

# IDENTIFICATION OF HYDROPHILIC PHENOLIC COMPOUNDS DERIVED FROM PALM OIL PRODUCTS

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## ABSTRACT

Palm oil is a source of water-soluble phenolic compounds, which have been proven to exhibit antioxidant effects. Phenolic compounds have great potential in the development of health-beneficial foods, feeds, cosmetic and pharmaceutical products. In this study, hydrophilic phenolic compounds were extracted from various palm oil products including crude palm oil (CPO), refined palm oil (RPO), refined palm olein (RPOo), crude palm kernel oil (CPKO), refined palm kernel oil (RPKO) and refined palm kernel olein (RPKOo). A simple reversed-phase high performance liquid chromatography (HPLC) equipped with ultra violet (UV)-visible detector was used to identify the phenolic compounds. The phenolic acids identified were benzoic acid derivatives; gallic, protocatechuic, *p*-hydroxybenzoic, vanillic and syringic acids, and cinnamic acid derivatives; caffeic, coumaric and ferulic acids. Benzoic acid derivatives exhibited higher concentration compared to cinnamic acid derivatives, with *p*-hydroxybenzoic acid being the predominant acid present in all sample extracts. The results suggested the presence of hydrophilic phenolics, with *p*-hydroxybenzoic level in palm oil products; CPO (1901.69 mg kg<sup>-1</sup>), RPO (19.13 mg kg<sup>-1</sup>), RPOo (7.87 mg kg<sup>-1</sup>), CPKO (1701.70 mg kg<sup>-1</sup>), RPKO (5.01 mg kg<sup>-1</sup>), and RPKOo (nil). The profiling of phenolic compounds in the palm oil products would provide basic information and understanding of their bioactivities. Besides, the potential of phenolics from palm oil products extracts as antioxidants can be further evaluated.

**Keywords:** phenolic compounds *p*-hydroxybenzoic acid, palm oil, palm kernel oil, total phenolic content.

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## INTRODUCTION

The oil palm (*Elaeis guineensis*) is the source of two important edible oils, *i.e.* palm oil from the mesocarp, and palm kernel oil from the kernel. Extraction of palm oil involves a milling process, where water is introduced into the system. It is reported that phenolic-rich fraction is isolated from the aqueous by-product obtained during the milling of the oil palm fruits (Balasundram *et al.*, 2005). Findings have shown the availability of hydrophilic phenolics from various palm products. Previous studies reported the presence of phenolics in the oil palm fruits, *i.e.* fresh fruit bunches (Sundram *et al.*, 2003; Neo *et al.*,

2008), empty fruit bunches (Han and May, 2012) and leaves (Hui *et al.*, 2017). Besides, palm oil mill effluent (POME) was reported to be a major source of palm phenolics (Sambanthamurthi *et al.*, 1998). A method was developed to recover the phenolics from POME. Phenolic compounds were also found in fibre of oil palm fruit nuts after screw pressing (Nang *et al.*, 2007). A study was carried to extract, analyse and quantify the soluble-free, insoluble-bound and esterified phenolic compounds extracted from the oil palm fruits (Neo *et al.*, 2010).

Phenolics are water-soluble compounds and they have been found to be powerful and rich source of antioxidants (Balasundram *et al.*, 2005; Szydłowska-Czerniak *et al.*, 2011). Phenolic compounds are natural compounds found in plants, comprised of groups such as phenolics acids, flavonoids and tannins. Phenolics are hydroxy derivatives of

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aromatic carboxylic acids, which arise from either the benzoic acid or the cinnamic acid groups (Neo *et al.*, 2010). They differ according to the number and position of hydroxylation and methoxylation of the aromatic ring. Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid and vanilic acid are the derivatives of the benzoic acid, whilst caffeic acid, *p*-coumaric acid and ferulic acid are the derivatives of cinnamic acid. The *p*-hydroxybenzoic acid is considered as the common monophenolic structure which has been shown to possess less efficient radical scavenging activity compared to polyphenols (Farhoosh *et al.*, 2016). Nevertheless, the functional groups substituted to the ortho or para positions of phenolic rings have been shown to be more effective than those attached to meta position in the performance of phenolic antioxidants. Moreover, *p*-hydroxybenzoic acid has received considerable attention due to its extensive applications in food, medicine and polymer industries (Cho *et al.*, 1998; Tomás-Barberán and Clifford, 2000; Miao *et al.*, 2002; McQualter *et al.*, 2005).

The extraction of water-soluble phenolics from oil samples have been carried out using solvents such as alcohols (methanol, ethanol), acetone, diethyl ether, and ethyl acetate (Stalikas, 2007; Tasioula Margari and Tsabolatidou, 2015; Abdullah *et al.*, 2018). The common quantitative determination of the extracted phenolic compounds is colorimetric assay based on reaction of Folin-Ciocalteu reagent (Hatami *et al.*, 2014; Abdullah *et al.*, 2018; Zhang *et al.*, 2018). The need for profiling and identifying the individual phenolic compounds necessitates the high-performance analyses. Separation of these phenolic compounds is commonly achieved by high performance liquid chromatography (HPLC) technique (Stalikas, 2007; Tasioula Margari and Tsabolatidou, 2015; Zhang *et al.*, 2018) which was developed by Wulf and Nagel in 1976. Reversed phase HPLC is commonly used and detection is usually by ultra violet (UV)-visible detector or a photodiode detector (Kim and Lee, 2002). Numerous mobile phases have been employed with different modifiers, which include methanol, acetonitrile or tetrahydrofuran and acids (acetic or formic). The most widely used solvents for phenolic HPLC analysis are aqueous methanol or ethanol and isocratic conditions are sufficient for the separation. Whereas the neutral polyphenolics are analysed by gradient elution. Shahidi and Naczki (2003) used HPLC for isolation and tentative identification of the major antioxidants present in the extracts of niger (*Guizotia abyssinica*), an oilseed which is phenolic-rich. This method was used for the study of phenolics extracted from palm oil and palm kernel oil. Detection of polyphenolics is usually in the region of 230 to 330 nm (Kim and Lee, 2002) with the latter being used for acidic phenolics. Detection is typically based on the measurement of ultra-violet UV absorption at characteristic wavelengths, usually at 280 nm, which

represents a suitable compromise as most phenols absorb considerably at this wavelength (Ryan and Robards, 1998). Therefore, the aim of this study is to identify the phenolic compounds extracted from palm oil products which included crude palm oil (CPO), refined palm oil (RPO), refined palm olein (RPO), crude palm kernel oil (CPKO), refined palm kernel oil (RPKO) and refined palm kernel olein (RPKOo). The identification of the phenolic compounds in the extracts was performed using reversed-phase HPLC equipped with UV-visible detector. Then, the quantification of the most predominant phenolic acid was conducted accordingly.

