PROXIMATE ANALYSIS, PHYTOCHEMICAL SCREENING AND PHYSICOCHEMICAL PROPERTIES OF *Cocos nucifera* OIL EXTRACT

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Abstract: Proximate analysis, physicochemical properties and phytochemical screening of *Cocos nucifera* oil were carried out using standard methods. Mineral composition of the oil was also determined. The results obtained showed that the percentage oil yield was 35.40%. The proximate analysis gave % moisture, ash, crude fiber, fat, protein and carbohydrate contents as 38.70±1.0, 3.15±0.1, 6.04±0.1, 35.10±0.1, 13.13±1.0 and 3.88±1.0, respectively. The physicochemical properties of the oil are as follow; specific gravity (0.95±0.05), iodine value (48.73±0.6), FFA (0.45±1.4), peroxide value (11.00±0.5 MeqH_2O_2), saponification value (10.24±0.3 MgKOH/g) and unsaponifiable matter (9.60±0.16%). Phytochemical result shows the presence of 0.21 mg of Flavanoid, 0.02 mg of saponin and 0.92 mg of alkaloid. Analysis of the mineral constituents of the oil extract shows the presence of Sodium, Potassium, Calcium, Magnessium and Iron in the order 3.2±1.0 mg/100g, 2.12±0.1 mg/100g, 1.48±1.1 mg/100g, 0.25±0.1 mg/100g and 1.64±0.1 mg/100g, respectively. These results may suggest the increased use of *Cocos nucifera* oil for food, medicinal, cosmetic and pharmaceutical purposes.

Keywords: *Cocos nucifera*, lipid, phytochemicals, minerals, rancidity

Introduction

Coconut (*Cocos nucifera* Linn; Family: Palmae) is one of the most extensively grown and used nuts in the world and it is rated as one of the most important of all palms (Onifade & Jeff- Agboola, 2003; Popenoe, 1969). Out of the 100 product that are directly or indirectly made from coconut, eighty are important in world trade. These are whole coconut, copra, coconut oil, coconut oil cake, coir (is a natural fiber extracted from the husk of coconut and used in products such as floor mats, door mats, mattresses, etc. it is the fibrous material found between the hard, internal shell and outer coat of coconut), desiccated shredded coconut, skim milk and coconut protein. It can be used to produce desired texture in cookies, candies, cakes, pies, salad and deserts. Coconut is commercially viable because of its rich nutritive values (Akubugwo *et al*., 2008; Kyari, 2008 & Child, 1964).

The oil and milk derived from it are commonly used in cooking and frying; Coconut oil is also widely used in soap making and cosmetics. The clear liquid coconut water within is portable; the husks and leaves can be used as material to make a variety of products for furnishing and decorating. It also has cultural and religious significance in many societies that use it.

Nutritional properties of coconut

Coconut is a very versatile and indispensable food item for most people under the tropical belt. It is a complete food rich in calories, vitamins and minerals. A medium-size nut carrying 400 g edible meat and some 30–150 ml of water may provide almost all the daily-required essential minerals, vitamins and energy of an average-sized individual. Coconut oil is made up of 100% fat. However, the structure of fat in coconut oil differs from the traditional saturated fat often found in animal products (primarily comprised of long-chain fatty acids). The important saturated fatty acid in coconut is Lauric acid (1:12 carbon fatty acid). Lauric acid increases good High density lipoprotein (HDL) cholesterol levels in the blood (Ware, 2015).

According to the USDA National Nutrient Database, one tablespoon of coconut oil contains 117 calories, 0 grams of protein, 13.6 grams of fat (11.8 saturated, 0.8 monounsaturated and 0.2 polyunsaturated) and 0 grams of carbohydrate (0 grams of fiber and 0 grams of sugar). It provides little to no vitamins or minerals.

Coconut water is a very refreshing drink to beat tropical summer thirst. The juice is packaged with simple sugar, electrolytes, minerals, and bioactive compounds such as cytokinin and enzymes such as acid phosphatase, catalase, dehydrogenase, peroxidase, polymerases. Although, these enzymes aid in digestion and metabolism; it is used in cooking, applied over scalp as hair nourishment, employed in pharmacy and in medicines (Rele & Mohile, 2003). It is also a very good source of B-complex vitamins such as folates, riboflavin, niacin, thiamin and pyridoxine. These vitamins are essential in the sense that body requires them from external sources to replenish. Coconut meat and water contain a very good amount of potassium. 100 g of fresh meat contains 356 mg% or 7.5% of daily required levels of potassium.

Antioxidant and health properties of coconut

Antioxidants are agents which scavenge free radicals and prevent the damage caused by Reactive oxygen species (ROS) and Reactive nitrogen species (RNS). Reactive oxygen species (ROS) is composed of superoxide anion (O^{2-})' hydroxyl (OH^{+}), hydroperoxyl (OOH^{-}), peroxyl (ROO^{-}), alkoxyl (RO^{-}) ions. Antioxidants can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells; prevent damage to lipids, proteins, enzymes, carbohydrates and DNA. Nowadays, search for natural antioxidant source is gaining much importance (Enig, 1999).

