Antioxidant Potential of Red Palm-Pressed Mesocarp Olein

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Abstract: Oxidative stress occurs due to the imbalance amount of the free radicals and antioxidants in human body which often associated with numerous chronic diseases. The antioxidant properties of red palm-pressed mesocarp olein (PPMO) have not been widely studied. Therefore, antioxidant properties of PPMO relative to commercially available edible oils, namely red palm olein (RPO), palm olein (PO), extra virgin olive oil (OO) and extra virgin coconut oil (CNO) were studied. PPMO exhibited significant higher phytonutrients which more than 2-fold compared to the edible oils. Overall, antioxidant screening indicated that PPMO has significantly higher antioxidant activities than RPO, PO and CNO in term of DPPH, H₂O₂, NO scavenging and FIC; and significantly higher H₂O₂ and FIC than OO. The outcomes of this study reveal that PPMO is as good as commercially available edible oil, also a good source for food applications and dietary nutritional supplements. More importantly, the utilization of PPMO could mitigate oil palm waste problem and results in positive environmental impact.

Key words: DPPH radical, hydrogen peroxide radical, nitric oxide radical, ferrous ion chelation

1 Introduction

Free radicals are highly reactive molecules, which are contributing to both beneficial and detrimental effects in the human body. They play important physiological roles such as cellular signalling, host defence mechanism, cell structures synthesis and blood pressure maintenance^{1,2}. However, excessive free radicals in the body will lead to oxidative stress where the essential macromolecules such as proteins, lipids, carbohydrates and nucleic acids are damaged and resulting in cell death³. Oxidative stress also promotes the process of aging, as well as decelerate cellular repairing process and collagen production. The phenomenon is often associated with various chronic diseases include inflammatory⁴, atherosclerosis⁵, cancer⁶, autoimmune disorder⁷, asthma⁸, diabetes⁵ and neuro-degenerative⁹. Antioxidants are capable to neutralize free radicals effectively through their scavenging property, subsequently inhibit its destructive effect to the human body. Antioxidants could act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist or metal-chelating agents to

inhibit cellular dysfunction¹⁰.

Literature data showed that although micronutrients such as vitamin C, vitamin E and beta-carotene are strong antioxidants, there is a concern on taking high doses of antioxidant supplements might do more harm than good. Scientific evaluation of natural products is challenging because of their immense complexity and variability. Natural products chemistry efforts are typically devoted to reducing complexity and identifying single active constituents for drug development. Many diseases are not regulated by a single molecular target, but often have a multi-factorial causality. Botanical extracts may contain hundreds or even more than thousands of individual metabolites at varying concentrations and identifying the compounds responsible for a given biological effect represents a significant challenge¹¹. The misconceptions of the behaviour of a mixture can be described by the presence of just a few known constituents are common. However, a number of studies have shown that the overall activity of botanical extracts can result from mixtures of phytochemicals with synergistic, additive, or antagonistic interactions, natural

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product chemists showed that it is very often where the isolation efforts on a botanical extract fail because activity is lost upon fractionation^{12, 13}. Young *et al.* 1994 reported that the use of vitamin C may cause hazardous in the human body if excessive iron exist due to the formation of ascorbate that could exert prooxidant effects¹⁴. Moreover, Bhat *et al.* 2006 reported that vitamin C at a concentration of 100-200 μM may cause oxidative DNA breakage in normal cells through Comet assay using human peripheral lymphocytes¹⁵. A case report published in 2011 also showed that high intake of vitamin C will lead to oxalate nephropathy, revealing the potential renal injury resulted from an excessive intake amount¹⁶. On the other hand, a meta-analysis carried out by Miller *et al.* 2005 showed a statistically significant relationship between vitamin E supplementation dosage and all-cause mortality, with an increased risk of dosages greater than 150 IU/d in a dose response analysis¹⁷. In addition, a study involving a total of 59,910 French women demonstrated that β -carotene supplementation intake is significantly associated with the risk of tobacco-related cancers among smokers¹⁸. There are a bunch of good evidences that consuming foods rich with antioxidants can help to boost immune system, protect against free radicals and may lower the risk of heart disease and cancer but it remains ambiguous and complicated when it comes to the intake of antioxidant supplements. Therefore, it urges researchers to find an alternative way to resolve the excessive production of free radicals in a safe manner which is from the foods that are rich in phytonutrients.

