Recovery and Utilization of Palm Oil Mill Effluent Source as Value-Added Food Products

Soek Sin Teh¹, Augustine Soon Hock Ong² and Siau Hui Mah³*

¹ Engineering and Processing Division, Malaysian Palm Oil Board, 43000 Kajang, Selangor, MALAYSIA
² Senior Fellow of the Academy of Sciences, MALAYSIA
³ School of Biosciences, Taylor’s University, Lakeside Campus, 47500 Subang Jaya, Selangor, MALAYSIA

Abstract: The environmental impacts of palm oil mill effluent (POME) have been a concern due to the water pollution and greenhouse gases emissions. Thus, this study was conducted to recover the value-added products from POME source before being discharged. The samples, before (X) and after (Y) the pre-recovery system in the clarification tank were sampled and analysed and proximate analysis indicated that both samples are energy rich source of food due to high contents of fats and carbohydrates. GCMS analysis showed that the oil extracts contain predominantly palmitic, oleic, linoleic and stearic acids. Regiospecific analysis of oil extracts by quantitative ¹³C-NMR spectroscopy demonstrated that both oil extracts contain similar degree of saturation of fatty acids at sn-2 and sn-1,3 positions. The samples are rich in various phytonutrients, pro-vitamin A, vitamin E, squalene and phytosterols, thus contributing to exceptionally high total flavonoid contents and moderate antioxidant activities. Overall, samples X and Y are good alternative food sources, besides reducing the environmental impact of POME.

Key words: antioxidant, fatty acid, food, palm oil mill, phytonutrients, polyphenolics, regiospecific

1 INTRODUCTION

Palm oil is one of the major oils in the world oils and fats trade. Palm oil derivatives such as palm olein, palm kernel oil, palm stearin, palm mid fraction, are typically obtained from palm oil refinery plants. A wide range of edible palm-based products are available in the current market including cooking oil, frying oil, margarines, shortenings, specialty fats, vanaspati and spray dried products. Besides, palm oil derivatives are important source of oleochemicals and other downstream derivatives such as soaps, surfactants, candles, cosmetics and personal care products, detergents, food emulsifiers, lubricants, paints, pharmaceutical, textiles and biofuels¹. Palm oil derivatives are also used extensively as food ingredients in ice-cream, salad dressings, snack foods, biscuits, mayonnaise, maggie mee, and condensed milk². Palm oils are known to exert precious nutritional values due to its high content of carotenoids, phytosterols, tocotrienols and tocophenols, which have been proven to exhibit anti-oxidant³, anticancer⁴ and anti-inflammatory⁵ properties. Moreover, crossover studies showed that normcholesterolemic subjects who were given palm olein-rich and olive oil-rich diets, reflected comparable effects of total, HDL and LDL cholesterol in their blood levels⁶. Similar trends were observed for the adults fed palm olein diets who demonstrated the same serum total and HDL cholesterol levels with those adults fed with canola oil in a double blind crossover study⁷.

Palm oils are extracted from oil palm fruit through a series of process, including sterilisation, threshing, pressing and clarification, in the palm oil mill. Several tanks are usually used in the clarification station in order to maximize the restoration of crude palm oil before discharging into watercourses⁸. The environmental impacts of waste water discharge from palm oil mill is known as palm oil mill effluent (POME). POME is usually released into open-air ponds for remediation, resulting in the emissions of greenhouse gases (GHG) including carbon dioxide and methane, which contribute to global climate change. POME has high acidity, biological oxygen demand (BOD), and chemical oxygen demand (COD). It is particularly harmful to aquatic communities by creating highly acidic environments or causing eutrophication if discharged into waterways, besides contaminating drinking water. This issue has urged researchers from worldwide to seek for the solutions and thus different holistic approaches have been used to treat POME in order to mitigate environmental impacts of POME, as well as the development of transformation products such as palm puree, methane, biodegradable plastics,
fertilizers and animal feeds.

Recent studies showed that the utilization of Bacillus cereus 103PB\textsuperscript{16}, ultra-filtration membrane\textsuperscript{11}, electrocoagulation reactor\textsuperscript{11} and vermicompost\textsuperscript{12} can reduce BOD, colour and total suspended solid in the waste water effectively. In the past decades, a number of studies reported that POME possessed high contents of polyphenolic compounds and these compounds demonstrated antioxidant activity, cardioprotective effect, reduce atherosclerosis, cytotoxicity\textsuperscript{13}, neuroprotective effect\textsuperscript{14}. As of today, there is no comprehensive research performed on the value-added products of palm oil mills. This indeed is a good approach to increase value adding of palm oil mills. In this study, we aim to recover and utilize value-added products from the palm oil mill, particularly from the POME source before being discharged into watercourses.

