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# PHYTOCHEMICAL INSIGHTS ON PALM OILS AND EXTRA VIRGIN OLIVE OIL

## (Tinjauan Fitokimia Minyak Sawit dan Pati Minyak Zaitun)

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**Abstract**

Plants are a major source of oils for food and oleochemicals. Oftentimes, plant oils are highly processed prior to consumer access to achieve certain criteria of appearance and adaptability at the expense of valuable natural components or phytochemicals. Palm oil is refined and fractionated into various end products and is one of the most globally consumed plant oils due to its versatility. In comparison, extra virgin olive oil (EVOO) is prized for its phytonutrients. Refined palm cooking oils, laboratory cold-pressed palm oil (CPPO) and EVOO were subjected to phytochemical analysis to detect the presence of phenolics and flavonoids and to assess their antioxidant activities. The extracts from oils with minimum treatment such as CPPO and EVOO showed higher phytochemicals presence of total flavonoid and phenolic contents, consistent with their chemical profiles observed in the liquid- and gas chromatography-mass spectrometry (LC-MS and GC-MS) analyses. The finding calls for more exploration on the palm oil phytochemical constituents and ultimately the enhancement of refining processes to seek balance between the industrial feasibility and the availability of valuable phytochemicals with potential health benefit.

**Keywords:** phytochemicals, palm oil, extra virgin olive oil, liquid and gas chromatography, mass spectrometry

**Abstrak**

Tumbuhan adalah sumber utama minyak untuk makanan dan bahan oleokimia. Sering kali, ianya menjalani pelbagai pemprosesan sebelum menemui pengguna untuk mencapai kriteria dan penampilan produk hiliran. Faktor ini menyebabkan hilangnya komponen semula jadi atau bahan fitokimia yang berharga daripada minyak tumbuhan tersebut. Minyak sawit ditapis dan mengalami pemeringkatan sebelum menjadi pelbagai produk akhir dan merupakan salah satu minyak tumbuhan yang paling banyak digunakan di dunia kerana ciri serba gunanya. Sebagai perbandingan, pati minyak zaitun (EVOO) mempunyai permintaan tinggi kerana kandungan fitonutriennya. Minyak masak sawit diproses, minyak dari perahan sejuk dari mesokarpa sawit (CPPO) dan EVOO dianalisis secara fitokimia untuk mengesan kehadiran fenolik dan flavonoid dan menilai aktiviti antioksidan mereka. Ekstrak dari minyak dengan proses yang minimum seperti CPPO dan EVOO menunjukkan kehadiran fitokimia flavonoid dan fenolik yang lebih tinggi, selaras dengan profil kimia mereka melalui analisis kromatografi cecair dan gas yang digabungkan dengan spektrometri jisim (LC-MS dan GC-MS). Penemuan ini menggalakkan lebih banyak penerokaan terhadap bahan fitokimia sawit dan seterusnya penambahbaikkan berterusan terhadap pemprosesan minyak sawit untuk

mendapatkan keseimbangan di antara kehendak industri dan keperluan bahan fitokimia yang bermanfaat untuk kesihatan pengguna.

**Kata kunci:** bahan fitokimia, minyak sawit, pati minyak zaitun, kromatografi cecair dan gas, spektrometer jisim

## Introduction

The natural chemical components of plants are responsible for their notable colour and organoleptic properties. These metabolites are of biological importance to plants; providing defence against pathogens, herbivores and solar radiation, and function as signalling molecules and regulators in addition to their role as structural components. Flavonoids (phytochemicals with 2-phenyl-1,4-benzopyrone backbone) and phenolics (natural chemicals with aromatic ring(s) with attached hydroxyl groups) are among the known plant chemicals that are bioactive against free radicals, cancerous cells, bacteria and viruses and possess therapeutic functions of anti-inflammatory, antimutagenic, antitumor and many other beneficial effects on human health [1, 2]. They are also the source of valuable commercial products of drugs, dyes, fragrances and flavours.

Plants oils and fats are consumed globally and are essential food supplies for the world's rising population. Among all the vegetable oils, palm oil ranks as the most widely produced and traded crop product [3]. It is extracted from the mesocarp flesh of the oil palm drupes and in its natural presence, contains phytochemicals such as pigments, flavonoids and other lipophilic compounds of tocols, carotenoids, phytosterols and squalene [4]. The oil palm fruit bunch undergoes mechanical processes of sterilization, fruit loosening, oil extraction and clarification to produce crude palm oil (CPO). It is further treated physically and chemically into refined, bleached and deodorized palm oil (RBDPO) or neutralized, bleached, and deodorized palm oil (NBDPO) before fractionation to obtain solid (stearin) and liquid (olein) phases for end use or downstream applications [5]. As a result, some natural components were removed in the pursuit of the versatility of colourless and odourless refined cooking oil. Olive oil is another important vegetable oil especially in the European market and is available in the market in several grades. The extra virgin olive oil (EVOO) is obtained from the first pressing and is untreated chemically during the oil extraction, allowing it to retain its phenolic compounds such as tyrosol, hydroxytyrosol, oleuropein and natural vitamins [6].

A holistic evaluation on palm oil natural chemical profile is warranted as previous investigations mainly focused on the palm oil industry waste valorization by harnessing phytochemicals from oil palm biomass and processing wastewater [7, 8, 9]. Thus, this work was undertaken to investigate and compare the phytochemical profiles of refined and unrefined palm oils using the EVOO as a benchmark, based on the phytochemical screening and liquid and gas chromatography-mass spectrometry (LC- and GC-MS) analyses. The information could promote substantial improvement in the palm oil processing efficiency and in due course encourage the palm oil industry to retain the natural phytonutrients in the palm oil and increase their value and significance for human consumption.

## Materials and Methods

### Chemicals

Solvents and acetic acid of high performance liquid chromatography (HPLC) grade, SeccoSolv dried pyridine (max. 0.0075% H2O), perfluorotributylamine (PFTBA), methoxyamine hydrochloride, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one (quercetin) (≥95%), 2,3-dihydrobenzoic acid (DHBA) (≥99%), 3,4,5-trihydroxybenzoic acid (gallic acid) (≥98.0%), 2,6-di-tert-butyl-4-methylphenol (BHT) (≥99.0%), Folin & Ciocalteu's phenol reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were purchased from Merck (Darmstadt, Germany). N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) plus 1% trimethylchlorosilane (TMCS) was obtained from Thermo Scientific (Bellefonte, PA, USA) while water was prepared from Milli-Q system water purification unit (Millipore Lab, Bedford, MA, USA).

