Phenolic Compounds and Antioxidant Potential of Nigerian Red Palm Oil (*Elaeis Guineensis*)

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Abstract

Polyphenols have been shown in both in vitro and in vivo experiments to induce responses that are consistent with the protective effects of diets rich in fruits and vegetables against degenerative conditions such as cardiovascular disorders and cancer. Red palm oil, extracted from the fruit mesocarp of *Elaeis guineensis* is very important in the diet of many Nigerians, and indeed many citizens of developing countries living around the tropics. It contains antioxidant components such as carotenoids (alpha, beta, and gamma carotenes) and vitamin E (tocophorols and tocotrienols), which are of known nutritional and health benefits, but the antioxidant polyphenol contents of this oil is not fully explored. To assess the total antioxidant potential due partly to the scavenging of reactive oxygen specie (ROS) and the inhibition of the enzyme, xanthine oxidase, the hypoxanthine/xanthine oxidase assay system and the 2-deoxyguanosine-assay model were utilized. These revealed that the oil possesses promising antioxidant and radical scavenging activities with IC50 values of 95 µM and 219 μ M for the hypoxanthine/xanthine oxidase and 2-deoxyguanosine assays, respectively. Liquid-Chromatography with Electrospray – Ionization Mass Spectrometry (LC-ESI) showed the presence of the antioxidant, 3,4 hydroxybenzyaldehyde, p-hydroxybenzoic acid, vanillic acid, syringic acid and ferulic acid in the red palm oil. Thus, in addition to antioxidant compounds like carotenoids, and vitamin E, the red palm oil also contains a rich mixture of phenolic compounds with potent antioxidant and radical scavenging capacities that might also account significantly for its widely reported capacity to modulate stress-related disorders.

Keywords: Palm oil, Elaeis guineensis, Antioxidant activity, Polyphenol

1. Introduction

Polyphenols have been shown in both *in vitro* and *in vivo* experiments to induce responses consistent with the protective effects of diets rich in fruits and vegetables against degenerative conditions such as cardiovascular

diseases and cancer (F'guyer *et al.*, 2003; Gupta and Mukhtar 2002; Linden *et al.*, 2003; Stoner and Mukhtar, 1995; Yang *et al.*, 2001; Nishino *et al.*, 2000; Park and Pezzuto, 2002). For this reason, many foodstuffs, including oils from the low-incidence Mediterranean region have been assessed for polyphenol content as well as antioxidant potential (Owen *et al.*, 2003; Owen *et al.*, 2000a; Owen *et al.*, 2000b). Oil that is very important in the diet of Nigerians and indeed many citizens of developing countries living around the tropics is the oil of the fruit mesopearp of *Elaeis guineensis*, popularly known as the red palm oil.

The red palm oil is extracted from the mesocarp of ripened fruit of oil palm tree, *Elaeis guineensis*. The oil palm tree is native to many West African countries, including, Nigeria, where the oil used for culinary and other purposes, the sap as a popular wine, called palm wine; the trunk for building, and the leaf stalks as broom for sweeping; and branch stalk for making baskets and roof rafters. However, large scale plantations and traditionally owned wild plantations, established principally in tropical regions of Africa, Asia and Latin America, are mostly aimed at the production of red oil (Wattanapenpaiboon and Wahlqvist, 2003; Edem, 2002) which is widely recognized the most popular vegetable cooking oil in tropical west Africa (Edem, 2002; Elson, 1992; Sundram *et al.*, 2003).

Red palm oil is different from other plant and animal oils in that it contains 50% saturated fatty acids, 40% unsaturated fatty acids, and 10% polyunsaturated fatty acids. The fruit also contains components such as carotenoids (alpha, beta, and gamma carotenes), vitamin E (tocophoreols and tocotrienols), sterols (sitosterol, stigmasterol and campesterol), phospholipids, glycolipids and squalene, which are of known nutritional and health benefits (Wattanapenpaiboon and Wahlqvist, 2003; Manorama and Rukmini (1991). In addition, it has been reported that certain powerful water-soluble antioxidants, phenolic acids and flavonoids can be recovered from the palm oil mill effluents (Abeywardena *et al.*, 2002; Balasundram *et al.*, 2003; Yun *et al.*, 2008).