2 EXPERIMENTAL

2.1 Sample collection and extraction

The samples of before pre-recovery system (X) and after pre-recovery system (Y) were collected freshly from clarification tanks of Tai Tak Palm Oil Mill, Kota Tinggi, Johor, Malaysia on 13\textsuperscript{th} July 2016. Sample X was collected from the settling tank which involves separation of oil and sludge by sedimentation while sample Y was collected from the settling tank which involves separation of oil and total suspended solid in the waste water effectively. In the past decades, a number of studies reported as recommended by Merrill\textsuperscript{et al.\textsuperscript{16}}. The total carbohydrate content of the samples were determined using the formula below as mentioned in Section 2.1. The total carbohydrate content of the samples were determined using the formula below as recommended by Merrill\textsuperscript{et al.\textsuperscript{17}}.

\[
\text{Total carbohydrate} = 100 \times \left[ \frac{\text{weight in grams (protein + fat + water)}}{100 \text{ g of sample}} \right]
\]

The energy value of individual macronutrients were calculated based on the of Atwater factors. The energy available from the samples were calculated by multiplying the number of grams of carbohydrate, protein, and fat by the Atwater factors of 4, 4 and 9 kcal/g, respectively\textsuperscript{17}. The test was carried out in triplicate for each sample.

2.2 Proximate analysis

The total protein concentration of the samples were measured using Dumas combustion method\textsuperscript{15}. The total water content of the samples were analyzed using MPOB Test Method p2.1 Part 1:2004. The total lipid of the samples were obtained using modified Folch method\textsuperscript{16} as mentioned in Section 2.1. The total carbohydrate content of the samples were determined using the formula below as recommended by Merrill\textsuperscript{et al.\textsuperscript{17}}.

2.3 Determination of Total Sterol, Carotene, Tocopherol and Tocotrienol contents

Total sterol of extracts were determined following the ISO standard ISO/FIDS 12228:1999 as described by Matthaus\textsuperscript{et al.\textsuperscript{16}}. In brief, 250 mg of sample was saponified with a solution of ethanolic potassium hydroxide by boiling under reflux. The unsaponifiable matter was isolated by solid-phase extraction on an aluminium oxide column. The sterol fraction was separated from unsaponifiable matter by thin-layer chromatography. The compounds were separated on a Gas-chromatography (GC) (Perkin Elmer) on a SAC fused capillary column (30 m (L) × 250 μm (D) × 0.25 μm (Film)) under isothermal condition at 265°C. Helium is used as carrier gas and the temperatures of injector and detector were set at 280°C. Peaks were identified by comparison of the retention time of the standard compounds, β-sitosterol, cholesterol, campesterol, stigmasterol and squalene.

Total carotene content of extracts were conducted using MPOB Test Method p2.6:2004. Approximately 0.1 g of oil extract was dissolved in 250 mL of n-hexane. The absorbance of oil extract was measured at 446 nm. The total carotene content of oil extracts were determined using the formula below.

\[
\text{Total Carotene Content} = 383 \frac{E}{Ic}
\]

where E is the observed difference in absorption between the oil extract and hexane, I is the path length of the cell (cm) and c is the concentration used for absorption measurement (g/100 mL).

The tocopherols and tocotrienols of extracts were analyzed using normal phase HPLC (Agilent 1100 series) on a Silica Hypersil column (250 mm (L) × 4.6 mm (D)) coupled to a fluorescence detector\textsuperscript{10}. The detection wavelengths for excitation and emission were 295 and 325 nm, respectively. The mobile phase used was n-hexane, 1-dioxane and 2-propanol at 97.5: 2.0: 0.5 v/v/v. The test was carried out in triplicate for each sample.

2.4 Products Characterisations

An amount of 50 μL of oil extract was dissolved in 1 mL of toluene, placed in a flask fitted with a condenser and treated with 2 mL of acidified methanol (2% H\textsubscript{2}SO\textsubscript{4}(v/v)) in methanol. The mixture was heated under reflux for 2 h at 80°C. After reflux process, 5 mL of 5% sodium chloride solution was added into the mixture, subsequently extracted with hexane (5 mL × 2) and washed with 2% sodium bicarbonate. The upper layer was then collected and concentrated under reduced pressure. The fatty acid methyl ester (FAME) was then kept for GC analysis. The FAME of oil extracts were determined using GC coupled to a mass spectrometer on a J&W DB-WAXetr column (30 m (L) × 250 μm (D) × 0.25 μm (Film); Agilent Technologies, US)\textsuperscript{20}. The carrier gas used was helium at a flow rate of 20 mL/
min. The injector and detector temperatures were set at 250°C and 280°C, respectively, while the oven temperature was set at 50°C for 1 min, 25°C/min to 200°C and 3°C/min to 230°C for 18 min.

Regio-specific analyses of oil extracts were executed using JEOL ECZ-600MHz NMR spectrometer\(^{23}\). Approximately 100 mg of oil extracts were dissolved in 500 µL of deuterated chloroform (CDCl\(_3\)) and analysed by quantitative \(^{13}\)C NMR. The fatty acid positional composition in the triacylglycerols of oil extracts were acquired under a relaxation delay of 15.0 s, 8192 data points, a 90° pulse angle and a spectral width of 1500 Hz at which the acyl chain carbonyl carbons resonate. The test was carried out in triplicate for each sample.

