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# Physiochemical and fatty acid analysis of *Virescens* (Ojukwu) oil and *Nigrescens* (ordinary) palm oil of *Eleaisguineensis*

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**Traditionally in Igbo land folklore medicine, *Virescens* (Ojukwu) palm oil of *Eleaisguineensis* is of value as anti-poison and miracle oil. The objective of the study was to evaluate the physiochemical properties with the identification of the fatty acids of the *Virescens* oil in comparison to *Nigrescens* oil of *Eleaisguineensis*. The result of the physiochemical properties shows that the values for meeting point (slip point) of the oils were found to be the same (33°C) while solidification (titer) point, 22°C, viscosity, 51.20 centistokes and moisture content, 1.6% of *Nigrescens* (ordinary) palm oil are higher than that of *Virescens* palm oil with solidification point (titer) point, 15°C; viscosity 29.89 centistokes and moisture content, 0.2%. Both *Virescens* oil, 83.82 and *Nigrescens* oil, 53.98 are non-drying oil (low iodine value) and have high saponification values (*Virescens*, 222.3 and *Nigrescens*, 223.7). The result of the peroxide value revealed that there are more peroxides in Virescens oil (15 and 18) than in Nigrescens oil (8 and 12.3) for a week and 4 weeks oil respectively. Results on Ester value revealed high ester value (*Virescens*, 265.78 and *Nigrescens*, 263.16) with percent Ester purity of 21.11% for *Virescens* and 21.32% for *Nigrescens*. *Nigrescens* have higher acid value (40.67) than Virescens (29.73). The fatty acid analysis result revealed the presence of oleic, stearic acid, tocopherol in both the *Virescens* and *Nigrescens* palm oil with $R_F$ values of 0.50 (oleic), 0.40 (stearic) and 0.29 (tocopherol) while lecithin was only observed in Virescens palm oil with $R_F$ value of 0.34. Some values of *Virescens* were found to agree with the same value of olive oil and the presence of lecithin suggests why *Virescens* is anti-poison and medicinal.**

**Key words:** *Virescens* and *Nigrescens* palm oil, physiochemical properties, anti-poison, medicinal.

## INTRODUCTION

Palm oil is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palms, primarily the African oil palm *Elaeisguineensis* (Reeves and Weihrauch, 1979). It is naturally reddish in colour due to high beta–carotene content. Palm mesocarp oil is 41% saturated and semisolid at room temperature and contain several saturated and unsaturated fats in the forms of glyceryllaurate (0.1% saturated), myristate (1%

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saturated), palmitate (44% saturated), stearate (5% saturated), oleate (39% monounsaturated) and linoleate (10% polysaturated) (Cottrell, 1991). Like all vegetable oils, palm oil does not contain cholesterol (US Federal Food, Drug and Cosmetic Act, 1990; UK Food Lebelling Regulation, 1984) although saturated fat intake increase both LDL and HDL (Mensink and Katan, 1992) cholesterols (Cha Sook et al., 2002; Tan, 1991). Palm oil is largely the cooking oil in the tropical belt of Africa, South East Asia, part of Brazil and South America. Its use in the commercial food industry in other parts of the world is due to its low cost and the high oxidative stability of the refined product when used for frying (Che Man et al., 1999; Matthaus, 2007). A large proportion of the oil is also consumed in the manufacture of soaps including native black soap, candles, lubricants and in tin plating industry (Onyegbado et al., 2002; Edgar, 1985). The folklore nutritional and healing properties have been recognized for generations. Red palm oil was the remedy of choice for nearly every illness in many parts of African. The taking of spoonful of palm oil when someone sick was common.