Owing to its high constituents of phytonutrients with antioxidant properties, the possibility exists that palm fruits and palm oil offers some health advantages by reducing lipid oxidation, oxidative stress and free radical-induced damage, which have been implicated in the etiology of several diseases, including cancer (Stoner and Mukhtar, 1995; Yang *et al.*, 2001; Nishino *et al.*, 2000) cardiovascular diseases gastrointestinal disorders diabetes neuro-degenerative disorders, Surh and Ferguson, 2003; Youdim and Joseph, 2000; Kinghorn et al 2004; Repetto and Llesuy, 2002), etc.

Tocotrienol-rich fraction of palm oil has been reported to activate p53, modulate Bax/BCl2 ratio and induce apoptosis independent of cell cycle association, protects against aspirin-induced gastric lesions, suppress pre-neoplastic mammary epithelial cell proliferation, inhibit tumour promotion and protects cellular membrane against oxidative damage (Manorama *et al.*, 1993; Balasundram *et al.*, 2005; Ebong *et al.*, 1999; Ong and Goh, 2002). The carotenoid fraction on the other hand, has been reported to chemoprevent and inhibits diesel exhaust-induced lung tumorigenesis, modulate the immune system by increasing peripheral blood natural killer (NK) cells and B-lymphocytes, and also suppress the growth of MCF-/ human breast cancer cells. Therefore, a great deal of studies on the antioxidant potential of red palm oil appears to have focussed on the tocopherol and carotenoids (Manorama *et al.*, 1993; Balasundram *et al.*, 2005; Ebong *et al.*, 1999; Ong and Goh, 2002).

However, in the last decade, Abeywardena and co-workers (2002) reported that polyphenol-enriched extract of oil palm fronts promotes vascular relaxation via endothelium-independent mechanisms, lending further support to the potential cardiovascular actions of plant polyphenols, while Balasundram and colleagues (2003) identified sugars, gallic acids and 4-hydroxybenzoate with antiradical power from the phenolic-rich aqueous by-product fraction of oil plam milling. Beside these, reports on the phenolic content and antioxidant potential of phenolic-rich fraction of the red palm oil itself, which is prepared differently in various countries and civilizations, are hard to find. Hence, we examined the phenol-rich fraction of red palm oil, prepared by the Igala-style double extraction, for the polyphenol constituents, as well as its antioxidant and radical scavenging capacities.

2. Materials and Methods

2.1 Samples

An Igala-type red palm oil prepared by double extraction method to maximally limit levels of water - soluble impurities and moisture, was acquired from Agaliga-Imani village in Olamaboro Local Government Area of Kogi State, central Nigeria. The Palm oil was stored in glass bottle and transported to the laboratory of analysis in Heidelberg, Germany.

2.2 Reagents

Acetic acid, EDTA, hypoxanthine, methanol, xanthine, and xanthine oxidase were obtained from Merck (Darmstadt, Germany). K₂HPO₄ and KH₂PO₄ were obtained from Serva (Heidelberg, Germany). Formic acid,

salicylic acid, and $FeCl_3 \cdot 6H_2O$ were obtained from Aldrich Chemie (Steinheim, Germany). *N*-Methyl-*N*-(trimethylsilyl) trifluoro-acetamide (BSTFA) was obtained from Fluka (Buchs, Switzerland). Tetrabutylammonium hydroxide was obtained from Sigma Chemie (Deisenhofen, Germany). Standard phenolic compounds were obtained from laboratory stock, acquired from commercial sources, or isolated, purified, and characterized from natural sources. All solutions were made in double-distilled water.

2.3 Extraction of phenolic compounds

The phenolic compounds in the oil were extracted from the oil, basically as described by Owen and co-workers (Owen *et al.*, 2003; Owen *et al.*, 2000a; Owen *et al.*, 2000b), but with minor modification. The oil (10g) was vortexed for 2 min at maximum speed in 50 ml polyphylene bottles with 2 ml methanol for 3 times. The mixture was centrifuged at 4000 rpm for 30 min, and methanol layer collected into graduated 20 ml glass test tubes. Pooled methanol fractions were dried under nitrogen, taken up in 1 ml acetonitrile, and lipid contaminants removed by vortexing with 3 X 3 ml hexane. The mixture was centrifuged at 3000 rpm for 15 min each, and the hexane layer discarded. The acetonitrile layer was made up to 2.5 ml, and used for subsequent analysis (Fig.1).