## MATERIALS AND METHODS

### Materials

CPO, RPO, RPOo, CPKO, RPKO, and RPKOo were obtained from refineries, palm oil mills and kernel crushers from different regions in Malaysia (Table 1). Oil palm extracts were prepared at the laboratories of the Malaysian Palm Oil Board (MPOB), Bandar Baru Bangi, Selangor, Malaysia. All oil samples were homogenised by melting in an oven set to 10°C above the melting point of the oils.

Analytical grade solvents used included *n*-hexane, methanol, ethanol and acetonitrile obtained from Merck obtained from Sigma Chemical Co. (St Louis, MO, USA). Phosphoric acid, salicylic acid, gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid and ferulic acid, all with 95% purity, were purchased from Sigma Chemical Co. (St Louis, MO, USA). Milli-Q water (18.2 MΩ.cm) was used in all experiments. Filter paper and polytetrafluoroethylene (PTFE) membrane filter were obtained from Whatman International Ltd (Maidstone, United Kingdom).

The apparatus used included vortex mixer, rotary evaporator and Perkin Elmer Turbochrom 4 HPLC system equipped with a UV-visible detector. The phenolic acids were separated on a pre-packed Crest Pack C-18S with dimensions 4.6 ID x 250 ml packed with 5 µl particle size using isocratic condition.

### Methods

**Preparation of standard solutions.** A total of 25 mg of each standard solution of phenolic acids including gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, *p*-coumaric acid and ferulic acid was weighed into a 50 ml volumetric flask and made up to 50 ml with acetonitrile:deionised water with a ratio of 4:1 v/v. These solutions were used as the reference for identification of phenolic acids in the extracts. Meanwhile, *p*-hydroxybenzoic acids were quantified based on the calibration curve

of respective standards. Other phenolic acids were identified by comparison of the retention time of the standard phenolic acids.

**Extraction of phenolic-rich compounds from palm oil and palm kernel oil.** The phenolics were extracted from the palm oil products according to the method

proposed previously (Gutfinger, 1981; Artajo *et al.*, 2006) with slight modification in the extracting solvent (Figure 1). Briefly, the phenolic extract was re-dissolved in 5 ml of acetonitrile and washed with 3 x 20 ml of n-hexane. The apolar phase was treated with 5 ml of acetonitrile. The acetonitrile solution was then rotary evaporated to dryness under vacuum

TABLE 1. TOTAL PHENOLIC CONTENT (TPC) OF EVOO, CPO, CPKO, DIFFERENT PO AND PKO FRACTIONS

Sample No.	Oil	TPC (mg kg <sup>-1</sup> GAE) *	Sources
1	CPO	31.20 ± 0.35	A
2	CPO	31.73 ± 0.40	B
3	CPO	33.41 ± 0.24	C
4	CPO	39.96 ± 0.32	D
5	CPO	65.07 ± 0.15	E
6	CPO	70.18 ± 0.33	F
7	BPO	18.36 ± 0.35	A
8	BPO	18.59 ± 0.27	B
9	BPO	21.47 ± 0.30	C
10	BPO	21.59 ± 0.10	D
11	BPO	22.05 ± 0.15	E
12	BPO	22.25 ± 0.15	F
13	RPO	16.90 ± 0.74	A
14	RPO	18.17 ± 0.50	B
15	RPO	21.24 ± 0.46	C
16	RPO	25.12 ± 0.33	D
17	RPO	25.91 ± 0.24	E
18	RPO	26.89 ± 0.32	F
19	RPO <sub>o</sub>	11.36 ± 0.60	A
20	RPO <sub>o</sub>	11.58 ± 0.47	B
21	RPO <sub>o</sub>	11.68 ± 0.80	C
22	RPO <sub>o</sub>	11.95 ± 0.31	D
23	RPO <sub>o</sub>	12.07 ± 0.44	E
24	RPO <sub>o</sub>	12.20 ± 0.40	F
25	CPKO	16.80 ± 0.44	a
26	CPKO	17.64 ± 0.30	b
27	CPKO	17.97 ± 0.22	c
28	CPKO	17.58 ± 0.34	d
29	CPKO	27.05 ± 0.21	e
30	CPKO	27.25 ± 0.35	f
31	RPKO	3.159 ± 0.30	a
32	RPKO	3.205 ± 0.17	b
33	RPKO	3.720 ± 0.20	c
34	RPKO	3.744 ± 0.35	d
35	RPKO	3.802 ± 0.30	e
36	RPKO	3.815 ± 0.20	f
37	RPKO <sub>o</sub>	2.521 ± 0.20	a
38	RPKO <sub>o</sub>	2.535 ± 0.35	b
39	RPKO <sub>o</sub>	2.697 ± 0.40	c
40	RPKO <sub>o</sub>	8.058 ± 0.30	d
41	RPKO <sub>o</sub>	8.185 ± 0.15	e
42	RPKO <sub>o</sub>	8.600 ± 0.25	f
43	EVOO	65.07 ± 0.45	A1
44	EVOO	68.83 ± 0.50	B2
45	EVOO	70.21 ± 0.30	C3
46	EVOO	70.85 ± 0.15	D4
47	EVOO	71.06 ± 0.25	E5
48	EVOO	73.32 ± 0.30	F6

Note: CPO - crude palm oil; BPO - degumming and bleaching oil; RPO - refined palm oil; RPO<sub>o</sub> - refined palm olein; CPKO - crude palm kernel oil; RPKO - refined palm kernel oil; RPKO<sub>o</sub> - refined palm kernel olein; EVOO - extra virgin olive oil.