Palm-pressed mesocarp fiber is a cellulosic fibrous material obtained after screw-pressing of palm fruitlets in the palm oil mills. It contains typically 5% to 6% of residual oil (dry basis), which is known as palm-pressed mesocarp oil¹⁹. The oil can be recovered using supercritical carbon dioxide (SC-CO_2)²⁰ and solvent extraction methods²¹. The high recovery rate of more than 90%²⁰ by using this extraction technology has generated extra revenue for the palm oil mills. Previous research studies indicated that the crude palm-pressed mesocarp oil is a rich source of naturally occurring phytonutrients such as carotenoids, vitamin E including tocopherol and tocotrienols, sterols, squalene¹⁹, phospholipids²² and water-soluble phenolic components²³. Thus, the nutritional value of mesocarp oil should be unveiled by transforming it into high value products and this is the first report on the antioxidant effects of red palm-pressed mesocarp olein (PPMO).

The crude mesocarp oil could be further refined by removing the impurities including gum, waxes, trace metals and free fatty acids. The refined mesocarp oil subsequently fractionated into PPMO and stearin for niche product applications. The refining process to produce PPMO was reported previously by Lau *et al.* 2014²⁴. Both crude and refined red palm-pressed mesocarp oil have greater

amount of phytonutrients if compared to other vegetable oils such as palm olein (PO), red palm olein (RPO), extra virgin olive oil (OO) and extra virgin coconut oil (CNO)²⁵⁻³³. Generally, phytonutrients are desirable in food consumption for health benefits. Phytosterols have been proven scientifically to reduce low-density lipoprotein (LDL) and total cholesterol (TC) levels³⁴, induce apoptosis in prostate³⁵, breast³⁶ and colon³⁷ cancer cells, interrupt testosterone metabolism³⁸, and inhibit inflammation³⁹. Likewise, carotenoids possess anti-inflammatory, antioxidant and anticancer activities⁴⁰, as well as decreasing the risk of age-related macular degeneration and cataracts⁴¹. Besides the anti-oxidant and anticancer properties, squalene is able to lower the serum cholesterol levels⁴². Furthermore, vitamin E (tocols), had been proven to exert beneficial roles on neurological function⁴³ and anticlotting⁴⁴ activities.

PO is known to exert precious nutritional values due to its high content of phytosterols, tocotrienols and tocopherols, which exhibited antioxidant, anticancer and anti-inflammatory properties^{40, 45, 46}. RPO is rich in carotenoids and vitamin E and its preclinical studies demonstrated health beneficial effects in terms of reduction in LDL level and TC to high-density lipoprotein ratio (TC:HDL)⁴⁷, as well as lowered atherogenic effect⁴⁸ if compared to that of PO. In addition, RPO supplementation exhibited heart protection against the consequences of ischemia/reperfusion injury⁴⁹. In contrast, the biological activities of the PPMO have yet to be assessed and compared with other vegetable oils. OO and CNO have been well studied for their biological activities and therefore these oils were selected for comparison purpose in this study. The evergreen olive cultivar (*Olea europaea* L., Oleaceae) is a valuable Mediterranean tree, which provides a unique flavour with nutritional benefits. It contains a number of bioactive components that showed antioxidant and anti-inflammatory properties such as polyphenols, phytosterols and vitamin E^{50, 51}. On the other hand, preclinical studies revealed that the CNO supplemented diet exhibited antioxidant activity by increasing antioxidant enzymes and reducing lipid peroxidation content⁵². CNO supplementation also exhibited antioxidant and hepatoprotective effects against oxidative stress and liver damage induced by anticancer drug methotrexate in rats. Thus, this study was conducted to assess antioxidant properties of PPMO, PO, RPO, OO and CNO.