2.5 Determination of total phenolic content (TPC)

The total phenolic content of extracts were measured using Folin–Ciocalteu method with some modifications as described by Teh et al.\(^{21}\). Gallic acid was used as the standard in the experiment. TPC of extracts were expressed in terms of gallic acid equivalents (GAE). The calibration equation for gallic acid was \( y = 0.261 \times -0.088 \) (R\(^2\) = 0.9962) where \( x \) is the gallic acid concentration in µg/mL and \( y \) is the absorbance reading at 725 nm. The values were presented as the means of triplicate analyses ± standard deviation.

2.6 Determination of total flavonoid content (TFC)

Total flavonoid content of extracts were measured using spectrophotometric method\(^{21}\). Rutin hydrate was used as the standard in the experiment. TFC of extracts were expressed in terms of rutin hydrate equivalents (RE). The calibration equation for rutin hydrate was \( y = 0.035 \times -0.0029 \) (R\(^2\) = 0.9941) where \( x \) is the rutin hydrate concentration in µg/mL and \( y \) is the absorbance reading at 415 nm. The values were presented as the means of triplicate analyses ± standard deviation.

2.7 Evaluation of Antioxidant Activity

2.7.1 DPPH Free Radical Scavenging Activity

Scavenging activity of extracts were assessed using DPPH free radical assay\(^{25}\). Ascorbic acid was used as the standard in the experiment. The average absorbance of each extract was calculated and the average value was used to determine the percentage of total radical scavenging activity by using the following formula:

\[
\text{Percentage of free radical scavenging activity} = \frac{A(\text{average}) - B(\text{average})}{A(\text{average})}
\]

where, \( A \) is the absorbance of blank, and \( B \) is the absorbance of extract. The absorbance values were recorded at 517 nm. The values were presented as the means of triplicate analyses ± standard deviation.

2.7.2 Nitric Oxide (NO) Scavenging Activity

NO scavenging activity of extracts were determined by the method as described in by Tsai et al.\(^{26}\). Gallic acid was used as the standard in the experiment. The average absorbance of each extract was calculated and the average value was used to determine the percentage of NO scavenging activity by using the following formula:

\[
\text{Percentage of free radical scavenging activity} = \frac{A(\text{average}) - B(\text{average})}{A(\text{average})}
\]

where, \( A \) is the absorbance of blank, and \( B \) is the absorbance of extract. The absorbance values were recorded at 560 nm. The values were presented as the means of triplicate analyses ± standard deviation.

2.7.3 Ferrous Ion Chelating Activity

The ferrous ion chelating activity of extracts were measured by the method described in Carter\(^{27}\). EDTA was used as the standard in the experiment. The iron chelating activity of extracts were calculated from the equation:

\[
\text{Percentage of chelation} = \frac{A(\text{average}) - B(\text{average})}{A(\text{average})}
\]

where, \( A \) is the absorbance of blank, and \( B \) is the absorbance of extract. The absorbance values were recorded at 562 nm. The values were presented as the means of triplicate analyses ± standard deviation.

3 RESULTS AND DISCUSSION

3.1 Proximate analysis

The science of food analysis has been refined constantly to ensure the safety, quality, and traceability of foods are in compliance with legislation and demands of consumers. Food analysis can be further subdivided into various parts in accordance to the characteristics of food such as composition, structure, physiological properties and sensory attributes. The principal structural components of foods comprise of lipids, proteins and carbohydrates. These are the main components listed in nutritional fact, which is an important piece of information for individuals to monitor their diets in order to maintain healthy diets. Hence, proximate analysis was carried out and the results are summarized in Table 1. The results indicated that the sample before pre-recovery system (X) has remarkably higher total fat content and marginally lower carbohydrate content if compared to the sample after pre-recovery system (Y). In fact, carbohydrates are one of the major classes of food components which serve as a major source of energy. Thus,
the high caloric content of X is implied to be contributed mainly by its significant high level of fat content (63.7 g/100g sample). Overall, the high caloric values of both samples (X = 596.65 and Y = 235.45 kcal/100 g) suggest that they are good sources of energy. Thus, the palm fruits are widely consumed as food by African tribes to help in boosting the energy level in their bodies. Refer to Table 1, the moisture content of sample X was seen to be lower than sample Y, indicating that X would have greater storage stability. This statement is supported by Joslyn who described that low moisture content of food is able to prevent the growth of food microorganism, as well as alleviate enzymatic reaction within the food. On the other hand, protein content of sample Y is slightly higher than sample X, revealing that Y is a good source of protein. Protein is an essential nutrient for human body as it promotes growth and maintenance. Moreover, it plays an important role in food and food products as functional ingredients. Functional characteristics of proteins refer to physical or chemical properties of proteins in foods during processing, storage, preparation, and consumption. The proximate analysis of samples was determined and subsequently suggesting both samples are nutritious foods.