**Oil samples and extraction of phytochemicals**

Palm cooking oil (refined, bleached and deodorized (RBD) palm olein), commercial red palm oil (RPO) and extra virgin olive oil (EVOO) (first mechanically pressed olive oil) were purchased from a supermarket shelf well within their production and expiry dates. Palm cooking oil spiked with 1 mg of quercetin and DHBA chemical standards was used as positive control after observation of their appropriate peak heights in mass spectrometry detection. Cold-pressed palm oil (CPPO) was prepared in the laboratory from *Elaeis guineensis* (oil palm) fresh fruit bunch harvested from the Malaysian Palm Oil Board (MPOB) Kluang Research Station, Malaysia. The flesh (mesocarp tissue) was removed from the kernel and cut into smaller pieces before being pressed in a stainless-steel manual oil extractor. The resulting oil was filtered and centrifuged at room temperature for 5 minutes at 4000 rotation per min (rpm) to separate the oil from mesocarp debris. These processes did not involve heating or the usage of chemicals. Each of the oil samples of 5 mL volume were extracted in triplicates with 30 mL methanol with 4 hours, 250 rpm shaking at room temperature based on previous works [10,11]. The mixture was then centrifuged for 10 min at 3000 rpm after which the supernatant was dried using N-EVAP nitrogen evaporator (Organomation, MA, USA). The dried residue was suspended in 10 mL acetonitrile and washed twice with hexane to remove lipid components. The mixture was vortexed and centrifuged for 10 min to discard the upper layer hexane and the lower layer of acetonitrile was again dried over nitrogen stream. The dried extract was reconstituted in the solvent of choice based on the assay(s) or the chromatographic mobile phase.

**Phytochemical and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays**

All assays were conducted in triplicates for reproducibility. Alkaline reagent test indicated the presence of flavonoids with the change of sample colour to colourless based on previous works [12,13]. The appearance of dark green colour for ferric chloride test performed according to Raaman [12] and Usman et al. [14] indicated the presence of phenols in the sample solution. For quantitation, aluminium chloride colorimetric technique modified from Aryal et al. [15] was used for flavonoid content estimation equivalent to quercetin while Folin-Ciocalteu assay was modified from Dudonne et al. [16] for phenol quantitation expressed in terms of gallic acid equivalent, GAE (mg/mL of dry mass). The antioxidant activity percentage (AA%) for DPPH radical scavenging was calculated from the absorbance values (Abs) recorded at 517 nm [17] as follows:

$$AA\% = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100 \quad (1)$$

The extract samples and BHT concentrations were prepared in a series of 50, 100, 150, 200 and 250 ppm concentrations. The absorbance of an analyte solution is proportional to the concentration of the absorbing species in the solution [18].

**Statistical analysis**

One-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) post hoc test were performed using IBM SPSS Statistics Version 20 for the total flavonoids, phenolics, and antioxidant assays. Statistical $p$ values (set at a threshold of $p < 0.01$) indicate levels of significant difference between the sets of data being analyzed.

**Liquid chromatography-mass spectrometry (LC-MS) analysis**

The dried extracts were reconstituted in methanol and 1 µL volume were injected and separated on a Thermo Fisher Scientific (MA, USA) C18 Reversed-Phase Acclaim® 120Å column of 4.6 mm internal diameter (ID), 150 mm length and 5 µm particle size at 35°C on a Thermo Scientific Ultimate 3000 HPLC. Gradient elution was performed with water containing 0.1% acetic acid (solvent A) and 100% methanol (solvent B) with the following gradient: 0-1.5 min: 5% solvent B, 1.5-15.0 min: B eluted up to 95%, 15.0-24.0 min: 95% B, 24.0-37.0 min: B eluted down to 5% and 37.0-39.5 min: 5% B. MS analysis was performed on MicrOTOF-Q™ quadrupole-time-of-flight (Q/TOF) MS (Bruker Daltonik GmbH, Bremen, Germany) for accurate mass measurement with 3.5 bar nebulizer gas (N2), 8.0 L/min dry gas, 200°C dry temperature, -3500 V capillary voltage and -500 V end plate offset in the

negative electrospray ionization (ESI) mode for a reduced background noise [19] in the mass-to-charge ratio (*m/z*) range of 50-1000. Data processing, molecular formula candidate computation and identity matching to available chemical public databases were performed on Data Analysis 3.4 and Compound Crawler 2.0 (Bruker Daltonik GmbH).

**Gas chromatography-mass spectrometry (GC-MS) analysis**

For derivatization, the dried samples were dissolved in 80 µL of 20 mg/mL methoxamine hydrochloride in pyridine and incubated at 37 °C for 90 minutes. The mixtures were added with 80 µL MSTFA, incubated for another 30 minutes at 37 °C and allowed to equilibrate at room temperature for an hour. Both the derivatized and underivatized samples in methanol of 1 µL volume were analysed on the 7200B Agilent GC Q/TOF MS with electron ionization (EI) at 70 eV energy, 1 mL/min constant helium carrier gas flow on Agilent J&W Scientific (CA, USA) HP5-MS UI column (0.25 mm ID, 30 m length, 0.25 µm film thickness). The oven temperature began at 50 ˚C, held for 1 minute, followed by an increase of 11˚C/min to 180˚C, held again for 3 minutes, ramped to 300 ˚C and maintained for 18 minutes with solvent delay set at 3.75 minutes. The raw data from 50-1000 atomic mass unit (amu) scanning range were deconvoluted on Mass Hunter Workstation Qualitative Analysis Software B.07.00. Peak identification was by mass spectra matching at ≥70% similarity to the National Institute of Standards and Technology (NIST, US) Library using MS Search 2.2 Software (John Wiley & Sons, Inc., NJ, USA) and G1676AA Agilent Fiehn 2013 GC-MS Metabolomics Library. The acquisition method was locked to the derivatized ribitol commercial standard at 17.44 min and *m/z* 319.1684 at 1 mL/min helium using the Retention Time Locking (RTL) setup.

## Results and Discussion

**Flavonoids and phenolics screening**

The extracts of extra virgin olive oil (EVOO), cold-pressed palm oil (CPPO), palm cooking oil spiked with quercetin and DHBA as positive control and red palm oil (RPO) showed positive changes for flavonoids and phenolics compared to palm cooking oil (Table 1). These results from manual observation exhibited colours and lucidity ranges and were further ascertained by their concentration equivalent to quercetin (flavonoid) and gallic acid (phenolic) chemical standards (Figure 1). The extract of CPPO was recorded with the highest total flavonoid content of 0.0984 quercetin equivalents (QE) mg/mL followed by EVOO with 0.0904 QE mg/mL flavonoids, RPO at 0.0489 QE mg/mL, positive control at 0.0417 QE mg/mL and lastly the palm cooking oil extract with 0.0324 QE mg/mL total flavonoids. The extract from EVOO was recorded to contain the highest phenolic compounds of 0.2771 mg/mL equivalent to gallic acid (GAE) compared to other samples, surpassing the positive control with 0.1449 GAE mg/mL total phenolics. This was followed by CPPO of 0.0774 GAE mg/mL phenolics, palm cooking oil with 0.0695 GAE mg/mL phenolics and lastly the RPO extract with 0.0651 GAE mg/mL total phenolics. In brief, for these tests, EVOO followed by CPPO demonstrated the highest phytochemical content among the oil extracts.

