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Determination of Antioxidants in Oil Palm Leaves (*Elaeis guineensis*)

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Abstract: Problem statement: Previous findings on the occurrence of water soluble antioxidants in palm oil has brought to the question on whether these compounds is also present in other parts of the oil palm; namely its leaves. **Approach:** It is now believed that the water soluble antioxidants are also present in other biomass of the oil palm, namely, the leaves. This study reported on the determination of the water soluble antioxidants in oil palm leaves. **Results:** The results showed the analyses of the antioxidants in oil palm leaves. **Conclusion:** This study is thus conducted to trace the availability of these antioxidants in the leaves of the oil palm of the *Elaeis guineensis* variety.

Key words: Antioxidant, *Elaeis guineensis*, oil palm leaves, phenolic compound

INTRODUCTION

Palm oil consists mainly of glycerides with ca. 1% non-glyceride components, also known as the minor components (Goh *et al*., 1985; Han *et al*., 2004; 2006; Ng *et al*., 2006; 2009; Choo *et al*., 2005). The minor components found to be present in palm oil, i.e., carotenes, tocols, squalene, coenzyme Q_{10} and phospholipids, are oil soluble (Goh *et al*., 1985; Han *et al*., 2004; 2006; Ng *et al*., 2006; 2009; Choo *et al*., 2005). Studies have shown that these oil soluble minor components exhibit beneficial properties such as antioxidative and anti cancer (Goh *et al*., 1994; Hamid *et al*., 1995; Kagan and Quinn, 2001; Nesaretnam *et al*., 1992; 1995; Sundram *et al*., 2003). Besides the oil soluble components, there are many other water soluble components present in the oil palm fruits as demonstrated by Neo *et al.* (2008). Neo *et al*. (2008) shown the presence of water soluble antioxidants or phenolic compounds in the oil palm fruits whereby these compounds were extracted in groups of insoluble bound, esterified free and free phenolics.

Investigations made in the past to trace the presence of water soluble antioxidants in oil palm revealed that they are concentrated in the sludge or the Palm Oil Mill Effluent (POME) (Sambanthamurthi *et al*., 2008). The sterilization process in the oil palm mill which uses steam to terminate the activity of the enzymes in the fruits has possibly removed most of the water soluble components from the fruitlets. The oil palm milling process has rendered the water soluble antioxidants to be washed away from the oil with water and ended up in the sludge. Attempts have been made to recover these water soluble antioxidants from the sludge with great success (Sambanthamurthi *et al*., 2008). These water soluble antioxidants found applications in the cosmeceutical industry (Sambanthamurthi *et al*., 2010).

Besides the palm oil mill effluent, the water soluble antioxidants were also retained in the pressed fiber of the oil palm fruits (Nang *et al*., 2007). Attempt to recover these components from palm pressed fiber has been carried out in the past using supercritical fluid extraction (Nang *et al*., 2007). It is now believed that the water soluble antioxidants are also present in other biomass of the oil palm, namely, the leaves. This study reports on the determination of the water soluble antioxidants in oil palm leaves.

MATERIALS AND METHODS

Chemicals and apparatus: All chemicals were purchased from Merck (Darmstadt, Germany). Gallic acid, catechin, ferulic acid, rutin, 2,2-Diphenyl-2-Picrylhydrazyl (DPPH) radical 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 a Thermo Spectronic Helios α UV-Visible spectrophotometer.

Sample preparation: Oil palm leaves were obtained from the Malaysian Palm Oil Board Palm Oil Milling Technology Centre (POMTEC), in Negri Sembilan,

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Malaysia. The leaves collected were separated into two whereby one part was left to dried overnight in the oven of temperature not more than 60°C. Both the fresh and dried leaves were subjected to the same deoiling process thereafter where the leaves were soaked overnight in hexane. The solvent was then filtered and the deoiled leaves were subjected to a series of extractions to extract the phenolics.

Extraction of soluble free, esterified and insoluble-bound phenolic compounds: Krygier *et al.* (1982) method for the isolation of the phenolics was adopted with few modifications. The deoiled palm leaves were extracted six times with methanol: acetone: water (7:7:6, v/v/v) at room temperature followed by concentration of the pool extracts by evaporation. pH of the mixture was then adjusted to 2 with HCl, followed by washing with hexane to remove lipid contaminants. The SF phenolics in the mixture was extracted by using diethyl ether: Ether acetate (1:1. v/v). EF phenolics in the aqueous fraction were hydrolysed with 4M NaOH for 20 h at room temperature under nitrogen blanketing. The resulting hydrolysate was then acidified o pH2. EF phenolics were obtained by extraction using diethyl ether: Ethyl acetate (1:1, v/v). ISB phenolics were obtained from the deoiled leaves following extraction by methanol: acetone: water, hydrolyses with NaOH and extraction using diethyl ether: Ethyl acetate. The SF, EF and ISB phenolics extracted were then redissolved in MeOH for subsequent analyses.