2.4 Analytical high-performance liquid chromatography (HPLC)

Analytical HPLC was conducted on a Hewlett-Packard (Palo Alto, CA, USA) model 10980 liquid chromatograph fitted with a C-18 reverse-phase (5- μ m) column (25 cm × 4 mm i.d.) (Latex, Eppelheim, Germany). For separation of individual compounds in the extract, 2% acetic acid in water (solvent A) and methanol (solvent B) were used as mobile phase when 20 μ L of the extract was injected.

The solvent gradient consisted of 95% A for 2 minutes, 75% A in 8 minute, 60% A in 10 minute, 50% A in 10 minutes, and 0% A until completion of the run at 45 minutes The flow rate of the mobile phase was maintained at 1 mL/minute, and phenolic compounds in the eluate were detected with an ultraviolet dual-array detector (HP1040M, Hewlett-Packard) set at 278 and 340 nm. Instrument control and data handling were by means of an HP Chemstation (Hewlett-Packard) operating in the Microsoft (Redmond, WA, USA) Windows software environment. The amount of phenolic compounds in the extracts was estimated by the external standard method (Owen *et al.*, 2003; Owen *et al.*, 2000a; Owen *et al.*, 2000b).

2.5 *Hypoxanthine/xanthine oxidase assay*

To assess the total antioxidant potential due partly to the scavenging of reactive oxygen species and the inhibition of the enzyme xanthine oxidase, the hypoxanthine/xanthine oxidase assay system was used. In this assay, the extent of diphenol (2,5-dihydroxybenzoic acid and 2,3-dihydroxybenzoic acid) produced by hydroxyl radical (HO) attack on salicylic acid was measured from standard curves of their respective diphenols (Owen *et al.* 2000a; Owen *et al.* 2000b). The assay involves the re-suspension of different extract residues (prepared in duplicates by drying 0–500 μ L of extract solution) in 1 mL of phosphate buffer (pH 6.6). After addition of 5 μ L of xanthine oxidase (20 μ U/1.09 mL), the tubes were incubated at 37°C for 3 h, after which the reaction was stopped by addition of 5 μ L of concentrated HCl. Where necessary, the reaction mixture was centrifuged at 10,000 rpm for 2 minutes in a Ficol Biofuge (Heraeus Instruments, Hanau, Germany), and 20 μ L of the mixture was analyzed by HPLC using the mobile phase and gradient condition noted above. The hydroxylation of hypoxanthine was monitored at 278 nm, whereas the hydroxylation of salicylic acid was monitored at an absorbance of 325 nm. The end products of the enzyme or free radical reaction were quantified against standard curves measured at the same wavelength.

2.6 Deoxyguanosine assay for radical scavenging potential

To evaluate the radical scavenging capacity of the extract, the 2-deoxyguanosine assay model was adopted. The buffer system is similar to that of the hypoxanthine/xanthine oxidase system, except that salicylic is replaced with 2-deoxyguanosine (2 mM). The generation of reactive oxygen species was initiated by addition of ascorbic acid (500 μ M). Dried residues (of 0–500 μ M of extract solution prepared in duplicates) were re-suspended in buffer and incubated at 37°C for 24 h. The assay of the 8-oxo-2-deoxyguanosine resulting from the reactive oxygen species attack on 2-deoxyguanosine was analyzed using an isocratic system consisting of 5% methanol and 95% aqueous buffer (5 m*M* tetra-butylammonium hydroxide, adjusted to pH 4.3 with 6% formic acid). The ultraviolet detector was set at an absorbance of 293 nm (Owen *et al.*, 2003; Owen *et al.*, 2000a; Owen *et al.*, 2000b).

