPLC-RID has a better accuracy for percentage area composition and peak-area ratio because the TAG components response equally to the detector.

Key words: triacylglycerol composition, triacylglycerol peak-area ratio, triacylglycerol HPLC profiles, palm oil

1 INTRODUCTION

Malaysian commercial crude palm oil (CPO) is extracted from mesocarps of the palm species Elaeis guineensis var. Tenera. The CPO has an iodine value of 50-54 and is comprised of >90% triacylglycerol (TAG), 5.3-7.7% diacylglycerol (DAG), 0.21-0.34% monoacylglycerol (MAG), 2.4 – 4.5% free fatty acids, and 1% minor components1. Crude palm oil is typically processed by a physical refining process into refined oil for end-use applications. Disaturated and mono saturated triacylglycerol can be fractionated into two products: liquid palm olein and solid palm stearin2. Palm olein and super olein are used as cooking oils, especially for deep fat or shallow frying. Palm stearin can be blended with other vegetable oils to obtain suitable functional products such as margarine fats, shortenings, and vanaspati, among others. Palm stearin is also a useful natural hard stock for the production of trans-free fats. Further fractionations of palm stearin/olein produces oils with other properties suitable for the various needs of the food industry e.g., palm mid fraction which can be used as cocoa butter replacers and extenders3 4. Palm stearin is an excellent raw material also used for non-food applications such as a starting material for oleo chemicals derivatives e.g. methyl ester sulfonate used in detergent5.

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The qualities of fats and oils are characterized by their TAG composition, which affects the melting point, crystallization properties, and their reaction to lipase-hydrolysis[1,2]. The TAG profile analyses of fats and oils provide information on the distribution of the fatty acids within the glyceride molecules. Two common methods found in the literature for palm oil and its fractions and palm kernel oil TAG composition analyses are GC-FID methods[5-8] and HPLC-RID/UV detectors[6,9-11]. The TAG composition as analyzed by GC-FID is based on the number of carbon atoms in the acyl chains of the TAG molecule. However, due to overlapping C54-triglycerides (SOS, SOO, SLS, SLO, OLO, OLL) and C48-TAG (MOP, MLP, PPP), the actual TAG compositions for each of the TAG cannot be determined. The HPLC method instead allows base separation of individual triglycerides with no overlaps[8,10].

The HPLC-RID method had some limitations due to its baseline, which is sensitive to temperature fluctuations. Furthermore, the mobile mixtures used with HPLC-RID such as acetone:acetonitrile (AOCS Official Method Ce 5b-89)[12] do have some solubilizing problems for those TAGs with more than 46 carbon atoms e.g. palm oil TAG. The use of an HPLC-ELSD for palm oil and its fractions for TAG composition analyses had yet to be explored in detail. The qualitative and quantitative use of HPLC-ELSD using a gradient elution for TAG profile analyses had been reported for palm oil, palm kernel oil[13] and other vegetable oils[14,15]. HPLC-ELSD has an advantage over RID for TAG profile analyses, because it allows flat baselines during gradient or isocratic runs. RID is unable to perform gradient analysis.

This work involved analyses of palm oil and its fractions using HPLC-RID and HPLC-ELSD using similar HPLC conditions e.g. the same column and mobile phase. However, the oil sample concentrations used were different due to the different sensitivity of the detectors for lipids[16]. The mobile phase used was a mixture of acetonitrile:methylene chloride 2:3 which allows better solubility for palm oil TAG. The oil samples were dissolved. The mixtures were filtered with a Polytetrafluoroethylene (PTFE) syringe filter (0.22 μm) prior to HPLC analysis. The peak area ratio of OOO:PPP is a good indicator of TAG quantification methods of these detectors. This comparison will then be used as a guideline for the use of HPLC-ELSD as an alternative to RID for fraction analysis.

