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Quality evaluation of low free fatty acid and high free fatty acid crude palm oil and variation of total fatty matter and fatty acid composition in Nigerian palm oil

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Quality of palm oil determines its consumer and market acceptability and price. This correlates directly to its moisture (%), free fatty acid (% FFA) and total fatty matter (%TFM) content. The aim of the present study was to evaluate the quality of low free fatty acid (LFFA) and high free fatty acid (HFFA) crude palm oil (CPO) samples aged 5 to 10 days, purchased from four locations in Southern Nigeria. HFFA CPO is produced using traditional methods while LFFA CPO is produced by modern milling methods. The oil samples were analyzed for quality and fatty acid using standard analytical methods. The results obtained showed that FFA and total contaminants were significantly (p<0.05) lower in LFFA and higher in HFFA CPO samples. The values of FFA were significantly (P<0.05) higher in HFFA (9.25±0.70-12.76±1.20%) when compared to LFFA CPO values (2.44±0.30-2.95±0.08%). No significant (p>05) difference was observed in the mean saponification value of LFFA (198.95±0.80 mg KOH/g oil) and HFFA CPO (198.62±0.40 mg KOH/g oil). TFM for LFFA CPO ranged between 91.94±0.40 - 92.45±0.75% suggesting no significant (p>0.05) variability in TFM values for LFFA CPO. TFM values for HFFA CPO were significantly (P<0.05) lower and varied between 81.06±0.64 and 85.16±1.05%. The palmitic acid in HFFA CPO was 44.670±0.85 and 45.641±1.77% in LFFA CPO. Oleic acid content was 37.370±0.92% in HFFA oil and 39.005±1.06% in LFFA oil. It was concluded that CPO is rich in SFAs, MUFAs and PUFAs. The ratio of TSFAs to TUFAs for both LFFA and HFFA CPO is 1:1.

Key words: Quality, crude palm oil, free fatty acid, total fatty matter, fatty acids, gas chromatography-mass spectrometry (GC-MS).

INTRODUCTION

The increasing demand for cooking oil and bio-fuels has made crude palm oil (CPO) the dominant globally traded vegetable oil (Baterman et al., 2010). In Nigeria, CPO is extracted from the fruit pulp of palm fruits (*Elaeis guineensis)* using either traditional or modern milling methods. The plantation varieties of oil palms are different from the wild varieties grown by traditional farmers in Nigeria. And the CPO extracted by modern

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methods and improved technologies (LFFA CPO) is different in quality from the traditional method of extraction based on cultural practices (HFFA CPO). The fresh fruit bunch (FFB) of the wild varieties is usually larger in size than the plantation varieties. In Nigeria, fruits from the wild varieties contain bigger kernel and less pulp (mesocarp) compared to the plantation variety with relatively smaller kernel and fleshy pulp. The composition of CPO includes triacylglycerols (TAGs), diacylglycerols (DAGs) and monoacylglycerols (MAGs) and free fatty acids (FFAs) which are the major components (95-99%), while the minor components (1-5%) consist of sterols, carotenoids, tocopherols, aliphatic alcohols, gums and phosphatides (Prasanth and Gopala, 2014).

Natural fats and oils vary widely in their physico-chemical properties, even though they are made up of the same constituents including fatty acid composition. This is because individual fats and oils vary over relatively large ranges in the proportion of the component fatty acids, and the structures of the individual component triglycerides (Sonntag, 1979). Although these two factors are interdependent, there are aspects of the overall effects that can more or less be attributed to one or the other. This is the case with CPO. The quality of CPO varies from one source to the other depending on several factors including age of fruits, age of oil, processing method, handling and storage conditions (Sonntag, 1979). A wide variation in the quality of locally extracted CPO (HFFA grade) sold in Nigerian markets in Lagos (Adebayo-Oyetoro et al., 2019) and Port Harcourt (Ohimain et al., 2012) has been reported.

Many hitherto unappreciated factors are now known to influence the fatty acid composition as well as the triglyceride structure of natural fats and oils. Among the factors which affect the composition of fats and oils in the vegetable kingdom are climatic conditions, soil type in which the parent plant was grown, geography of the growing location, hydrology of the area, maturity of the plant, health of the plant, environmental conditions, processing culture and traditions, handling and storage conditions and most importantly, genetic variations in the plant (Sonntag, 1979). For instance, the plantation varieties of oil palms are different from the wild varieties grown by traditional farmers in Nigeria. And the CPO extracted by modern methods and improved technologies (LFFA CPO) showed different quality attributes from the CPO obtained by traditional methods of extraction (Mba et al., 2015).

Traditionally, the oil is isolated by several methods including boiling the fruit, pounding and pressing or suspending the sludge in hot water. Modern methods of extraction of palm oil from the fruits are more efficient and include the following steps: cooking, pressing, centrifugation and filtration under vacuum. Palm fruits may be subjected to strong enzymatic hydrolysis and microbial degradation during harvest and handling prior to extraction of CPO. This scenario is very common with traditional practices in Nigeria, and has been responsible for high FFA in HFFA CPO. The estate palm oil (LFFA CPO) which is produced by modern milling technology has low FFA values and impurities because the fruits are harvested and immediately processed and extracted in the factory without long periods of storage (Mba et al., 2015).