2 Materials and Methods

2.1 Materials

PPMO was obtained according to the method described by Sulihatimarsyila *et al.* 2019²¹. In brief, crude palm-pressed mesocarp oil was obtained from a local palm mill in Malaysia. The oil was degummed, deacidified, deodorized and fractionated to obtain the refined red palm-

pressed mesocarp oil. The refining condition was using hot distilled water and phosphoric acid for degumming and natural bleaching earth for bleaching, as well as deodorization at temperature lower than 200°C. The refined red palm-pressed mesocarp oil was then fractionated to obtain two fractions which were red palm-pressed mesocarp stearin (solid fraction) and PPMO (liquid fraction). PO, RPO, OO and CNO were purchased from local supermarket in Malaysia. All the chemicals, reagents and media were purchased from Sigma Aldrich (Switzerland), Merck Sdn. Bhd. (Germany), R&M Sdn. Bhd. (Malaysia) and Team Medical & Scientific Sdn. Bhd. (Malaysia).

2.2 Methods

2.2.1 Chemical constituents of PPMO

2.2.1.1 Sterol Composition and Squalene Content

The sterol composition and squalene content were determined simultaneously⁵³. The gas chromatograph (GC, 7890B Agilent, Agilent Technologies, USA) on a CP9078 – select biodiesel for glycerides, UM + 2m RG column of 15 m (L) × 320 µm (D) × 0.1 µm (Film) coupled with a FID was used. The flow rate of carrier gas, helium was 5 mL/min. The injector, detector and oven temperatures were set at 50°C, 380°C and 370°C, respectively. Identification of sterol composition and squalene content in the oils was done by comparing their retention times with commercial standards, Mono C19, Di C38, Tri C57, squalene and cholesterol.

2.2.1.2 Total Carotene Content

Total carotene content of the oils was conducted using MPOB Test Method p2.6:2004⁵⁴. Approximately 0.1 g of oil was dissolved in 250 mL of *n*-hexane. The absorbance of oil extract was measured at 446 nm. The total carotene content of the oils was expressed in mg/kg, was defined and calculated as β-carotene.

2.2.1.3 Vitamin E Composition

The tocopherols and tocotrienols of the oils were analysed using a HPLC (Agilent 1100 series, Agilent Technologies, USA), equipped with a normal phase Supelco Ascantis® Si column (150 mm × 4.6 mm × 5 µm particle size) coupled to a fluorescence detector according to Che *et al.*⁵⁵ with some modifications. The detection wavelengths for excitation and emission were 270 and 315 nm, respectively. The mobile phase used was a mixed *n*-heptane and ethyl acetate at 97: 3% (v/v).

2.2.2 Preparation of Oil Extracts

The polar compounds from oil samples were extracted according to the methods as described by Radhia *et al.* 2017⁵⁶ with minor modifications. Approximately 1 g of oil sample was mixed with 1 mL of extraction solvent, which was methanol:water (80:20 v/v). The oil-solvent mixture was vortexed for 10 min and left to stand for approximately 1 h until the two layers were formed. The upper layer was collected through careful pipetting. The extraction process

was repeated twice. The oil extracts were combined and stored in a scintillation vial at 4°C for subsequent bioassays. The concentration of the oil extracts was noted as 330 µg/mL.

2.2.2.1 Total Phenolic Content (TPC)

TPC analysis of the oil extracts was carried out using Folin-Ciocalteu (FC) method as described by Abdullah *et al.* 2018⁵⁷. Gallic acid (GA) was used to plot the standard curve that was constructed by a range of concentrations (20, 40, 60, 80 and 100 µg/mL) dissolved with the extraction solvent. The equation of $y = 0.7996x + 0.1162$ with a R^2 value of 0.9949 was obtained, where y is the absorbance at 765 nm and x is the GA concentration with a unit of µg/mL. TPC of the oil extracts was expressed as GA equivalent per gram of oil extract (µg GAE/ g oil extract).

2.2.2.2 Total Flavonoid Content (TFC)

TFC of the oil extracts was determined using the aluminium chloride (AlCl₃) colorimetric method as described by Radhia *et al.* 2017⁵⁶ where rutin hydrate was used as the standard compound. A standard curve of rutin hydrate was constructed using a series of concentrations of 20, 40, 60, 80 and 100 µg/mL by serial dilution using the extraction solvent. The equation of $y = 0.5248x - 1.1534$ ($R^2 = 0.9982$) was obtained where y is the absorbance at 420 nm and x is the rutin concentration in µg/mL. TFC of the oil extracts was presented as rutin hydrate equivalent per gram of oil extract (µg RE/ g oil extract).

