

Determination of Antioxidants in Oil Palm Empty Fruit Bunches

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ABSTRACT

The oil palm Fresh Fruit Bunches (FFB) undergoes sterilization before being threshed to separate the fruits from the bunch. Upon threshing, the fruits were pressed for its oil while the now Empty Fruit Bunch (EFB) will be discarded or used as biomass. It is believed that the EFB contains small amount of oil as well as phytonutrients which contain antioxidative property. This study reports on the extraction and analyses of various types of phenolic compounds, which have been known to exhibit antioxidant property, from the EFB. Different methods were employed in order to extract the Soluble Free (SF), Insoluble Bound (ISB) and Esterified Free (EF) phenolics in the EFB. Analyses of these phenolics were carried out spectrophotometrically. The concentrations of the SF, ISB and EF phenolics varies among the wet and dried EFB extracts. All the extracts from both wet and dried EFB exhibit radical scavenging activities.

Keywords: Palm, Fresh Fruit Bunches (FFB), Antioxidant, Phytonutrients, Empty Fruit Bunches (EFB)

1. INTRODUCTION

The oil palm fruits grow in bunches weighing in average 40-80 kg when fully ripe. These bunches are termed Fresh Fruit Bunches (FFB). When the fruits ripen, the FFB is harvested and sent to the palm oil mills for processing. At the palm oil mill, the FFB first undergo sterilization to halt the enzymatic lypolysis which otherwise, will cause rises in the free fatty acids content and reduces the quality of the oil. The sterilized FFB then goes through a threshing process to separate the fruit nuts from the Empty Bunches (EFB). The fruit nuts were then pressed for its oil while the EFB, which consists of ca. 20-25% stalk and 75-80% spikelets is discarded or used as biomass.

The oil obtained from the pressing of the fruit nuts contains vast variety of phytonutrients, i.e., carotenes, tocopherols, tocotrienols, squalene, sterols and coenzyme Q (Goh *et al.*, 1985; Ng *et al.*, 2004; 2006; 2009; Choo *et al.*, 2005). These phytonutrients have been found to exhibit beneficial properties such as anti cancer and antioxidative (Goh *et al.*, 1994; Ong *et al.*, 1995; Kagan and Quinn, 2001; Nesaretnam *et al.*, 1992; 1995). The EFB on the other hand, contains small amount of oil and it is now believed that some of the phytonutrients found in the oil are retained in the EFB. A particular group of compounds that are of interest is the phenolics.

The phenolics are water soluble and they have been found to be powerful antioxidants. They have been found to have potential applications as nutraceuticals and as cosmetics. Neo *et al.* (2008) and Sundram *et al.* (2003) reported the presence of the phenolics in the oil palm fruits in FFB. The sterilization process in the oil palm mill which uses steam has possibly caused the phenolics in FFB to be washed away with water and ended up in the Palm Oil Mill Effluent (POME) (Sambanthamurthi *et al.*, 1998). Sambanthamurthi *et al.* (1998; 2007) and co-workers reported on found in the POME and developed a method to recover them from the sludge. Besides POME, the phenolics have also been found to be retained in the fibre of the oil palm fruits nuts after screw pressing (Lau *et al.*, 2007). As such, it is not surprising if the phenolics are also found in the EFB. This study reports the extraction and analyses of various types of phenolics in EFB.

2. MATERIALS AND METHODS

2.1. Chemicals and Apparatus

All chemicals were purchased from Merck (Darmstadt, Germany). Standards of 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

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used were of analytical grade unless otherwise stated. Spectrophotometric analyses were performed using a Thermo Spectronic Helios α UV-Visible spectrophotometer.

2.2. Sample Preparation

Empty Fruit Bunches (EFB) were obtained from the Malaysian Palm Oil Board Palm Oil Milling Technology Centre (POMTEC), in Negri Sembilan, Malaysia. The EFB collected were chopped and 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 EFB were subjected to the same deoiling process thereafter where the EFB were soaked overnight in hexane. The solvent was then filtered and the deoiled EFB were subjected to a series of extractions to extract the phenolics.