### 3.2 Total Sterol, Carotene and Squalene contents

Crude palm oil is known to be comprised of minor nutritious constituents including sterols, carotenoids, squalene, tocotrienols and tocopherols. Phytosterols are natural occurring steroids that are ubiquitous in plant species. It is widely used in enriched food supplements and commercially added to foods for its beneficial properties. Phytosterols have been proven scientifically that they are able to reduce low density lipoprotein (LDL) and total cholesterol levels, induce apoptosis in prostate, breast and colon cancer cells, interrupt testosterone metabolism by enzymatic reactions, as well as inhibit the production of pro-inflammatory and matrix degradation mediators. The concentrations of the total sterols, in addition to its individual sterols (cholesterol, campesterol, stigmasterol and β-sitosterol) for lipid extracts were examined and tabulated in the Table 2. The results show that the total sterols present in extracts X and Y are 508.7 and 160.8 ppm, respectively with β-sitosterol present as the dominant sterol. It is observed that the concentrations of all individual sterols are relatively higher in extract X. This is possibly due to several stages of skimming processes designed in the clarification tanks of the mill that results in the valuable components to be skimmed off during the process. These components are well retained in the clarification tank where extract X was collected. In addition, the concentrations of total sterols, together with β-sitosterol of both extracts found in this study are similar to that of crude palm oil, reported by Siew. By comparing to the same source, extract Y shows lower concentration of campesterol, stigmasterol and cholesterol than crude palm oil, while extract X shows higher concentrations of cholesterol and stigmasterol. The difference of the amounts of sterols between crude palm oil and extracts is mainly due to the oil extraction processes in refinery.

Carotenoids are orange pigments that are widely available in plants. Some of the carotenes can be converted into vitamin A in the human body. Previous research findings showed that the foods enriched with carotenoids possessed various protective health benefits such as anti-inflammatory, antioxidant and anticancer activities. An increase in the consumption of carotenoids-rich food are claimed to be

### Table 1 Nutrition facts of samples.

| Nutrition Information | Total Water (g) | Total Protein (g) | Total Fat (g) | Total Carbohydrate (g) | Energy (kcal)/100 g |
|-----------------------|----------------|------------------|--------------|-----------------------|--------------------|
| Before pre-recovery (X) | 30.45 ± 2.71 | 0.78 ± 0.01 | 63.70 ± 2.47 | 5.07 ± 0.22 | 596.65 ± 12.51 |
| After pre-recovery (Y) | 67.60 ± 1.98 | 1.53 ± 0.12 | 21.17 ± 1.92 | 9.70 ± 0.17 | 235.45 ± 14.62 |

### Table 2 Total sterols, carotenoids and squalene contents of oil extracts.

| Components | Before pre-recovery system (X) (ppm) | After pre-recovery system (Y) (ppm) |
|------------|----------------------------------|----------------------------------|
| Total Sterols | 508.7 ± 10.5 | 160.8 ± 8.8 |
| Cholesterol | 19.8 ± 2.7 | 5.4 ± 1.6 |
| Campesterol | 71.1 ± 3.7 | 4.2 ± 1.2 |
| Stigmasterol | 71.5 ± 4.7 | n.d. |
| β-sitosterol | 346.3 ± 9.6 | 151.2 ± 8.1 |
| Total Carotenoids | 519.0 ± 10.5 | 569.0 ± 7.8 |
| Total Squalene | 706.5 ± 12.4 | 513.5 ± 11.3 |

n.d. - Not detected
correlated with a decrease in the risk of age-related macular degeneration and cataracts\textsuperscript{32}. Likewise, squalene has been reviewed to exert a number of beneficial effects such as antioxidant, anticancer, as well as lowering serum cholesterol levels\textsuperscript{33}. The extracts were evaluated for total carotenoids and squalene contents and the results are presented in Table 2. The total carotenoids contents of X and Y are 519.0 and 569.0 ppm, respectively, while total squalene contents are 706.5 and 513.5 ppm. The carotenoids contents shown were similar to the crude palm oil that was reported by Jacobsberg\textsuperscript{34} whereas the squalene contents were comparable with that of the literature data reported by Gapor \textit{et al.}\textsuperscript{35} for crude palm oil. The results indicated that the mechanical and physical extraction processes in the refinery where the crude palm oil is produced do not interfere the amount of carotenoids and squalene contents, unlike the fractionation process that reduced the concentration of carotenoids tremendously\textsuperscript{34}. In this study, extract Y exhibited higher carotenoid content than extract X. Heat treatment and centrifugation that took place in the clarification tank are responsible for the changes in carotenoid contents. This is supported by previous studies which showed that the carotenoid content in cherry tomatoes can be enhanced by thermal and mechanical processes due to higher lycopene extractability from the vegetable matrix\textsuperscript{36}.