The quality of palm oil can be poor unless the source of palm fruits are handled carefully and promptly to minimize the impact of agents of spoilage such as air, water, enzymes and micro-organisms (Sonntag, 1979). It has been reported that poor quality palm oil can be processed and cleaned by washing the oil with hot water, followed by filtration and centrifugation. This process eliminates impurities, high FFA and moisture content and improves the stability of the final product (Igile et al., 2013). As was earlier stated, the quality of palm oil determines its market acceptability and price, and this correlates directly with the free fatty acid (% FFA) concentration and the total fatty matter (% TFM) content of the commodity. Therefore, businesses using palm oil for production are very particular about the moisture content, FFA, impurity and TFM contents of CPO. Total contaminants including moisture, unsaponifiable matter and other impurities add to the free fatty acid content of any oil to significantly reduce the available % TFM content of the oil (Saad et al., 2007). It has been reported that the degradation action of lipase increases FFA levels in CPO, which is considered a very important quality parameter because FFA concentration is one of the most important characteristic quality index for the storage time, marketing, production, and price of palm oil (Saad et al., 2007; Baterman et al., 2010).

Palm oil contains several saturated and unsaturated fatty acids. Irrespective of the grade of CPO, the fatty acids composition of crude palm oil have been reported to include capric, caproic, caprylic, lauric, myristic, palmitic, stearic, oleic, linoleic and linolenic acids. Palmitic acid has been reported to be the dominant fatty acid in CPO (40-45%) and to be the most widely distributed saturated fatty acid in vegetable oils and animal fats to the extent of at least 5%. It was reported that the oleic acid content in CPO is also significant (36-40%) (Japir et al., 2017).

The lower members of saturated fatty acids are liquids at room temperature, whereas those containing 10 or more carbon atoms are solids having progressively higher melting points (titre, °C) with increasing length of carbon chain. It has been reported that the even progression of melting points and the length of the carbon chain gives a smooth curve only if the even-membered homologues are considered. When all the fatty acids are included, lower than that of the even-chain acid immediately before it (Sonntag, 1979).

Palm oil is rich in carotenoids including β-carotene which is a precursor of Vitamin A. Palm oil has been used for Vitamin A deficiency interventions and has therefore been used for preventing Vitamin A deficiency, cancer,

brain disease, and aging. It is also used to treat malaria, high blood pressure, high cholesterol level, and dementia and cyanide poisoning. Palm oil is used for weight loss and for increasing the body's metabolism (Mancini et al., 2015). As food, palm oil is used as cooking oil and for frying, and it is also an ingredient in many processed foods. Industrially, palm oil is used for the manufacture of cosmetics, soaps, detergents, toothpaste, waxes, lubricants and ink. However, excessive intake of palm oil has been reported to predispose hypercholesterolemia, hyperlipidemia and diabetes with attendant high concentrations of low density lipoprotein cholesterol (LDH-Cholesterol) (Mancini et al., 2015).

The aim of the present study was to assess the quality of LFFA CPO and HFFA CPO samples from different locations in Southern Nigeria produced through traditional and modern extraction methods, and to determine their respective TFM content aimed at assisting statutory regulatory activities.

MATERIALS AND METHODS

Collection of plant

Fresh LFFA CPO samples aged 5 to 10 days from estate palm oil mills were purchased from four locations in Nigeria including, Port Harcourt (SPORP), Calabar (SPOCAL), Benin (SPOOK), and Ibadan (SPOIB) and stored in 2-L brown bottles under refrigeration until required. Also samples of HFFA CPO were purchased from the same locations in an open retail market after ensuring that they were traditionally processed. The HFFA CPO samples were also 5 to 10 days old and were labelled Port Harcourt (TPORP), Calabar (TPOCAL), Benin (TPOOK), and Ibadan (TPOIB). All samples were properly labelled and identified, and stored in brown glass bottles under refrigeration (16±2.0°C) to minimize further deterioration and production of FFA.

Sample preparation for physico-chemical analysis

Two hundred grams of each sample of LFFA and HFFA CPO was measured and labelled according to the labelling earlier described and stored under refrigeration (16±2.0°C) for physico-chemical analysis including, moisture content, free fatty acid, impurity, unsaponifiable matter, saponification value, peroxide value, iodine value, hydroxyl value, acid value, total fatty matter, titre or melting point and the lovibond colour of oil samples (using 51/4" cell at 55°C). All parameters were analysed using to standard analytical procedures.

Determination of moisture content of LFFA and HFFA CPO sample

The moisture content of the LFFA and HFFA CPO samples was determined using the Karl Fischer method (AOCS, Tb 2-64; Emery, 1983). The fundamental principle in this method is to determine the actual water content of the oil by titration of the oil sample with Karl Fischer reagent which reacts quantitatively with water.

Determination of impurity/dirt in LFFA and HFFA CPO samples

Five (5) grams of oil sample was heated to a temperature of 105°C in a Gallenkamp oven. The oil was filtered through Whatmann No. 4 Filter paper, previously dried and weighed to a constant weight. The filter paper was then dried after oil filtration to constant weight. The impurity content was determined from the equation:

$$\text{Impurity (\%)} = (W_2 - W_3 / W_1) \times 100$$

where W_1 = weight of oil sample, W_2 = initial weight of filter paper, and W_3 =final weight of filter paper.