2.3. Extraction of Soluble Free, Esterified and Insoluble-Bound Phenolic Compounds

Extractions of different groups of phenolics from EFB were performed by adopting the method as described by Krygier *et al.* (1982) with few modifications. The deoiled EFB 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. Lipid contaminants in the mixture was removed by hexane after adjusting the pH of the mixture to 2 by HCl. The Soluble Free (SF) phenolics in the mixture was extracted by using diethyl ether: ether acetate (1:1, v/v). Esterified Free (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 to pH2. EF phenolics were obtained by extraction using diethyl ether: ethyl acetate (1:1, v/v). The Insoluble Bound (ISB) phenolics were obtained from the deoiled EFB 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.

2.4. Extraction of Total Phenolics

Total phenolic compounds regardless of groups were extracted from the deoiled EFB according to the method described by Wang and Helliewel (2001) as well as Neo *et al.* (2008). The deoiled EFB 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.

2.5. Determination of Phenolics Indices

The different types of phenolics extracted from the deoiled EFB were analysed by using a UV

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

2.6. Determination of Total Phenolics

Total Phenolic Compounds (TPC) in the EFB were determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). 0.1 mL of the total phenolic extract was added with 0.5 mL Folin-Ciocalteu 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 added and the mixture was left at room temperature for another 2 h. UV absorbance at 765nm was measured with gallic acid as reference.

2.7. 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 1 mL 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.

2.8. 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 10 mL 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.

3. RESULTS

The results from the analyses of phenolics in EFB was carried out by several hydrolytic procedures. Total Phenolic Compounds (TPC), Flavonol Indices (FI), Hyxroxycinnamic Acid Indices (HCAI), O-Diphenol Indices (ODPI) and Total Flavonoid Compounds (TFC) are depicted in **Fig. 1-6**.

The results of the radical scavenging activities of the different samples are depicted in **Fig. 7**.

