Quantification of Polyphenolic Acids and Antioxidant Capacity of Palm Puree from Different Tenera Breeds of *Elaeis Guineensis*

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***Abstract*—In this study, two Tenera breeds were used in the preparation of Palm Puree (PP), namely T24 and T99. Each breed was formulated with two different PP formulations which made of 2% mesocarp fibre with 98% crude palm oil (labeled as A) and 5% mesocarp fiber with 95% crude palm oil (labeled as B). The four formulations were named as PP24A, PP24B, PP99A and PP99B. Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant capacity of four Palm Puree formulations were determined using spectrophotometric methods. Identification and quantification of individual polyphenolic acid compounds were performed using reversed phase high performance liquid chromatography. TPC of all samples varied from 486.33 to 778.29 mg GAE/100 g EW. The amount of TFC ranged between 30.08 and 52.01 mg CE/100 g EW. The FRAP value varied from 585.58 to 1234.06 mM TE/g EW. While for the IC50 ranged between 100.24 and 156.38 µg/ml. A strong correlation was found between the TPC and the antioxidant capacity, indicating that polyphenolic acid would be responsible in scavenging the free radicals. Syringic acid was found to be the most predominant phenolic acid in all formulations.**

***Index Terms*—Antioxidant, crude palm oil, mesocarp fibre, palm puree.**

I. Introduction

In recent years, there has been much demand on the use of natural antioxidant instead of synthetic antioxidant. Natural antioxidant known to be as safe and possesses role as protecting human body against free radicals and protect human body from any progress of chronic diseases. In fats and oils, there are abundant of naturally occurring antioxidant that present which include the water soluble antioxidant as well as lipid soluble antioxidant. Antioxidant component that contributed to the antioxidant activities in palm oil is water soluble antioxidant. Flavonoids and phenolic acids are commonly found rich in palm oil that has the capacity to function as antioxidants [1]. The fleshy mesocarp which has fiber in nature produces palm oil which is used mainly for its edible properties. Antioxidants are chemical that can delay the onset of the oxidative rancidity that can extend the shelf life of edible fats and oil and provide longer oxidative stability. This has led to many of researchers' interest on the various studies on the effect of antioxidant activities of the palm fruit and its by-product.

Palm Puree (PP) is a new food product that was developed from the combination of certain percentage of mesocarp fiber and crude palm oil. It undergoes a series of processing steps that include sterilisation, pressing and canning to form canned Palm Puree. The fleshy mesocarp which has fiber in nature produces palm oil which is used mainly for its edible properties. Reference [3] reported that one of the minor components of interest present is the high content of phenolic acid and flavonoids. Up to date, no study was conducted on the PP and hence this study aim to provide antioxidant activities information of PP using various assays such as 2,2-diphenyl-2- picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) as well as total phenolic content (TPC) and total flavonoid content (TFC) by spectrophotometric analyses. The amount of individual phenolic acid compounds in PP was also carried out using high performance liquid chromatography (HPLC) assay.

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II. Materials and Methods

A. Chemical and Apparatus

Ethanol and methanol were purchased from J.Kollin, UK. Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), ferric chloride hexahydrate ($FeCl_3.6H_2O$), starch powder was purchased from Merck, Germany. B-carotene, 2,4,6-tris-2,4,6-tripyridyl-*s*-triazine (TPTZ), sodium acetate trihydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), (+) – catechin hydrate, gallic acid and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97%) standards were purchased from Sigma Chemical Co. (St Louis, MO, USA). All reagents used were of analytical grade unless otherwise stated. Spectrophotometric analyses were performed using Perkin Elmer Lambda 35, USA.

B. Sample Preparation

Two tenera breeds (T24 and T99) of *Elaies Guineensis* Jacq. were obtained from Sime Darby Plantation Sdn. Bhd Banting. Samples were then collected and processed to make Palm Puree. Palm Puree in this research was developed based on two formulations which were combination of 2% mesocarp fiber with 98% of crude palm oil part (CPO) which labeled as 'A' and 5% mesocarp fibre with 95% CPO labeled as 'B' each name given as PP24A, PP24B, PP99A and P99B which were then packaged in cans

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and stored at room temperature until used for further analysis.

C. Sample Extraction

All PP samples were subjected to *n*-hexane extraction by using soxhlet technique for 3 days to deoil the samples. Samples were then extracted according to method by [4] with modification. A 200 mg of sample was extracted for 2 hours with solvent mixture containing 2 ml of 80% methanol containing 1% hydrochloric acid at room temperature on incubator shaker set at 200 rpm. The mixture was centrifuged at 3500 rpm for 15 minutes and the supernatant was kept in amber bottle at -20^{o}C for further analyses.

