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Total Phenolic Content and Antioxidant Activities of Palm Puree Prepared From Various Tenera Varieties

Haswani Maisarah Mustafa, Noriham Abdullah and Zainon Md Noor
Faculty of Applied Sciences
Universiti Teknologi MARA
40450Shah Alam, Selangor Darul Ehsan, Malaysia
E-mail: dextrous_panda84@yahoo.com and noriham985@salam.uitm.edu.my

Abstract **- Four palm puree samples were used in this study namely Palm Puree 1 (PP1), Palm Puree 2 (PP2), Palm Puree 3 (PP3) and Palm Puree 4 (PP4). All samples were prepared from the combination of mesocarp fiber and crude palm oil of Tenera varieties. Total Phenolic Content (TPC) OF Palm Puree was determined by Folin-Ciaocalteu assay while antioxidant activities were determined by Ferric Reducing Antioxidant Power (FRAP), beta-carotene content and 1,1-Diphenyl-2-2picrylhydrazyl (DPPH) assays. Palm Puree 4 (PP4) showed highest TPC and FRAP values of 412.194 mg GAE/100 g extract and 282.03 x 10^3µM Trolox/g dry weight respectively as compared to other samples. Palm Puree 3 (PP3) exhibited the highest percentage of free radical scavenging (DPPH) (95.48%) and the beta-carotene content (1116.61 mg/kg) among all Palm Puree samples studied.**

Keywords- antioxidant activity; Palm Puree; mesocarp fiber; crude palm oil

I. INTRODUCTION

The pericarp of oil palm fruit comprises of three layers: the exocarp, mesocarp and endocarp [6]. Mesocarp when undergoes pressing process, it will produce crude palm oil and leaving behind the fiber part. Fiber part that contain high amount of lignin normally used as live feedstock. Palm Puree in this research was developed from the combination of certain percentage of fiber and crude palm oil part. Palm Puree is a new food product developed from the combination of certain percentage of mesocarp fiber and crude palm oil. It undergoes a series of processing steps that include sterilization, pressing and canning to form canned palm puree. Crude palm oil is considered as one of the world's richest natural plant sources of carotenoids which are responsible for the bright red-orange in color of palm fruit. The major carotenes that present in crude palm oil namely α-carotene and β-carotene that account for 36% and 54% respectively [9].

The fleshy mesocarp which has fiber in nature produces palm oil which is used mainly for its edible properties. Reference [9] reported that one of the minor components of interest present is the high content of phenolic acid and flavonoids. 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.

Reference [7] has reported on the total phenolic content (TPC) and 2,2-diphenyl-2- picrylhydrazyl (DPPH) of the phenolic extract of oil palm fruit which contain 83.97±20.08 gL^{-1} GAE per gram extract and 61.98 gL^{-1} TE per gram extract respectively. Up to date, no study was conducted on the Palm Puree and hence this study aim to provide antioxidant activities information of Palm Puree using various assays such as 2,2-diphenyl-2- picrylhydrazyl (DPPH), β-carotene content, ferric reducing antioxidant power (FRAP) as well as total phenolic content (TPC).

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), thiobarbituric acid reagent, (+) – 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). Glacial acetic acid and hexanes were purchased from J.T.Baker. Hydrochloric acid (HCl), chloroform, iso-octane were purchased from R&M chemicals. Potassium iodide (KI) and sodium thiosulphate-5-hydrate were purchased from Bendosen, UK. All reagents used were of analytical grade unless otherwise stated. Spectrophotometric analyses were performed using Perkin Elmer Lambda 35, USA.

B. Sample Preparation

Tenera varieties of palm fruits 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 from the combination of 2% fiber and 98% of crude palm oil part namely PP1, PP2, PP3 and PP4 which were then packaged in cans and stored at room temperature until used for further analysis. Samples were subjected to Soxhlet extraction using *n*-hexane until the Palm Puree was free from oil [7].

