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 Q₁₀ 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,
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 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% NaNO2 solution. The mixture was then left standing for 6 min at room temperature before adding in 0.3 mL 10% AlCl3.6H2O 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](image-url)
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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**REFERENCES**

Bonoli, M., V. Verardo, E. Marconi and M.F. Caboni, 2004. Antioxidant phenols in barley (Ordeum vulgare L.) flour: Comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. J. Agri. Food Chem., 52: 5192-5200. DOI: 10.1021/jf040075c

Choo, Y.M., M.H. Ng, A.N. Ma, C.H. Chuah and M.A. Hashim, 2005. Application of supercritical fluid chromatography in the quantitative analysis of minor components (carotenes, vitamin E, sterols and squalene) from palm oil. Lipids, 40: 429-432. PMID: 16028723

Dvorakova, M., L.F. Guido, P. Dostalek, Z. Skuliova and M.M. Moreira et al., 2008. Antioxidant properties of free, soluble esters and insoluble-bound phenolic compounds in different barley varieties and corresponding malts. J. Inst. Brew., 114: 27-33. http://cat.inist.fr/?aModele=afficheN&cpsidt=20292077

Goh, S.H., N.F. Hew, A.W. Norhanom and M. Yadar, 1994. Inhibition of tumor growth promotion by various palm-oil tocotrienols. Int. J. Cancer, 57: 529-531. PMID: 8181855

Goh, S.H., Y.M. Choo and S.H. Ong, 1985. Minor constituents of palm oil. J. Am. Oil Chem. Soc., 62: 237-240. DOI: 10.1007/BF02541384

Hamid, H.A., Y.M. Choo, S.H. Goh and H.T. Khor, 1995. The Ubiquinones of Palm Oil in Nutrition, In: Lipids, Health and Disease, Ong, A.S.H., E. Niki and L. Packer, (Eds.). AOCS Press, Champaign, IL., pp: 122-128.

Han, N.M., C.Y. May, M.A. Ngan, C.C. Hock and M.A. Hashim, 2004. Isolation of palm tocols using supercritical fluid chromatography. J. Chrom. Sci., 42: 536-539. PMID: 15768840

Han, N.M., C.Y., M.A. Ngan, C.C. Hock and M.A. Hashim, 2006. Separation of coenzyme Q10 from palm oil by supercritical fluid chromatography. Am. J. Applied Sci., 3: 1929-1932. http://www.scipub.org/fulltext/ajas/ajas371929-1932.pdf

Kagan, V.E. and P.J. Quinn, 2001. Coenzyme Q: Molecular Mechanism. In: Health and Disease, Kagan, V.E. and P.J. Quinn (Eds.). CRC Press, Boca Raton, FL., pp: 109-118.

Krygier, K., F. Sosulski and L. Hogge, 1982. Free, esterified and insoluble bound phenolic acids I. Extraction and purification procedure. J. Agric. Food Chem., 30: 330-334. DOI: 10.1021/jf00110a028

Liu, M., X.Q. Li, C. Weber, C.Y. Lee and J. Brown et al., 2002. Antioxidant and antiproliferative activities of raspberries. J. Agric. Food Chem., 50: 2926-2930. PMID: 11982421

Maillard, M.N., M.H. Soum, P. Boivin and C. Berset, 1996. Antioxidant activity of barley and malt: Relationship with phenolic content. Lebensmittel Wissenschaft und-Technol., 29: 238-244. DOI: 10.1006/fstl.1996.0035
Nang, H.L.L., C.Y. May, M.A. Nang and C.C. Hock, 2007. Extraction and identification of water soluble compounds in palm pressed fiber by SC-CO2 and GC-MS. Am. J. Environ. Sci., 3: 54-59. http://www.scipub.org/fulltext/ajes/ajes3254-59.pdf

Neo, Y.P., A. Ariffin, C.P. Tan and Y.A. Tan, 2008. Determination of oil palm fruit phenolic compounds and their antioxidant activities using spectrophotometric methods. Int. J. Food Sci. Technol., 43: 1832-1837. DOI: 10.1111/j.1365-2621.2008.01717.x

Nesaretnam, M.S.K., H.T. Khor, J. Ganesan, Y.H. Chong and M.S.K. Sundram et al., 1992. The effect of vitamin E tocotrienols from palm oil on chemically-induced mammary carcinogenesis in female rats. Nutr. Res., 12: 879-892. DOI: 10.1016/s0271-5317(05)80645-1

Nesaretnam, K., N. Guthrie, A.F. Chambers and K.K. Carroll, 1995. Effect of tocotrienols on the growth of human breast cancer cell line in culture. Lipids, 30: 1139-1143. DOI: 10.1007/BF02536615

Ng, M.H., Y.M. Choo, A.N. Ma, C.H. Chuah and M. Ali Hashim, 2006. Isolation and identification of individual palm carotenes using supercritical fluid chromatography. Malaysian J. Sci., 25: 139-145.

Ng, M.H., Y.M. Choo, A.N. Ma, C.H. Chuah and M.A. Hashim, 2009. Analyses of coenzyme Q9 and Q10 in developing palm fruits. J. Am. Oil Chem. Soc., 86: 201-205. DOI: 10.1007/s11746-009-1345-z

Sambanthamurthi, R., Y.A. Tan and K.S. Manickam, 2008. Treatment of vegetation liquors derived from oil-bearing fruits. PatentStorm. http://www.freepatentsonline.com/y2009/0053333.html

Sambanthamurthi, R., Y.A. Tan, K.S. Manickam and M. Basri Wahid, 2010. Botanical extracts from oil palm vegetation liquor for cosmeceutical application. Freepatentsonline. http://www.freepatentsonline.com/y2010/0209544.html

Singleton, V.L. and J.A. Rossi, Jr., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Ecol. Viticult., 16: 144-158. http://ajevonline.org/cgi/content/abstract/16/3/144

Sundram, K., R. Sambanthamurthi and Y.A. Tan, 2003. Palm fruit chemistry and nutrition. Asia Pac. J. Clin. Nutr., 3: 355-362. PMID: 14506001

Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D.H. Byrne, 2006. Comparison of ABTS, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extract. J. Food Composit. Anal., 19: 669-675. DOI: 10.1016/j.jfca.2006.01.003

Wang, H. and K. Helliwell, 2001. Determination of flavonols in green and black tea leaves and green tea infusion by high-performance liquid chromatography. Food Res. Int., 34: 223-227. DOI: 10.1016/S0963-9969(00)00156-3

Waterhouse, A.L., 2002. Polyphenolics: Determination of Total Phenolics. In: Current Protocols in Food Analytical Chemistry, Wrolstad, R.E (Ed.). John Wiley and Sons, Inc., New York, ISBN: 0471325651, pp: 1-4.

Yen, G.C. and P.D. Duh, 1994. Scavenging effect of methanolic extracts of peanut hulls on the free-radical and active oxygen species. J. Agric. Food Chem., 42: 629-632. DOI: 10.1021/jf00039a005