D. Determination of Total Phenolic Content (TPC)

Samples were measured colorimetrically using the Folin-Ciocalteu method [5] with modification. A 100 µl of supernatant was mixed with 0.5 ml Folin-Ciocalteu reagent. The solution was added with 7 ml of distilled water and allowed to stand at room temperature for 5 minutes. Then, 1.5 ml sodium bicarbonate solution was added to the mixture and left in dark place for 2 hours. Absorbance was read at 725 nm against blank using UV-Visible spectrophotometer. Results were expressed as gallic acid equivalents mg GAE/100 g extract weight.

E. Determination of Total Flavonoid Content (TFC)

Total flavonoid content assay of samples were determined by [6] with modification. 1 ml of sample was added to 4 ml distilled water. Then, 0.3 ml sodium nitrite ($NaNO_2$) was added and left to stand in a dark place for 5 minutes. The solution was then, added with 0.3 ml aluminium chloride ($AlCl_3$) left at dark place for 6 minutes. 2 ml sodium hydroxide was added to the solution and made to a final volume of 10 ml. Absorbance was read at 510 nm against blank using UV-Visible spectrophotometer (Perkin Elmer Lambda 35, USA). Results were expressed as catechin equivalents mg CE/100 g extract weight.

F. DPPH Free Radical Scavenging Assay

The antioxidant activity was carried out through evaluation of free radical scavenging effect on 1,1 – diphenyl-2-picrylhydrazyl (DPPH). The determination was based on the method described by [7] with some modifications. An aliquot (600µl) sample was added to 4.5 ml of 0.1 mM DPPH ethanolic solution. The mixture was then thoroughly vortex and incubated for 20 minutes in dark condition at room temperature. The absorbance was measured at 517 nm against a blank of ethanol. Results were then expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of DPPH radical was calculated according to the following equation:

$$\% \text{ inhibition of DPPH} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

where Abs control is the absorbance of DPPH without sample.

G. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out previously as describe by [8]. The mechanism of this method is based on the reduction of ferric 2, 4, 6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous form (Fe^{2+}-TPTZ) in the presence of antioxidants. The FRAP reagent contain 20 mM $FeCl_3.6H_2O$, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40mM HCl and 0.3M acetate buffer, pH 3.6. It was prepared freshly and incubated at 37 ^{0}C for 10 minutes. The FRAP reagent was mixed and aliquot of 100 µl sample was mixed with 2.9 ml of FRAP reagent. The absorbance of the reaction mixture was measured spectrophotometrically at 593 nm after incubation at room temperature for 1 hour. The results were expressed as µM of TE/g EW.

H. Quantification of Flavonoid Compounds by HPLC Analysis

Phenolic acid standards were quantified by preparing in methanol solution and serial dilutions were carried out by diluting with distilled water. Various standard concentrations were injected into HPLC system to establish standard calibration curves. Phenolic acids in PP samples were analysed by HPLC Agilent 1200 (Agilent Technologies, Baudrats, Germany) equipped with Zorbax Eclipse SB-C18 (i.d 4.6 × 250 mm, 5 µm) column. Analysis was performed according to [2] with modification. A mobile phase was prepared which consist of ratio acidified water to acetonitrile of 90:10 (v/v) containing acid, at a flow rate of 1ml/min. Detection of phenolic acids using HPLC were coupled using a diode array detector (DAD) at 295nm. All volume samples were injected at 20 µl. Quantitation in each gram of sample was carried out using external standard method. The amount of each phenolic acid was expressed as milligram per gram of extract weight (EW) of the deoiled PP (mg/g).

I. Statistical Analysis

All data were analyzed using SAS 9.0 software. Analysis of variance (ANOVA) and Duncan's multiple range method were used to compare any significant differences between samples. Values were expressed as means ± standard deviations. Differences were considered significant at $p<0.05$. All analysis were carried out in triplicates.