2 EXPERIMENTAL PROCEDURES

2.1 Materials

HPLC grade acetonitrile and methylene chloride were obtained from Fischer Scientific and Lichrosolv Merck, respectively. Refined palm olein, super olein, and palm stearin were obtained from local commercial companies. Triolein (OOO), tripalmitin (PPP), dioleoyl palmitoyl glycerol (POO), dipalmitoyloleoylglycerol (POP), palmitoyl oleylinooleylglycerol (PLO) and palmitoyldilinoleyl glycerol (PLL) standards with >98% purities were purchased from Laradon Fine Chemicals, Solna, Sweden.

2.2 Sampling procedure for HPLC analyses

The standard mixtures, comprised of OOO, PPP, POO, PLO, and POP, were prepared at a concentration of 1 mg/mL using solvent mixture of acetonitrile:methylene chloride (2:3). Ten milligrams (HPLC-ELSD) and hundred twenty mg (HPLC-RID) of each of the palm oil fractions were weighed into 5-mL volumetric flasks and filled with acetonitrile:methylene chloride (2:3). Each mixture was shaken on a vortex mixture until the oil samples were dissolved. The mixtures were filtered with a Polytetrafluoroethylene (PTFE) syringe filter (0.22 μm) prior to HPLC analysis. The palm oil and fraction samples and the TAG standard mixtures were analyzed in four replicates.

2.3 HPLC conditions for triacylglycerol profile analyses

The HPLC system (1100 series, Agilent Technologies) was equipped with a quaternary pump, auto-injector system and a thermo stated column compartment. Two types of detectors were linked to the same system which was Agilent Technologies refractive index detector and an Alltech ELSD 800. The oil samples (10 μL) were analyzed using a Phenomenex Luna reverse phase C18 column (250 × 4.6 mm, 5 μm particle size) operating at 35°C. Both methods used isocratic analyses with mobile phase mixtures of acetonitrile:methylene chloride (2:3 v/v). For ELSD, analysis was conducted with an evaporator temperature of 40°C and an air pressure of 2.3 bars. For the RID, the detector temperature was set at 35°C. The flow rates for the mobile phase were 0.8 mL/min (HPLC-ELSD) and 1.0 mL/min (HPLC-RID).

3 RESULTS AND DISCUSSION

3.1 HPLC TAG profiles of palm oil and its fractions using HPLC-RID and HPLC-ELSD

Figures 1, 2 and 3 show the typical HPLC TAG profiles of refined palm olein, palm stearin, and palm super olein,
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respectively, obtained using HPLC with both ELSD and RID. The mixtures of triglycerides (whichever available) were used to provide retention time comparison and tentative identifications of the TAG peaks. Other peaks were tentatively identified based on their elution sequence, as reported in Muhammad et al. A qualitative examination of the chromatograms of the palm oil and its fractions showed that analyses with the two different detectors identified similar TAG compositions. However, the chromatograms for HPLC-ELSD for refined palm olein and super olein showed a slightly better baseline resolved separations than the HPLC-RID method. This is however not observed for palm stearin. In an observation of the HPLC-ELSD chromatograms (Figs. 1a and 3a), the OOO peak was baseline resolved and stand-alone from the POO peak. However, for HPLC-RID (Figs. 1b and 3b), the OOO peak was not baseline resolved and merged with POO peak at the base. The POO and POP peaks of palm olein and super olein were resolved at baseline (Figs. 1a and 3a) for HPLC-ELSD compared to HPLC-RID. Figures 1b and 3b showed that POO and POP nearly merged at the base.

There was a report of an acetone:acetonitrile gradient elution using two reverse phase columns (length 15 cm x 4.6 mm i.d., 4 μm particle size) HPLC-ELSD analysis for palm oil. However, when compared to chromatograms

Fig. 1 TAG chromatographic profile of refined palm olein using (a) HPLC-ELSD (b) HPLC-RID.

Fig. 2 TAG chromatographic profile of refined palm stearin using (a) HPLC-ELSD (b) HPLC-RID.