C. Determination of Total Phenolic Content

Samples were measured for total phenolic content colorimetrically using the Folin-Ciocalteu method [8] with

modification. A 200mg of sample was extracted for 2 hours with 2 ml of 80% methanol containing 1% hydrochloric acid at room temperature on incubator shaker and set at 200rpm. The mixture was centrifuged at 3500rpm for 15 minutes and the supernatant was kept in amber bottle for further analysis. A 100µl of supernatant was mixed with 0.5ml Folin-Ciocalteu reagent (diluted 10 times with distilled water). The solution was added with 7ml of distilled water and allowed to stand at room temperature for 5 minutes. Then, 1.5ml sodium bicarbonate (60mg/ml) solution was added to the mixture and left and room temperature in dark place for 2 hours. Absorbance was read at 725nm against blank using UV-Visible spectrophotometer (Perkin Elmer Lambda 35, USA). A calibration curve was prepared, using a standard solution of gallic acid (0.2, 0.4, 0.6, 0.8 and 1mg/ml). Results were expressed as gallic acid equivalents mg GAE/100g.

D. 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 [11] with some modifications. An aliquot (600µl) sample was added to 4.5ml of 0.1mM 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 517nm 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:

% inhibition of DPPH =

[(Abs control – Abs sample) / Abs control] x 100

Where Abs control is the absorbance of DPPH without sample.

E. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was carried out previously as describe by [2]. 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 20mM $FeCl_36H_2O$, 10mM 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 37^0C for 10 minutes. The FRAP reagent was mixed in the ratio of 1: 1: 10. Aliquot of 100µl sample was mixed with 2.9ml of FRAP reagent. The absorbance of the reaction mixture was measured spectrophotometrically (Perkin Elmer Lambda 35, USA) at 593nm after incubation at room temperature for 1 hour. Trolox (1000µM) was used for calibration curve and the results were expressed as µM of Trolox equivalents per mg fresh weight.

F. β-carotene Content

The amount of β-carotene content in sample was determined according to [1]. Initially, 0.1g ± 0.001g of the oil sample was weighed into a 25 ml volumetric flask. Test portion was dissolved with few milliliters of solvent and diluted to the mark. Sample was measured using 10mm path length glass cells and reading was taken at 446nm. Original solution was diluted to a measured volume and further reading was taken so that the observed absorptions are between 0.2 and 0.8 optical density. Result was expressed as β-carotene, in mg/kg.

β-carotene content was calculated according to calculation below :

383E / Ic

Where :

E - Observed difference between sample solution and solvent
I - Path length in cm
c - Concentration used for absorption measurement (in g per 100ml)

G. 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 was carried out in triplicates.

III. RESULTS AND DISCUSSION

A. Total Phenolic Content (TPC)

The total phenolic content (mg/100g) in sample was determined from equation of calibration curve ($y = 0.1855x + 0.253$, $R^2 = 0.9814$) and expressed in gallic acid equivalent. Results showed that the levels of phenolic compounds in different palm purees were significantly ($p < 0.05$) different from each other. The results in TABLE I revealed that the phenolic content was in order of PP4 > PP3 > PP2 > PP1 with values of 412.19, 267.25, 195.61 and

TABLE I. AMOUNT OF TOTAL PHENOLIC CONTENT IN PALM PUREE SAMPLES

Sample	TPC (mg GAE/100g extract)
PP1	155.30^{d}
PP2	195.61^{c}
PP3	267.25^{b}
PP4	412.19^{a}

Note: Analysis data were obtained from triplicate samples
[a-d] Means with the different letter within the column are significant different ($p < 0.05$)

155.30 mg GAE/ 100g extract, respectively. The total phenolic contents of PP4 possessed much higher total phenolic contents than other samples.

According to [3], total phenolic compound that exceeded 16.71 mg GAE/100 g samples gave high antioxidant activity. Hence, all of the Palm Puree samples which were obtained from this study gave high antioxidant activity.

B. 2,2 –diphenyl-1-picrylhydrazyl (DPPH)

Butylhydroxyanisole (BHA) is a common commercial synthetic antioxidant that has been added into food products. BHA is also use as standards for comparing antioxidant potential. Fig. 1 showed that the percentage scavenging effect of samples over BHA and Trolox as standards. All samples were capable of scavenging DPPH free radicals. The scavenging effect were as the following order: Trolox (97.22%), palm puree 3 (95.48%), palm puree 4 (95.31%), palm puree 2 (94.99%), palm puree 1 (94.68%) and BHA (94.34%) at concentration 200µg/ml (200ppm). This result showed that PP3 could be another potential radical scavenger which can be utilized as food antioxidants replacing the commercial synthetic antioxidant such as BHA.