Besides, the vitamin E contents of both extracts were studied and presented in Table 3. The results indicated that X and Y possessed 743.42 and 705.98 ppm of total tocols, respectively which were comparable with that of palm olein which are 72.57\textsuperscript{`s} and 14.43\textsuperscript{`s} ppm, respectively, while plam olein contains 64.1\textsuperscript{`s} ppm. These values are comparable with that of palm olein which are 10.4\textsuperscript{`s}, 14.21\textsuperscript{`s}, and 69.67\textsuperscript{`s} ppm\textsuperscript{37}. The saturated fatty acids (SFA) are tabulated in Table 4. Both extracts are found to be containing these minor components, which possess numerous health beneficial effects, proposing both extracts as good sources of nutritional food.

### Table 2

| Components Before pre-recovery system (X) | After pre-recovery system (Y) |
|-----------------------------------------|-------------------------------|
| Alpha-tocopherol (α-T)                  | 133.12 ± 1.01                |
| Alpha-tocotrienol (α-T3)                | 177.16 ± 1.52                |
| Beta-tocopherol (β-T)                   | 106.67 ± 0.55                |
| Beta-tocotrienol (β-T3)                 | n.d.                          |
| Gamma-tocopherol (γ-T)                  | n.d.                          |
| Gamma-tocotrienol (γ-T3)                | 243.76 ± 1.55                |
| Delta-tocopherol (δ-T)                  | 82.71 ± 0.38                 |
| Total T                                 | 239.79                        |
| Total T3                                | 503.63                        |
| Total (T+T3)                            | 743.42                        |

n.d. - Not detected

### Table 3

| Components Before pre-recovery system (X) | After pre-recovery system (Y) |
|-----------------------------------------|-------------------------------|
| Alpha-tocopherol (α-T)                  | 133.12 ± 1.01                |
| Alpha-tocotrienol (α-T3)                | 177.16 ± 1.52                |
| Beta-tocopherol (β-T)                   | 106.67 ± 0.55                |
| Beta-tocotrienol (β-T3)                 | n.d.                          |
| Gamma-tocopherol (γ-T)                  | n.d.                          |
| Gamma-tocotrienol (γ-T3)                | 243.76 ± 1.55                |
| Delta-tocopherol (δ-T)                  | 82.71 ± 0.38                 |
| Total T                                 | 239.79                        |
| Total T3                                | 503.63                        |
| Total (T+T3)                            | 743.42                        |

n.d. - Not detected

### 3.3 Products characterizations

The GCMS analysis for the lipid extracts are summarized in Table 4 and the results indicated that both extracts contain similar fatty acid composition. The extracts X and Y contain predominantly palmitic acid (∼44%) and oleic acid (∼40%), followed by linoleic acid (∼9%) and stearic acid (4.19%). These results were comparable with the fatty acid profile of palm oil\textsuperscript{38}. As a comparison with palm olein, both extracts were found to contain slightly higher palmitic acids than palm olein (40.9%) but lower oleic acids than palm olein (41.5%)\textsuperscript{39}. This is due to the refinery process where the palm oil is fractionated into two fractions, which are palm stearin with higher melting point components and palm olein with lower melting point components\textsuperscript{30}.

The positional distribution of fatty acids in extracts X and Y were determined with NMR analysis and the results are tabulated in Table 5. Both extracts demonstrated comparable saturation levels at either sn-1,3 or sn-2 positions. The saturated fatty acids (SFA) of extracts X and Y at sn-2 position are 14.43% and 14.21%, respectively, while sn-1,3 position are 69.67% and 72.57%. These values are comparable with that of palm olein which are 10.4% and 64.1%, with respect to sn-2 and sn-1,3 positions\textsuperscript{41}. Previous studies reported that different positions of fatty acids situ-
ated at triglycerides could influence absorption, metabolism and fat distribution in the human body. Small reviewed that fatty acids at sn-2 position plays an important role in regulating serum cholesterol levels. A number of clinical studies reported that adults who consumed palm olein and olive oil diets, palm olein and canola oil diets, and palm olein and groundnut oils diets, showed comparable serum cholesterol profiles. The reason is that all of the tested oils contain similar saturation levels at sn-2 position with less than 20 discrepancies among each other, as reported by Teh et al. Previous literature also demonstrated that the adults fed with palm olein diets have significantly lower cholesterol levels than that of lard diets, even though both diets have similar total fatty acid compositions. Thus, it is notably that the significant differences of their fatty acids at sn-2 position contributed to the outcomes. Meanwhile, saturated fatty acids at sn-1,3 positions play an important role in the reduction of fat deposition. This is due to the saturated fatty acids at sn-1,3 positions cannot readily digest through enzymatic reaction in the body and will bind to calcium or magnesium in the body to form insoluble calcium or magnesium soaps, subsequently expelled from the body in the form of excretal. Therefore, extracts X and Y are good sources of food in terms of cholesterol levels and fat deposition.