**2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay**

Table 2 lists the average antioxidant activity (AA%) for the oil sample extracts and the antioxidant standard of BHT in a series of concentration ranging from 100 to 1000 ppm.

Among the oil sample extracts, the positive control sample recorded the overall highest antioxidant activity (AA%) of 27.82 to 80.60% across the series of concentration. At 100 ppm, EVOO extract recorded the second highest antioxidant activity after positive control while RPO extract had the lowest. At 250 and 500 ppm, EVOO maintained its high activity and CPPO and palm cooking oil exhibited the lowest activity among the oil extracts. At 750 ppm, RPO surpassed that of EVOO extract after positive control and lastly, at 1000 ppm, RPO extract topped the other oil extracts at 62.01% antioxidant activity. Overall, EVOO displayed a constant and high antioxidant activity among all samples up to 750 ppm concentration. Despite its lowest activity at 100 ppm concentration, RPO extract rose to high antioxidant capacity parallel to that of EVOO at 750 ppm and

exhibited the highest activity at 1000 ppm, with two-fold value compared to the rest of the samples. Antioxidant activities of CPPO were low, comparable to that of palm cooking oil extract. The fact that the antioxidant activity of CPPO extract was lower than the other oils require some deliberation as illustrated by several studies of flavonoids that exert both antagonistic and synergistic activities in a tested oxidation system [20, 21]. The average antioxidant activity (AA%) values across the series of concentration were plotted into a graph in Figure 2.

Table 1. Flavonoid and phenolic contents in extra virgin olive oil (EVOO), cold-pressed palm oil (CPPO), red palm oil (RPO) and palm cooking oil extracts

| Sample | Flavonoids | | | | Phenolics | | | |
|---|---|---|---|---|---|---|---|---|
| | **Visual screening** | **Concentration (mg QE/mL)** | **Average (ppm) ±SD** | **Relative Standard Deviation (RSD)** | **Visual screening** | **Concentration (mg GAE/mL)** | **Average (ppm) ±SD** | **Relative Standard Deviation (RSD)** |
| EVOO | + | $0.0904^{b}$ | 90.38 ±4.31 | 4.77 | Cloudy light green solution | $0.2771^{a}$ | 277.07 ±10.71 | 3.86 |
| CPPO | Colourless solution with a top layer of precipitate | $0.0984^{a}$ | 98.44 ±1.31 | 1.33 | Cloudy orange solution with a top layer of precipitate | $0.0774^{c}$ | 77.38 ±7.89 | 10.20 |
| RPO | + | $0.0489^{c}$ | 48.91 ±0.72 | 1.46 | Cloudy yellow solution | $0.0651^{c}$ | 65.13 ±7.52 | 11.54 |
| Palm cooking oil | White cloudy solution | $0.0324^{e}$ | 32.35 ±0.66 | 2.03 | Cloudy light yellow solution | $0.0695^{c}$ | 69.47 ±4.88 | 7.02 |
| Positive control | + | $0.0417^{d}$ | 41.74 ±0.28 | 0.68 | + | $0.1449^{b}$ | 144.90 ±8.17 | 5.64 |

*+ = positive change; QE = quercetin equivalents; GAE = gallic acid equivalents, SD = standard deviation ($n$=3)
*Flavonoids ($F_{4,\ 10}$ = 642.01, $p < 0.0001$), phenolics ($F_{4,\ 10}$ = 366.24, $p < 0.0001$), superscript letters a, b, c, d and e denote significance ($p < 0.01$) from highest to lowest at each subset at $\alpha = 0.05$ according to Tukey's HSD

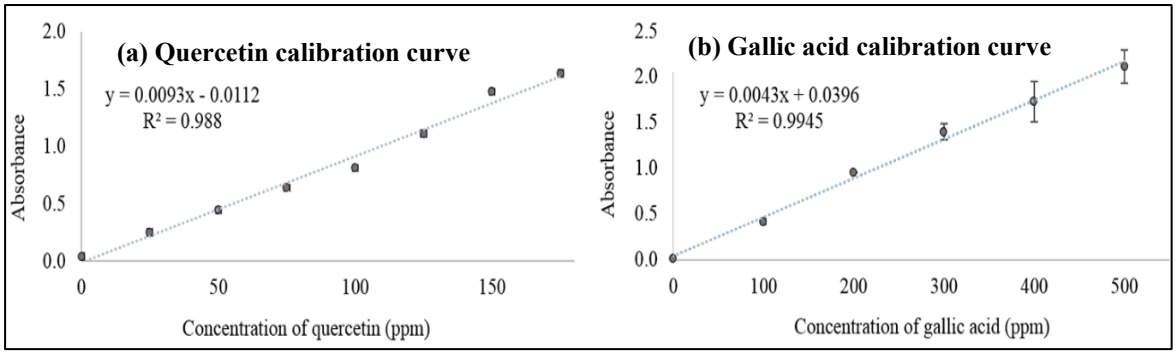


Figure 1. (a) Quercetin and (b) gallic acid standards calibration curves for flavonoid and phenolic quantitation.

Table 2. Average antioxidant activity (AA%±SD) according to sample concentration (ppm)

| Sample | 100 ppm | 250 ppm | 500 ppm | 750 ppm | 1000 ppm |
|---|---|---|---|---|---|
| BHT | 36.95 ±0.69[a] | 44.31 ±0.80[a] | 59.10 ±7.77[a] | 86.14 ±0.17[a] | 96.61 ±1.14[a] |
| Positive control | 27.82 ±0.00[b] | 43.66 ±0.57[a] | 56.60 ±4.23[a] | 70.17 ±2.40[a] | 80.60 ±0.69[a] |
| EVOO | 24.18 ±1.37[c] | 27.90 ±6.40[b] | 30.41 ±3.77[b] | 35.10 ±1.26[b] | 39.22 ±4.12[c] |
| RPO | 16.08 ±0.03[d] | 22.24 ±4.80[b] | 26.29 ±8.00[b] | 35.50 ±9.37[b] | 62.01 ±1.37[b] |
| CPPO | 17.90 ±0.48[d] | 19.25 ±1.26[b] | 25.70 ±2.20[b] | 29.55 ±1.65[b] | 31.62 ±3.43[c] |
| Palm cooking oil | 16.16 ±0.13[d] | 19.02 ±0.22[b] | 26.04 ±7.89[b] | 28.28 ±9.45[b] | 29.52 ±11.20[c] |