2.2.3 Antioxidant Assays

2.2.3.1 α, α-diphenyl-β-picrylhydrazyl (DPPH) Radical Scavenging Assay

The DPPH radical scavenging assay of the oil extracts was conducted according to Teh *et al.* 2013⁵⁸. Ascorbic acid (vitamin C) was used as a standard drug in this assay. The absorbance of each well was recorded at 517 nm using a microplate reader. The percentage of total radical scavenging activity was calculated by using the formula below:

$$\text{Percentage of DPPH scavenging activity (\%)} = (A - B) / A \times 100, \text{ where, A equals to absorbance of control and B equals to absorbance of oil extract}$$

2.2.3.2 Hydrogen Peroxide (H₂O₂) scavenging assay

The H₂O₂ scavenging assay was performed according to the method reported by Fernando and Soysa⁵⁹ with some modification. Ascorbic acid was used as a standard drug and the absorbance was determined immediately at 230 nm against a blank solution by using UV spectrophotometer (Lambda XLS, Perkin Elmer). The H₂O₂ scavenging activity was calculated using the formula below:

$$\text{Percentage of H}_2\text{O}_2 \text{ Scavenging Activity (\%)} = (A - B) / A \times 100$$

where, A equals to absorbance of control and B equals to

absorbance of oil extract

2.2.3.3 Nitric Oxide (NO) Radical Scavenging Assay

The NO radical scavenging activity of samples was determined by referring to the method described by Parul, Kundu and Saha⁶⁰ with minor modifications. Ascorbic acid was used as a standard and the absorbance was read at 546 nm using a microplate reader. The NO radical scavenging activity was calculated using the formula:

$$\text{Percentage of NO Scavenging Activity (\%)} = (A - B) / A \times 100$$

where, A equals to absorbance of control and B equals to absorbance of oil extract

2.2.3.4 Ferrous Ion Chelating (FIC) Assay

The FIC activity of the oil extracts was determined according to the method described by Mah *et al.* 2017⁶¹. Ethylenediaminetetraacetic acid (EDTA) was used as the standard compound and the absorbance of each well was then measured at 595 nm.

The percentage inhibition of the ferrozine-Fe²⁺ complex formation was calculated as $[(A_0 - A_s) / A_s] \times 100$, where A_0 is the absorbance of the control and A_s is the absorbance of the sample.

2.2.3.5 Ferric Reducing Antioxidant Power (FRAP)

The FRAP of the oil extracts was determined according to the method reported by Mah *et al.* 2017⁶¹ where ascorbic acid (AA) was used as the standard compound. A standard curve was constructed by using AA with a series of concentrations (200, 400, 600, 800 and 1000 µg/mL) in the extraction solvent. The equation $y = 0.0121x + 0.7417$ with R^2 value of 0.9967 was obtained where y is the absorbance at 650 nm and x is the concentration of AA in µg/mL. The results of FRAP are expressed as AA equivalent per gram of oil extract (µg AAE/ g oil extract). The experiments were carried out in triplicates.

2.2.4 Statistical Analysis

All the data were presented in mean and statistical analyses were performed by using GraphPad Prism 7. Data were analysed by one-way ANOVA and two-way ANOVA, followed by Tukey's Multiple Comparison Test. In addition, Pearson correlation was performed to determine the relationship between phytonutrients in oils and the antioxidant activities where "0" indicates no association, " $0 < \text{correlation coefficient value } (r) \leq 0.2$ " indicates weakly association, " $0.2 < r \leq 0.5$ " indicates moderately association, " $0.5 < r \leq 0.8$ " indicates strongly association, and lastly " $0.8 < r \leq 1.0$ " indicates perfectly association. The p value ≤ 0.05 was considered to be statistically significant.

3 Results and Discussion

PPMO was extracted with hexane where the breakdown of phospholipid bilayer in mesocarp was involved. Crude palm oil was extracted with mechanical pressing method, also known as screw pressing where the oil was extracted without disrupting the membrane layer, subsequently subjected to refining process including degumming, deacidification and fractionation to obtain RPO. Meanwhile, bleaching process was performed on RPO to obtain PO. Both OO and CNO were obtained through cold pressing method. Different extraction methods could lead to different levels of phytonutrients in the oils.