Determination of colour of raw LFFA and HFFA CPO samples

The colour of LFFA and HFFA raw oil samples was determined using the Lovibond method described by the British Standard Institute method BS 684 using the Lovibond Tintometer Model E AF900 (Tintometer Ltd, Salisbury, UK). In this method, 100 ml of raw oil was heated to 70°C in a Gallenkamp oven at 105°C. The oil was allowed to cool to 55°C, and measured at 55°C using $5\frac{1}{4}$ inch cell in a lighted Lovibond Tintometer against known standard glass colours. The colour of oil was expressed as (RYBN), meaning Red (R), Yellow (Y), Blue (B) and Neutral (N) for both LFFA and HFFA CPO samples.

Determination of free fatty acid (% FFA) of LFFA and HFFA CPO samples

Free fatty acid (FFA) of LFFA and HFFA CPO samples was determined using the AOCS method Ca 5a-40 (1989). In this method 5 g of oil was placed in a 250 ml Erlenmeyer flask. Then 50 ml pre-neutralized isopropyl alcohol (IPA) was added and mixed thoroughly and 3 drops of phenolphthalein was added and mixed. The mixture was placed on a hot plate with a magnetic stirrer and heated to 40°C, and then titrated with 0.1N NaOH to the phenolphthalein end point of a stable pink solution.

$$\%\text{FFA} = \frac{\text{Vol. of titrant} \times (N) \text{ of titrant} \times 25.6}{\text{Weight of oil sample (g)}} \times \frac{\text{mg NaOH}}{\text{Sample (g)}}$$

where, 25.6 is the FFA conversion factor for palmitic acid.

Determination of acid value (AV) of LFFA and HFFA CPO samples

Acid values (AV) of LFFA and HFFA CPO samples were determined by multiplying individual % FFA with 2.19 which is the conversion factor for palmitic acid.

$$\text{Acid Value (AV)} = \% \text{ FFA} \times 2.19;$$

where 2.19 is the conversion factor for palmitic acid.

Determination of iodine value (IV) of LFFA and HFFA CPO samples

Iodine value (IV) of the LFFA and HFFA CPO samples was determined using the Wij's method (BS 684; Section 2.13, 1976) as described by Abdullah et al. (2013). Briefly, 4 g of oil sample was placed in a 500 ml Erlenmeyer flask and 15 ml of cyclohexane was added and shaken to dissolve the oil. Then 25 mL of Wij's reagent was added and mixed and place in the dark with periodic mixing for 1 h, and 150 mL of distilled water was added to the mixture and shaken to mix. Finally, 20 mL of 10% KI solution was added to the mixture and titrated against 0.1N Na2S2O3 to obtain a stable

yellow colour to which 1 mL of 1% starch solution was added. The titration continued to obtain a stable blue colouration. The procedure was repeated using a blank solution of distil water.

Iodine value (IV) was calculated according to the equation:

$$IV = \frac{(Vb\text{-}Vt) \times (N) \text{ of titrant} \times (12.69)}{\text{Weight of sample (g)}} \times \frac{I2\ (g)}{\text{Sample (100 g)}}$$

where Vb = mL of blank, Vt = mL of titrant, and 12.69 is the equivalent conversion factor from thiosulphate to I2.

Determination of hydroxyl value (HV) of LFFA and HFFA CPO samples

The method described by Fernandes et al. (2014) was adopted. Briefly, 5 g of oil sample was placed in a 250 mL conical flask and 5 mL of a reagent mixture of acetic anhydride/pyridine, 1:4 v/v was added and mixed. The mixture was refluxed at 100°C for 1 h with constant stirring using a magnetic bar, and then cooled to ambient room temperature (27±0.50°C) and 10 ml of deionized water was added to complete the hydrolysis of excess acetic anhydride in the mixture. The mixture was further refluxed for about 10 min, then cooled to room temperature, followed by the addition of 25 mL neutralized ethanol and 3 drops of phenolphthalein indicator. This was titrated against 0.5N methanolic KOH to obtain a light pinkish colouration. Hydroxyl value was obtained according to the equation:

$$IV = \frac{(V_b\text{-}Vt) \times (N) \text{ of titrant}) \times (12.69)}{\text{Weight of sample (g)}} \times \frac{I_2\ (g)}{\text{Sample (100g)}}$$

where Vb = mL of blank, Vt = mL of titrant; AV= Acid Value, and 56.1 is a factor for the conversion of hydroxyl value.

Determination of saponification value (SV) of LFFA and HFFA CPO samples

The SV of the oil samples was determined using the BS 684 2.6 (1977) method described by Abdullah et al. (2013). In this method 3 g of oil sample was placed in a 250 mL conical flask, and 25 mL 0.5 N ethanolic KOH was added and mixed by shaking and refluxed for 1 h by boiling over a hot plate and stirring with a magnetic bar. The mixture was allowed to cool slightly and 3 drops of phenolphthalein was added and mixed and then titrated against 0.5 N HCL to a pink colouration end point. Saponification value (SV) was calculated using the equation:

$$SV = \frac{(V_b\text{-}Vt) \times (N) \text{ of titrant}) \times (56.10)}{\text{Weight of sample (g)}} \times \frac{mgKOH}{g\ sample}$$

where Vb = mL of blank and Vt = mL of titrant.