The roots of coconut are astringent, diuretic and anthelmintic and are useful in pharyngodyria, uterine disorders, olennorrhagia, bronchitis, hepatopathy, strangury and helmenthiasis. The juice of the young spadie when fresh is sweet, refrigerant. The shell is cooling and is good for hyperdipsia, strangury and halitosis. The kernel is sweet, cooling, and is useful in bronchitis, vitiated condition of pitta, tumors, skin diseases, fever and general diebility. The water is sweet, cooling, and digestive. The objectives of this research is to do the proximate analysis, study the physicochemical properties and screen coconut

oil extract phytochemically so as to unravel useful properties of the oil as it relates to health.

Materials and methods

Materials

Cocos nucifera known as coconut was used as sample and was bought from Anyigba main market, Kogi State, Nigeria. The coconut was cracked open by hitting the shell on a hard surface. This separated it into several parts; the flesh was then removed from the shell using a knife. The flesh was washed, cleaned after which the sample was chopped into smaller pieces and further ground using mortar and pestle. The sample was oven dried until required for analysis. Wijs reagent, Carbon tetrachloride (CCl_4), Potassium hydroxide, Hydrochloric acid, Phenolphthalein indicator, Acetic acid and chloroform solvent mixture, Potassium iodide, Sodium thiosulphate, Starch indicator, Diethyl ether, Ethanol, Sodium hydroxide were all from BDH in the UK. Distilled water used was fully characterized.

Methods

The method used for the extraction and determination of the percentage oil yield is the Franz von soxhlet extractor method described by AOAC (2000). Oven-dried coconut samples of fifty grams (50.00 g) was weighed and placed in a thimble to extract the oil using petroleum ether (boiling point 40-60℃). There was continuous extraction for six hours. The solvent was recovered and the defatted oil sample was removed and cooled in a desiccator. The oil content was reweighed to determine the weight of the oil. The percentage oil yield was obtained by expressing the oil weight as a percentage of the weight of the sample.

Percentage oil yield% = weight of sample + thimble before extraction - weight of sample + thimble after extraction x 100/weight of sample.

Percentage oil yield (%) = weight of oil x 100/weight of sample

The **moisture content** was determined by a procedure described by Pearson (1991)

$$Moisture\ content\ \% = \frac{weight\ loss\ of\ oil}{weight\ of\ oil} \times 100$$

Ash content was determined using the procedure described by Oyeleke (1984).

$$Ash\ content\ \% = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

Determination of specific gravity

A clean and dried density bottle with a stopper was weighed. The density bottle was then filled with cold distilled water and kept in a water bath for 30 min at 25^0C. The weight of the bottle together with the water was taken. After weighing, the bottle was emptied and then dried in an oven for 2 min. The dried bottle was then filled with the oil and then weighed. The specific gravity was calculated using the formula below:

$$Specific\ gravity = \frac{W_2 - W_0}{W_1 - W_0}$$

Where: W_o= weight of empty bottle (g)
W_1= weight of bottle (g)
W_2= weight of bottle and oil (g)

Determination of crude fiber

Defat 2 g of sample with petroleum ether (if the fat content is more than 10%). Boil under reflux for 30 min with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100 ml of solution. Filter the solution through linen or several layers of cheese cloth on a fluted funnel. Wash with boiling water until the washings are no longer acid. Transfer the residue to a beaker and boil for 30 min with 200 ml per 100 ml. Filter the final residue through a thin but close pad of washed and ignited asbestos in a Gooch crucible. Dry in an electric oven and weigh. Incinerate, cool and weigh. The loss in weight after incineration x 100 is the percentage crude fiber. The crude fiber content of the sample is given by the expression:

$$\frac{b-c}{a} \times 100$$

Where: a = mass of sample in g; b = loss of mass after ashing during the determination in g; c = loss of mass after ashing during the blank test in g.

Determination of fat content

Dry 250 ml clean boiling flasks in oven at 105–110^0C for 30 min. Transfer into a desicator and allow cooling. Weigh about 2 g of samples accurately into labeled thimbles. Weigh corresponding labeled, cooled boiling flasks. Fill the boiling flasks with about 300 ml of petroleum ether (boiling point 40°C–60°C). Plug the extraction thimble lightly with cotton wool. Assemble the soxhlet apparatus and allow refluxing for about six hours. Remove thimble with care and collect petroleum ether in the top container of the set up and drain into a flask for re-use. When flask is almost free of petroleum ether, remove and dry at 105°C–110°C for one h. Transfer from the oven into a dessicator and allow cool and then weighing.