All the edible oils were extracted for their polar components and evaluated for their TPC, TFC and antioxidant activities. The TPC and TFC results of the oil extracts were summarized in Table 1. The TPC results showed that PPMO has comparable values with PO and RPO. However, the TPC value of RPO is significantly higher than PO, which might be due to the removal of polar carotenoids such as zeaxanthin, violaxanthin, and lutein during the bleaching process. Results showed that the TPC value of

Table 1 Total phenolic contents (TPC) and total flavonoid contents (TFC) of the oil extracts.

Oil Extract	TPC (µg GAE/g oil extract)	TFC (µg RE/g oil extract)	Significant difference between TPC and TFC
PPMO	3.81 ± 0.37 ^{b,c}	5.58 ± 0.29 ^b	$p > 0.05$
PO	3.26 ± 0.17 ^{a,b}	4.76 ± 0.64 ^{a,b}	$p > 0.05$
RPO	5.72 ± 0.10 ^c	4.80 ± 0.80 ^{a,b}	$p > 0.05$
OO	61.87 ± 1.53 ^d	7.28 ± 2.01 ^b	$p < 0.05$
CNO	1.74 ± 0.12 ^a	2.49 ± 0.19 ^a	$p > 0.05$

Note: Each data represents the mean ± SD of three independent experiments where means with different letters in the same column indicate significant differences ($p < 0.05$). PPMO: red palm-pressed mesocarp olein; PO: palm olein; RPO: red palm olein; OO: extra virgin olive oil; CNO: extra virgin coconut oil.

PPMO is significantly lower than OO but relatively higher than CNO, also the TPC value of OO is significantly higher than CNO even though both OO and CNO were processed using the same method, which is cold pressing method. The remarkably high TPC value of OO is in good agreement with the literature data where OO is rich in phenolics, which are constituted of both lipophilic and hydrophilic compounds⁶². The hydrophilic phenolic compounds include phenolic acids, phenolic alcohols, flavonoids, lignans, secoiridoids and their derivatives⁶². The results postulate that the amount of TPC in oil extracts is highly correlated to the naturally occurring metabolites but not solely to the processing methods.

Flavonoid is one type of the phenolic compounds that are widely distributed in the plants. There was no significant difference observed for the TPC and TFC values of PPMO, PO, RPO and CNO. The results postulate that the phenolic compounds exist in the oil extracts are mostly presented as polyphenolic compounds, flavonoids. Meanwhile, the TPC value in OO was found to be significantly higher than its TFC value owing to most of the phenolic compounds present are different classes from flavonoid. The findings are in good agreement with the literature data where the main constituents are hydroxytyrosol (class of phenolic acid) and oleuropein (class of glycosylated secoiridoid)⁶³.

DPPH, H₂O₂ and NO radicals scavenging assay were conducted to assess the radical scavenging capacity of the edible oils. Ascorbic acid was selected as the standard drug for these antioxidant assay due to its well-known strong antioxidant activities⁶⁴. The overall results, which were presented in **Table 4**, demonstrated that the PPMO possess good antioxidant property in terms of radicals scavenging capability. OO recorded the highest DPPH scavenging activity, followed by PPMO, RPO, PO and CNO. The outcome can be explained by the phytonutrients in each oil where squalene and TPC are significantly associated with DPPH scavenging activity as shown in **Tables 1, 2 and 5**. There is

almost all the phytonutrients are strongly associated either positively or negatively with H₂O₂ scavenging effect, except for TFC which correlated moderately (**Tables 1-5**).

Therefore, even OO exhibited highest DPPH scavenging effect but it marked the lowest H₂O₂ scavenging activities as the high concentration of TPC and squalene are strongly negatively associated with the activity which in turn diminished H₂O₂ scavenging effect possessed by other phytonutrients. The finding showed that PPMO exhibited the strongest scavenging activity towards H₂O₂ radical among the oil extracts with 50.5% at the concentration of 50 µg/mL (**Tables 1-5**). The diminishment effect by squalene and TPC towards the activity was not significantly affected PPMO as it has the remarkably high levels of carotene and vitamin E, where both are perfectly associated with H₂O₂ scavenging effect ($r > 0.8$), which in turn counterbalance the diminishment effect. Literature data showed that carotenoids could scavenge H₂O₂ radicals effectively⁶⁵. A previous study also acknowledged the antioxidant capacity of the extracts of oil palm fruit through H₂O₂ scavenging assay⁶⁶.