Fig. 3 TAG chromatographic profile of refined super olein using (a) HPLC-ELSD (b) HPLC-RID.
from both modes of separation, it was found that the isocratic mode allowed better baseline separation for OOO, POO, and POP compared to the gradient method with HPLC-ELSD\(^\text{13}\). The profiles obtained in this study also show better baseline resolved separation than the method using C22 and C30 silica columns coupled with HPLC-RID\(^\text{11}\). The gradient method was unable to achieve baseline separation for OOO, POO, POP for palm oil\(^\text{10}\).

This HPLC-RID method\(^\text{8}\) has shown that using mixtures of acetonitrile:acetone together with reverse phase column was found to operate at a longer retention time (75 mins) compared to this study (about 30 - 35 mins). Furthermore, the solvent mixtures of acetonitrile:acetone often have a poor solubility for TAG samples with more than 46 carbon atoms in the alkyl chain\(^\text{14}\) as in the case of palm oil and its fractions. In terms of TAG elution profiles, both the HPLC-RID methods show comparable performance, as the same amount of TAG components was detected. The HPLC-RID method developed in this work was also found to show better separation for palm oil compared to the one using a C22 and C30 silica columns with isocratic runs with the mobile phase of acetonitrile:acetone reported by Endo et al.\(^\text{11}\). For these columns, the OOO, POO, POP, and PPP were completely unresolved at the base.\(^\text{11}\)

3.2 Comparison of TAG compositions obtained from HPLC-ELSD and HPLC-RID for palm oil and fractions

Percentage area normalization is a common method of use to describe the palm oil TAGs composition. The percentage area method usually expressed the area of each peak in the chromatogram as a percentage of the sum of all the selected peaks. In this method, the area percentage does not require prior calibration and is usually not dependent on the amount of sample injected within the limits of the detector\(^\text{15}\). The response factor is also not used in this case, as it assumes equal detector response for all the TAG components\(^\text{18}\). Table 1 shows analyses of standards mixtures representing the major components of palm oil using the two detectors. A mixture of 1 mg mL\(^{-1}\) for each TAG standards was analyzed using both detectors, and the injection volume was maintained at 10 μL for both analyses. Comparing the percentage area for mixtures of five TAG for HPLC-ELSD, it was observed that POP showed only slightly higher response (25.4%) compared to the other TAGs (17.8 – 20.2%). The percentage area for POP was also slightly higher for ELSD than RID. Overall, at sample concentration of 1 mg/mL, both methods showed similar responses from the five TAG components and their percentage area compositions were almost comparable. In terms of sensitivity of the detectors, the peak areas of the TAG standards was larger for ELSD as compared to RID, showing that ELSD has a higher sensitivity compared to RID.

The same sample concentrations used cannot be applied in both methods due to the extremely low sensitivity of HPLC-RID for TAG components in palm oil and fractions. Table 2 showed the limit of detection (LOD) for several TAG standards. The LOD was found on analysing the TAGs standard mixtures at different concentrations levels (closest to the chromatogram baseline). The LOD was selected as the minimum concentration where the TAG peaks can be integrated from the baseline. The optimum sample concentrations used to detect the TAG profile with a good response (while not compromising on the separation for palm oil TAG profile) were 2 mg/mL and 24 mg/mL for HPLC-ELSD and HPLC-RID, respectively. The sample concentrations used were within the limit of linearity for HPLC-ELSD. Rombaut et al.\(^\text{10}\) found that irrespective of the TAG species, at concentrations of 0.25 – 2 mg/mL, HPLC-ELSD response is linear and fairly uniform. Buchgraber et al.\(^\text{20}\) showed that the response factors for TAG as analysed with an ELSD did not deviate from unity when 10 μL of test solutions with concentrations of 10, 50 and 100 mg of total TG/mL were injected. HPLC-RID has a very low sensitivity to TAG components of palm oil and its fractions.