Determination of peroxide values (PV) of LFFA and HFFA CPO samples

Peroxide values (PV) of the LFFA and HHFFA CPO samples were determined using the improved AOAC mFOX method described by Burat and Bozkurt (1996). Briefly, 0.2 g of oil sample was weighed into 100 ml conical flask into which a mixture of 9.8 m < chloroform/methanol (7:3) was added and mixed briefly. The mixture was transferred into a screw-capped vial and 100 µL of 10 mM xylenol orange was added and mixed by shaking for about 20s. Then 50 µL of 36 mM iron (111) chloride solution was added and mixed. The solution obtained was allowed to stand for 5 min at room temperature (27±0.50°C). The absorbance of each sample was read at 560 nm wavelength using a UV/VIS spectrophotometer (Model; Schimadzu UV-1700). The PV was extrapolated from a previously prepared standard curve. The standard calibration curve was prepared from a solution of iron (111) chloride (10 ug/ml) in 9.8 mL chloroform/methanol (7:3) mixture. A serial dilutions ranging from 5 to 30 ug/L was prepared and plotted against the absorbance to produce a linear curve from which the respective PV of the oil samples was extrapolated.

Determination of ester value (EV) of LFFA and HFFA CPO samples

The ester values (EV) of the LFFA and HFFA CPO were calculated for each oil sample according to the equation,

EV = SV-AV

where SV is the saponification value and AV is the acid value of the oil sample, respectively.

Determination of %TFM of LFFA and HFFA CPO samples

Total fatty matter (as %TFM) was calculated for each oil sample according to the equation:

%TFM = 100% – (FFA + Moisture + Impurity + Unsaponifiable Matter)% × (95/100)

where 95% is the absolute TFM of clean Oils and conversion factor for % TFM of oil.

Composite oil samples preparation for GC-MS analysis of fatty acids

Four grams of oil was measured from each LFFA CPO sample and mixed to produce 20 g of composite LFFA CPO sample for the derivatization of FAMEs for GC-MS analysis. The same procedure was replicated with HFFA CPO samples for GC-MS analysis.

Fatty acids methyl esters (FAMEs) derivatization of the oil samples for GC-MS analysis

Twenty five (25) ng of oil sample was weighed into 10 mL micro-reaction vessel and 2 mL BCl3-MeOH 12% w/w was added. This was followed by the addition of 1 mL 2, 2-dimethoxyproprane. The mixture was mixed thoroughly and then heated for 5 min at 60°C. It was then cooled to below 30°C and 1 mL distilled water and 1 mL n-hexane was added and mixed thoroughly again, then allowed to stand for 10 min. The upper (organic) layer was pipetted into a sterile and clean vial and covered with a stopper and stored for GC-MS analysis. This procedure was replicated in all the LFFA and HFFA CPO samples.

GC-MS analysis of FAMEs for fatty acid composition of SPO and TPO samples

The method reported by Dodds et al. (2005) and modified by Igile et al. (2020) was used to carry out the GC-MS analysis. The sample of FAME prepared from each oil sample, was injected manually through the injector pot of the Agilent 6890 GC coupled

Table 1. Physico-chemical quality characteristics of Nigerian crude palm oil samples (LFFA and HFFA).

Parameter	SPOCAL	SPOIB	SPORP	SPOOK	TPOCAL	TPOPH	TPOBN	TPOIBA
Moisture (%)	0.53±0.04^{a}	0.60±0.05^{a}	0.55±0.02^{a}	0.45±0.02^{a}	1.02±0.25^{c}	1.63±0.07^{b}	1.47±0.40^{b}	1.65±0.05^{c}
FFA (% Palmitic acid)	2.44±0.30^{a}	2.81±0.21^{a}	2.95±0.08^{a}	2.65±0.20^{a}	9.25±0.70^{bc}	12.54±0.45^{b}	10.52±0.85^{b}	12.76±1.20^{c}
Impurity (%)	0.05±0.01^{a}	0.11±0.03^{a}	0.04±0.02^{ac}	0.02±0.01^{ac}	0.24±0.05^{c}	0.44±0.03^{b}	0.39±0.03^{b}	0.35±0.02^{b}
Unsap Matter (%)	0.27±0.04	0.30±0.05	0.29±0.07	0.24±0.03	0.41±0.11	0.59±0.09	0.47±0.05	0.45±0.06
Sap Value (mgKOH/g oil	199.95±0.8^{a}	199.87±0.5^{a}	199.92±0.6^{a}	199.88±0.9^{a}	197.57±0.8^{a}	197.52±0.7^{a}	196.55±0.5^{a}	196.62±0.4^{a}
Peroxide Value (meq/kg)	5.25±0.45^{a}	5.44±0.30^{a}	5.27±0.25^{a}	4.79±0.11^{a}	1.77±0.03^{c}	1.45±0.02^{b}	1.56±0.05^{b}	1.90±0.06^{c}
Iodine Value (Wijs)	56.25±0.85^{b}	57.25±0.92^{b}	57.83±0.75^{b}	56.55±0.64^{b}	52.11±1.20^{a}	53.32±1.95^{c}	52.65±1.13^{a}	53.42±1.25^{a}
Acid Value (mgKOH/g)	6.72±0.12^{c}	7.87±0.35^{a}	7.92±0.45^{a}	7.21±0.37^{a}	9.77 ± 0.17^{b}	9.32 ± 0.25^{b}	8.57 ± 0.45^{b}	9.68 ± 0.29^{b}
Hydroxy Value	17.22±0.55^{a}	16.41±0.39^{a}	16.55±0.15^{a}	16.92±0.42^{a}	24.28±0.25^{b}	27.45±0.34^{c}	25.62±0.46^{c}	27.35±0.09^{b}
Ester Value (mgKOH/g)	193.23±1.1^{a}	190.00±0.9^{a}	192.00±0.92^{a}	192.67±1.4^{a}	187.80±1.8^{a}	188.20±1.2^{a}	187.98±0.7^{a}	186.94±1.5^{a}
Titre (^{o}C)	45.05±0.41^{a}	45.00±0.05^{a}	44.95±0.04^{a}	44.90±0.05^{a}	43.75±0.25^{b}	44.11±0.45^{b}	44.05±0.07^{b}	43.85±0.15^{b}
TFM (%)	92.45±0.75^{a}	91.95±0.82^{a}	91.94±0.40^{a}	92.39±0.37^{a}	85.16±1.05^{b}	81.07±0.31^{bc}	83.32±0.92^{bc}	81.06±0.64^{c}
Lovibond Colour (5^{1}/4" Cell)	18R12Y7B^{a}	20R13Y7B^{a}	19R13Y6B^{a}	18R12Y5B^{a}	23R15Y9B	23R14Y12B	24R15Y12B	25R15Y10B