Calculation:

$$\%Fat = \frac{weight\ of\ fat}{weight\ of\ sample} \times 100$$

Protein content was according to AOAC (2010).

The conversion is done using the formula below:

$$\%\ protein = \%\ N\ x\ F = \frac{100}{\%\ Nitrogen\ in\ food\ protein}$$

Where: F= conversion factor= 100/ (% Nitrogen in food protein). The common factor used for most foods and food mixture is 6.25.

Carbohydrate content was determined using carbohydrate by difference (AOAC, 2010).

$$\%\ Carbohydrate = 100 - [protein\ content + moisture\ content + ash\ content + crude\ fiber + fat\ content].$$

Total **alkaloid** content was measured using the method described by Harborne, (1998).

Formula for calculation of alkaloid:

$$Alkaloid\ (mg/g) = \frac{Weight\ of\ residue}{Weight\ of\ sample}$$

Total **flavonoid** content was determined using the method described by Harborne, (1993).

$$Flavonoid\ (mg/g) = \frac{Weight\ of\ residue}{Weight\ of\ sample}$$

The total **saponin** content was measured using the method described by Obadoni and Ochuko, (2001).

$$Saponins\ (mg/g) = \frac{Weight\ of\ residue}{Weight\ of\ sample}$$

The method was described by Pearson, (1976). The **tannin** content was given as follows:

%Tannin= An/As x C x 100/W x Vf/Va

An= absorbance of test samples, As = absorbance of standard solution

C= concentration of standard solution, Vf = total volume of extract, Va = volume of extract analyzed.

The **phenol** content was determined using the method described by Trease and Evans, (2002).

Saponification value was determined according to Pearson (1991)

$$Saponification\ value\ (mgKOH/g) = \frac{(S-B)\ x\ M\ x\ 56.1)}{Weight\ of\ oil\ sample\ (g)}$$

Where S= sample titre value (ml)
B= Blank titre value (ml)
M= Molarity of HCL
56.1= Molecular weight of KOH

Unsaponifiable matter was determined using Pearson, (1991).

$$\text{Unsaponifiable matter (\%)} = \frac{Weight\ of\ residue}{Weight\ of\ sample}\ x\ 100$$

Peroxide value ($mEqH_2O_2$) was determined according to Pearson (1991)

$$Peroxide\ value\ (mEqH2O2) = \frac{(S-B)x\ M\ x\ 100}{W} x\ 100$$

Where, S= Sample titre vale (cm^3), B= Blank titre value (cm^3), M = Molarity of $Na_2S_2O_3$ solution (mEq/Cm^3), 1000 = Conversion of unit (g/kg), W= Weight of oil sample.

Iodine value was carried out using A.O.A.C, (1997).

$$Iodine\ value\ \left(\frac{gI2}{100g}\right) = \frac{12.69\times c\times(V_1-V_2)}{w}$$

Where, C= Concentration of sodium thiosulphate
V_1= Volume of sodium thiosulphate used for blank
V_2= Volume of sodium thiosulphate for test
W= Weight of sample

FFA was determined using the method described by Pearson (1991).

$$\%FFA = \frac{V\times M\times 282}{w}\ x\ 100$$

Where: %FFA= Percentage free fatty acid (Oleic acid)
V= Average volume of NaOH used (cm^3)
M= Molarity of NaOH
282 g/mol= Molecular weight of Oleic acid, W= Weight of oil.

Results and Discussion

The coconut oil appeared colourless and since the oil has no smell, any case of adulteration can be easily detected. The extraction procedure revealed that *Cocos nucifera* oil extract had a high percentage of oil yields. The percentage oil yield for *Cocos nucifera* oil extract was 35.40% which is higher compared to the value obtained by Onyeike & Acheru (2002) which was (23.13±0.44%), indicating that the oil is rich in Lipids. The moisture content of the *Cocos nucifera* oil was gotten to be 38.70±1.0% (Table 1); it is desirable to keep the moisture content low as it will prevent oxidative rancidity. This is because any oil that has a moisture level greater than 15% is subject to rapid deterioration. Thus, the *Cocos nucifera* with high moisture content suggests that the oil is subject to rapid deterioration from mold growth and early sprouting. The specific gravity of *Cocos nucifera* which is the ratio of the density of a substance, which amounts for the concentration of the oil, has the value of 0.95±0.05g/L. From Table 2, the iodine value of *Cocos nucifera* oil which is used to determine the amount of unsaturation in fatty acids was gotten to be 48.73±0.63%. This value indicates that the oil is highly saturated and fall into the class of lipids called non-drying oils (oils with iodine value below 100). The oil will be suitable for margarine manufacture, since it is non-drying oil, they can be used in perfume, food, medicine, pharmaceutical and chemical industries.