### 3.4 Total phenolic content (TPC) and total flavonoids content (TFC)

TPC and TFC of extracts X and Y were evaluated and summarized in Table 6. TPC of the extracts are presented as gallic acid equivalent (μg of gallic acid/g of sample). Sample X (3545.39 μg/g) exhibited a TPC value, which is three times higher than that of sample Y (1162.99 μg/g). However, both samples X and Y possess lower TPC values as compared to that of palm olein (control) with the variations of 27.5% and 76.2%, respectively. On the other hand, TFC of extracts were expressed as rutin equivalent (μg of...
3.5 Antioxidant Activity

DPPH radical scavenging, nitric oxide (NO) scavenging and ferrous ion chelating (FIC) assays have been performed to evaluate the antioxidant activities of extracts X and Y. The results as presented in Table 7 indicated that both extracts possess stronger antioxidant activities in FIC assay. FIC activities of both extracts are presented in half maximal effective concentration (EC₅₀) with EDTA as a control. The EC₅₀ values obtained for extracts X and Y in FIC assay are 31.09 and 15.15 mg/mL, respectively. Both extracts exhibited comparable weak DPPH scavenging activities at the concentration of 1 mg/mL. For NO scavenging assay, only extract X is able to exhibit 13.2% scavenging effect at the concentration of 5 mg/mL, indicating weak scavenging effects of both extracts. The results implied that the oil extracts possess antioxidant activities in different mechanisms extent.

The ion chelating properties is important due to the accumulation of transition metal ions causes tissue damage and leads to inflammation and cancer[40]. Thus, FIC results highlighted the therapeutic importance of the chelation capacities of both samples collected from palm oil mill. The ion chelating capacity is correlated to the electron-donating ability[40], suggesting that this ability attributed to the observed antioxidant properties in the extracts. The components that contributed to the antioxidant properties of extract are possibly due to the tocols and flavonoids. Vitamin E compounds, including tocopherols and tocotrienols are well known for its antioxidant properties due to their phenolic hydrogen donating ability with less contribution from singlet oxygen quenching[37]. Samples X and Y are proven to be tocols-rich as shown in Table 3, suggesting that the antioxidant properties are mainly contributed by tocopherols and tocotrienols. Previous studies also revealed that flavonoids possess various potent antioxidant activities, besides anti-atherosclerotic, anticancer and anti-inflammatory activities[40]. Therefore, the antioxidant activity of the extracts are also possibly due to the presence of flavonoids, supported by TFC values presented in Table 6.

4 CONCLUSION

As a summary, both of the samples are good source of food, attributed of their nutritional values. Indeed, these samples are not solely served as an alternative food source for human but also providing a solution for diminishing or mitigating the environmental impacts of POME, and possible lead to zero discharge.

---

Table 6 Total phenolic content (TPC) and Total flavonoid content (TFC) of samples.

| Samples                   | TPC of Samples in GAE (μg of gallic acid/g of sample) | TFC of Samples in RE (μg of rutin/g of sample) |
|---------------------------|------------------------------------------------------|-----------------------------------------------|
| Before pre-recovery system (X) | 3545.39 ± 14.38                                     | 698.93 ± 18.65                               |
| After pre-recovery system (Y)   | 1162.99 ± 7.17                                      | 258.09 ± 11.34                               |
| Palm Olein                  | 4893.04 ± 12.67                                     | 138.99 ± 9.96                                |

Table 7 Antioxidant activities of oil extracts.

| Samples                  | DPPH (%) | NO (%) | FIC |
|--------------------------|----------|--------|-----|
|                          | At 1 mg/mL | At 5 mg/mL | EC₅₀ (mg/mL) |
| Before pre-recovery system (X) | 3.64 ± 0.12 | 13.20 ± 0.11 | 31.09 ± 0.34 |
| After pre-recovery system (Y)   | 4.09 ± 0.34 | n.d.   | 15.15 ± 0.21 |
| Ascorbic Acid             | 100.00 ± 0.09 | –     | –   |
| Gallic Acid               | –        | 100.00 ± 0.12 | –   |
| EDTA                      | –        | –     | 3.56 ± 0.16 |

n.d. - Not detected
ACKNOWLEDGEMENTS

The authors acknowledge Taylor’s Research Grant Scheme (TRGS) - Major Grant Scheme (MFS/1/2015/SBS/001) for financial supports. We would like to acknowledge Tai Tak Palm Oil Mill for providing the samples, before and after pre-recovery system in the palm oil mill, as well as the Director General of MPOB for permission to publish these data. None of the authors had a conflict of interest.