*Superscript letters a, b, c, and d denote significant different activities ($p < 0.01$) from highest to lowest at each subset at $\alpha = 0.05$ according to Tukey's HSD

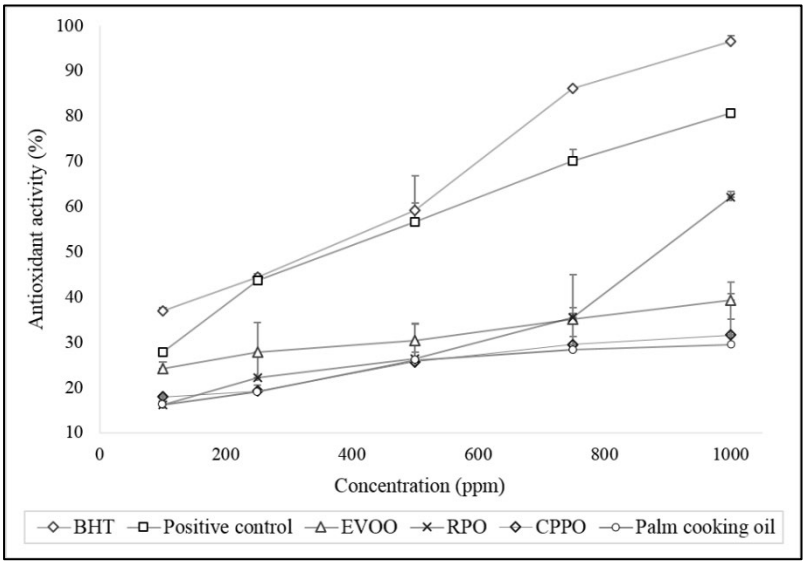


Figure 2. Average antioxidant activities (AA%) of BHT standard, EVOO and palm oil extracts

**Liquid chromatography-mass spectrometry (LC-MS) analysis of palm oil extracts**

Figure 3(a) shows the LC-MS chromatograms of the investigated oil extracts with numbered peaks and identities of the phytochemicals. The details of retention times ($t_R$), molecular formulae, molecular ion masses (*m/z*, $[M-H]^-$) with mass errors (mDa), ESI MS/MS fragments for the numbered phytochemical peaks and references to previous investigations are listed in Table 3. The availability of accurate mass measurement by TOF mass spectrometers with resolving power from 8,000 to 20,000 (m/Δm, full width at half maximum; FWHM) allows the determination of elemental composition of monoisotopic mass [22,23]. The identity of peak **1** (*m/z* 187.0992 $[M-H]^-$, $C_9H_{15}O_4$, err [mDa] 1.6) and its tandem MS (MS/MS) fragments of *m/z* 169.0894 ($[M-H]^-$, $C_9H_{13}O_3$, err [mDa] 2.4) and 125.0970 ($[M-H]^-$, $C_8H_{13}O$, err [mDa] 0.2) matched that of nonanedioic acid (azelaic acid) [24], a natural dicarboxylic acid plant signalling metabolite [25]. Peak **2** of *m/z* 171.1048 ($[M-H]^-$, $C_9H_{15}O_3$, err [mDa] 2.1) was identified as oxononanoic acid, a medium-chain fatty acid also found in Camellia (tea seed) oil and grapes [26,27]. Peak **3** (*m/z* 285.0794 $[M-H]^-$, $C_{16}H_{13}O_5$, err [mDa] 2.5) was fragmented by MS/MS into *m/z* 165.0209, 119.0463 and 93.0296, consistent to those of sakuranetin ((2S)-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-2,3-dihydrochromen-4-one) [28, 29].

Peak **4** (*m/z* 329.2344 $[M-H]^-$, $C_{18}H_{33}O_5$, err [mDa] 1.0) corresponded to trihydroxyoctadecenoic acid, which was discovered using LC-MS in olive oil [30]. This trihydroxy fatty acid was also screened in Japanese elm and proposed as one of the anti-inflammatory compounds tested [31]. Peak **5** of *m/z* 283.0637 ($[M-H]^-$, $C_{16}H_{11}O_5$, err [mDa] 2.5) was fragmented into *m/z* 268.0405 with 15 amu neutral loss, denoting a loss of methyl group ($CH_3$) from the molecular ion. A multitude of candidates from flavone and isoflavone classes of flavonoids correspond to the generated molecular formula of $C_{16}H_{12}O_5$, but the loss of $CH_3$ observed in the MS/MS spectra of peak **5** narrowed the candidates to those with *O*-methylated group. Based on earlier reports of LC-MS and MS/MS data, peak **5** was proposed as 5,7-dihydroxy-8-methoxy-2-phenyl-4H-chromen-4-one or wogonin, an *O*-methylated flavone [32,33]. Flavonoids with methyl and methoxy groups found in the CPPO are known to exhibit the least antioxidant properties compared to other flavonoids with hydroxyl groups [34,35], consistent with the results of the DPPH radical scavenging assay. Nevertheless, flavonoids with these functional groups are of superior bioavailability and their beneficial properties are well introduced into human systemic circulation [36].

Peak **6** (*m/z* 311.2254 $[M-H]^-$, $C_{18}H_{31}O_4$, err [mDa] 2.6) was a hydroxy-oxooctadecenoic acid oxylipin, a group of plant oxygenated fatty acids. The loss of HO-$CH{=}CH(CH_2)_3CH_3$ side-group from the oxylipin molecule during MS/MS as illustrated in Figure 4 can be detected by monitoring 100 amu neutral loss in the LC/MS chromatogram as shown in Figure 5. This deduction was ascertained with the observation of the three common oxylipin molecular ions of *m/z* 329 (as found for peak **4**), *m/z* 311 (as recorded for peak **6**) and *m/z* 295 (peak **8,** *m/z* 295.2319 $[M-H]^-$, $C_{18}H_{31}O_3$, err [mDa] 4.0) that were all fragmented to yield *m/z* 171, a fragment that contains a carboxyl and an OH-group which are characteristics to oxylipins -ESI MS/MS fragments [37]. These natural hydroperoxides were found in several plants such as barley and wheat and are involved in plant development and growth, and protection against stressors [38, 37, 39].