III. Results and Discussion

A. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Total phenolics and total flavonoids in all Palm Puree are shown in Table I. The TPC of different batches of Palm Puree ranged from 486 to 778 mg GAE/100g extract. All samples were found to have TPC significantly ($p<0.05$) different from each other with PP24A has the highest amount of TPC and the least was PP99B. Different TPC exhibited by PP samples due to different breeds used and different amount of phenolic compounds that present and released from T24 puree matrix which were higher and capable to react with Folin Ciocalteu reagent than T99 [9], [10]. PP samples from different breeds (T24 and T99) were found to have higher TPC (486.33 to 778.29 mg GAE/100 g EW) when compared to previous researcher had reported on purees from different apple varieties used which were Sampion and Idared (20 mg GAE/100 g EW and 47 mg GAE/100 g EW respectively) [10] and different frozen

acelora purees from different clones (113.0 to 229.9 mg GAE/100 g FW) [9]. Results showed that that amount of TPC in all PP formulations except PP99B were higher that that oil palm leaves (500 mg GAE /100 g EW) studied by [11]. The Folin-Ciocalteu reagent is applied in order to estimate the presence of phenolic compound in an extract [12]. The assay does not focus on specific polyphenols but also to any other substance that could be oxidised by the Folin reagent [13].The flavonoid content of different breeds of PP ranged from 30.08 to 52.01 mg CE/100 g extract weight (EW). All samples were found to have significantly ($p<0.05$) different amount of TFC from each other being PP24A the highest amount and the least amount being was PP99B with 30.08 mg CE/100 g extract. It was believed that different breeds [14]. PP samples from different breeds (T24 and T99) were also found to have higher TFC (30.08 to 52.01 mg CE/100g EW) when compared to previous researcher such as frozen purees from different acelora clones (6.91 to 14.73 mg CE/100 g FW) [9]. However lower TFC was found when compared to study on optimisation of extraction from a pink guava puree (184.46 to 344.99 mg CE/100 g FW). This could be due to in that study, flavonoid compound was found to be predominant in pink guava puree studied. PP samples were found to possess double amount of TFC from [11] had reported in their study on water soluble antioxidant in oil palm leaves (*Elaeis guineensis*) which were 5 to 25 mg CE/100 g EW). Different amount of TPC and TFC present in all PP samples could be attributed by breeds, geographic origin, growing condition and other agricultural practices [15].

TABLE I: TPC AND TFC IN PALM PUREE

Sample	TPC (mgGA/100g EW)	TFC (mg CE/100g EW)
PP24A	778.29 ±23.99a	52.01 ±2.49a
PP24B	709.97 ±8.53b	49.85 ±1.24b
PP99A	537.97 ±3.76c	34.55 ±0.31c
PP99B	486.33 ±17.36d	30.08 ±2.62d

B. *DPPH Free Radical Scavenging Assay and FRAP Value*

TABLE II: THE SCAVENGING EFFECT OF PALM PUREE SAMPLE

Sample	IC_{50} (μg/ml)	FRAP (mM TE/g EW)
BHA	100.24 ±0.01^{e}	-
PP24A	142.17 ±0.12^{d}	1234.06 ±17.50^{a}
PP24B	145.04 ±0.24^{c}	1180.14 ±27.97^{b}
PP99A	151.15 ±0.13^{b}	646.24 ±14.84^{c}
PP99B	156.38 ±0.28^{a}	585.58 ±28.54^{d}

Table II shows the amount of free radical scavenging and FRAP value. Results showed that BHA exhibited significantly ($p<0.05$) higher scavenging effect than sample PP24 samples. At 50% inhibition PP24A exhibited the strongest antioxidant activities followed by PP24B, PP99 and the weakest scavenging activities was PP99B. The significant different of scavenging activities between PP samples were attributed to their TPC and TFC. Previous study by [16] reported high scavenging activities that were found in flowering tope and fruiting tope oil of 19.8 and 29.2 μg/ml respectively. This result indicated that both oils were 10 times higher scavenging activities than what has been obtained in PP samples. This assay act is a concentration-dependent manner whereby a sample which can scavenge free radical at the lowest concentration level act as an indicator of a good antioxidant activity [17]. Based on FRAP assay results, the PP samples demonstrated significantly high antioxidant activity of FRAP ranged between 585.58 to 1234 mM Trolox Equivalent/g extract weight. PP24A shows the highest ability of reducing antioxidant power, followed by PP24B, PP99A and PP99B. Limited information on FRAP value with regard to previous studies of puree. However, FRAP value for PP samples were found lower than strawberry purees (80,600 – 95,542 mM TE/g sample) [18]. FRAP is a method to screen the ability of cells and plant tissue to be able for maintaining the redox status. It cannot act by hydrogen atom transfer for quenching radical particularly protein. Adjusting to pH 3.6 which is in acidic medium, it is able to maintain the solubility of the iron in the solution. Due to this medium, it drives the electron transfer and increases the redox potential [19]. Negative linear relationships as shown in Fig. 1 and Fig. 2 with correlation coefficient of $R^2 = 0.964$ and $R^2 = 0.957$ respectively were displayed between TPC and DPPH as well as between TFC and DPPH indicating that the lower amount radical scavenging activities present in samples the more effective it act as a free radical scavenger. Meanwhile, positive linear relationships as shown in Fig. 3 and Fig. 4 were displayed between TPC and TFC with respective FRAP value indicating that, higher the amount of phenolic and flavonoid content present in samples, higher antioxidant activities it possessed. Based from all linear relationship, it can be predicted that strong antioxidant properties may be attributed to the phenolic compounds present in the PP sample. Antioxidant activities may be evaluated with different antioxidant assays due to the fact that different antioxidant activities may show different mechanism and act in various ways such as lipid peroxidation decomposition, radical scavenging abilities and metal ions chelation [20]. Different breed of plant may also shows results in the TPC, TFC and their antioxidant activities. This was also in agreement with previous research as reported by [21] hydrogen atom transfer (HAT) and single electron transfer (SET) mechanism are known to be the reaction that occurred during antioxidant analysis. Generally, HAT acts by donating hydrogen atom from antioxidant to quench free radicals while SET act by tranferring single electron of a potential antioxidant to free radicals for reduction. Both mechanism are simple and relevant, however the presence of metal ions may lead to the erraneous reactivity and results [19]. Therefore no single assay that can accurately determined the probable multiple mechanism of antioxidant reactions in a mixed or complex system [19].