### Table 2 Limit of detection for TAG standards for HPLC-ELSD and HPLC-RID.

| TAG*     | LOD (μg/mL) HPLC-RID | HPLC-ELSD |
|----------|---------------------|-----------|
| PLO      | 97.5                | 0.90      |
| OOO      | 84.4                | 0.81      |
| POO      | 108.1               | 0.90      |
| POP      | 83.5                | 0.64      |
| PPP      | 113.8               | 0.92      |

Note: LOD : limit of detection

* Triacylglycerols (TAG) are abbreviated using L – Linoleoyl, O – oleoyl, P – palmitoyl, S – Stearoyl, M – Myristoyl fatty acids

### Table 1 Peak Area of 1 mg/ mL TAG standard mixtures with the same injection volume analysed using HPLC ELSD and HPLC-RID.

| Detector | TAGs standard Mixtures Peak Area* (% Peak Area*) |
|----------|-------------------------------------------------|
|          | PLO | OOO | POO | POP | PPP |
| ELS      | 614,480 (18.0) | 676,127 (20.2) | 610,122 (18.2) | 848,282 (25.4) | 596,409 (17.8) |
| RI       | 89,361 (19.6) | 103,316 (22.7) | 80,596 (17.7) | 104,276 (23.0) | 76,593 (16.9) |

*Average of four replicates with RSD < 5%
and needed a much higher concentration e.g. 15 mg/mL and 50 mg/mL for detection.

The TAG composition based on percentage area normalization for palm oil (Table 3) and its fractions (Tables 4, 5 and 6) were not comparable for both detectors. The ELSD detector showed a higher percentage area with regards to POP and POO compared to the RID. The concentration used was within the limits of the ELSD detector and equal response of all of the TAG components was expected. However, the large differences in percentage area normalization between the detectors showed that the TAG components in the samples do not respond equally to the ELSD detector.

In this work, four commercial sources of palm stearin were analyzed. These palm stearins showed different compositions with the same detector (Table 5), likely due to the different fractionation processes. A similar trend as the refined palm olein and palm oil fractions was also observed for palm stearin. This is because the percentage area for the major TAG peaks e.g. POP and PPP from the ELSD was observed to be higher than the RID. This means that the overall area normalization for palm stearin was different for both detectors.

For the two different commercial sources with different

### Table 3

| Detector | Company | OLL | PLL | MLP | OLO | PLO | PLP | OOO | POO | POP | PPP | SOO | POS | Others |
|----------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| ELS      | 1       | 0.08** | 0.62* | 0.10* | 0.70* | 8.30* | 7.80* | 2.30* | 30.30* | 41.40* | 3.40* | 0.87* | 2.80* | 1.33** |
|          | 2       | 0.09* | 0.63* | 0.11* | 0.70* | 8.10* | 7.70* | 2.30* | 30.10* | 41.80* | 3.50* | 0.87* | 2.80* | 1.30** |
| RI       | 1       | 0.52* | 2.60* | 0.60* | 2.10* | 9.80* | 9.10* | 5.60* | 24.40* | 28.40* | 5.60** | 2.80** | 5.00* | 4.38** |
|          | 2       | 0.50** | 2.50* | 0.64* | 2.10* | 9.60* | 9.20* | 5.70* | 24.30* | 28.20* | 5.10* | 2.60** | 4.90 | 4.66** |

Note: TAGs % composition of refined palm olein from each company were average of 4 replicates. * RSD < 10.0 % ** RSD ≤ 10.00 – 16.00%

Triacylglycerols are abbreviated using L – Linoleoyl, O – oleoyl, P – palmitoyl, S – Stearoyl, M – Myristoyl fatty acids

### Table 4

| Detector | Company | OLL | PLL | MLP | OLO | PLO | PLP | OOO | POO | POP | PPP | SOO | POS | Others |
|----------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| ELS      | 1       | 0.42* | 2.63* | 0.62* | 2.18* | 10.50* | 9.30* | 6.30* | 28.10* | 28.20* | 0.36* | 3.40* | 5.36* | 2.43** |
|          | 2       | 0.58* | 3.30* | 0.59* | 2.52* | 12.00* | 10.40* | 5.80* | 28.00* | 26.95* | 0.00* | 2.91* | 4.94* | 2.01** |
|          | 3       | 0.56* | 3.27* | 1.19* | 2.30* | 10.80* | 9.86* | 5.38* | 26.70* | 28.85* | 0.00* | 2.92* | 5.37* | 2.80 |
|          | 4       | 0.58* | 3.30* | 0.59* | 2.52* | 12.00* | 10.40* | 5.80* | 28.00* | 26.95* | 0.00* | 2.91* | 4.94* | 2.01** |
|          | 5       | 0.43* | 2.37* | 0.70* | 1.97* | 9.90* | 8.80* | 6.10* | 29.70* | 29.10* | 0.15* | 3.26* | 5.17* | 2.35 |