For the NO radical scavenging activities, PPMO showed comparable effect with OO with 26.3% and 27.5%, respectively as the TFC and squalene exhibited strongest association among all the phytonutrients where OO exhibited the highest, followed by PPMO. However, there is no significant different between NO scavenging effect of PPMO and OO which might owing to the high concentration of sterol and α -tocopherol in PPMO (**Tables 1-5**). The findings are in line with literature data where α -tocopherol and phytosterol have been reported as antioxidants by breaking free-radical chain reactions⁶⁷ and stabilizing liposomal membranes⁶⁸, respectively.

Even though PPMO has a certain degree of radical scavenging properties, it is not as effective as the standard drug, ascorbic acid. Generally, the radicals scavenging activity of oil palm based edible oils, including PPMO, PO and RPO are stronger than CNO. The activities are postulated

Table 2 Total carotene, squalene and sterol contents of the oils.

	Concentration (ppm)			
	Carotene	Squalene	Sterol	Vitamin E
PPMO	1312 ± 14 ^b	401 ± 14 ^b	519 ± 8 ^d	1643 ± 13 ^d
PO	ND	106 ± 11 ^a	160 ± 5 ^a	761 ± 3 ^b
RPO	571 ± 35 ^a	83 ± 10 ^a	185 ± 7 ^b	894 ± 9 ^c
OO	ND	1323 ± 15 ^c	235 ± 12 ^c	223 ± 2 ^a
CNO	ND	ND	ND	ND

Note: Each data represents the mean ± SD of three independent experiments where means with different letters in the same column indicate significant differences ($p < 0.05$). PPMO: red palm-pressed mesocarp olein; PO: palm olein; OO: extra virgin olive oil; CNO: extra virgin coconut oil; ND: not detectable

Table 3 Vitamin E composition of the oils.

	PPMO	PO	RPO	OO	CNO
α -tocopherol	857 $\pm$ 12 ^c	159 $\pm$ 2 ^a	156 $\pm$ 2 ^a	210 $\pm$ 2 ^b	ND
α -tocotrienol	363 $\pm$ 6 ^c	241 $\pm$ 1 ^a	296 $\pm$ 16 ^b	ND	ND
β -tocopherol	ND	ND	ND	ND	ND
β -tocotrienol	34 $\pm$ 1 ^a	25 $\pm$ 3 ^a	31 $\pm$ 3 ^a	ND	ND
γ -tocopherol	ND	ND	ND	13 $\pm$ 1	ND
γ -tocotrienol	343 $\pm$ 5 ^a	293 $\pm$ 2 ^a	327 $\pm$ 8 ^a	ND	ND
δ -tocopherol	ND	ND	ND	ND	ND
δ -tocotrienol	46 $\pm$ 1 ^a	43 $\pm$ 1 ^a	84 $\pm$ 4 ^b	ND	ND
Total tocopherol, T (ppm)	857 $\pm$ 12 ^c	159 $\pm$ 2 ^a	156 $\pm$ 2 ^a	223 $\pm$ 3 ^b	ND
Total tocotrienol, T3 (ppm)	785 $\pm$ 13 ^c	602 $\pm$ 6 ^a	738 $\pm$ 31 ^b	ND	ND
Total T+T3 (ppm)	1643 $\pm$ 15 ^d	761 $\pm$ 3 ^b	894 $\pm$ 8 ^c	223 $\pm$ 2 ^a	ND

Note: Each data represents the mean $\pm$ SD of three independent experiments where means with different letters in the same row indicate significant differences ($p < 0.05$). PPMO: red palm-pressed mesocarp olein; PO: palm olein; OO: extra virgin olive oil; CNO: extra virgin coconut oil; ND: not detectable

Table 4 Antioxidant properties of oil extracts.