Extraction of total phenolics: Phenolic compounds were extracted from the deoiled palm leaves according to the method described by Wang and Helliwell (2001) and Neo *et al.* (2008). The deoiled palm leaves was mixed with 40 mL 60% ethanol. Five milliliters of 6M HCl was added to the mixture and refluxed for 2 h. The cooled extract was then made up to 50 mL with 60% ethanol.

Determination of phenolics: Bonoli *et al*. (2004) method for the determination of total phenolics was adopted. The extract was diluted with methanol and UV absorbance at 280, 320 and 370 nm were recorded.

Determination of total phenolics: Total phenolic compounds in the oil palm leaves were determined using the Folin-Ciocalteau method (Singleton and Rossi, Jr., 1965). About 0.1 mL of the total phenolic extract was added with 0.5 mL Folin-Ciocalteau reagent, followed by 7 mL distilled water. The mixture was left standing at room temperature, in the dark for 5 min. Thereafter, 1.5 mL sodium carbonate solution was addied and the mixture was left at room temperature for another 2 h. UV absorbance at 765 nm was measured. Gallic acid was used as reference.

Determination of total flavonoids content: Liu *et al.* (2002) method for the determination of total flavonoids content was adopted. 0.5 mL extract was added with 2.5 mL distilled water, followed by 0.15 mL 5% $NaNO_2$ solution. The mixture was then left standing for 6 min at room temperature before adding in 0.3 mL 10% $AlCl_3.6H_2O$ solution. The mixture was left to stand for a further 5 min, added with 1mL 1M NaOH and made up to 5 mL with distilled water. The solution was vortexed and the UV absorbance at 510 nm was recorded with catechin as reference.

DPPH radical scavenging assay: DPPH radical scavenging assay was carried out according to the method by Thaipong *et al*. (2006) and Yen and Duh (1994) with slight modifications. DPPH stock was prepared by dissolving 24 mg DPPH in 100 mL methanol. The working solution contained 10mL stock solution and 45 mL methanol. 0.15 mL extract was added to 2.85 mL DPPH working solution and left to react in the dark for 24 h. The absorbance at 515 nm was measured.

RESULTS AND DISCUSSION

The results from the analyses of the antioxidants in oil palm leaves are depicted in Fig. 1-6. Figure 7 depicts the radical scavenging activity of each of the extract.

Four extracts were obtained in this study, the SFP, EFP, ISBP and Total Phenolics Extract (TPE). With the exemption of TPE, each of the extracts were subjected to different analyses to determine the total flavonol index, hydroxycinnamic acid index, o-diphenol indeces and total flavonoid content.