Note: TAGs % composition of refined palm olein from each company were average of 4 replicates. * RSD < 10.0 % ** RSD ≤ 10.00 – 20.00%

### Table 5

| Detector | Company | OLL | PLL | MLP | OLO | PLO | PLP | OOO | POO | POP | PPP | SOO | POS | Others |
|----------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------|
| ELS      | 1       | 0.00 | 0.10* | 0.00 | 0.12** | 1.28* | 3.25* | 1.07* | 7.05* | 39.40* | 41.60* | 0.15* | 1.89* | 4.09* |
|          | 2       | 0.03** | 0.15* | 0.05* | 0.20** | 2.80* | 4.30* | 0.77* | 14.50* | 50.95* | 20.50* | 0.35* | 1.52* | 3.88* |
|          | 3       | 0.03** | 0.14** | 0.04* | 0.16* | 1.98* | 4.24* | 0.62* | 10.30* | 56.90* | 18.20* | 1.20* | 6.43* | 3.21 |
|          | 4       | 0.07** | 0.15** | 0.47* | 0.25* | 3.04* | 4.71* | 0.86* | 12.48* | 54.00* | 17.50* | 0.35* | 4.88* | 1.24* |

Note: TAGs % composition of refined palm stearin from each company were average of 4 replicates. * RSD < 10.0 % ** RSD ≤ 10.00 – 16.00%

### Table 6

Note: TAGs % composition of refined palm stearin from each company were average of 4 replicates. * RSD < 10.0 % ** RSD ≤ 10.00 – 16.00%

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processing technologies) of super olein, Company 1 and Company 2, it was found that their TAG percentage area composition from the same detector (Table 6) was also different. The major TAG composition for super olein from Company 1 and Company 2 was OOO, and POO, respectively. It was observed that the percentage area for their major TAG, OOO (Company 1) and POO (Company 2) was higher for ELSD. But these results were unlike those found for palm oil, refined palm olein, and stearin where the percentage area for OOO and POO (only for palm stearin) was lower for ELSD. The major TAG component responded differently to the ELSD as compared to the minor TAGs. Again, due to differences in the major TAG response to the ELSD detector, the percentage area composition for super olein from ELSD was not comparable to that of RID.

These results show a significant difference in the ELSD response for various TAGs from edible oil but this was not observed for the standard mixtures (Table 1). The concentration of the oil samples used for ELSD (2 mg/mL) was almost equivalent to the TAG standards (1 mg/mL) (Table 1). However, unlike the TAG standard mixtures, the major TAGs in the actual oil samples may show unequal response compared to the other minor TAG components. Due to the unequal responses of TAG components in the oil samples to ELSD, the percentage area composition was found to be different than that of RID. Therefore, the usage of percentage area for quantification in the case of ELSD was not accurate. Instead, quantification using available standards with relative response factors is recommended for HPLC-ELSD technique (5). Also, comparison of the percentage area composition generated from HPLC-ELSD and HPLC-RID of TAG components for palm oil and its fractions would not be accurate without considering the response factor.

### 3.3 Comparison of peak-area ratios

The peak-area ratio of OOO:PPP is a good indicator of the quality of fractionated refined palm olein and refined super olein. A peak area ratio of 1:0 indicates that the sample is free from tripalmitin (PPP) (3). The table shows that the peak-area ratios for OOO:PPP for both ELSD and RID were 1:0 for both refined palm olein (Table 8) and refined super olein (Table 9). This indicates that the HPLC-ELSD method could still be used as an indicator for the presence of tripalmitin. However, for palm stearin, due to the higher response of the ELSD for PPP and low response for OOO, the peak area ratios for OOO:PPP showed large differences compared with results from RID (Table 10).