\*Values followed by capital letter (A-F) from different palm oil refineries. While mean values with lowercase (a-f) from different palm oil mills.

All results presented as mean values ± standard deviation (S.D) of triplicate analyses.

Values were based on regression analysis ( $r^2 = 0.9991$ ) expressed as mg kg<sup>-1</sup> gallic acid equivalent (GAE).

and the phenolic residue was dissolved in 5 ml of acetonitrile. Finally, an aliquot of 2 ml was obtained under a nitrogen stream. The phenolic residue was then filtered through a 0.45 µm PTFE filter.

The remaining residue containing the phenolic extract was then re-dissolved in 1 ml ethanol for analysis of phenolic content using the Folin-Ciocalteu assay. Some polyphenols were degraded during sample preparation and extraction due to light and oxidation. Using nitrogen gas purging during sample preparation prevented oxidation of polyphenolics. The samples were analysed within one day of extraction to prevent degradation. The amount of total phenolic content was calculated as  $\text{mg kg}^{-1}$  [gallic acid equivalent, (GAE)] from the calibration curve of gallic acid standard solution. For the gallic acid, the absorbance versus concentration is described by the equation  $y = 0.010x - 0.0067$  ( $R^2 = 0.999$ ) where  $y$  = absorbance and  $x$  = concentration. The obtained extract was then used for identification of phenolic compounds.

**Determination of total phenolic content (TPC) by the Folin-Ciocalteu method.** After the aqueous methanol extraction, the amount of phenolic content from the oil samples was determined colorimetrically according to the Folin-Ciocalteu protocol by Gutfinger (1981) and Vazquez *et al.* (1973). Folin reagent is a mixture of tungsten and molybdenum oxides. In this assay, the reagent is reduced and the product of the metal oxide reduction has a blue colour that has a broad light absorption with a maximum at 765 nm. The intensity of the light absorption follows the Beer Lambert Law, where absorption is proportional to the concentration of the phenolics.

Briefly, the Folin-Ciocalteu reagent was diluted with distilled water at 1:10 ratio and was used as stock solution. Then, 1 ml of diluted Folin-Ciocalteu was added to 0.2 ml of phenolic extract from the oil. This was followed by addition of 0.8 ml of 7.5% sodium carbonate solution which was prepared earlier. After 30 min, the phenolic extract was mixed

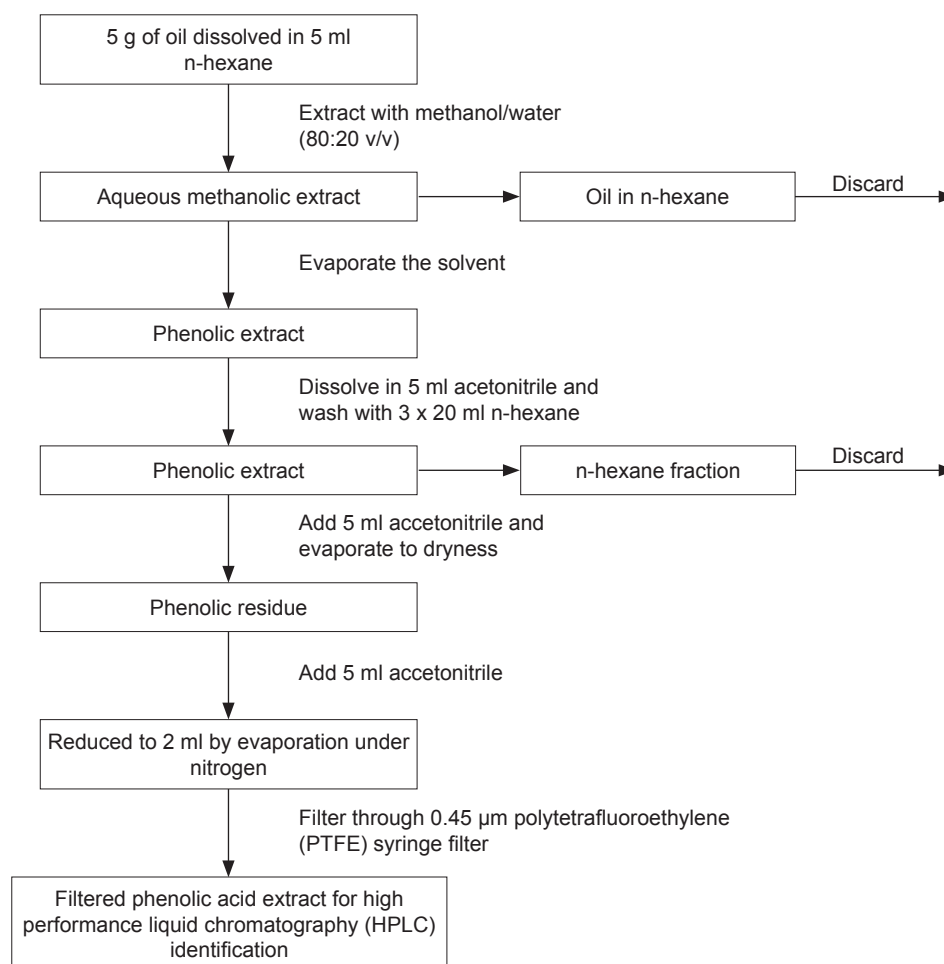


Figure 1. Extraction of phenolics from oil for subsequent identification.

well and the absorbance of blue coloured mixtures was read at 765 nm (Perkin Elmer LS 50B UV-Vis spectrophotometer). The TPC was calculated from a calibration curve of gallic acid standard solutions and expressed as GAE per gram of the sample. The regression coefficient of calibration curve is  $> 0.99$ . All measurements were done in triplicate and the results were expressed as means  $\pm$  standard deviation. While the analytical determination was being carried out, it is best that the prepared standard solution be kept under refrigeration until used. Standard solutions will retain 98% of their potency for two weeks if kept closed under refrigeration (4°C), but this potency is retained for only five days at room temperature (Wrolstad *et al.*, 2002).

**Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity.** The potential antioxidant activity of the oil samples was assessed on the basis of the scavenging activity of the stable DPPH free radical, as previously described by Brand-Williams *et al.* (1995). A 0.1 mmol litre<sup>-1</sup> solution of DPPH in methanol was prepared as stock solution. The radical stock solution was freshly prepared every day. Various concentration of 0.5 ml phenolic extract of each sample solution was added to 3.5 ml of DPPH radical solution. The mixture solution was then shaken vigorously and then allowed to stand at room temperature for 30 min in a dark room. The antioxidant activity was measured by decreased absorbance at 517 nm using a UV-Vis spectrophotometer until the reading reached a plateau. A low absorbance of the reaction mixture indicates a high free radical scavenging activity.

A blank solution consisted of 0.5 ml of methanol and 3.5 ml of DPPH radical solution. Standard (0.5 ml) gallic acid solution at different concentrations was added to 3.5 ml of 1 mg ml<sup>-1</sup> DPPH radical solution. These solutions were used as reference to determine the antioxidative potential of the phenolic extracts from the test oils.

The capability of extra virgin olive oil (EVOO), palm oil and palm kernel oil phenolic extracts to scavenge the DPPH radical was expressed as the inhibition percentage and was calculated using Equation (1):

$$\text{Radical-scavenging activity (\%)} = \left( \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \right) \times 100 \quad \text{Equation (1)}$$

where, Control OD was the optical density/absorbance of the control (blank) reaction and Sample OD was the optical density/absorbance in the presence of the sample extracts.

The IC<sub>50</sub> (inhibitory concentration) value was determined from the plotted graph of scavenging activity versus the concentration of oil extracts, which

is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out and the antioxidant activity was calculated based on the percentage of DPPH scavenged and the data measurements were expressed as means  $\pm$  standard deviation.

**Identification of extracted hydrophilic phenolic compounds.** The extracted sample was then prepared for identification of phenolic compounds using HPLC. Both sample and blank were filtered through PTFE membrane filter to achieve clarity. Nylon or other membranes that absorb phenolics should not be used. Membranes can be tested for phenolic absorption by comparing absorbance after single and double filtration. A Perkin Elmer Turbochrom 4 HPLC system equipped with a UV-Visible detector and a pre-packed Crest Pak C-18 (4.6 ID x 250 ml) was used for separation of phenolic acids. The isocratic mobile phase was water/ acetonitrile/ acetic acid glacial (88:10:2 by volume), the flow rate was 0.8 ml min<sup>-1</sup> and the injection volume was 20  $\mu$ l. The peaks were monitored at 320 nm. Peaks representing different phenolics were tentatively identified by comparison of their retention times with those of commercial standards. All measurements were from three replicates, and the results were expressed as mean values.

## RESULTS AND DISCUSSION

### TPC

Quantitative extraction of phenolic compounds in oils was difficult. Therefore, recovery studies using spiked samples were carried out to determine the efficiency of the procedure used for extracting phenolic compounds from oils (Abdullah *et al.*, 2018). In our previous study, the amount of TPC in palm oil products was quantified using Folin-Ciocalteu method (Abdullah *et al.*, 2018). The TPC, expressed as GAE, was found the highest in CPO compared to other extracted oil samples; RPO, RPOo, CPKO, RPKO, RPKOo. A significant amount of the phenolics was probably lost during the refining process through absorption of bleaching earth, volatilisation and degradation. Herein, the phenolic compounds in the palm oil products extracts were identified accordingly.

Table 1 shows the TPC in commercial palm oil samples from different palm oil mills and refineries (Abdullah *et al.*, 2018). In our previous study, the results showed that the extract of CPO was found to have the highest amount (31.20-70.18 mg kg<sup>-1</sup>) of phenolics compared to refined palm products and the amounts of phenolics in the oils are in the order: EVOO > CPO > CPKO > RPO > RPOo > RPKOo >

RPKO (Abdullah *et al.*, 2018). There were significant differences in TPC between all samples. EVOO was used as reference oil in this investigation as it is known to contain phenolic compounds and hydrophilic phenols (Boskou, 1996; Hafidi *et al.*, 2005).

On the other hand, Tan and Howe (2005) studied the hydrophilic phenolic compounds in CPO, refined, bleached, deodourised (RBDPO) and neutralised, bleached, deodourised palm oil (NBDPO). TPC of CPO ranged from 40-70 mg kg<sup>-1</sup> GAE, 5-17 mg kg<sup>-1</sup> GAE in RBDPO and 1 mg kg<sup>-1</sup> GAE in NBDPO. For comparison purpose, EVOO was also analysed and found to contain 70.9 mg kg<sup>-1</sup> of TPC. Besides TPC, the refining process also removes free fatty acids, phosphatides, odouriferous matter, water, as well as impurities such as dirt and traces of metals from the CPO. Phenolic compounds, which are present in significant amounts in virgin olive oil, are almost completely destroyed during the refining process of the oils (Nergiz and Unal, 1991). Carotenoids and phenolic compounds are removed almost totally, while vitamin E-active compounds and phytosterols are reduced by about 10% to 40% (Matthaus and Spener, 2008). According to Carrasco-Pancorbo *et al.* (2005), polyphenols are significantly related to the quality of virgin olive oil and their contribution to the oxidative stability of the oil is widely accepted. This indicated that the values obtained by the colorimetric assay can provide direct information on the amount of antioxidant phenolic groups in olive oil extracts. They are consequently related to the oxidative stability of virgin olive oil (Blekas *et al.*, 2002). It is also strongly affected by the agronomic and technological conditions of its production.