Peak **7** (*m/z* 171.1401 $[M-H]^-$, $C_{10}H_{19}O_2$, err [mDa] 1.0) was identified as decanoic acid, a saturated fatty acid also known as capric acid [40]. Peaks **9** (*m/z* 199.1734 ($[M-H]^-$, $C_{12}H_{23}O_2$, err [mDa] 3.0), **11** (*m/z* 277.2145 $[M-H]^-$, $C_{18}H_{29}O_2$, err [mDa] 2.8), **12** (*m/z* 253.2204 $[M-H]^-$, $C_{16}H_{29}O_2$, err [mDa] 3.1), **13** (*m/z* 279.2310 $[M-H]^-$, $C_{18}H_{31}O_2$, err [mDa] 1.9), **14** (*m/z* 255.2307 $[M-H]^-$, $C_{16}H_{31}O_2$, err [mDa] 2.2), **15** (*m/z* 281.2466 $[M-H]^-$, $C_{18}H_{33}O_2$, err [mDa] 2.0) and **16** (*m/z* 283.2610 $[M-H]^-$, $C_{18}H_{35}O_2$, err [mDa] 3.3) were deduced as acids of dodecanoic (lauric), octadecatrienoic (linolenic, ALA), (9Z)-hexadec-9-enoic (palmitoleic, POA), (9Z,12Z)-octadeca-9,12-dienoic (linoleic, LA), hexadecanoic (palmitic, PA), (9Z)-octadec-9-enoic (oleic, OA) and octadecanoic (stearic, SA) respectively as previously discovered by LC-MS analyses while

peak **10** (*m/z* 413.2909 $[M-H]^-$, $C_{23}H_{41}O_6$, err [mDa] 3.6) was identified as glyceryldiacetate 2-palmitate with MS/MS fragment of acetate ion of $[C_2H_3O_2]^-$ (*m/z* 59.0139 $[M-H]^-$, err [mDa] 2.2) [41,42]. Similar to the CPPO extract, peaks **1** and **10** in the palm cooking oil and RPO extracts LC-MS chromatograms in Figure 3(b) was identified as nonanedioic acid and glyceryldiacetate 2-palmitate. The processed palm oil extracts also contain linolenic, linoleic, palmitic, oleic and stearic acids.

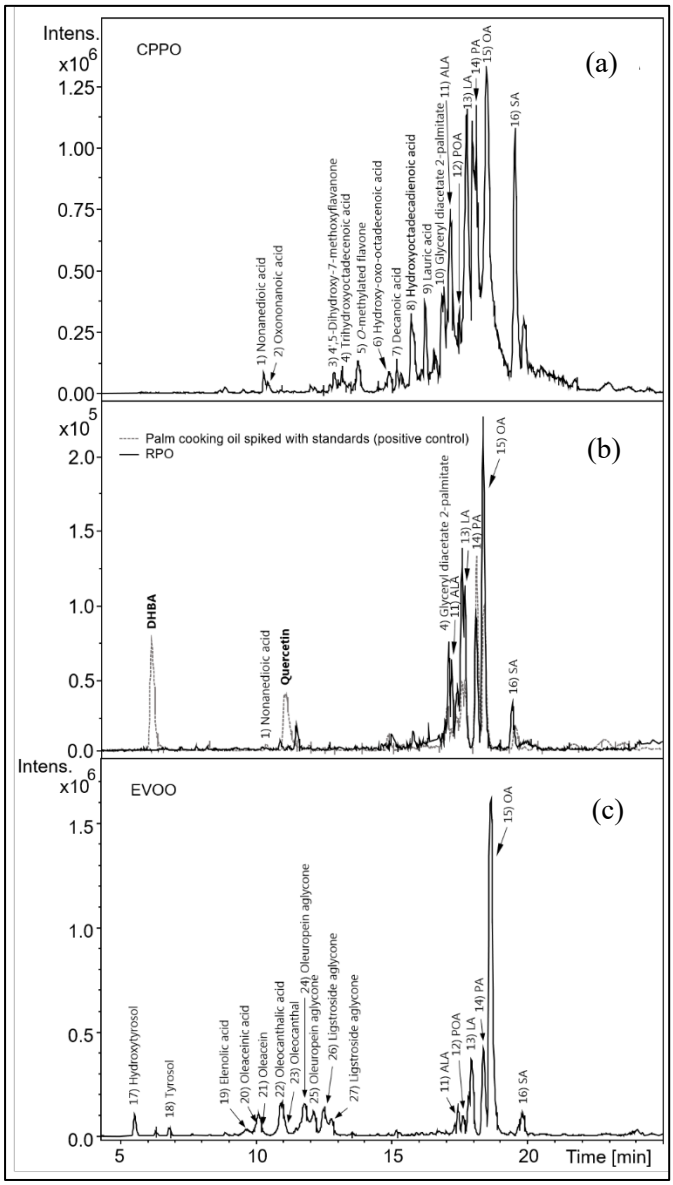


Figure 3. LC-MS chromatograms of CPPO (a), palm cooking oil spiked with DHBA and quercetin, overlapped with RPO extracts (b) and EVOO extract (c). Abbreviation: DHBA, 2,3-dihydrobenzoic acid; ALA, linolenic acid; POA, palmitoleic acid; LA, linoleic acid; PA, palmitic acid; OA, oleic acid; SA, stearic acid).

Table 3. Identified phytochemicals in palm oils and extra virgin olive oil using LC-MS/MS