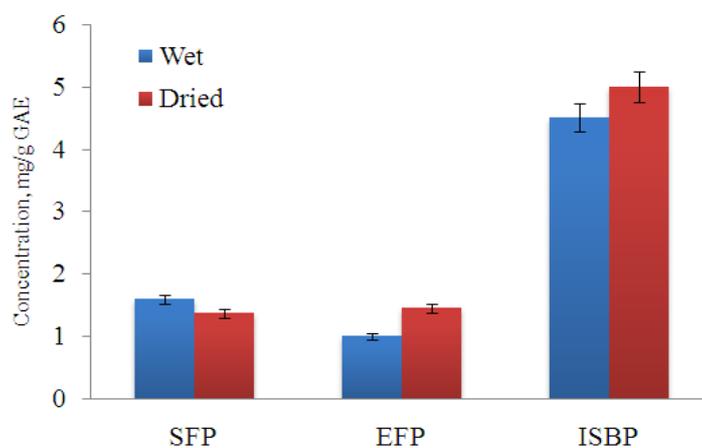


Fig. 1. Phenolic indices of empty fruit bunches extracts

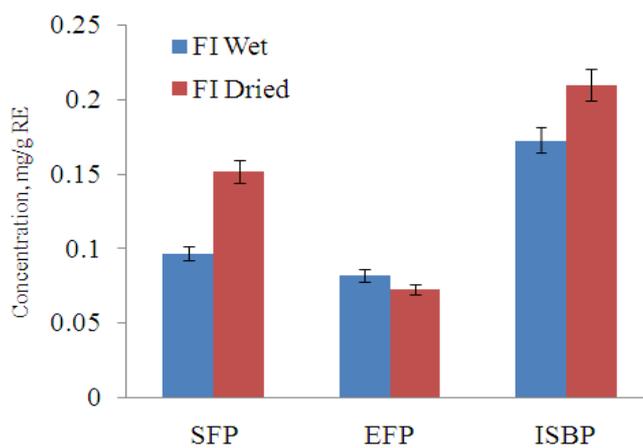


Fig. 2. Flavonol indices of empty fruit bunches extracts

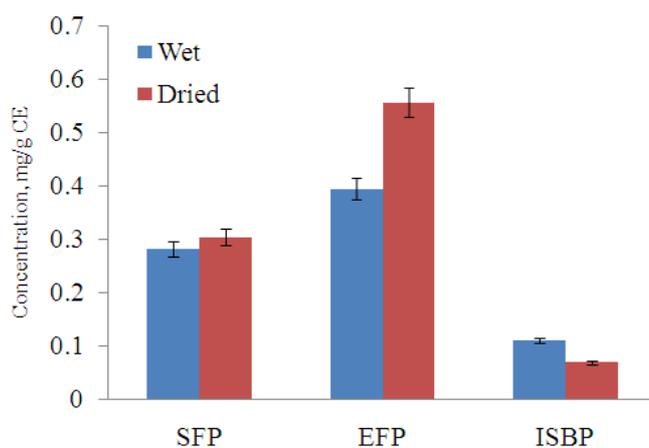


Fig. 3. Total flavonoid content of empty fruit bunches extracts

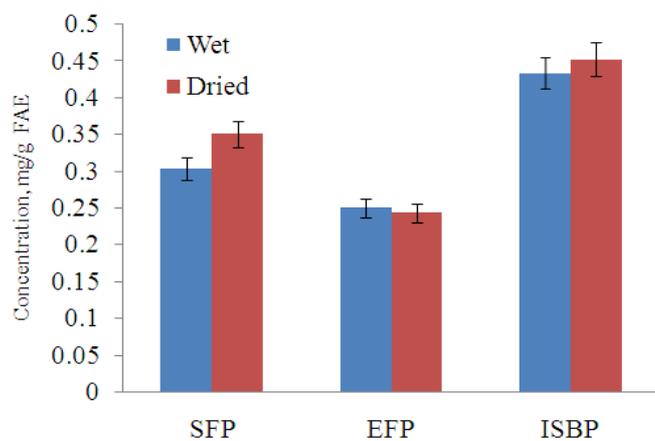


Fig. 4. Hydroxycinnamic acid indices of empty fruit bunches extracts

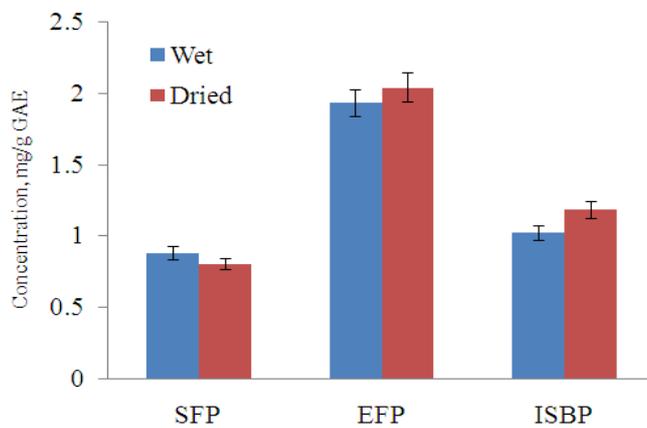


Fig. 5. o-Diphenol indices of empty fruit bunches extracts

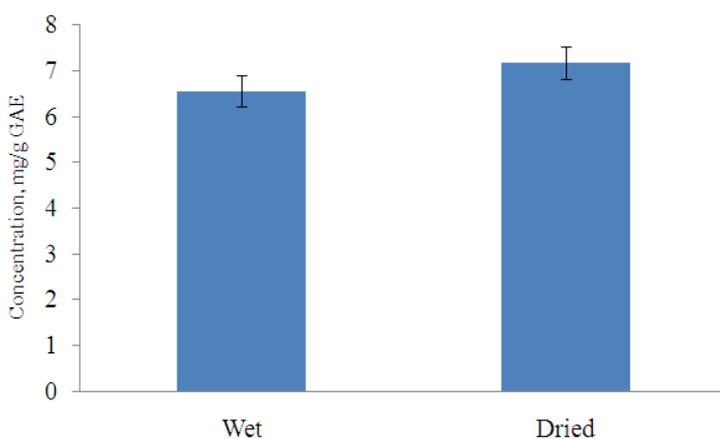


Fig. 6. Total phenolic content of empty fruit bunches extract

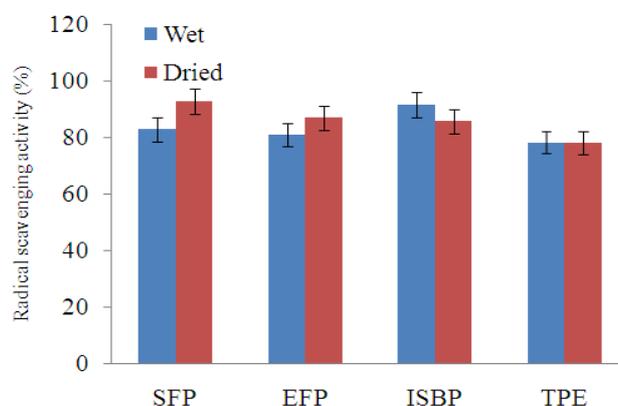


Fig. 7. DPPH radical scavenging activities of empty fruit bunches extracts

4. DISCUSSION

Quantification of phenolics in EFB was carried out by several hydrolytic procedures. Total Phenolic Compounds (TPC), Flavonol Indices (FI), Hydroxycinnamic Acid Indices (HCAI), O-Diphenol Indices (ODPI) and Total Flavonoid Compounds (TFC) were quantified by measuring absorbance at different wavelengths. Each group of phenolic compounds was quantified with reference to a certain standard of the same group. TPC was quantified as Gallic Acid Equivalents (GAE), FI as Rutin Equivalent (RE), HCAI as Ferulic Acid Equivalents (FAE), ODPI as Gallic Acid Equivalents (GAE) and TFC as Catechin Equivalent (CE).

Each of the extracts (SF, EFP, ISBP) with the exception of TPE was subjected to different analyses to determine the type of phenolic compounds present. Each type of phenolics does not respond to the spectral analysis in the similar way. In this respect, ODPIs may provide more accurate analyses as it is carried out based on the reaction of a specific phenolic structure. **Figure 1-6** depict the results from these analyses.