REFERENCES

1) Gunstone, F.D. The Chemistry of Oils and Fats. Blackwell Publishing, Oxford, Chapter 8, 11 (2004).
2) Gunstone, F.D. Vegetable Oils in Food Technology: Composition, Properties, and Uses. Blackwell Publishing, Oxford, Chapter 3 (2002).
3) Packer, L.; Weber, S.U.; Rimbach, G. Molecular aspects of α-tocotrienol antioxidant action and cell signalling. J. Nutr. 131, 369S-373S (2001).
4) Chew, B.P.; Park, J.S.; Wong, M.W.; Wong, T.S. A comparison of the anticancer activities of dietary beta-carotene, canthaxanthin and astaxanthin in mice in vivo. Anticancer Res. 19, 1849-1853 (1999).
5) Loden, M.; Andersson, A.C. Effect of topically applied lipids on surfactant-irritated skin. Br. J. Dermatol. 134, 215-220 (1996).
6) Choudhury, N.; Tan, L.; Truswell, A.S. Comparison of palm olein and olive oil: effects on plasma lipids and vitamin E in young adults. Am. J. Clin. Nutr. 61, 1043-1051 (1995).
7) Sun, G.; Xia, H.; Yang, Y.; Ma, S.; Zhou, H.; Shu, G.; Wang, S.; Yang, X.; Tang, H.; Wang, F.; He, Y.; Ding, R.; Yin, H.; Wang, Y.; Yang, Y.; Zhu, H.; Yang, L. Effects of palm olein and olive oil on serum lipids in a Chinese population: a randomized, double-blind, cross-over trial. Asia Pac. J. Clin. Nutr. doi:10.6133/apcn.032017.12 (2017).
8) Sundram, K.; Hayes, K.C.; Siru, O.H. Both dietary 18:2 and C16:0 may be required to improve serum LDL/ HDL cholesterol ratio in normocholesterol men. J. Nutr. Biochem. 6, 179-187 (1995).
9) Ma, A.N.; Ong, A.S.H. Palm oil processing- new development in effluent treatment. Water Sci. Technol. 18, 35-40 (1986).
10) Bala, J.D.; Lalung, J.; Ismail, N. Biodegradation of palm oil mill effluent (POME) by bacterial. Int. J. Sci. Res. Publ. 4, 502-511 (2014).
11) Mao, X.; Hong, S.; Zhu, H.; Lin, H.; Wei, L.; Gan, F. Alternating pulse current in electrocoagulation for wastewater treatment to prevent the passivation of al electrode. J. WUHAN UNIV. TECHNOLOG. 23, 239-241 (2008).
12) Rupani, P.F.; Singh, R.P.; Ibrahim, M.H.; Esa, N. Review of current palm oil mill effluent (POME) treatment methods: vermicomposting as a sustainable practice. World Appl. Sci. J. 11, 70-81 (2010).
13) Sambanthamurthi, R.; Tan, Y.A.; Sundram, K.; Hayes, K.C.; Abeywardena, M.; Leow, S.S.; Sekaran, S.D.; Sambadan, T.G.; Rha, C.K.; Sinskey, A.J.; Subramaniam, K.; Fairus, S.; Wahid, M.B. Positive outcomes of oil palm phenolics on degenerative diseases in animal models. Br. J. Nutr. 106, 1664-1675 (2011).
14) Leow, S.S.; Sekaran, S.D.; Tan, Y.A.; Sundram, K.; Sambanthamurthi, R. Oil palm phenolics confer neuroprotective effects involving cognitive and motor functions in mice. Nutr. Neurosci. 16, 207-217 (2013).
15) Thierry, S.D.; Jacques, G. Optimization of a nitrogen analyser based on the Dumas Method. Anal. Chim. Acta 515, 191-198 (2004).
16) Folch, J.; Lees, M.; Stanley, G.H.S. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. 226, 497-509 (1957).
17) Merrill, A.L.; Watt, B.K. Energy value of foods - Basis and derivation. U.S. Department of Agriculture, Agriculture Handbook. No. 74 (1973).
18) Matthaus, B.; Ozcak, M.M. Determination of fatty acid, tocopherol, sterol contents and 1,2 and 1,3-diacylglycerols in four different virgin olive oil. J. Food Process. Technol. 2, 117 (2011).
19) Che, H.L.; Tan, D.M.Y.; Meganathan, P.; Gan, Y.L.; Razak, G.A.; Fu, J.Y. Validation of a HPLC/FLD Method for quantification of tocotrienols in human plasma. Int. J. Anal. Chem. 2015, doi:10.1155/2015/3576091-7 (2015).
20) Teh, S.S.; Voon, P.T.; Ong, A.S.H.; Choo, Y.M. Incorporation of palmitic acid or stearic acid into soybean oils using enzymatic interesterification. J. Oleo Sci. 65, 797-802 (2016).
21) Teh, S.S.; Voon, P.T.; Ng, Y.T.; Ong, S.H.; Ong, A.S.H.; Choo, Y.M. Effects of fatty acids at different positions in the triglycerides on cholesterol levels. J. Oil Palm Res. 28, 211-221 (2016).
22) Teh, S.S.; Ee, G.C.L.; Mah, S.H.; Yong, Y.K.; Lim, Y.M.; Raluman, M.; Ahmad, Z. In vitro cytotoxic, antioxidant, and antimicrobial activities of Mesua beccariana (Baill.) Kosterm., Mesua ferrea Linn., and Mesua congestiflora Extracts. BioMed Res. Int. 2013, doi:10.1155/2013/5170725 (2013).
23) Quettier, D.; Gressier, B.; Vasseur, J.; Dine, T.; Brunet, C.; Luyckx, M.; Cayin, J.; Bailleul, F.; Trotin, F. Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. J. Ethnopharmacol. 72, 35-42 (2000).
24) Tsai, P.I.; Tsai, T.H.; Yu, C.H.; Ho, S.C. Comparison of NO-scavenging and NO-suppressing activities of different herbal teas with those of green tea. Food
Value-Added Food Products from Palm Oil Mill Effluent

25) Carter, P. Spectrophotometric determination of serum iron at the submicrogram level with a new reagent (ferrozine). Anal. Biochem. 40, 450-458 (1971).