2.7 Determination of 50% inhibition of oxidation (IC_{50})

The amount of extracts producing the IC_{50} using the hypoxanthine/xanthine oxidase model system as well as the 2-deoxyguanosine assay methods was determined using the Table curve program (Jandel Scientific, Chicago, IL, USA).

2.8 Gas chromatography-mass spectrometry

Analyses were performed on a HP 5973 mass spectrometer coupled to a HP 6890 gas chromatograph. Prior to gas chromatography-mass spectrometry analysis, dried methanolic extracts (1 μ M) were derivatized by addition of BSTFA (100 μ M) at 37°C for 30 min. Separation of the analytes was achieved using an HP 5MS capillary column (30 m × 0.25 mm i.d., 0.25 μ m film thickness). Helium was used as the carrier gas with a linear velocity of 0.9 mL/second. The oven temperature program was as follows: initial temperature, 100°C; 100–270°C at 4°C/min; and maintenance at 270°C for 20 min. The gas chromatograph injector temperature was maintained at 250°C; the transfer line temperature was held at 280°C. The mass spectrometer parameters for electrospray ionization mode were as follows: ion source temperature, 230°C; electron energy, 70 eV; filament current, 34.6 μ A; electron multiplier voltage, 1,200 V (Owen *et al.* 2003; Owen *et al.* 2000a; Owen *et al.* 2000b).

2.9 Liquid chromatography-electrospray ionization mass spectrometry

Liquid chromatography-electrospray ionization was conducted on an Agilent (Palo Alto) 1100 HPLC apparatus coupled to an Agilent LC/MSD apparatus (HP1101). Chromatographic separation was conducted using a C-18 reverse-phase (particle size, 5 μ m) column (25 cm × 2mm i.d.; Latex) using the same mobile phase and gradient as described for analytical HPLC, except that the flow rate was maintained at 0.5 mL/min. The analyses were conducted in the negative ion mode under the following conditions: dry gas (nitrogen) flow rate, 10 L/min; nebulizer pressure = 30 psi, drying gas temperature = 350°C; capillary voltage = 2,500 V; fragmenter voltage = 100 V; mass range = 50–3,000 Da.2 (Owen *et al.* 2003; Owen *et al.* 2000a; Owen *et al.* 2000b).

3. Results

3.1 Polyphenol contents and antioxidant activity

HPLC analysis revealed the presence of 3, 4-hydroxybenzyaldehyde, p-hydroxybenzoic acid, vanillic acid, syringic acid and ferulic acid in the red palm oil. Results of assay for antioxidant potential revealed that the oil possesses promising antioxidant and radical scavenging activities with IC50 values of 95 μ M and 219 μ M for the hypoxanthine/xanthine oxidase and 2-deoxyguanosine assays, respectively (Fig.2).

4. Discussions and Conclusions

The nutritional and health benefits of red palm oil have been a subject of intense research, especially because of its relevance to disease prevention in developing countries of the tropics. However, whereas, previous studies have concentrated on the antioxidant potential of the carotenoids and tocotrienols components of the oil (Wattanapenpaiboon and Wahlqvist, 2003; Edem, 2002; Elson,1992; Sundram *et al.*, 2003; Ebong *et al.*, 1999; Ong and Goh, 2002) the current results have demonstrated unequivocally, for the first time, that phenolic compounds with antioxidant properties, particularly, 3,4-hydroxybenzyaldehyde, p-hydroxybenzoic acid, vanillic acid, syringic acid and ferulic acid are also present in red palm oil, at least the Igala-style double extraction type obtained from Nigeria (Fig.2).

The presence of these phenolic compounds in palm oil is of major significance in disease prevention in the tropics, where this oil is widely consumed, since these compounds are potent antioxidants as reflected by the IC50 values of the oil when tested with the hypoxanthine/ xanthine and the 2-deoxyguanosine assay models (Fig.2). These phenolic acids are synthesized by a common pathway from phenylalanine involving polyketide pathway condensation reactions, and are credited for the health effects of several foodstuffs. This is not surprising, since individually the phenolic acids have been demonstrated to possess significant antioxidant properties. Owen and colleagues (2003) have demonstrated that p-hydroxybenzoic acid and ferulic acids are potent antioxidants with IC50 values of 1.69 μ M and 1.56 μ M respectively, while IC50 values for syringic acid and vanillic acids were 3.19 μ M and 2.7 μ M respectively.