Red palm is rich in vitamin A precursors and can be used in place of cod-lover oil (Zeba et al., 2006; ChaSooket al., 2002). It was reported in some research work that oleic acid, a monounsaturated fatty acid in palm oil is as effective as the polyunsaturated fatty acids in lowering blood cholesterol (Mattson and Grundy, 1985; Qureshi et al., 1995). Palm oil can not only improve coronary blood flow and remove plaque buildup in arteries, it also reverses the process of atherosclerosis and improves cholesterol values and also helps maintain proper blood pressure (Homstia, 1987; Yuen et al., 2011; Edem, 2002). Both crude and refined palm oil helps to maintain proper blood pressure due to the high antioxidant content of the oil. These antioxidants quenches free radicals and keep inflammation that causes swelling that narrows artery passage way restricting blood flow to vital organs such as the heart under control (Esterhuyseet al., 2005). Tocopherol one of the phyto nutrients of palm oil are beneficial to consumers who want to maintain healthy brain (neuroprotection), blood lipid level, arterial compliance (reducing arterial stiffness), liver health, skin nutrition, immune protection and inhibit the growth of skin, stomach pancreas, liver, lung, colon, prostate, breast and other cancers (Rink et al., 2011; Patel et al., 2012; Qureshi et al., 1995; Yano et al., 2005).

The antioxidant power of red palm oil is of help in protecting against a variety of health problems including osteoporosis, asthma, cataract, macular degeneration, arthritis and liver diseases. It also stunts the processes that promote premature aging (Khanna et al., 2003).

## MATERIALS AND METHODS

### Sample collection

The fresh samples of *Nigrescens* (ordinary) and *Virescens* (Ojukwu) types of *E. guneensis* palm fruit were collected from Oghe community in Ezeagu LGA, Enugu state on March 10, 2014 and authenticated by Prof. JC Okafor of Applied Biology and Biotechnology Department, Enugu State University of Science and Technology.

### Isolation of oil from the samples

The riped fruits of *Nigrescens* and *Virescens* types of *E. guineensis* were each boiled in water and pounded to disintegrate the pulp, thus freeing the nut. The traditional method of oil expression in some parts of West African was used. The fresh pulp was re-cooked with a large volume of water (1:5 v/v). The oil floated on top and was skimmed off and stored in different container for analysis, respectively.

### Physical characterization

#### *Ubbbelohde melting point determination*

Each oil in a capillary tube was allowed to freeze in a freezer for 1 h and heated slowing in a water bath. The temperature at which the oil began to slip in the capillary tube was recorded as the slip point or melting point (Ubbolohde melting point method).

### Solidification point (titre value) determination

The oil in a capillary tube was allowed to flat in watch glass on a water trough. Blocks of ice were added continuously until the oil solidified and the temperature recorded.

### Viscometric studies

The time taken for the oil to fall between the two graduation Marks on Oswards viscometer at room temperature was recorded (Ikhuoria and Maliki, 2007).

### Moisture content

The percent loss in weight of oil sample heated in a drying oven at 105°C for 2 h and weighed at an interval of 30 min till a constant weight was obtained. Initial weight of oil sample = a gm, Final weight of oil sample after drying = b gm, the dry weight percent = (a/b)*100.

Moisture content (x %) = (1-(a/b))*100 (AOAC, 1980).

### Chemical characterization

#### *Determination of iodine value*

Twenty five (25) milliliters of iodine monochloride was added to 1 01 g of the oil, stoppered and left to stand in the dark alongside a blank without the oil sample and 10 mL of chloroform added instead; for 1 h. The flask was rinsed with 50 mL of distilled water and 10 ml of 10% KI solution was added. The liberated Iodine was immediately titrated with 0.1 M $Na_2S_2O_3$ until the iodine solution was brownish yellow then 1 mL of starch solution indicator was added. The titration was continued until the developed blue colour disappeared. The volume of the $Na_2S_2O_3$ was used to calculate the iodine value.

$$\text{Iodine value} = \frac{(\text{Blank- Titre value}) * \text{molarities of } Na_2S_2O_3 * 12.69}{\text{Weight of sample gm}}$$

**Determination of saponification value (JIS K 007- 1992)**

Two (2) grams of the oil was refluxed with 25 mL of alcoholic potassium hydroxide solution (0.5 M) for 1 h with frequent shaking. The excess alkali was titrated with 0.5 M hydrochloric acid and 1 mL of phenolphthalein indicator. A Blank titration was carried out alongside and the Saponification value calculated thus:

$$\text{Saponification value} = \frac{(\text{Blank-Titre value})*28.05}{\text{Weight of oil (g)}}$$

**Acid value determination (Ejim and Kamen, 2013)**

Two grams of the oil sample was dissolved in 25mL diethyl either with 25mL ethanol was titrated with 0.1M NaOH solution and 1mL of phenolphthalein indicator until a faint pink colour persisted for 15 seconds.