| Peak | Phytochemicals | $t_R$ (min) | Molecular Formula | Measured mass-to-charge Ratio (*m/z*), $[M-H]^-$ | Mass Error [mDa] | MS/MS Fragments (*m/z*) | Classification Details | CPPO | Palm Cooking Oil | RPO | EVOO | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Nonanedioic acid (azelaic acid) | 10.8 | $C_9H_{16}O_4$ | 187.0992 | 1.6 | 169.0894, 125.0970 | Dicarboxylic acid, plant signalling metabolite | √ | √ | √ | | [24,25] |
| 2 | Oxononanoic acid | 10.9 | $C_9H_{16}O_3$ | 171.1048 | 2.1 | 127.1163 | Medium-chain oxo-fatty acid | √ | | | | [26,27] |
| 3 | Dihydroxy-methoxyflavanone (sakuranetin) | 13.3 | $C_{16}H_{14}O_5$ | 285.0794 | 2.5 | 165.0209, 119.0463, 93.0296 | Methoxyflavanone | √ | | | | [28,29] |
| 4 | Trihydroxy-octadecenoic acid | 13.6 | $C_{18}H_{34}O_5$ | 329.2344 | 1.0 | 229.1504, 171.1023 | Trihydroxy fatty acid | √ | | | | [30,31] |
| 5 | Dihydroxy-methoxyflavone (wogonin) | 14.2 | $C_{16}H_{12}O_5$ | 283.0637 | 2.5 | 268.0405 | *O*-methylated flavone | √ | | | | [32,33] |
| 6 | Hydroxy-oxooctadecenoic acid | 15.3 | $C_{18}H_{32}O_4$ | 311.2254 | 2.6 | 293.2152, 211.1358, 171.1043 | Plant oxygenated fatty acid, natural hydroperoxide (oxylipin) | √ | | | | [37,38,39] |
| 7 | Decanoic acid (capric acid) | 15.6 | $C_{10}H_{20}O_2$ | 171.1401 | 1.0 | 153.1436, 127.0374 | Saturated fatty acid | √ | | | | [40] |
| 8 | Hydroxyoctadecadienoic acid | 16.2 | $C_{18}H_{32}O_3$ | 295.2319 | 4.0 | 277.2198, 195.1421, 171.1048 | Plant oxygenated fatty acid, natural hydroperoxide (oxylipin) | √ | | | | [37,38,39] |
| 9 | Dodecanoic acid (lauric acid) | 16.6 | $C_{12}H_{24}O_2$ | 199.1734 | 3.0 | 181.0247 | Saturated fatty acid | √ | | | | [40] |
| 10 | Glyceryldiacetate 2-palmitate | 17.1 | $C_{23}H_{42}O_6$ | 413.4420 | 3.6 | 59.0117 | Diglyceride derivatives | √ | √ | √ | | [41,42] |
| 11 | Octadecatrienoic acid (linolenic, ALA) | 17.5 | $C_{18}H_{30}O_2$ | 277.2145 | 2.8 | 233.2288 | Polyunsaturated omega-3 fatty acid | √ | √ | √ | √ | [41,42] |
| 12 | Hexadecenoic acid (palmitoleic, POA) | 17.6 | $C_{16}H_{30}O_2$ | 253.2204 | 3.1 | 209.1381 | Monounsaturated omega-7 fatty acid | √ | | | √ | [41,42] |
| 13 | Octadecadienoic acid (linoleic, LA) | 18.0 | $C_{18}H_{32}O_2$ | 279.2310 | 1.9 | 261.2226 | Polyunsaturated omega-6 fatty acid | √ | √ | √ | √ | [41,42] |
| 14 | Hexadecanoic acid (palmitic, PA) | 18.4 | $C_{16}H_{32}O_2$ | 255.2307 | 2.2 | 211.2005 | Saturated fatty acid | √ | √ | √ | √ | [41,42] |
| 15 | Octadecenoic acid (oleic, OA) | 18.6 | $C_{18}H_{34}O_2$ | 281.2466 | 2.0 | 237.3641 | Monounsaturated omega-9 fatty acid | √ | √ | √ | √ | [41,42] |
| 16 | Octadecanoic acid (stearic, SA) | 19.8 | $C_{18}H_{36}O_2$ | 283.2610 | 3.3 | 239.2309 | Saturated fatty acid | √ | √ | √ | √ | [41,42] |

Table 3 (cont'd). Identified phytochemicals in palm oils and extra virgin olive oil using LC-MS/MS

| Peak | Phytochemicals | $t_R$ (min) | Molecular Formula | Measured mass-to-charge Ratio (*m/z*), $[M-H]^-$ | Mass Error [mDa] | MS/MS Fragments (*m/z*) | Classification Details | CPPO | Palm Cooking Oil | RPO | EVOO | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | Hydroxytyrosol | 5.6 | $C_8H_{10}O_3$ | 153.0567 | 1.0 | 123.0454 | Phenylethanoid phenolic compound | | | | √ | [43,44] |
| 18 | Tyrosol | 6.8 | $C_8H_{10}O_2$ | 137.0619 | 1.1 | 119.0484, 106.0420 | Phenylethanoid phenolic compound | | | | √ | [44] |
| 19 | Elenolic acid | 9.7 | $C_{11}H_{14}O_6$ | 241.0726 | 0.9 | 165.0736, 139.0079, 127.0422 | Phenolic compound | | | | √ | [45] |
| 20 | Oleaceinic acid | 10.1 | $C_{17}H_{20}O_7$ | 335.1160 | 2.4 | 181.0520, 155.0716, 111.0836 | Phenolic secoiridoids | | | | √ | [46] |
| 21 | Oleacein | 10.1 | $C_{17}H_{20}O_6$ | 319.1187 | 2.6 | 275.1028, 249.0820, 183.0694, 165.0572, 69.0333 | Phenolic secoiridoids | | | | √ | [47,48] |
| 22 | Oleocanthalic acid | 10.9 | $C_{17}H_{20}O_6$ | 319.1246 | 1.4 | 199.0646, 181.0531, 111.0105 | Phenolic secoiridoids | | | | √ | [46] |
| 23 | Oleocanthal | 11.1 | $C_{17}H_{20}O_5$ | 303.1243 | 0.5 | 285.1155, 217.0054, 183.0693, 165.0605, 141.0183 | Phenolic secoiridoids | | | | √ | [47,48] |
| 24 | Oleuropein aglycone isomer | 11.8 | $C_{19}H_{22}O_8$ | 377.1252 | 1.0 | 307.0874, 275.0870, 149.0267, 139.0371 | Phenolic secoiridoids | | | | √ | [49,50] |
| 25 | Oleuropein aglycone isomer | 12.1 | $C_{19}H_{22}O_8$ | 377.1249 | 0.7 | 307.0874, 275.0855, 149.0268, 139.0371 | Phenolic secoiridoids | | | | √ | [49,50] |
| 26 | Ligstroside aglycone | 12.5 | $C_{19}H_{22}O_7$ | 361.1304 | 1.1 | 291.0920, 259.0954 | Phenolic secoiridoids | | | | √ | [47,50,51] |
| 27 | Ligstroside aglycone | 12.8 | $C_{19}H_{22}O_7$ | 361.1329 | 3.6 | 291.0924, 259.1012 | Phenolic secoiridoids | | | | √ | [47,50,51] |

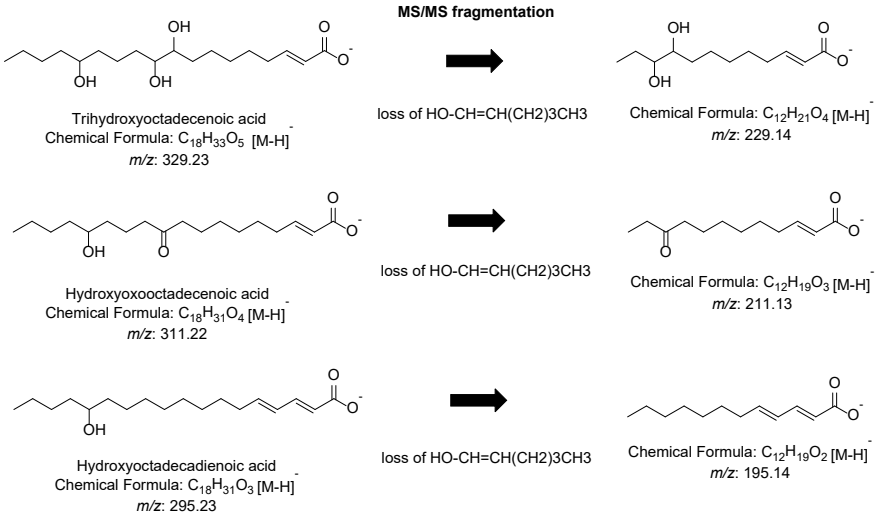


Figure 4. Proposed MS/MS fragmentation of peaks 4, 6 and 8 with the neutral loss of 100 atomic mass unit (amu) corresponding to HO-CH=CH($CH_2$)$_3$$CH_3$ moiety