Values are expressed as Mean ± SD from triplicate samples test results (n=3). Different superscripts letters within the same row are significantly ($p<0.05$) different.

with a 5973i mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) which was connected to a ChemStation Integrator to interpret data. The GC was equipped with a HP-5MS capillary column (30 m × 250 µm i.d. × 0.25 µm, Agilent Technologies). Helium was used as the carrier gas with a constant flow rate of 1 mL/min to the column. The initial oven temperature was set at 40°C, holding for 2 min, then raised to 150°C at 5°C/min; and finally raised to 280°C at 15°Cmin^{-1}, holding for 2 min. The injection pot was maintained at splitless mode. The mass detector was operated at 150°C in electron impact (EI) mode at 70 eV. The ion source temperature was at 230°C and the transfer line temperature was maintained at 250°C. The chromatograms were recorded by monitoring the total ion currents in the 15 to 450 mass range. MS was detected with 2 min solvent delay. Analysis of each sample at each condition was repeated twice to ensure consistency. C6-C24 *n*-alkanes were run under the same chromatographic conditions in order to calculate the retention indices (RI) of detected compounds. Identification of fatty acids and other volatile constituents were based on retention indices relative to n-alkanes (C6-C24), and computer matching with the WILLEY 275.L library, and those contain in the NIST08 database; and confirmed by comparison of the retention times reported in literature (Igile et al., 2020).

Statistical analysis

Physico-chemical determinations were carried out in triplicates while GC-MS determinations were done in duplicates and results were expressed as Mean ± SEM, for n=2 or n=3, and subjected to analysis of variance (ANOVA) to check for statistical significance ($p<0.05$).

RESULTS AND DISCUSSION

Physico-chemical evaluation of LFFA and HFFA CPO

The comparative results of the physico-chemical evaluation of LFFA and HFFA CPO samples are presented in Table 1. The physico-chemical properties of CPO including, appearance, odour and taste determine its quality and consumer acceptability (Igile et al., 2013). The length of time between fruits harvest and processing, type of processing method employed, storage and handling conditions, may significantly affect the moisture content, impurities, unsaponifiable matter and free fatty acid content of both LFFA and HFFA CPO. These parameters contribute significantly to the overall quality of palm oil in the market place (Fakou et al., 2009; Igile et al., 2013; Japir et al., 2017). Modern palm oil (PO) mills and improved technologies have successfully been applied to produce high grade palm oil of low FFA, impurity and moisture values, and this type of oil has been graded as LFFA CPO (Table 1). The FFA values of the LFFA CPO samples were significantly ($p<0.05$) lower and stable than values for HFFA CPO (Figure 1). The range of FFA% values for LFFA CPO was 2.44±0.30 -2.95±0.08%, whereas mean FFA values for HFFA CPO was in the range 9.25±0.70 - 12.76±1.20%. The moisture and FFA values obtained for HFFA CPO in the present study were consistent with the values reported by Ohimain et al. (2012). However,

Figure 1. Map of Nigeria showing cities sampling and study locations. Sampling areas are circled in the map. SPORP = SPO Rison Palm, SPOCAL = SPO Calaro Palm, TPOCAL = TPO Calabar Market, TPOBN = TPO Benin Market, SPOOK = SPO Okomu, SPOIBA = SPO Ibadan Palm, TPOPH = TPO Port Harcourt Market, TPOIBA = TPO Ibadan Market.