Acid value = %FFA (as Oleic)* 1.99

**Peroxide value determination (Eddy et al., 2011)**

One (1) gram of the oil sample was allowed to boiled with 1 g potassium iodide and 20 mL of solvent mixture (Glacial acetic acid and chloroform [2:1] v/v) for 30 s and then vigorously for another 30 s. This was poured into 20 mL of 5% potassium iodide and the boiling tube washed twice with 25 mL of distilled water. This was titrated with 0.002 M of the $Na_2S_2O_3$ using starch indicator, a blank was similarly titrated. Calculation

$$\text{Peroxide value} = \frac{1000(V_2-V_1)T}{M}$$

Where M = mass of oil taken (1 g); $V_2$ = volume of 0.1 N $Na_2S_2O_3$; $V_1$ = volume of 0.1 N $Na_2S_2O_3$ used I blank and T = nomlity of $Na_2s_2O_3$ (0.1 N)

**Determination of ester value (JIS K 0070-1992)**

Two (2) grams of the oil sample was refluxed for 1 h with 25 mL of aqueous sodium hydroxide in a water bath. The condenser was washed down with 5 mL of distilled water and the content allowed to cool down to room temperature. The excess alkali was titrated with 0.5 M HCl using phenolphthalein as indicator. A blank titration was repeated without the oil sample.

Ester value = Saponification value – Acid value.

**Protein determination (nitrogen) through Kjedahls method**

Half a gram of the oil sample was digested by heating in an inclined position with 1 g of digestion catalyst mixture and 5 mL of concentrated sulphuric acid. The flask was stoppered loosely with cotton wool. After fronting subsided, then it was heated vigorously until the solution became clear. The digest was allowed to cool down to room temperature, then 25 mL of distilled water was added with 20 mL of 10 M NaOH through a funnel with tap which was closed after the addition to act as a seal with little water. The kjedahls flash content was heated and the stream absorbed into 25 mL of 0.04 M HCl for 3 min. This was titrated with 0.02 M sodium hydroxide using phenolphthalein as indicator. A blank determination was carried out side by side using glucose in place of the oil sample. The value obtained in the computation was multiplied by 6.25 (Chaco et al., 1993).

**Extimation of hydroxyl group**

Half a gram of the oil sample was refluxed with 10 mL of acetylating mixture (pyridine and acetic anhydride [3:1v/v]) in a water bath for 1 h. The condenser was washed with 20 mL of distilled water into the mixture and gently shaken. This was allowed to cool for 10 min and titrated with 1 M NaOH using phenolphthalein as indicator. A black was similarly titrated (Chaco et al., 1993).

***Protein hydrolysis***

Half a gram of the oil sample was refluxed with 20 mL of 20% hydrochloric acid with two pieces of anti-bump for 45 min using a very low flame. Three milliliter of the hydrolysates was carefully neutralized with 10% sodium hydroxide. The solution was made alkaline with 1 mL of 2 M NaOH (Linstronberg and Ballmgaten, 1966).

***Protein hydrolysis***

Half a gram of the oil sample with 5 mL water and 5 mL hydrochloric acid was sealed in 25 mL ampoule. The ample was wrapped in a cotton wool and heated in an electric over at 135°C for 5 h. After cooling down to room temperature the ampoule was opened and the content heated in a crucible to dryness until the odor of hydrochloric acid is no longer detectable in water bath. Then the residue was dissolved in 1 mL of distilled water (Beckett and Stenlake, 1974).