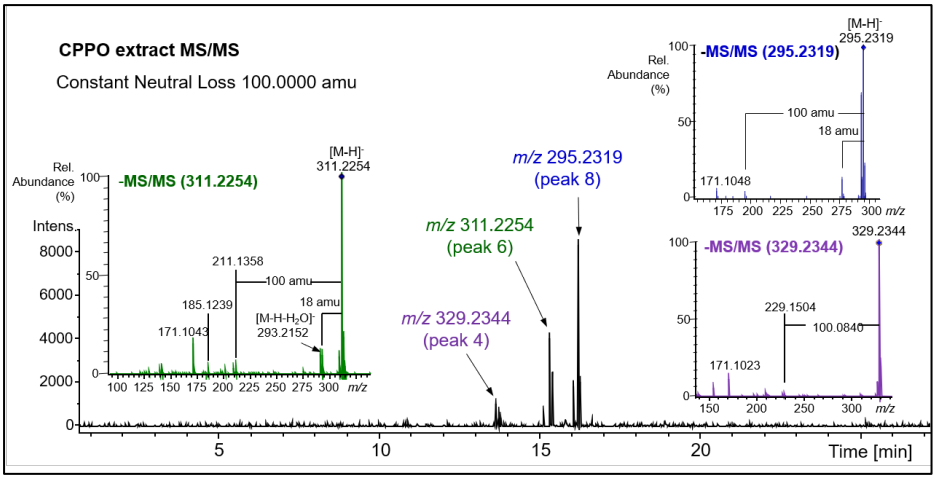


Figure 5. CPPO extract 100.000 amu neutral loss chromatogram and MS/MS spectra of peaks 4, 6 and 8

**LC-MS/MS analysis of extra virgin olive oil (EVOO) extract**

Peak **17** of *m/z* 153.0567 ([M-H]$^-$, $C_8H_9O_3$, err [mDa] 1.0) observed in the EVOO chromatogram in Figure 3(c) was identified as hydroxytyrosol (4-(2-hydroxyethyl)-1,2-benzenediol), a phenylethanoid phenolic compound with MS/MS fragment of *m/z* 123.0454 ($C_7H_7O_2$, err [mDa] 0.2) after losing $CH_2O$ from its molecular structure [43,44]. Peak **18** (*m/z* 137.0619 [M-H]$^-$, $C_8H_9O_2$, err [mDa] 1.1) that was fragmented into *m/z* 119.0484 after loss of $H_2O$ and *m/z* 106.0420 due to removal of methoxy radical ($CH_3O$•) was none other than tyrosol (4-(2-hydroxyethyl)phenol), another significant phenolic constituent of olive oil [44]. Peak **19** (*m/z* 241.0726 [M-H]$^-$, $C_{11}H_{13}O_6$, err [mDa] 0.9) was elenolic acid (2-

[(2S,3S,4S)-3-formyl-5-methoxycarbonyl-2-methyl-3, 4-dihydro-2H-pyran-4-yl]acetic acid), a phytochemical indicator for olive ripening with its common MS/MS fragments of *m/z* 165, 139 and 127 [45]. Peak **20** (*m/z* 335.1160 $[M-H]^-$, $C_{17}H_{19}O_7$, err [mDa] 2.4) together with peak **21** (*m/z* 319.1187 $[M-H]^-$, $C_{17}H_{19}O_6$, err [mDa] 2.6), and also peaks **22** (*m/z* 319.1246 $[M-H]^-$, $C_{17}H_{19}O_6$, err [mDa] 1.4) and **23** (*m/z* 303.1243 $[M-H]^-$, $C_{17}H_{19}O_5$, err [mDa] 0.5) were coeluting respectively with both peaks **21** and **22** were of equivalent *m/z* 319. However, peak **21** yielded different MS/MS spectra with fragment ions of *m/z* 275.1028, 249.0820, 183.0694, 165.0572 and 69.0333, while peak **22** was fragmented into *m/z* 199.0646, 181.0531 and 111.0105. Based on their molecular formulae and respective MS/MS fragments, peaks **20** and **22** were coherent to those of oleaceinic acid ((E)-3-(2-(3,4-dihydroxyphenethoxy)-2-oxoethyl)-4-formylhex-4-enoic acid) and oleocanthalic acid ((E)-4-formyl-3-(2-(4-hydroxyphenethoxy)-2-oxoethyl)hex-4-enoic acid) discovered recently [46].

Peak **21** was deduced as oleacein (2-(3,4-dihydroxyphenyl)ethyl (Z)-4-formyl-3-(2-oxoethyl) hex-4-enoate) and peak **23** was identified as oleocanthal (2-(4-hydroxyphenyl)ethyl (3S,4E)-4-formyl-3-(2-oxoethyl)hex-4-enoate) [47,48]. The molecular ion mass of peaks **24** (*m/z* 377.1252 $[M-H]^-$, $C_{19}H_{21}O_8$, err [mDa] 1.0) and **25** (*m/z* 377.1249 $[M-H]^-$, $C_{19}H_{21}O_8$, err [mDa] 0.7) were of similar mass-to-charge (*m/z*) ratio of 377 in addition to their analogous MS/MS fragments of *m/z* 307, 275, 149 and 139. These molecular and fragment ions were identical to those found for oleuropein aglycone isomers (methyl (2R,4S,E)-4-(2-(3,4-dihydroxyphenethoxy)-2-oxoethyl)-3-ethylidene-2-hydroxy-3,4-dihydro-2H-pyran-5-carboxylate) [49]. Peaks **26** (*m/z* 361.1304 $[M-H]^-$, $C_{19}H_{21}O_7$, err [mDa] 1.1) and **27** (*m/z* 361.1329 $[M-H]^-$, $C_{19}H_{21}O_7$, err [mDa] 3.6) were another pair of molecular ion mass of *m/z* 361 with similar MS/MS fragments. These metabolites are secoiridoid isomers known as ligstroside aglycone (methyl(2R,E)-3-ethylidene-2-hydroxy-4-(2-(4-hydroxyphenethoxy)-2-oxoethyl)-3,4-dihydro-2H-pyran-5-carboxylate) which always yield *m/z* 291 and *m/z* 259 MS/MS fragments as exemplified in the olive and ash tree from the Oleaceae family [47,51]. Peaks 11-16 in Figure 3(c) were of linolenic, palmitoleic, linoleic, palmitic, oleic and stearic acids similar to those observed in LC-MS chromatogram of palm oil extracts.