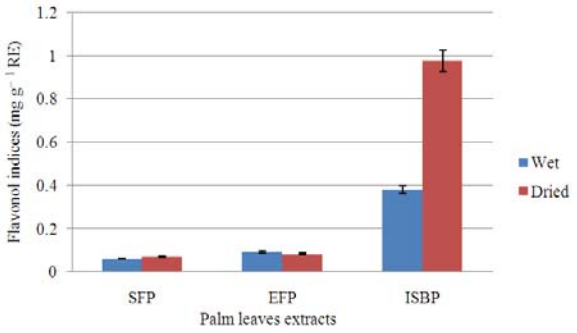

Fig. 1: Flavonol indices of palm leaves extracts

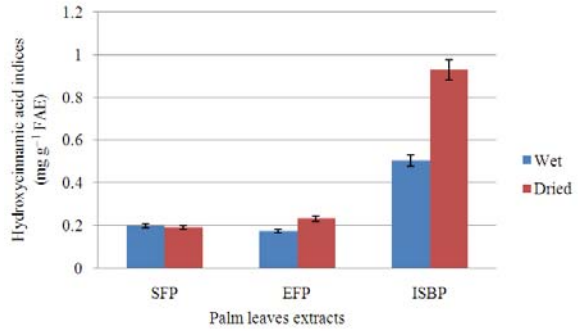

Fig. 2: Hydroxycinnamic acid indices of palm leaves extracts

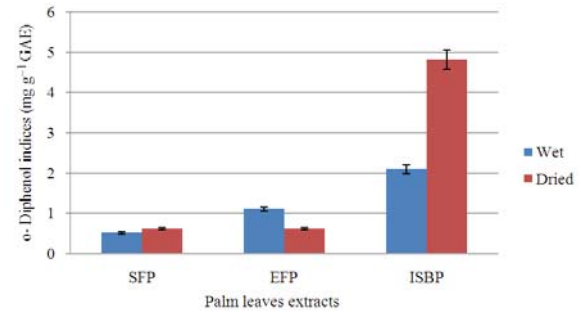

Fig. 3: o-Diphenol indices of palm leaves extracts

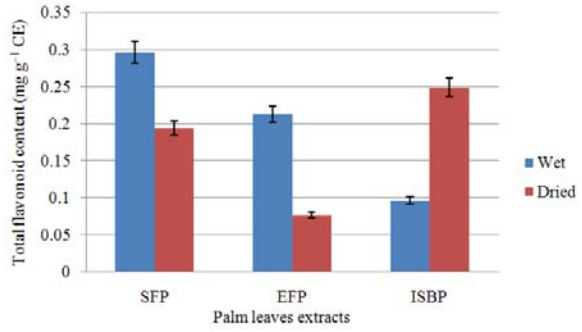

Fig. 4: Total flavonoid content of palm leaves extracts

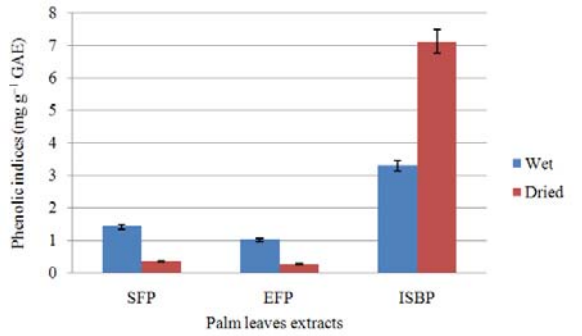

Fig. 5: Phenolic indices of palm leaves extracts

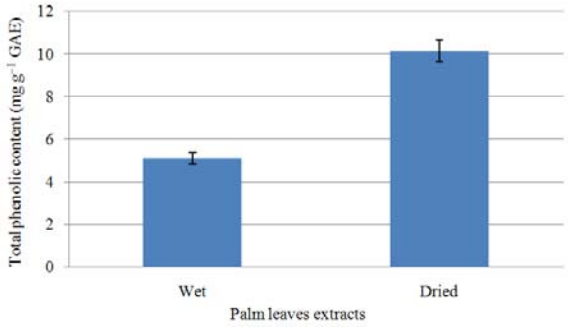

Fig. 6: Total phenolic content in palm leaves

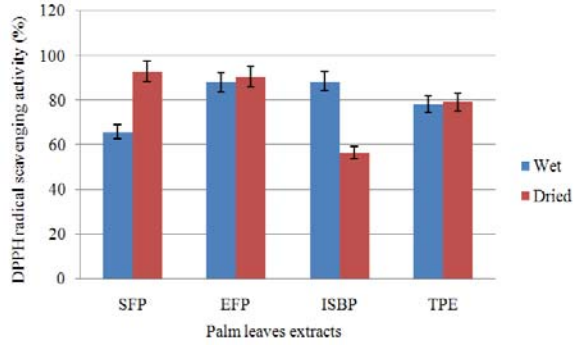

Fig. 7: DPPH radical scavenging activity of palm leaves extract

Antioxidants or phenolic structured antioxidants are detected in a number of food stuff (Dvorakova *et al*., 2008). The majority of the free phenolics are flavonols while the bound phenolics are phenolic acids. The free form of phenolic compounds is rarely present in comparison with esters, glycosides and bound complexes. Several hydrolytic procedures are used to quantify the phenolics. Bonoli *et al.* (2004) indicated that different groups of phenolics compounds can be quantified by measuring absorbance at different wavelengths.

As seen in Fig. 1, the Flavonol Indices (FI) of the ISBP extract from dried leaves is much higher than the SFP and EFP extracts of both dried and wet leaves. The FI of the wet leaves extracts ranged from 0.060-0.38 mg Rutin Equivalent (RE) per gram extract while the FI of dried leaves extracts ranged from 0.07-1.00 mg RE per g extract. The Hydroxycinnamic Acid Indices (HCAI) of the wet leaves extracts ranged from 0.17-0.50 mg Ferulic Acid Equivalents (FAE) per g extract while the

HCAI of dried leaves ranged from 0.19-0.92 g FAE per g extract. Measurements of O-Diphenol Indices (ODPI) were carried out after reacting the extracts with molybdate to form yellow solutions (Maillard *et al*., 1996). Measuring the absorbance at 370nm, the ODPI of wet leaves extracts were found ranging from 0.50-2.10 mg Gallic Acid Equivalent (GAE) per g extract. ODPI of dried leaves extracts were found to be higher at 0.62-4.80 mg GAE per g extract.

Waterhouse (2002) indicated that the Folin-Ciocalteau method provides a more accurate measurement of Total Phenolic Content (TPC) compared with the Phenolic Indices (PI) as the Folin reagent reacts equally with various groups of phenolic compounds. The Folin method is based on the reduction of the reagent where the product of reduction exhibits a blue color with maximum absorption at 765 nm (Singleton and Rossi, 1965). Thus, the TPC could be used as an indicator of the amount of total phenolic compounds present in the oil palm leaves. In the wet and dried oil palm leaves extracts, the TPC of wet and dried leaves extracts ranged from 5.1-10.2 mg GAE per g extract.

All four extracts of the oil palm leaves, SFP, ISBP, EF and TPE showed antioxidant activities when determined by the DPPH assay (Fig. 7). The antioxidative activities of the wet leaves extract ranged from 65-88% while the dried leaves extracts showed activities from 56-93%. With the exception of the ISBP extract, all other dried leaves extract showed higher antioxidative activities than the wet leaves extracts.

CONCLUSION

The oil palm leaves contains water soluble antioxidative compounds with varying concentrations. The dried leaves extracts showed higher antioxidative power than the wet leaves extracts.

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