Generally, the dried EFB extracts showed higher concentrations of TPC. TPC is an indication of the amount of phenolics present regardless of their respective groups. The concentration of specific groups of the phenolics in EFB varies between the SFP, EFP and ISBP extracts.

The phenolic indices is higher in dried EFB compared to the wet ones in the EFP and ISBP extracts. However, different scenario is seen for the SFP extract. The Total Phenolic Extract (TPE) is considered to represent the Free and Bound (FB) phenolics present in EFB as it is extracted by reflux with acid. In this respect, the FB is the dominant form of phenolics in EFB, followed by ISBP, EFP and SFP. Although the TPE is able to give a general idea on the concentration of

phenolics, the SFP, ISBP and EFP nevertheless, provided a more complete picture of the phenolic content in EFB. The flavonol indices of the SFP extract, amounting from 0.1-0.15 mg g⁻¹ RAE showed that the flavonoid in SFP consists mainly of flavonols. The EFP and ISBP extracts however, do not show similar pattern.

All the extracts of both wet and dried EFB showed radical scavenging activity. The total phenolic extract showed similar radical scavenging activity between the wet and dried EFB. Similar scenario was detected for the EFP and SFP extracts. However, the opposite is true for the ISBP extract whereby the wet EFB showed higher radical scavenging activity than the dried EFB. The ISBP extracts for both the wet and dried EFB showed higher radical scavenging activity than the other extracts of the EFB. In general, the DPPH radical scavenging activities for the dried and wet EFB extracts followed the order of SFP > EP > ISBP > TPE and ISBP > SFP > EFP > TPE respectively.

5. CONCLUSION

The EFB contains various types of phenolic compounds which exhibit radical scavenging activities. Different methods need to be employed to extract the phenolics from the EFB and UV spectrophotometry is able to quantify these phenolics.

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