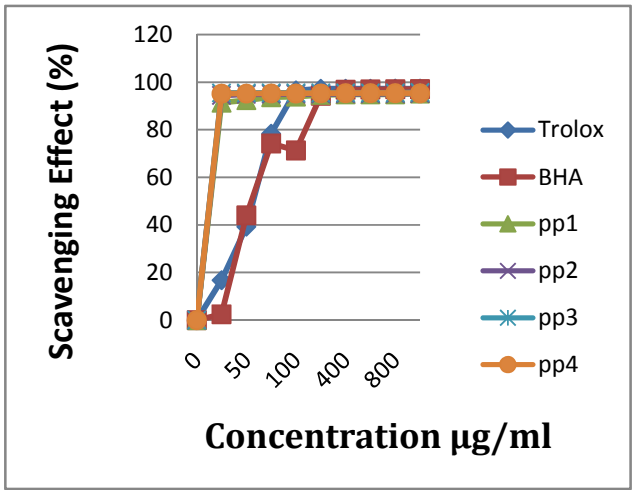

Figure 1. Percentage free radical scavenging activity of PP1, PP2, PP3, PP4, BHA and Trolox

C. Ferric Reducing Antioxidant Power (FRAP)

FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+} –TPTZ) complex and producing a colored ferrous tripyridyltriazine (Fe^{2+} –TPTZ) [2]. Generally, the reducing properties are associated with the presence of compounds, which exert their action by breaking the free radical chain through donating a hydrogen atom [4], [5]. According to [2], the reduction of Fe^{3+} –TPTZ complex to blue colored of Fe^{2+} –TPTZ occurs at low pH.

TABLE II. THE VALUE OF FRAP IN PALM PUREE SAMPLES

Sample	FRAP (µM Trolox/100g fresh weight)
PP1	12.02×10^{3d}
PP2	29.90×10^{3c}
PP3	140.87×10^{3b}
PP4	282.03×10^{3a}

Note: Analysis data were obtained from triplicate samples.
[a-d:] Means with the different within the column are significant different ($p < 0.05$)

Results from TABLE II showed that samples were identified according to descending order of PP4 > PP3 > PP2 > PP1 with values of 282.03×10^3, 140.87×10^3, 29.90×10^3 and 12.02×10^3 µM Trolox/100g fresh weight respectively. Reference [10] classified medicinal plants into their antioxidant capacity as very low FRAP (< 10 µM/100g), low FRAP (10 – 50 µM/100g), good FRAP (50 – 100 µM/100g), high FRAP (100 – 500 µM/100g) and very high FRAP(>500 µM/100g). From this experiment, we can consider that PP4 possessed much higher FRAP than PP3, PP2 and PP1.

D. β-carotene Content

β-carotene present in oil palm fruit in high amount compared to α carotene, γ carotene and other carotene profiles in palm oil. β-carotene that present in canned palm puree and expressed in mg/kg. Table III showed the results of palm purees that contain β-carotene content in descending order: PP3 > PP4 > PP2 > PP1 with values of 1116.61, 492.35, 304.21 and 286.27 mg/kg fresh weight, respectively.

TABLE III. THE VALUE OF B-CAROTENE CONTENT IN PALM PUREE SAMPLES

Sample	β-carotene content (mg/kg)
PP1	286.27^{d}
PP2	304.21^{c}
PP3	1116.61^{a}
PP4	492.35^{b}

Note: Analysis data were obtained from triplicate samples
[a-d:] Means with the different letter within the column are significant different ($p < 0.05$)

IV. CONCLUSION

This study on total phenolic content and antioxidant activities on palm puree from various Tenera varieties has beneficial input to the crude palm oil industry as well as food industry because it provides new information which can lead to the new food product development. The screening of phenolic compound and the antioxidant activities were found to be very useful tools to provide in depth the characteristic of phenolics that are present in crude palm oil. These results can initiate the food industry to develop a new food product and also stimulates the use of palm puree as a new value added food ingredients.

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