Ohimain et al. (2012) reported very high impurity levels of between 5.48 and 12.52% when compared with the values (0.24-0.44%) obtained for HFFA CPO in this study. We compared the values of some physico-chemical parameters obtained in this study with those obtained by Adebayo-Oyetoro et al. (2019). Their study reported unusually high values of FFA (14.70- 21.45%) and unusually low moisture values (0.38-2.41%) which did not correlate with one another. Such low moisture values cannot give such high FFA values. They also reported IV values of 84.94-179.71 which was inconsistent with the entire physico-chemical results in that study. The IV values did not correlate with the FFA and moisture values reported and known structural and ageing chemistry parameters of CPO.

Modern mills from Calabar gave the lowest FFA values for LFFA (2.44±0.30%) and HFFA CPO (9.25±0.70%), respectively. Port Harcourt LFFA CPO (SPORP) sample gave the highest mean FFA% value (2.95±0.08%), while Ibadan HFFA CPO (TPOIB) gave the highest mean FFA value (12.76±1.20%). Traditional milling and processing methods among the natives in Nigeria are known to be crude and thus produce HFFA CPO grades with significantly ($p<0.05$) high FFA values (>5.00%) (Figure 1). The factors affecting the traditional methods of processing include delays in processing harvested fruits and the use of crude processing and extraction methods such as boiling of fruits, extraction with hot water under unhygienic conditions, resulting in enzymes and microbial-aided fruits spoilage, bad odour, high moisture, poor colour, high impurity and unsaponifiable matter, as well as high FFA values of CPO (Table 1).

HFFA CPO samples from traditional production always present with significantly ($p<0.05$) low available TFM (81.06±0.64-85.16±1.05%), when compared with LFFA CPO with significantly high available TFM values (91.94±0.40 - 92.45±0.75%) (Table 1) processed from modern mills with improved technologies. Therefore, the concentration of FFA, moisture, impurity and unsaponifiable matter significantly contributes to available %TFM of both LFFA and HFFA CPO (Figure 2), and this affects the industries utilizing the oil as raw material for production (Igile et al., 2013). Poor quality oil results in increased cost of processing. High values of the quality parameters of CPO give rise to lower available %TFM in HFFA oil (Figure 2). Thus, production of palm olein, butter and salad dressings in the food industry will incur losses because of low available TFM resulting from high concentrations of key physico-chemical parameters in HFFA CPO. Also manufacturers of soap, detergents and cosmetics will incur losses because of the availability of low TFM in HFFA oil. Consequently, organizations around the world utilizing CPO for production insist on buying LFFA grade CPO. LFFA CPO contains significantly ($p<0.05$) low concentrations of physico-chemical parameters including FFA, moisture, impurity and unsaponifiable matter (Figure 1) resulting in high available TFM content (Figure 2). It has been reported that high

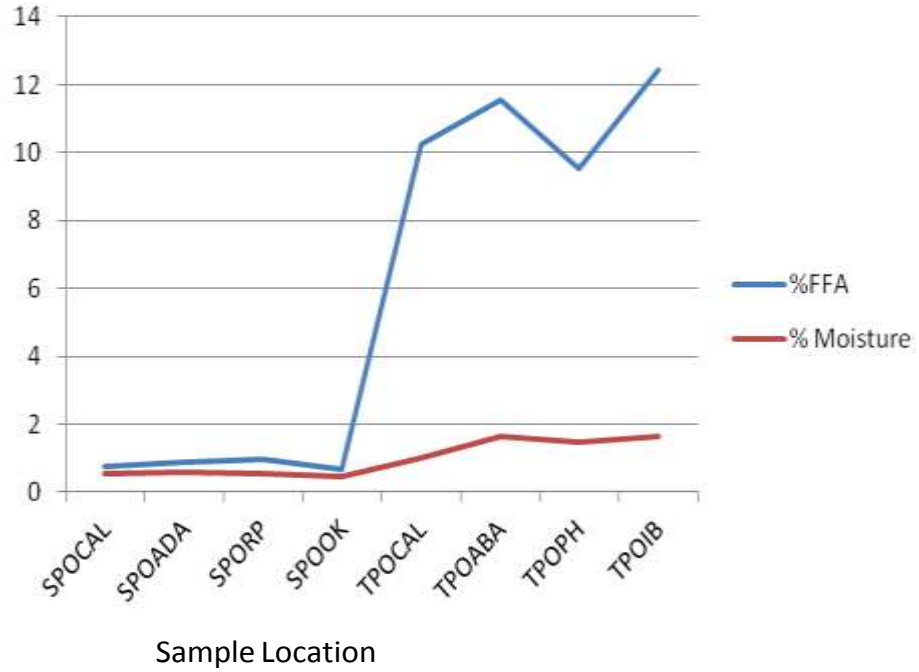

Figure 2. Variation of %FFA and moisture values with oil type and sample location.

hydrocarbons, tocopherols, carotenoids, plant sterols, gums and tocotrienols constitute what is referred to as unsaponifiable matter. These are substances which are dissolved in the CPO but cannot be saponified by caustic alkali (Prasanth and Gopala, 2014).

The saponification value (SV) of oil is a measure of the average molecular weight of oil or all fatty acids in the oil. It can be defined as the number of milligrams of KOH required to saponify 1 g of oil or fat under the conditions specified (Salimon et al., 2012). The acid value of oil just like the FFA% is an indication of the ageing state or degradation of the oil (Saad et al., 2007). It is therefore the number of mg of KOH required to neutralize the free fatty acid in 1 g of sample. Thus SV (or, precisely, the AV of oil or fatty acids) and the fatty acid ratio are both measures of the average equivalent mass, and the average chain length of the mixture of fatty acids in the oil.