**Separation of $\alpha$- amino acid by paper chromatography**

A rectangular piece of Whatman no.1 chromatography paper (30 cm x 10 cm) was used to separate the $\alpha$-amino acid present in the hydrolysate using 80% phenol solution as solvent system (Linstromberg and Ballmgaten, 1966). The chromatogram after development are visualized after washing with acetone and allowed to dry with 2% solution of ninhydrin in 95% ethanol. The $R_F$ values computed were compared with those given in literature (Linstromberg and Ballmgaten, 1966).

**Two dimensional separation of amino acid by paper chromatography**

The chromatogram, developed with 6% acetic acid solvent system by the method of ascending chromatography in one dimension using 6% acetic acid. The paper was dried and turned at right angles to the first and developed with a second solvent system either n-butanol- acetic acid - water (4:1:5). The chromatogram developed was dried and sprayed with 0.1% nihydrin in n-butanol saturated with water and heated at 90°C for 10 min. Each spot was circled (Hartley, 1988).

**TLC analysis of fatty acids**

TLC plate (F-254 type E) was used to separated the fatty acids in the *Virescens* and *Nigrescens* oils in comparison to standard fatty acids using n-hexane: ethyl ether: acetic acids [80:20:1] solvent system. The chromatogram after development is visualized by

**Table 1.** The results of the physicals and chemical properties of Nigrescens and Virescens palm oils.

| Property | *Nigrescens* | Virescens |
|---|---|---|
| **Physical property** | | |
| Specific gravity | 0.9002 | 0.9116 |
| Melting point [slip point] | 33°C | 33°C |
| Solidification [titre] point | 22°C | 15°C |
| Viscosity [centistokes] | 51.20 | 29.89 |
| Moisture content [%] | 1.6 | 0.2 |
| **Chemical property** | | |
| Iodine value | 53.98 | 83.82 |
| Saponification value | 223.7 | 222.3 |
| Acid value | 40.46 | 29.73 |
| Peroxide value [1 wk old] | 8.0 | 15.0 |
| Peroxide value [4 wk old] | 12.3 | 18.0 |
| Hydroxide group [%] | 2.55 | 3.23 |
| Ester value | 263.16 | 265.78 |
| %purity Ester | 21.32 | 21.11 |
| Phospholipids [µg/ml] | 0.35 | 0.76 |

spraying with 10% phosphomolybdic acid solution in a fume hood and heated in an electric oven at 70°C for 20 min (Moran et al., 1994; Moran and Serimgeour, 1994; Plumer, 1971).

#### Quantitative determination of phospholipids

Colorimetric determination through an acidic digestion method was used. One milliliter of the oil sample was digested by heating with 0.65 mL of 70% perchloric acid until the yellow colour disappeared alongside the standard (0.10 to 0.90 µg/mL $KH_2PO_4$). The digests were diluted with 3.5 mL of distilled water followed by 0.5 mL of ammonium molybdate solution and 0.5 mL of ascorbic acid solution. The content was shaked very well and heated in a boiling water for 30 min for colour development. The absorbances of cool samples (including the standards) are read at 800 mm. (Rouser et al., 1970).

## RESULTS AND DISCUSSION

Table 1 shows the results of the physical and chemical parameters of *Nigrescens* and *Virescens* palm oils as determined. The result of the iodine value presented in this study shows that *Virescens* and *Nigrescens* (ordinary) palm oil belongs to a class of oil known as non-drying oil. This evident in the iodine number of the oils which is less than 110 for non-drying oils. However, the *Virescens*oil can be grouped with olive oil because the iodine value and peroxide value are relatively within the same range (80 to 90 and 10 to 20), respectively. They are also both liquid below room temperature, the specific gravity of both are within the same range (0.910 to 0.916) (Thomas, 2002; Codex Stan 33 to 1981; 2001). Furthermore, the rather very high iodine number than *Nigrescens* (ordinary) palm oil well suggested that *Virescens* palm oil have components which may have higher degree of unsaturation than *Nigrescens* (Ordinary) palm oil. Moreover, it also suggested that oleic acid may be more than palmitic acid in *Virescens* palm oil. It becomes possible that *Virescens* palm oil could be very easily hydrogenated and used in cosmetics and creams than *Nigrescens.* The degree of unsaturation of the two oil suggested that the melting point will not be low. This is true because unsaturation lowers melting point, *Virescens* and *Nigrescens* palm oils are only a little unsaturated. This much agrees with the fact that palmitic and oleic acid have boiling and melting points which are generally high. These oils cannot therefore be used in making paints and varnishes. Interestingly, *Virescens* and *Nigrescens* palm oils have high saponification values. In agreement with many previous studies, this implies that the oil contains few carbon chains and produces very large acid per gram of fatty acid (Chemical and Process Technology Encyclopedia, 1974). It contains very large glycerides and can be easily sapoinfied-for use in soap production. This is further supported by the titre (solidification) value of the oils which is 22°C for *Nigrescens* and 15°C for *Virescens*; for oils that are good for making soap must have high titre value.