**Gas chromatography-mass spectrometry (GC-MS) analysis of oil extracts**

The identified phytochemicals from the oil extracts analysed using GC-Q/TOF are listed in Table 4. In the shotgun GC-MS analysis of underivatized oil extracts, phytochemical **28** of 1,2-benzenediol was detected only in EVOO extract possibly from the breakage of catechol moiety in the olive oil, e.g., oleuropein, hydroxytyrosol, during the analysis. The aromatic 2,4-decadienal (phytochemical **29**) was identified in palm cooking oil, EVOO and RPO, with significant abundance in the RPO extract. Phytochemical **30** was observed only in the CPPO and was identified as 2-phenylethan-1-ol or benzeneethanol, a natural chemical found in plant essential oils and is suggested to possess medicinal properties [52]. Another important phytochemical detected only in the CPPO extract is squalene (phytochemical **31, (**6E,10E,14E,18E)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene). Squalene is a triterpene involved in the synthesis of plant sterols and was comprehensively investigated for anticancer activity [53]. The detection of all these natural chemicals in the CPPO and not in the refined palm oil extracts suggests that they were unavailable after refining.

Another batch of the oil extracts were derivatized to improve the GC-MS detection and more constituents were found to be abundance in CPPO and EVOO extracts compared to that of refined oils. Several phenolics were uniquely detected in EVOO namely tyrosol (phytochemical **18**), hydroxytyrosol (phytochemical **17**) which were also observed in the LC-MS analysis, and 4-ethenylphenol or 4-vinylphenol (phytochemical **32**). Coniferyl alcohol (phytochemical **33,** 4-[(E)-3-Hydroxyprop-1-enyl]-2-methoxyphenol), an important monolignol for the synthesis of lignin and lignans in plants was only detected in the CPPO extract indicating that the unrefined palm oil contains phytochemicals that could indirectly improve human health by lowering blood pressure, reducing

cardiovascular risk and suppressing the development of diabetes by its ingestion [54]. Tetradecanoic (myristic) (phytochemical **34**), palmitic, linoleic and oleic acids were detected in all of the oil extracts along with xanthoxin (phytochemical **35, (**2Z,4E)-5-[(1S,4S,6R)-4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl]-3-methyl penta-2,4-dienal), 2-palmitoylglycerol (phytochemical **36**, 1,3-dihydroxypropan-2-yl hexadecanoate), monopalmitin (phytochemical **37**, 2,3-dihydroxypropyl hexadecanoate) and glyceryl monooleate (phytochemical **38**, 2,3-dihydroxypropyl octadecanoate).

Table 4. Phytochemicals detected in palm cooking oil, CPPO, RPO and EVOO extracts by GC-Q/TOF

| No. | Phytochemicals* | $t_R$ (min) | Molecular Formula | Palm Cooking Oil | CPPO | RPO | EVOO | Classification |
|---|---|---|---|---|---|---|---|---|
| *Underivatized extract* | | | | | | | | |
| 28 | 1,2-Benzenediol | 8.40 | $C_6H_6O_2$ | - | - | - | √ | Phenol |
| 29 | 2,4-Decadienal | 9.71 | $C_{10}H_{16}O$ | √ | - | √ | √ | Aromatic compound |
| 30 | Benzeneethanol | 11.26 | $C_8H_{10}O$ | - | √ | - | - | Alcohol and terpene |
| 31 | Squalene | 24.07 | $C_{30}H_{50}$ | - | √ | - | - | Triterpene |
| *Derivatized extract* | | | | | | | | |
| 18 | Tyrosol | 15.11 | $C_8H_{10}O_2$ | - | - | - | √ | Phenolic |
| 17 | Hydroxytryrosol | 18.14 | $C_8H_{10}O_3$ | - | - | - | √ | Phenolic |
| 34 | Myristic acid | 19.47 | $C_{14}H_{28}O_2$ | √ | √ | √ | √ | Fatty acid |
| 33 | Coniferyl alcohol | 20.80 | $C_{10}H_{12}O_3$ | - | √ | - | - | Phenolic |
| 14 | Palmitic acid | 22.26 | $C_{16}H_{32}O_2$ | √ | √ | √ | √ | Fatty acid |
| 13 | Linoleic acid | 24.39 | $C_{18}H_{32}O_2$ | √ | √ | √ | √ | Fatty acid |
| 15 | Oleic acid | 24.46 | $C_{18}H_{34}O_2$ | √ | √ | √ | √ | Fatty acid |
| 35 | Xanthoxin | 24.76 | $C_{12}H_8O_4$ | √ | √ | √ | √ | Furocoumarin |
| 36 | 2-Palmitoylglycerol | 28.31 | $C_{19}H_{38}O_4$ | √ | √ | √ | √ | Diglycerol |
| 37 | Monopalmitin | 28.68 | $C_{19}H_{38}O_4$ | √ | √ | √ | √ | Monoglyceride |
| 32 | 4-Vinylphenol | 29.54 | $C_8H_8O$ | - | - | - | √ | Phenolic |
| 38 | Glyceryl monooleate | 30.46 | $C_{21}H_{40}O_4$ | √ | √ | √ | √ | Monoacylglycerol |

*≥70% similarity to the NIST Library and Agilent Fiehn 2013 GC-MS Metabolomics RTL Library

## Conclusion

The constituents of palm cooking oil (RBDO), red palm oil (RPO), cold-pressed palm oil (CPPO) and extra virgin olive oil (EVOO) were examined and compared using phytochemical screening and also liquid and gas chromatography-mass spectrometry. The total flavonoid and phenolic quantitation were encouraging for EVOO and CPPO extracts albeit direct correlation could not be drawn from the results of DPPH radical scavenging assay. From the LC- and GC-MS profiling, EVOO followed by CPPO extracts exhibited more content and metabolite peaks compared to the processed palm oil extracts. A total of 38 phytochemicals were identified from all the oil samples in this investigation. As the total phytochemical content is not accurately reflective of the bioactivities

in a complex mixture for instance its antioxidant activity, more efficient assessment of individual identification and assay of specific metabolite component need to be carried out to fully grasp the status, stability and optimum potential of the palm oil constituents. In the long run, to attain the gold standard of edible oil packed with phytonutrients as attained by the EVOO, the palm oil processing methodologies requires a revisit. Nevertheless, the change or improvement in the physical extraction and milling technology to obtain premium grade palm oil may only be achieved after the phytochemical composition of the unprocessed palm oil is fully profiled and identified.

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