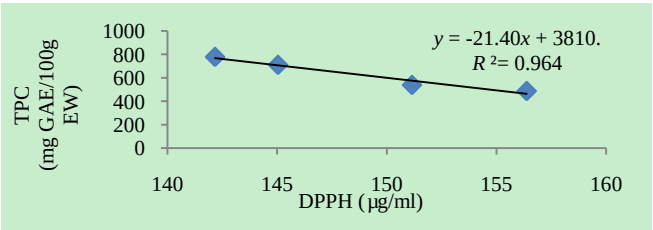

Fig. 1. Relationship between TPC and DPPH.

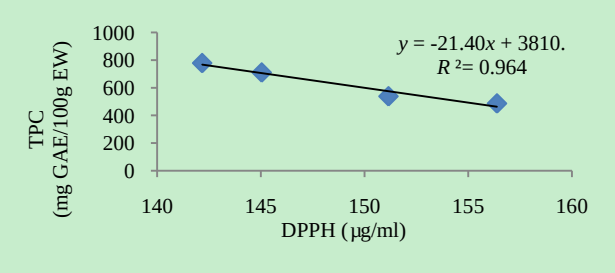

Fig. 2. Relationship between TFC and DPPH.

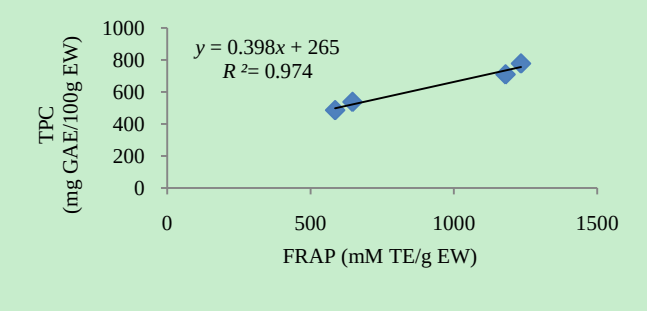

Fig. 3. Relationship between TPC and FRAP.

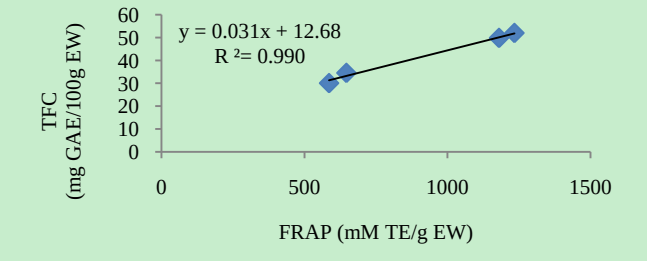

Fig. 4. Relationship between TFC and FRAP.