Generally, acid value gives idea about the purity of oil. High value implies high content of fatty acids which in turn implies low purity. On the other hand, low value means that the oil contains low amount of fatty acids and is pure. Acid value may also indicate the age of oil. The acid value obtained for *Nigrescens*palm oil is high (40.67) while that of *Virescens* palm oil is low (29.73) and is therefore of high purity than *Nigrescens*palm oil. From the specific gravity determination it may be inferred that the density of *Virescens* palm oil (0.9116) is within the range of specific gravity of olive oil which is between 0.910 to 0.916 while that of *Nigrescens* palm oil is 0.9002. The peroxide value of the *Nigrescens* palm oil is 8 within the first week old and 12.3 after four weeks. While that of the *Virescens*palm oil is 15 with one week old and 18 after four weeks old. This is probably due to the age and degree of unsaturation (higher iodine value) of *Virescens* palm oil, because the greater the degree of unsaturation, the greater is the liability of the oil or fat to oxidative rancidity (Pearson, 1976). The peroxide value of *Virescens* palm oil is closer to that of olive oil which is always less than 20 (Tayeb, 2013). It is worthy to note that both oils have high ester value probably due to the high saponifiable ester content of the oils. They have also the same percent purity ester. Both oils have alanine amino acid while *Nigrescens*palm oil has proline amino acidand *Virescens* palm oil has cystine and phenylalanine amino acids Viscosity of the *Nigrescens* (51.20 Centistokes) palm oil (Table 1) appears to be nearly double that of *Virescens* palm oil (29.89 centistokes). This is probably due to the high titre (solidification value of the *Nigrescens*palm oil than *Virescens* palm oil.

**Table 2.** TLC analysis of Fatty acids/other lipids in *Nigrescens* and *Virescens* palm oils with standards.

| **Fatty acids/ other lipids** | | |
|---|---|---|
| Oleic acid | + | + |
| Stearic acid | + | + |
| Palmitic acid | + | + |
| Lecithin | - | + |
| Tocopherol | + | + |

**Table 3.** Amino acids present in Nigrescens and Virescens palm oils.

| **α-Amino acid** | | |
|---|---|---|
| Cystine | - | + |
| Alanine | + | + |
| Proline | + | - |
| Phenylalanine | - | + |

The result of the thin layer chromatographic identification of fatty acids and other lipids (Tables 2 and 3) with standards revealed that *Nigrescens* palm oil contain Tocopherol, Oleic acid, stearic acid, palmitic acid, while *Virescens*palm oil contain tocopherol, oleic acid, stearic acid, palmitic acid and lecithin. Cholesterol was not identified in the two oil samples. The estimation of the total amount of phospholipids in the oil samples revealed that *Virescens* palm oil contains 0.76 mg/ml while *Nigrescens* (ordinary) palm oil contains 0.35 mg/ml. With the results of the study, it becomes obvious that *Virescens* palm oil is more healthy oil than *Nigrescens* palm oil. This is probably due to the large proportion of unsaturated fatty acids that is heart friendly. Finally, it can be concluded that the relationship of the *Virescens*palm oil and olive oil, more so, the presence of cystine amino acid in both of them and the amount of phospholipids in *Virescens* palm oil accounts for the anti-poison and medicinal characteristics of the Virescens palm oil.

## Conflict of interests

Theauthor(s) did not declare any conflict of interest.

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