Oil Extract	DPPH Scavenging (%)	H ₂ O ₂ Scavenging (%)	NO Scavenging (%)	Ferrous Ion Chelation (%)
PPMO	30.5 $\pm$ 1.7 ^c	50.5 $\pm$ 0.2 ^d	26.3 $\pm$ 0.8 ^{c,d}	29.5 $\pm$ 3.2 ^b
PO	9.5 $\pm$ 0.4 ^a	34.0 $\pm$ 0.0 ^c	24.4 $\pm$ 1.1 ^{b,c}	5.9 $\pm$ 0.9 ^a
RPO	20.9 $\pm$ 1.7 ^b	34.0 $\pm$ 0.1 ^c	22.5 $\pm$ 1.0 ^{a,b}	26.2 $\pm$ 0.7 ^b
OO	76.6 $\pm$ 1.1 ^d	18.0 $\pm$ 0.1 ^a	27.5 $\pm$ 0.7 ^d	7.5 $\pm$ 2.8 ^a
CNO	6.9 $\pm$ 0.9 ^a	31.5 $\pm$ 0.0 ^b	21.9 $\pm$ 0.8 ^a	7.3 $\pm$ 0.6 ^a
AA	100.0 $\pm$ 0.0	92.59 $\pm$ 1.27	51.91 $\pm$ 1.51	—
EDTA	—	—	—	100 $\pm$ 0.00

Note: Each data represents the mean $\pm$ SD of three independent experiments where means with different letters in the same column indicate significant differences ($p < 0.05$). PPMO: red palm-pressed mesocarp olein; PO: palm olein; OO: extra virgin olive oil; CNO: extra virgin coconut oil; AA: ascorbic acid; EDTA: Ethylenediaminetetraacetic acid. '—' means not tested. ND: not detectable. The concentration of the oil extract used: DPPH = 333 μ g/mL; H₂O₂ = 50 μ g/mL; NO = 5 μ g/mL; Ferrous ion chelation = 333 μ g/mL.

to be attributed to the presence of TPC, carotene, sterol, squalene and vitamin E that are known for their potent antioxidant activity⁶⁹). Moreover, this finding further confirms that the oil palm-based edible oil, is rich of antioxidants that act on radicals.

Alternatively, antioxidant can act on the chelation of iron by binding directly with iron so that the iron-chelator complex will then be excreted from human body in the forms of urine or faeces. Iron plays an essential role in promoting the formation of blood but excessive iron in the body will result in iron-mediated oxidative stress¹⁴). Free iron is a potent promoter of hydroxyl radical formation that

can cause increased lipid peroxidation and depletion of antioxidants, eventually leading to increased susceptibility to infection, veno-occlusive disease and chronic liver diseases⁷⁰). FIC assay was performed to determine the chelation ability of the oil extracts. EDTA, which was used a standard drug in this assay, exhibited strong chelating activity as supported by previous study⁷¹). PPMO and RPO oil extracts exhibited comparable FIC activity and their activities were significantly stronger than OO, CNO and PO. The significantly lower chelating of PO than RPO might due to the removal of carotene compounds from RPO during bleaching process and the lower vitamin E content than RPO

Table 5 Pearson correlation analysis between phytonutrients and antioxidant activities of the oils.

Phytonutrients	Correlation Coefficient Value (<i>r</i>)				
	DPPH Scavenging	H ₂ O ₂ Scavenging	NO Scavenging	Ferrous Ion Chelation	Ferric Ion Reducing Antioxidant Power
TPC	0.95*	−0.75	0.70	−0.34	0.98*
TFC	0.88	−0.26	0.89*	0.13	0.84
Carotene	−0.04	0.84	0.21	0.93*	−0.26
Squalene	0.99*	−0.54	0.85	−0.18	0.94*
Sterol	0.35	0.60	0.68	0.70	0.13
Vitamin E	−0.12	0.84	0.28	0.82	−0.28
α-tocopherol	0.19	0.73	0.57	0.70	−0.07
α-tocotrienol	−0.33	0.81	0.01	0.78	−0.41
β-tocotrienol	−0.36	0.77	−0.03	0.76	−0.41
γ-tocotrienol	−0.39	0.74	−0.05	0.70	−0.42
δ-tocotrienol	−0.36	0.50	−0.27	0.71	−0.31
Total T	0.20	0.72	0.58	0.69	−0.05
Total T3	−0.37	0.76	−0.04	0.75	−0.41

Note: * indicates significant differences ($p < 0.05$). T: tocopherol; T3: tocotrienol.