As most phenolics are water-soluble, they are probably removed during the refining process where live steam is used for stripping of free fatty acids from the oil during the de-acidification and deodourisation process. This accounted for the lower TPC in refined oils compared to crude oils. Phenolics are also being carried over into the fatty acid distillate during the deodourisation step (Scrimgeour, 2005).

After the first step of Seed Crushers' and Oil Processors Association (SCOPA) process, the degummed and bleached oil (BPO) contained a lower amount of TPC compared to the CPO (4.4%-6.4%) reduction of TPC due to the oil was subjected to SCOPA bleach. This is attributed to the adsorption of some phenolics by the earth used and to remove colouring matters as well as to absorb any metal ions by the bleaching earth during the bleaching stage. In the final refined product, the RPO, the TPC has been reduced considerably (9.57%-14.02%). After going through the refining process, the oils still contain phenolic compounds but in a small amount of TPC.

Analysis of the palm fatty acid distillate (PFAD) samples obtained after the SCOPA process of CPO samples showed that a portion of the phenolics end

up in the PFAD fraction. According to Scrimgeour (2005), phenolics are also being carried over into the fatty acid distillate during the deodourisation step. The TPC content was reduced by 9.2%-11.34% after the refining process of CPO. The experiment showed that the refining process can remove phenolics and most probably the phenolics are volatilised. The phenolics were also carried over into the fatty acid distillate during the deodourisation step. Thus, in refined, bleached and deodourised palm oils, the content of TPC was reduced. The decrease in TPC with various stages of refining was also reported for rapeseed oil, silkworm pupa oil and kenaf seed oil (Szydłowska-Czerniak and Laszewska, 2015; Liu *et al.*, 2016; Chew *et al.*, 2016).

### DPPH Scavenging Capacity

The DPPH scavenging activities of different oils are shown in Figure 2 (Abdullah *et al.*, 2018). In our previous study the results showed that there were significant differences in DPPH scavenging activities among all samples. EVOO was used as reference oil in this investigation and it showed the highest inhibition (70% inhibition). The CPO extract exhibited significantly high DPPH scavenging activity (45% inhibition) followed by CPKO (30% inhibition), RPO (29% inhibition), RPOo (20% inhibition), RPKO (15% inhibition), RPKOo (8% inhibition). The results are indicative of the hydrogen donating ability of CPO phenol extract, since the effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability (Grassmann *et al.*, 2002).

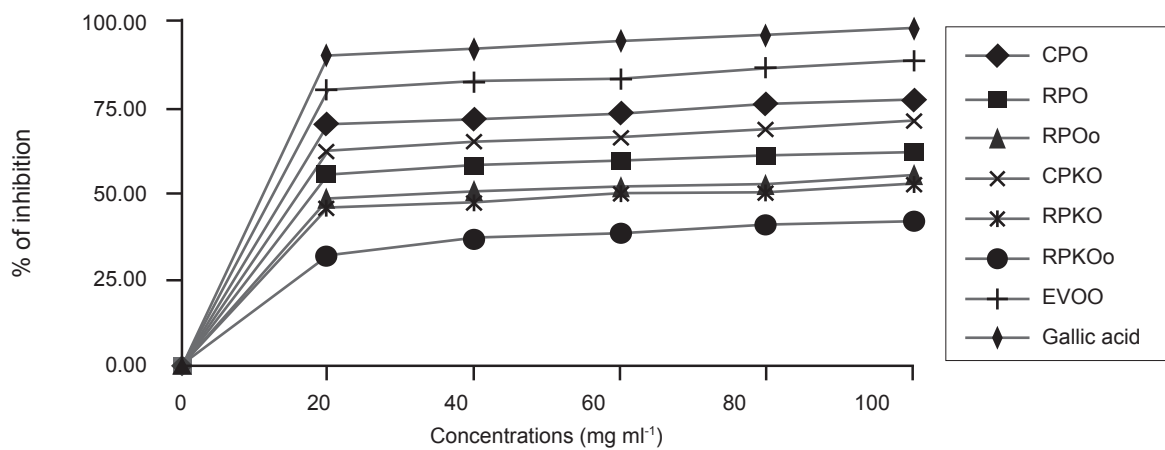
Figure 3 shows the IC<sub>50</sub> of different oil DPPH scavenging capacity of all the phenolic extracts from the test (Abdullah *et al.*, 2018). In our previous study the results also showed that IC<sub>50</sub> value was determined from the plotted graph of scavenging activity versus the concentration of oils extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. It is noted that a 20 mg ml<sup>-1</sup> gallic acid standard solution exhibits 90% with the value IC<sub>50</sub> 13.47 mg ml<sup>-1</sup>. This is followed by EVOO extract capacity exhibiting significant DPPH scavenging activity (13.50 mg ml<sup>-1</sup>), CPO (13.39 mg ml<sup>-1</sup>), RPO (13.23 mg ml<sup>-1</sup>), RPOo (12.05 mg ml<sup>-1</sup>), and CPKO (12.88 mg ml<sup>-1</sup>). RPKO and RPKOo were present at very low concentrations, so their definitive quantification was not attempted.

It is known that a number of natural extracts from selected fruits, herbs, spices and some vegetables are stable to autoxidation due to the presence of natural phenolic compounds (Pokorny *et al.*, 2001). From the previous analyses, it is proven that CPO contains the highest level of TPC compound compared to the other oils. The results of the present study reveal that there is a strong correlation

between antioxidant activity and phenolic content. A lower concentration of phenolic compounds could lead to lower antioxidant capacities.