C. Quantification of Phenolic Acid Compounds in Palm Puree

There were seven phenolic acids identified using HPLC in PP samples namely, gallic acid (GA), hydroxybenzoic acid (HBA), caffeic acid (CA), vanillic acid (VA), syringic acid (SA), ferulic acid (FA) and *p*-coumaric acid. The amount of individual phenolic acid compounds was calculated and shown in Table III. Based on the total amount of phenolic acids in all PP samples there were four major phenolic acids were abundantly present in tenera breeds which were syringic acid (218.64 mg/g EW), vanillic acid (161.05 mg/g EW), gallic acid (58.15 mg/g EW) and *p*-hydroxybenzoic acid (56.33 mg/g EW). From the study, there were no significant different of syringic acid in PP samples that comes from the same tenera breeds of PP24A and PP24B (66.65 ± 1.82 and 65.05 ± 1.49) and PP99A and PP99B (44.93 ± 1.50 and 42.01 ± 1.60) respectively. Vanillic acid of both PP samples from different tenera breeds where PP24A and PP24B (46.91 ± 2.43 and 45.76 ± 1.13 respectively) were significantly different from PP99A and PP99B (35.40 ± 1.51 and 32.98 ± 0.70 respectively). All of the seven phenolic acids identified are known to be the hydrophilic antioxidant. The different amount of the same compound present in all samples were due to the different breed of tenera been used. Different breeds which cause the different amount of phenolic acids in samples were also supported by [22]. Results from this study were in agreement with [2] who stated that *p*- hydroxybenzoic acid is one of the phenolic compounds that abundantly present in oil palm fruit (*E. guineensis*). Other factors that contributed to different amount of phenolic acids in different breeds could be due to the environmental conditions in growing phase such as location or growing region, geographical factor, climate (temperature and humidity) and soil type [23]. In plant, phenolics are found in different location of cellular and tissue levels. Phenolics such as lignin and hydroxycinnamic acid are responsible for enhancing the mechanical strength of the cell wall, regulate the plant growth and morphogenesis. Major phenolic acids such as ferulic and and *p*-coumaric are found in the form of dimers which esterified to pectins and arabinoxylans or cross-linked to cell wall polysaccharides. Phenolic compounds are reported to be soluble by the type of polarity of the solvent used, degree of the polymerisation of phenolics present and complexes of phenolics with other food constituents [24].

TABLE III: TOTAL AMOUNT OF PHENOLIC ACIDS IN PP SAMPLES DETERMINED BY HPLC ANALYSIS

Compound	Sample (mg/g extract weight)				
	PP24A	PP24B	PP99A	PP99B	Total
Gallic acid	15.81 ± 0.55^{a}	16.45 ± 0.21^{a}	13.23 ± 0.18^{b}	12.66 ± 0.36^{b}	58.15 ± 1.30^{C}
p- Hydroxybenzoic acid	8.37 ± 0.45^{c}	7.98 ± 1.38^{c}	17.55 ± 0.49^{b}	22.43 ± 2.98^{a}	56.33 ± 5.30^{C}
Caffeic acid	4.73 ± 0.31^{a}	4.39 ± 0.33^{a}	3.92 ± 0.08^{b}	2.74 ± 0.01^{c}	15.78 ± 0.73^{D}
Vanillic acid	46.91 ± 2.43^{a}	45.76 ± 1.13^{a}	35.40 ± 1.51^{b}	32.98 ± 0.70^{c}	161.05 ± 5.77^{B}
Syringic acid	66.65 ± 1.82^{a}	65.05 ± 1.49^{a}	44.93 ± 1.50^{b}	42.01 ± 1.60^{b}	218.64 ± 6.41^{A}
p-Coumaric acid	4.26 ± 0.21^{a}	4.24 ± 0.28^{a}	3.48 ± 0.25^{b}	3.50 ± 0.02^{b}	15.48 ± 0.76^{D}
Ferulic acid	9.83 ± 2.57^{a}	6.30 ± 1.97^{a}	trace	trace	16.13 ± 4.54^{D}
Total	156.56 ± 8.54^{a}	150.17 ± 7.69^{a}	118.51 ±4.61^{b}	116.32 ± 5.67^{b}	

Note: Results are mean for three replicates ± standard deviation

^{ABCD}Means with the different capital letter within the same column are significant different ($p < 0.05$)

^{abc}Means with the different letter within the same row are significant different ($p < 0.05$)

IV. CONCLUSION

In conclusion, different breeds used in the PP formulations showed different amount of total phenolic content, total flavonoid content and antioxidant capacity. There was strong relationship between total phenolic and flavonoid content in the PP with their antioxidant capacity. Syringic acid was found to be the major phenolic acid compound found in all PP formulations. The results in this study might suggest that PP as a natural antioxidant can be use as functional food ingredient with health-promoting effects in human.

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