The SV, AV and EV are related by the equation:

EV = SV–AV;

Where EV is the ester value which is a measure of the glyceride present in the oil sample. Also SV and AV are important parameters in the estimation of the glycerol content of a fat or oil. The average degree of unsaturation of a fat or mixture of fatty acids is measured by the iodine number or iodine value (IV) and is expressed in terms of the number of centigrams of iodine absorbed per g of sample in a Wijs reaction (British Standards: BS 684 Section 2.13; 1976).

Variation of TFM in LFFA and HFFA CPO samples

The %TFM was expectedly found to be higher in LFFA CPO samples and significantly lower ($p<0.05$) in the HFFA CPO samples studied (Figure 3). Calabar SPOCAL gave the highest mean TFM value of 92.45±0.75% while Port Harcourt SPOPH gave the lowest mean TFM value of 91.94±0.40%. With respect to the %TFM values of HFFA CPO samples, Calabar (TPOCAL) gave the highest mean %TFM value of 85.16±0.05 while Ibadan (TPOIBA) gave significantly lower mean %TFM value of 81.06±0.64% ((Figure 3).

Total fatty matter was relatively stable in the LFFA CPO samples and was found within the range (91.94±0.40 92.45±0.75%) when compared with the large variation of mean %TFM in HFFA CPO samples (81.06±0.64 - 85.16±0.05%) (Figure 2). It was observed that the differences in processing methods and impurities contents in both grades of CPO accounted for the difference in %TFM found between the two grades of CPO studied. Differences in cultural practices and processing methods between eastern and western Nigeria may also have contributed to the variation in the TFM of the HFFA CPO samples studied.

Fatty acid composition of LFFA and HFFA CPO samples

The fatty acid composition of both grades of CPO samples was determined by GC-MS method. The

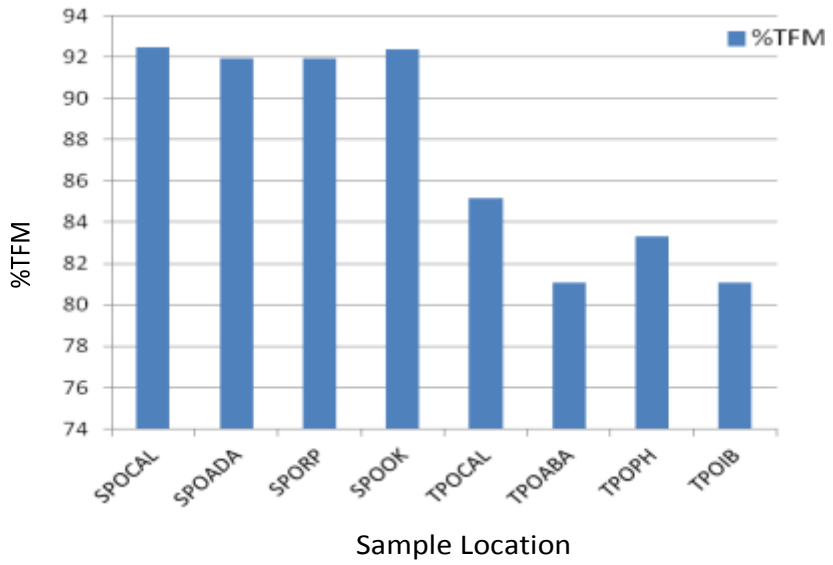

Figure 3. Variation of %TFM values with oil type and sample location.

Table 2. Fatty acid distribution of composite sample of LFFA CPO from *Elaeis guinensis* (Jacq.) purchased from retail market stores in Nigeria.

Peak no.	RT (min)	Name of compound	Molecular formular	Abundance (%)
1	1.441	Caprylic acid	$C_8H_{16}O_2$	0.119±0.05
2	2.652	Capric acid	$C_{10}H_{20}O_2$	0.355±0.03
3	3.112	Lauric acid	$C_{12}H_{24}O_2$	0.591±1.02
4	3.524	Myristic acid	$C_{14}H_{28}O_2$	0.792±0.45
5	4.710	Palmitic acid	$C_{16}H_{32}O_2$	45.641±1.77
6	10.625	Stearic acid	$C_{18}H_{36}O_2$	3.452±0.08
7	11.911	Palmitoleic acid	$C_{16}H_{30}O_2$	0.491±0.55
8	14.260	Oleic acid	$C_{18}H_{34}O_2$	38.005±1.06
9	14.871	Linoleic acid	$C_{18}H_{32}O_2$	10.440±0.25
10	15.035	Linolenic acid	$C_{18}H_{30}O_2$	0.511±0.04
			Total	100.465±3.11

Values are expressed as mean ± SEM, n = 2.

fatty acid composition of fats and oils is generally determined by conversion of the fat or oil by methanolysis to mixed fatty acid methyl esters (FAMEs) followed by analysis with GC-MS. The summary of the GC-MS results for the LFFA CPO composite sample is presented in Table 2, while that of HFFA CPO sample is presented in Table 3.