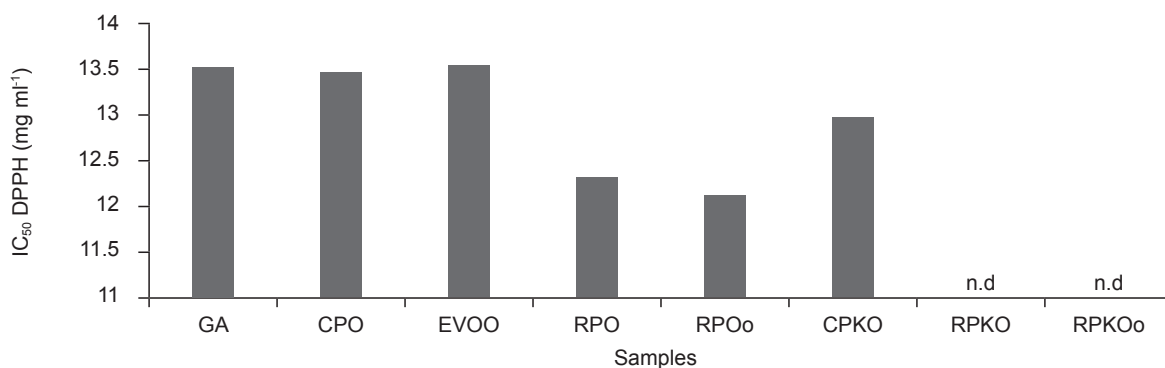
It is believed that the antioxidant properties of phenolics are a result of their ability to act as reducing agents, hydrogen donors and free radical quenchers and phenolics can also act as metal chelators which prevent the catalytic function of metal in the process of initiating radicals (Prior *et al.*, 2005). Thus, phenolic compounds have an important role in antioxidant activities (Harborne, 1998). A good correlation between antioxidant activity and phenolic compounds was found in Bulgarian medicinal plants (Ivanova *et al.*, 2005). The phenolic hydroxyl groups present in the plant antioxidants have redox properties (Shahidi *et al.*, 1992; Pietta, 2000) allowing them to act as a reducing agent and a hydrogen donor.

The order of scavenging activity of the oil extracts obtained in this study was EVOO > CPO > CPKO > RPO > RPOo > RPKO > RPKOo (Figure 3). Almost all of the oil extracts showed that the TPC was related to the scavenging activity (DPPH assay). Refined products of palm and palm kernel oils were less effective in quenching the DPPH radical than the crude extract at the same level. However, their effectiveness increased at higher concentrations. The activity was lower in the refined products of palm and palm kernel oils. Figure 3 suggested that during the refining process some antioxidant compounds are degraded or transformed (Abdullah *et al.*, 2018). The reduction in antioxidant activity with refining processes were also reported for rapeseed oil, silkworm pupa oil and kenaf seed oil (Szydłowska-Czerniak and Laszewska, 2015; Liu *et al.*, 2016; Chew *et al.*, 2016).



Note: CPO - crude palm oil; RPO - refined palm oil; RPOo - refined palm olein; CPKO - crude palm kernel oil; RPKO - refined palm kernel oil; RPKOo - refined palm kernel olein.

Figure 2. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of extra virgin olive oil (EVOO), palm and palm kernel oils extract. Each value is expressed as the mean ± standard error (S.E.) of triplicate analyses.



Note: n.d - not detected; GA - gallic acid; DPPH - 2,2-diphenyl-1-picrylhydrazyl; EVOO - extra virgin olive oil; CPO - crude palm oil; RPO - refined palm oil; RPOo - refined palm olein; CPKO - crude palm kernel oil; RPKO - refined palm kernel oil; RPKOo - refined palm kernel olein.

Figure 3. The IC<sub>50</sub> (inhibitory concentration) of different oil DPPH scavenging capacity of extra virgin olive oil, palm and palm kernel oils extract. Each value is expressed as the mean ± standard error (S.E.) of triplicate analyses.

The extract of crude palm and palm kernel oils showed high phenolic contents and correspondingly higher antioxidant activity. Concentration significantly affects the free radical scavenging activity for all samples. The results, thus, confirm that phenolic compounds rich in vegetable oils possess strong antioxidant activity. A strong correlation has been observed between antioxidant activity and total phenolics (Cao *et al.*, 1996; Wang *et al.*, 1996; Prior *et al.*, 1998).

### Identification of Phenolic Acids in Palm Oil Products

Figure 4 shows the HPLC chromatograms of the standard phenolic acid mixture. The phenolic acids; gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid and ferulic acid were separated under the HPLC conditions used. The identification of phenolic acids extract of palm and palm kernel oils and their respective retention time in HPLC profile are shown in Table 2.

TABLE 2. IDENTIFICATION AND RETENTION TIME OF PHENOLIC ACIDS IN STANDARD SOLUTIONS

Peak No.	Retention time (min)	Phenolic acids
1	4.544 ± 0.11	Gallic acid
2	7.693 ± 0.25	Protocatechuic acid
3	13.475 ± 0.15	<i>p</i> -hydroxybenzoic acid
4	17.884 ± 0.85	Vanillic acid
5	18.064 ± 1.15	Syringic acid
6	20.859 ± 1.02	Caffeic acid
7	35.580 ± 1.89	<i>p</i> -coumaric acid
8	51.266 ± 2.99	Ferulic acid

Figure 5 presents the HPLC chromatographic profiles of phenolic compounds in CPO, RPO, RPOo, CPKO, RPKO and RPKOo, respectively. Table 3 summarises the phenolic acids found in the phenolic extracts of the analysed oils. The results indicate that CPO and CPKO contained almost all the phenolic acids while the refined products have

lost majority of the phenolics through the refining/milling process. This supports the fact that palm oil mill effluent has become the major source of palm phenolics as reported previously (Sambanthamurthi *et al.*, 1998).

In this study, the acids found in the oil extracts were benzoic and cinnamic acid derivatives. The benzoate derivatives are vanillic acid, protocatechuic acid and *p*-hydroxybenzoic acid. Meanwhile, the cinnamic acids/hydroxycinnamic acid derivatives which were present in lower concentrations were caffeic acid, *p*-coumaric acid and ferulic acid. Other unknown compounds were also found in CPO and CPKO extracts. Lower concentration of hydroxycinnamic acid derivatives was also reported on a study of phenolic compounds in apples and ciders (Cilliers *et al.*, 1990). Nonetheless, the content of hydroxycinnamic acid derivatives are relatively constant while the flavonoids vary depending on the maceration and cultivar being processed (Cilliers *et al.*, 1990). Hydroxycinnamates derived from phenylalanine are the precursor of flavonoid.