(Tables 3 and 5). Previous studies on iron uptake by human colorectal adenocarcinoma cells, Caco-2 showed that carotenoids significantly increased iron uptake from ferrous fumarate and NaFe-EDTA, and were capable of partially overcoming the inhibition produced by tannic acid⁷²). Thus, the weaker activities shown by the oil extracts in the FIC assay is possibly due to lack of the carotenoids that are efficient in ferrous ion binding activity.

The total antioxidant capacity of the oil extracts was examined by FRAP method, which relies on the reduction of the complex ferric ion-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) by antioxidants. An intense blue color solution will be formed when ferric tripyridyl triazine (Fe^{3+} TPTZ) complex is reduced to the ferrous (Fe^{2+}) form, where the absorbance can be measured to quantify the amount of ferric ion being reduced and correlate it to the amount of antioxidants present. The results were presented in Fig. 1. OO has the strongest reducing power among the oil extracts with the value of 1411 μg AAE/g oil extract. This value is significantly higher than the other oil extracts, which are in the FRAP value order of $\text{RPO} > \text{PO} > \text{PPMO} > \text{CNO}$. The findings follow the same trend of the difference between TPC and TFC (Table 1). Literature data showed that FRAP method has limitation which is unable to measure polyphenolic compounds and thiols in which the total reducing power was significantly correlated with the phenolic compounds⁷³) and squalene (Table 5). The results are in good agreement with the literature data where most of the compounds in OO are not phenolic compounds as a huge difference was observed between the TPC and TFC

Fig. 1 Ferric ion reducing antioxidant power (FRAP) of oil extracts.

Note: Each data represents the mean $\pm$ SD of three independent experiments where means with different letters indicate significant differences ($p < 0.05$). Each data represents the mean $\pm$ SD of three independent experiments. PPMO: red palm-pressed mesocarp olein; PO: palm olein; RPO: red palm olein; OO: extra virgin olive oil; CNO: extra virgin coconut oil. AA: ascorbic acid.

values, therefore, a great discrepancy was observed between OO and the other oil extracts.

In summary, PPMO possesses the strongest H_2O_2 scavenging and FIC activities among the oil extracts. Thus, PPMO is a good source of antioxidants that are worth to be considered as a nutritious food due to its phytonutrients including carotenoids, vitamin E and phytosterols.

4 Conclusion

In conclusion, PPMO exhibited significant higher phytonutrients which more than 2-fold compared to the edible oils. The results indicated that PPMO has comparable TPC and TFC levels when compared with PO, RPO and CNO but significantly lower levels than in the OO ($p < 0.05$). The DPPH scavenging activity of oil extracts was in the order of OO > PPMO > RPO > PO > CNO whereas different order was recorded by FRAP method which was OO > RPO > PPMO > PO > CNO. In addition, PPMO oil extract exhibited comparable FIC activity with RPO and had significantly higher FIC activity than OO, CNO and PO ($p < 0.05$). The correlation analysis showed that different photometric measurement plays an important role in determining antioxidant activity as synergistic and antagonistic effects between the components in the oil extracts existed. Overall, antioxidant screening indicated that PPMO has significantly higher antioxidant activities (DPPH, FIC and FRAP) than PO and CNO; and significantly higher FIC activity than OO. The outcomes of this study reveal that PPMO is as good as commercially available cooking oil and could be a good source of phytonutrient to be applied in food industry. More importantly, value discovery of PPMO could encourage the recovery and refining of residual pressed-mesocarp fibre oil in the palm oil mills and increase revenue of the oil palm industry.

Conflict of Interest

None of the authors had a conflict of interest.

Author Contributions

All authors were responsible for the design and execution of the study. Teh S.S. performed the hands-on experiments, namely: conceptualization, methodology, validation, formal analysis, investigation, writing- original draft preparation. Mah S. H. performed the hands-on experiments, namely: conceptualization, methodology, validation, formal analysis and investigation. Lau H.L.N., Teng K.T. and Loganathan R. reviewed, read and approved the final version.

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