p-Hydroxybenzoic acid and 3,4-dihydroxybenzaldehyde are important antioxidants in foods. With the exception of certain red fruits, black radish and onions, the hydroxybenzoic acid content of edible plants is generally low, but they are component of complex structures such as hydrolysable tannins (gallotannins in mangoes, and ellagitannins in strawberries and raspberries, respectively (Hannum, 2004; Chun *et al* 2003; Bravo 1998; Manach *et al*. 2004) Raspberries ellagitannins have been shown to inhibit 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase, TPA-stimulated hydroperoxide production and TPA-stimulated DNA synthesis, and inhibited formation of the promutagenic adduct O⁶-methlyguanine by 73% and 80% respectively, after a single dose of N-nitrosomethylbenzyl-amine at 0.25 mg/kg (Park and Pezzuto, 2002). Despite this, it has not received attention with respect to its nutritional benefit until recent decades.

Other phenolic acid detected in palm oil are syringic acid and vanillic acid. These phenolic acids have been found to act synergistically with vitamin C to enhance human and hamster LDL resistance to oxidation (Chen et

al., 2004) and indeed, inhibit the activities of two forms of phenolsulfotransferase enzymes involved in carcinogen sulfate conjugation (Yeh *et al.*, 2004). They contribute to the antioxidant and anti-inflammatory properties of wine, cereals and other foodstuffs (Bravo, 1998; Lopez *et al.*, 2003 Ross and Kasum, 2002; Hollman, 1996; Middleton, 2000). Other workers have demonstrated their ability to protect cultured skin cells from oxidative damage, inhibition of pancreatic lipase, protect against LDL oxidation, and possess antiproliferative and apoptotic effects on human breast cancer cells (Youdim and Joseph 2000; Kinghorn *et al.*, 2004; Repetto and Llesuy *et al.*, 2002).

According to Manach and colleagues (2004), ferulic acid, another phenolic found in palm oil, is also found most abundant in outer parts of cereal grains, like wheat (0.8-2g/kg dry weight), where it is associated with the health benefit of barley, wheat and oat bran and rice bran (Ross and Kasum, 2002; Hollman *et al.*, 1996; Middleton *et al.*, 2000). An evaluation of ferulic acid and twenty three related compounds showed that ferulic acid possess stronger antioxidant activity than any of these related compounds. Other studies demonstrate that ferulic acids alleviates lipid peroxidation in diabetic rats, synergistically interact with other antioxidants to preserve physiological integrity of cells exposed to free radicals (Mathew and Abraham, 2004; Graf, 1992; Anselmi, 2004) and attenuates the processes that drive the pathology associated with Alzheimer's disease if the treatment is initiated before the neuroinflammatory processes can develop. In addition, ferulic acid protects against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems, as well as strongly inhibits arylamine N-acetyltransferase activities in human gastrointestinal microflora (Mathew and Abraham 2004; Graf, 1992; Anselmi, 2004)

In conclusion, palm oil contains a rich mixture of phenolic compounds with potent antioxidant and radical scavenging activity to suggest that the widely reported capacity of palm oil to modulate the effect of oxidative stress on serum and antioxidant enzymes in membranes of liver and kidney, oxidative stress hypertension, suppress pre-neoplastic mammary epithelial cell proliferation, inhibit diesel-exhaust induced lung tumorigenesis, inhibit tumour promotion as well as modulate the immune system (Wattanapenpaiboon and Wahlqvist, 2003; Edem, 2002; Elson,1992; Sundram *et al.*, 2003; Abeywardena *et al.*, 2002; Balasundram *et al.*, 2003; Yun *et al.*, 2008) cannot be ascribed to their carotenoids and tocopherol components alone, but also to their antioxidant polyphenol contents.

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Figure 1. Analytical scheme for extraction, identification and in vitro antioxidant activity of Elaeis guineensis oil



Figure 2. *In vitro* evaluation of the antioxidant and radical scavenging potential of methanol extract of *Elaeis* guineensis using the xanthine oxidase and 2-deoxyguanosine assay models