In general, the most abundant phenolic acid identified in this study was *p*-hydroxybenzoic acid and it was the predominant acid in all samples. The *p*-hydroxybenzoic acid contents in the extracts of palm and palm kernel oils were quantified accordingly (Table 4). The separation of phenolics compounds by HPLC analysis revealed that gallic acid, protocatechuic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid, ferulic acid were present at very low concentrations, thus, their definitive quantification was not attempted. The results reflect the change of phenolics content in the sequence of CPO > CPKO > RPO > RPOo > RPKO > RPKOo. The trend of *p*-hydroxybenzoic acid contents in palm oil products is the same with the trend of TPC in palm oil products as reported previously (Abdullah *et al.*, 2018). It is suggested that the amount of phenolic decreases throughout the refining process and consists of water-soluble (highly polar) compounds in decreasing levels of polarity.

TABLE 3. PHENOLIC ACIDS IN PALM OIL PRODUCTS

Phenolic acids	Palm oil products					
	CPO	RPO	RPOo	CPKO	RPKO	RPKOo
Gallic acid	√	√	√	√	√	√
Protocatechuic acid	√	ND	√	√	ND	ND
<i>p</i> -hydroxybenzoic acid	√	√	√	√	√	√
Vanillic acid	√	ND	ND	√	ND	ND
Syringic acid	√	ND	ND	√	ND	ND
Caffeic acid	√	ND	ND	ND	ND	ND
<i>p</i> -coumaric acid	√	ND	ND	√	ND	ND
Ferulic acid	ND	ND	ND	√	ND	ND

Note: √ - detected, ND - not detected.

CPO - crude palm oil; RPO - refined palm oil; RPOo - refined palm olein; CPKO - crude palm kernel oil; RPKO - refined palm kernel oil; RPKOo - refined palm kernel olein.



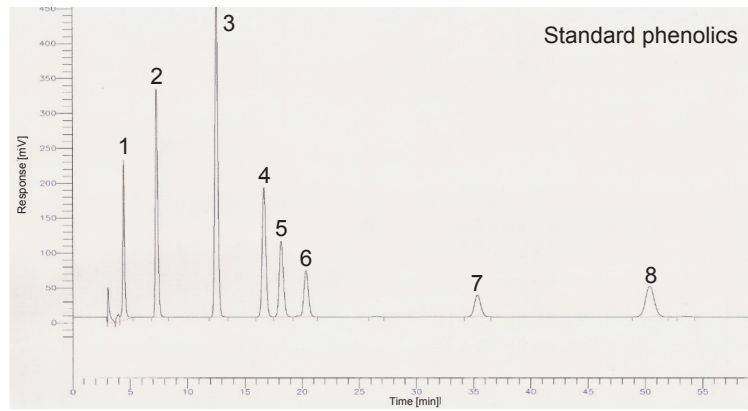
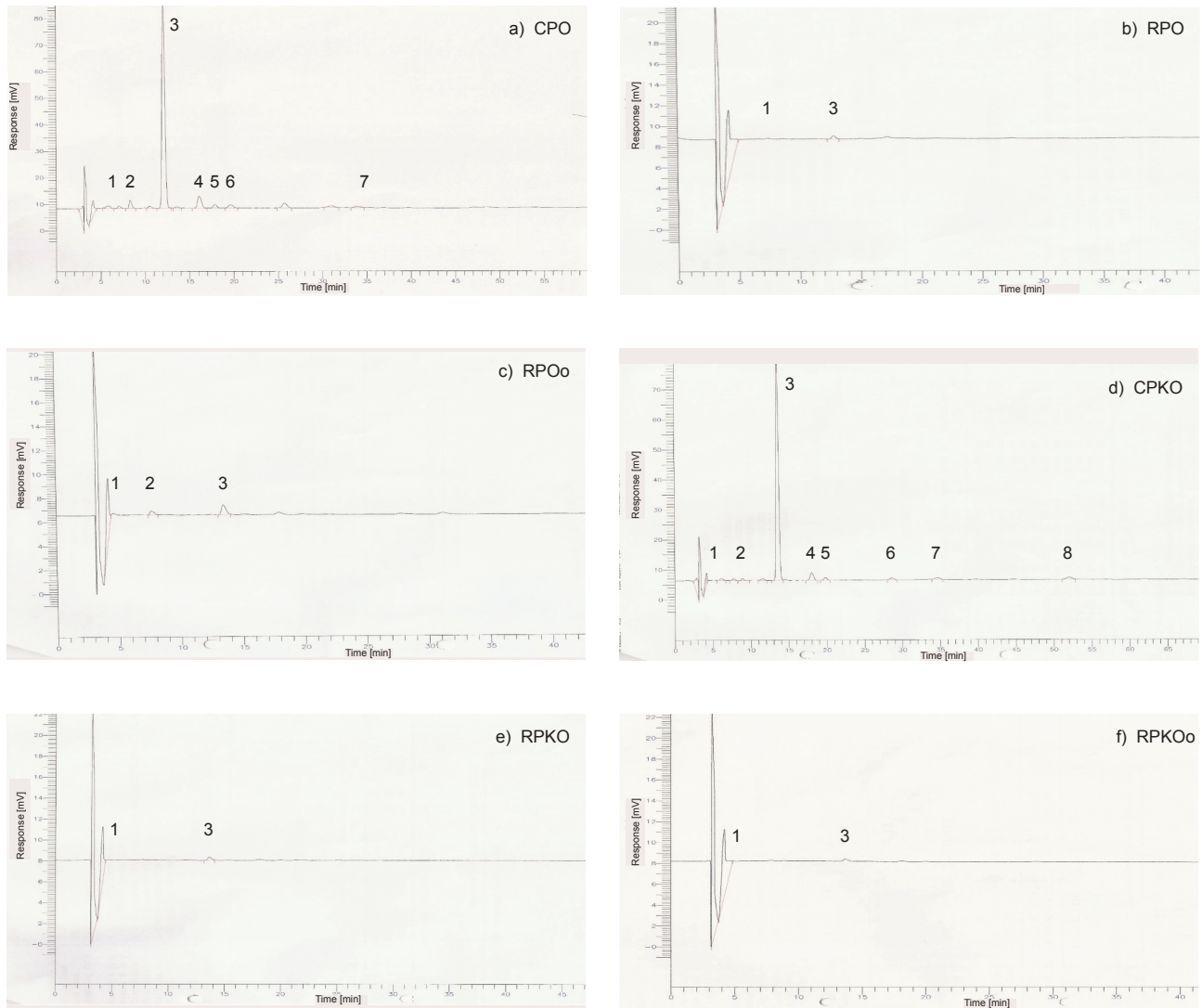