The GC-MS analysis gave the fatty acid composition for LFFA CPO as follows: caprylic acid (0.119±0.05%), capric acid (0.355±0.03%), lauric acid (0.591±1.02%), myristic acid (1.792±0.45%), palmitic acid (45.641±1.77%), stearic acid (3.452±0.08%), palmitoleic acid (0.491±0.55%), oleic acid (39.005±1.06%), linoleic acid (10.440±0.25%), and linolenic acid (0.511±0.04%). The GC-MS results for HFFA CPO composite sample were, caprylic acid (0.167±0.07%), capric acid (0.438±0.05%), lauric acid (1.114±1.25%), myristic acid (1.725±0.65%), palmitic acid (44.670±0.85%), stearic acid(3.050±0.55%), palmitoleic acid (0.450±0.72%), oleic acid (37.370±1.06%), linoleic acid (10.420±0.40%), and linolenic acid (0.250±0.05%).

The fatty acid composition and distribution in the palm oil samples studied were consistent with results earlier reported (Derawi et al., 2014; Japir et al., 2017). The saturated fatty acids (SFAs) found in this study were common to both grades of CPO and included capryllic, capric, lauric, myristic, palmitic and stearic acid. These fatty acids constituted the TSFAs

Table 3. Fatty acid Distribution of Composite sample of HFFA CPO from Elaeis guinensis (Jacq.) purchased from Retail Market Stalls in Nigeria.

Peak no.	RT (Min)	Name of compound	Molecular formular	Abundance (%)
1	1.279	Caprylic acid	$C_8H_{16}O_2$	0.167±0.07
2	2.124	Capric acid	$C_{10}H_{20}O_2$	0.438±0.05
3	2.677	Lauric acid	$C_{12}H_{24}O_2$	1.114±1.25
4	3.908	Myristic acid	$C_{14}H_{28}O_2$	1.725±0.65
5	4.522	Palmitic acid	$C_{16}H_{32}O_2$	44.670±0.85
6	10.390	Stearic acid	$C_{18}H_{36}O_2$	3.050±0.55
7	11.490	Palmitoleic acid	$C_{16}H_{30}O_2$	0.450±0.72
8	13.591	Oleic acid	$C_{18}H_{34}O_2$	37.370±0.92
9	13.972	Linoleic acid	$C_{18}H_{32}O_2$	10.420±0.40
10	14.515	Linolenic acid	$C_{18}H_{30}O_2$	0.250±0.05
			Total	100.955±3.782

Values are expressed as mean ± SEM, n = 2.

Table 4: Composition of Saturated, Monounsaturated and Polyunsaturated Fatty Acids in LFFA and HFFA CPO Samples.

Fatty Acid Type	Type of Oil	
	SPO	TPO
Total SFAs (%)	49.950±1.72	51.164±2.05
Total MUFAs (%)	38.496±1.43	37.820±0.75
Total PUFAs (%)	10.951±0.45	10.670±0.52
Summary		
Total SFAs (%)	49.950±1.72	51.164±2.05
Total TUFAs (%)	49.447±1.09	48.490±1.25
TSFAs **:** TUFAs	1:1	1:1

Values are expressed as mean ± SEM, n = 2
SFAs = Saturated fatty acids; MFUs = Monounsaturated fatty acids; PUFAs = Polyunsaturated fatty acids; TSFAs = Total saturated fatty acids; TUFAs = Total unsaturated fatty acids

of 49.950±1.72% in LFFA CPO and 51.164±2.05% in HFFA CPO, respectively (Table 4). In both grades of CPO samples, the major saturated fatty acid was palmitic acid (C16:0).

Oleic acid (C18:1) was the major monounsaturated fatty acid (MUFA) found in RPO in this study, accounting for 38.496±1.43% in LFFA CPO and 37.820±0.75% in HFFA CPO. Linoleic and linolenic acids were common to both grades of oil samples as polyunsaturated fatty acids (PUFAs). The total PUFAs in LFFA CPO was 10.951±0.45% and 10.670±0.52% in HFFA CPO, and showed no significant difference in concentration between the two grades of CPO (Table 4). The total unsaturated fatty acids (TUFAs) in LFFA CPO were 49.447±1.09 and 48.490±1.25% for HFFA CPO. Linoleic acid (C18:2) and linolenic acid (C18:2) which are essential FAs accounted for over 10% of the fatty acid contents of both grades of CPO. The presence of linoleic acid at about 10% has favourable nutritional implications and beneficial physiological effects in humans.

Linoleic acid prevents coronary heart disease and cancer and provides lipids necessary for cell membrane repair and cellular respiration (Oomah et al., 2000). The ratio of total saturation to total unsaturation in both LFFA CPO and HFFA CPO was found to be 1:1 (Table 4). The dietary and nutritional significance of this balance in ratio between total saturation and total unsaturation in CPO is not very clear and has not been investigated or reported in literature, and this may be a subject of further investigation.

Conclusion

It was concluded that CPO is rich in SFAs, MUFAs and

PUFAs and the ratio of TSFAs to TUFAs for both LFFA and HFFA CPO is 1:1, and this may have dietary and nutritional significance. The available TFM in LFFA CPO was significantly higher than in HFFA CPO. The quality of both grades of oils did not affect their fatty acid composition and distribution; neither did it affect the SV, EV and IV but affected AV (an indicator of the FFA content of vegetable oils).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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