Table 1: Proximate analysis of cocont oil extract

Parameters	Value (%)
Moisture content	38.70±1.0
Ash content	3.15±0.1
Crude fibre	6.04±0.1
Fat content	35.10±0.1
Protein content	13.13±1.0
Carbohydrate content	3.88±1.0

Table 2: Physicochemical properties of coconut oil extract

Percentage oil yield (%)	35.40
Specific gravity	0.95± 0.05
Iodine value (gI_2/100g)	48.73±0.63
Free fatty acid (%)	0.45±1.46
Peroxide value ($MEqH_2O_2$)	11.00±0.50
Saponification value (Mg/KOH/g)	10.24±0.3
Unsaponification matter (%)	9.60±0.16

Free fatty acid is the amount of free acids present per gram of the sample. The percentage of free fatty acid obtained from Table 2 was 0.45 ± 1.46%, this shows that the higher the amount of fatty acid present in the oil, the higher the smoke, flash and fire point of the oil. High quality oils are low in free fatty acids (AOAC, 1997). The result of free fatty acid in this work that is 0.45 ± 1.46%, suggests that the oil is of a high quality. Peroxide value is a measure of oxidative rancidity of oil. Oxidative rancidity is the addition of oxygen across the double bounds in unsaturated fatty acids in the presence of enzymes or certain chemical compounds. High peroxide values are associated with higher rate of rancidity. The value obtained was 11.00 ± 0.05 $mEqH_2O_2$ which indicate the coconut oil has high chance of becoming rancid (Bligh & Dyer, 2007), the value is also higher than that obtained and reported earlier by Bligh and Dyer, (2007) which was (0.32 ± 0.12 $mEqH_2O_2$). The coconut oil showed it has saponification value of 10.24 ± 0.3 mgKOH/g which is lower than the saponification values of the following oils; *Citrus lanatus* 189.35 mgKOH/g, *Adansonia digitata* Linn 230.01 mgKOH/g, *Elaeis guineensis* 246.60 mgKOH/g (Ibironke, 2010). The low value obtained indicates that this oil is a poor ingredient for soap making as compared with the saponification value of other oils. The *Cocos nucifera* oil showed it has low unsaponifiable matter content of 9.60 ± 0.16%, this shows that the levels of sterols, carbohydrate and higher alcohols are very low thereby classifying the oil as of normal purity. Unsaponifiable constituent are an important consideration when selecting oil mixtures for the manufacture of margarine, food, medicine, pharmaceutical and chemical industries, as a component of drug and as an antioxidant agent.

The results of the qualitative pytochemical screening of the oil extract indicate that flavonoids, saponnins and alkaloid are present in moderately high concentrations while tannins and phenols were undetected (Table 3)

Table 3: Phytochemical screening of coconut oil extract

Phytochemicals	(Mg/100g)
Flavanoid	0.21
Tannin	Not detected
Alkaloid	0.92
Saponin	0.02
Phenol	Not detected

Table 4: Mineral content of coconut oil extract

Minerals	(Mg/100g)
Sodium (Na)	3.20±1.0
Potassium (K)	2.12±0.1
Calcium (Ca)	1.48±1.1
Magnesium (Mg)	0.25±0.1
Iron (Fe)	1.64±0.1

The analysis of the mineral element composition of coconut oil as shown in Table 4 revealed the presence of Iron, Potassium, Sodium, Magnesium and Calcium. Calcium is an important component of a healthy diet and one of the essential minerals necessary for life. Calcium plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life. The RDA for calcium per day is 1000 to 1500 mg (Larsen, 2007). The concentration of calcium in the oil was found to be 1.48 ± 0.1 mg/100g. It is essential to support growth, it prevents life threatening hemorrhage. The value obtained from the Coconut oil extract shows it is poor source of calcium as compared with the daily requirement.

Magnesium serve as a co-factor of many enzyme, involved in energy metabolism, protein synthesis, RNA and DNA synthesis, maintenance of electrical potential of nerve cells and cell membrane. The total concentration of magnesium in the oil was found to be 0.25 ± 0.1 mg/100g. The daily requirement of 150 to 500 mg is needed (FAO/WHO, 1987), indicating that this oil is a poor source of magnesium since it is below the daily requirement.

Conclusion

Cocos nucifera is a good source of oil because it has moderate oil content with % yield of 35.40. The phytochemical constituents present in the oil are generally moderate in concentration. Therefore, this has the advantage of interring pharmacological attributes in the oil. Although, the oil is a fair source of iron and a poor source of sodium, calcium, potassium and magnesium, which is because the concentrations of these elements in the oil may not meet the adequate requirement needed by the body daily. The oil also contains important phytochemicals like flavonoids in very high amounts. These flavonoids provide protection against cardiovascular diseases by contributing to the total antioxidant defense system of the human body. Thus, coconut oil can be said to have nutritional and pharmacological benefits.

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