Figure 4. The high performance liquid chromatography (HPLC) profile of the standard phenolic compound. Peak identification: (1) gallic acid; (2) protocatechuic acid; (3) *p*-hydroxybenzoic acid; (4) vanillic acid; (5) syringic acid; (6) caffeic acid; (7) *p*-coumaric acid and (8) ferulic acid.



Note: Peak identification: (1) gallic acid; (2) protocatechuic acid; (3) *p*-hydroxybenzoic acid; (4) vanillic acid; (5) syringic acid; (6) caffeic acid; (7) *p*-coumaric acid and (8) ferulic acid.

Figure 5. The high performance liquid chromatography (HPLC) profile of phenolic compounds in (a) crude palm oil (CPO), (b) refined palm oil (RPO), (c) refined palm olein (RPOo), (d) crude palm kernel oil (CPKO), (e) refined palm kernel oil (RPKO), and (f) refined palm kernel olein (RPKOo).

TABLE 4. QUANTIFICATION OF *p*-HYDROXYBENZOIC ACID IN THE EXTRACT OF PALM OIL AND PALM KERNEL OILS (mg kg<sup>-1</sup> extract)

Palm oil products	<i>p</i> -hydroxybenzoic acid (mg kg <sup>-1</sup> ) AV + SD (RSD%)
CPO	1901.69 ± 1041.54 (54.7%)
RPO	19.13 ± 10.49 (54.7%)
RPOo	7.87 ± 4.34 (54.7%)
CPKO	1701.70 ± 932.19 (54.8%)
RPKO	5.01 ± 2.78 (54.8%)
RPKOo	n.d

Note: CPO - crude palm oil; RPO - refined palm oil; RPOo - refined palm olein; CPKO - crude palm kernel oil; RPKO - refined palm kernel oil; RPKOo - refined palm kernel olein.

AV – average; SD – standard deviation; n.d - not detected.

Relative standard deviation (RSD) - (standard deviation / mean) × 100%, (n = 3).

Phenolics are known to scavenge DPPH radical effectively. In previous study, we have reported the antioxidant activity of palm oil products extracts determined using DPPH assay (Abdullah *et al.*, 2018). Results showed that the effect of antioxidants on DPPH radical scavenging activity decreases in the order of oil extracts CPO > CPKO > RPO > RPKO > RPOo > RPKOo. Phenolic acids also play an important role in combating oxidative stress in the human body by maintaining a balance between oxidants and antioxidants. It is reported that caffeic acid has shown better activity in scavenge DPPH radicals compared to other phenolic acids (Brand Williams *et al.*, 1995). In this study, caffeic acid appeared in sample extracts of CPO and CPKO.

Several studies have reported the relationship between structure of phenolic acids and antioxidant activity (Hsieh *et al.*, 2005; Farhoosh *et al.*, 2016). Monophenols have been shown to be less efficient as phenolic antioxidants than polyphenols (Farhoosh *et al.*, 2016). The antioxidative activity of polyphenol is related to the hydroxyl group. The functional groups attached to the ortho or para positions of phenolic rings have been shown to be more effective than those attached to meta position in the performance of phenolic antioxidants. This is due to the additional resonance stability, and *o*-quinone or *p*-quinone formation (Chen and Ho, 1997; Bouchet *et al.*, 1998). Besides, the introduction of different electron donating or withdrawing groups at various positions of the phenolics which give structural variations is the basis for enhancement of the antioxidant activity (Shahidi *et al.*, 1992). As reported herein, *p*-hydroxybenzoic acid was found to be present in all sample extracts.

The palm fruit is a rich source of water-soluble phenolic antioxidant (Sambanthamurthi *et al.*,

2000). During the palm oil milling process, these antioxidants find their ways into the POME. Preliminary investigations have shown that the extract from POME has potent anti-cancer and antioxidant properties while inhibiting progression of atherosclerosis lesions as reported by Sundram *et al.* (2003). The studies also discovered the protective effects of palm phenolic antioxidants on vegetable oils. As a result of investigation of the antioxidant efficacy of palm phenolics, it was suggested that phenolic from POME is a rich source of natural antioxidants that can be used in the field of food preservation (Sundram *et al.*, 2003). Today's heightened interest in these compounds is due to possible health benefits that are attributed to their antioxidant properties. The possible health benefits include reduced risk of coronary heart disease, cancer, stroke and diabetes.

## CONCLUSION

The present investigation revealed that palm oil products; CPO, RPO, RPOo, CPKO, RPKO, and RPKOo contain phenolic compounds (albeit in a small quantities). Phenolics in extracts analysed by HPLC show the presence of benzoic and cinnamic acid derivatives, with *p*-hydroxybenzoic acid being predominant and present in all sample extracts. The profiling of hydrophilic phenolic compounds would provide information on the possible role of these compounds such as in oil stability, colour stability and other possible beneficial properties.

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