For POP/(PPP + OOO) ratios, both refined super olein (Table 9) and palm stearin (Table 10) showed slight differences due to the higher percentage area of OOO (Table 6) and PPP (Table 5), respectively. However, for refined palm olein, Table 8 shows that the ELSD has much higher values (12.92 – 16.90) compared to RID (4.23 to 5.36). Again, a trend similar to that of refined palm olein was observed for refined palm oil, as ELSD had higher values than RID (Table 7). For the other types of peak-area ratios shown in Table 7 to Table 10, the values were only slightly different for both detectors. The overall results show that the ELSD and RID method were not equivalent in terms of peak-area ratio.

### Conclusions

The two HPLC methods for TAG analysis using two different detectors showed very similar elution profiles for palm oil and its fractions. However, better and more stable baseline separation was achieved using ELSD. TAG standard mixtures comprising POL, PPP and OOO showed

![Table 6](https://example.com/table6.png)

| Detector | Company | OLL | PLL | MLP | OLO | PLO | PLP | OOO | POO | POP | PPP | SOO | POS | Others |
|----------|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--------|
| ELSD     | 1       | 1.75** | 0.00 | 0.00 | 5.40** | 1.51** | 0.77** | 52.60 | 22.90 | 11.52 | 0.00 | 2.10 | 1.19 | 0.26** |
|          | 2       | 0.12*  | 1.12* | 0.15* | 1.14* | 14.60 | 8.80 | 4.70 | 53.50 | 12.7 | 0.00 | 1.80 | 0.65* | 0.72** |
| RI       | 1       | 4.51** | 0.00 | 0.00 | 8.46* | 3.37* | 1.73* | 39.50 | 20.30 | 12.80 | 0.00 | 5.41** | 2.95** | 0.97** |
|          | 2       | 0.78*  | 4.05* | 0.82* | 3.12* | 15.10 | 10.33 | 7.71 | 37.65 | 12.85 | 0.00 | 3.90* | 2.25* | 1.44** |

*Note: TAGs % composition of refined palm super olein from each company were average of 4 replicates. * RSD < 10.0 % ** RSD ≤ 10.00 – 23.00%*

![Table 7](https://example.com/table7.png)

| Detector | Company | POP:PPP | POP:000 | POP:(PPP+OOO) | POP:POO | OOO:PPP | OOO:PLO | POP | POP/(PPP+OOO) | 2x PLP/(OOO + PLO) |
|----------|---------|---------|---------|---------------|---------|---------|---------|-----|---------------|-------------------|
| ELSD     | 1       | 1.008   | 1.006   | 1.014         | 1.073   | 1.146   | 1.364   | 1.092 | 7.23          | 1.44              |
|          | 2       | 1.008   | 1.006   | 1.014         | 1.072   | 1.148   | 1.366   | 1.091 | 7.29          | 1.43              |
| RI       | 1       | 1.020   | 1.020   | 1.020         | 1.086   | 1.099   | 1.174   | 1.094 | 2.52          | 1.19              |
|          | 2       | 1.020   | 1.020   | 1.040         | 1.087   | 1.099   | 1.172   | 1.092 | 2.48          | 1.16              |
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nearly equal responses to the HPLC-ELSD but slightly higher responses for POP. However, the POP and PPP for palm oil and its fractions do not respond equally to HPLC-ELSD. The peak-area ratios and percentage area were found to differ for RID and ELSD. It is recommended that authentic standards be used when HPLC-ELSD is used for the quantification of TAGs in palm oil and its fractions. Otherwise, when percentage area is used, the relative response factor for each TAG component should be included. The HPLC-RID gave more accurate results for area composition(%) and peak-area ratio because of the equal detector response from the TAGs components.

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