26) Atinmo, T.; Bakre, A.T. Palm fruit in traditional African food culture. Asia Pac. J. Clin. Nutr. 12, 350-354 (2003).

27) Joslyn, M.A. Methods in food analysis: Applied to Plant Products. Academic Press Inc. Publishers, New York, pp. 47-86 (1950).

28) Tolstoguzov, V.B. Functional properties of food proteins and role of protein-polysaccharide interaction. Food Hydrocoll. 4, 429-468 (1991).

29) Ogbe, R.J.; Ochalefu, D.O.; Mafuhul, S.G.; Olaniru, O.B. A review on dietary phytoestrogens: their occurrence, metabolism and health benefits. Asian J. Plant Sci. Res. 5(4), 10-21 (2015).

30) Siew, W.L. Palm Oil Developments. Malaysian Palm Oil Board, Malaysia, pp. 18-19 (1990).

31) Ciccone, M.M.; Cortese, F.; Gesualdo, M.; Carbonara, S.; Zito, A.; Ricci, G.; De Pascalis, F.; Sciechitano, P.; Riccioni, G. Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. Mediators Inflamm. 2013, 782137 (2013).

32) Mayne, S.T. Beta-carotene, carotenoids, and disease prevention in humans. FASEB J. 10, 690-701 (1996).

33) Kelly, G.S. Squalene and its potential clinical uses. Altern. Med. Rev. 4, 29-36 (1999).

34) Jacobsberg, B. Palm oil characteristics and quality. In Proceedings of the 1st Mardi Workshop on Oil Palm Technology (Chai, O.S.; Awalludin, A. eds.), Malaysian Agriculture Research and Development Institute (MARDI), Kuala Lumpur, pp. 48-68 (1974).

35) Gapor, A.M.T.; Hazrina, A.R. Squalene in oils and fats. Palm Oil Devel. 32, 36-40 (2000).

36) D’Evoli, L.; Lombardi-Boccia, G.; Lucarini, M. Influence of heat treatments on carotenoid content of cherry tomatoes. Foods 2, 352-363 (2013).

37) Gapor, A.M.T. Content of vitamin E in palm oil and its antioxidant activity. Palm Oil Devel. 12, 25-27 (1990).

38) Grimn, M.O.W.; Mett, J.; Hartmann, T. The impact of Vitamin E and other fat-soluble vitamins on Alzheimer’s Disease. Int. J. Mol. Sci. 17, 1785-1802 (2016).

39) Tan, B.K.; Oh, F.C.H. Malaysian palm oil: Chemical and physical characteristics. PORIM Technology pp. 1-5 (1981).

40) Small, D.M. The effects of glyceride structure on absorption and metabolism. Annu. Rev. Nutr. 11, 413-434 (1991).

41) Zhang, J.; Wang, P.; Wang, C.; Chen, X.S.; Ge, K. Non-hypercholesterolemic effects of a palm oil diet in Chinese adults. J. Nutr. 127, 509S-513S (1997).

42) Mattson, F.H.; Volpenhein, R.A. The digestion and absorption in triglycerides. J. Biol. Chem. 239, 2772-2777 (1964).

43) Mattson, F.H.; Nolen, G.A.; Webb, M.R. The absorbability by rats of various triglycerides of stearic and oleic acid and the effect of dietary calcium and magnesium. J. Nutr. 109, 1682-1687 (1979).

44) Sambanthamurthi, R.; Tan, Y.A.; Sundram, K. Treatment of vegetation liquors derived from oil-bearing fruit. U S Pat. 2009/0053333 A1: Malaysian Palm Oil Board (2008).

45) Ercal, N.; Gurur-Orhan, H.; Aykin-Burns, N. Toxic metals and oxidative stress part I: Mechanism involved in metal induced oxidative damage. Curr. Top. Med. Chem. 1, 529-539 (2001).

46) Aduroko, O.I. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat Res. 523-524, 9-20 (2003).

47) Eitenmiller, R.R.; Lee, J. Oxidation and the role of vitamin E as an antioxidant in foods. In Vitamin E: Food Chemistry, Composition, and Analysis (Eitenmiller, R.R.; Lee, J. eds.), Marcel Dekker Inc. New York, NY, pp. 89-135 (2004).

48) Perez-Cano, F.J.; Castell, M. Flavonoids, inflammation and immune system. Nutrients 8, 659-662 (2016).