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Evaluation of physiochemical, antioxidant, proximate and nutritional values of virgin coconut oil (*Cocus nucifera)*.

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Abstract

Virgin coconut oil can be a good food supplement due to its high medium – chain fatty acids unlike other cooking oils which contain long – chain fatty acids. This research is to investigate the physiochemical, antioxidant properties, proximate and nutritional values of Virgin coconut oil (VCO). The extract of virgin coconut oil was investigated for its proximate and nutritional composition showed that coconut oil can be good food supplement. The physiochemical parameters showed low iodine, saponification, peroxide, and acid value of 0.5918, 134.50, 1.5 and 0.673 meq/kg respectively. The antioxidant activity was investigated using DPPH free radical scavenging, the result showed that the VCO inhibits in the range of (37.0 – 61.0) % while Vitamin C (control) inhibits within (93-97) %. The proximate and nutritional values analyses showed that the moisture content, ash content, crude fibre, crude protein, crude lipid, and carbohydrate were 14.28, 1.075, 7.22, 9.255, 39.72 and 28.45 respectively. Minerals found were K, C, Na, Fe, Ca, Zn and P 138.15, 0.015, 58.6, 1.82, 38.55, 5.35 and 0.79 mg/kg respectively.

Keywords: Virgin coconut oil, Iodine value, saponification value, rancidity, peroxides Value.

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1. Introduction

There seems to be continues decline in the health status of most persons in most developing countries, especially Nigeria. Several studies have linked this challenge to the kind of food they consume and general lifestyle [1-5]. A lot of studies have blamed the nature of oil mostly consumed in Nigeria, and have linked oil consumption to diseases like cardiovascular diseases, liver diseases, stroke and so on. However, to this end, experts, industrialists and scholars have been looking for alternative oils that would be healthier for the Nigeria population. One of the sources of oil that have been given a reasonable consideration in this regard is the virgin coconut oil (VCO). VCO can be defined as "an edible oil obtained from the fresh, mature kernel of coconut by mechanical or natural means, with or without the use of heat, without undergoing chemical refining, bleaching or deodorising, which does not lead to the alteration of the nature of the oil" [6-10]. Coconut is the source of virgin coconut oil. Its botanical name is *Cocos nucifera*, of the family *Arecaceae*.

In Nigeria, different tribes, has different names they call coconut, the Hausas, call it *Kwakwa*, the Yorubas call it *Agbon*, the Igbo call it *Akioyibo* and the Igalas from the Eastern part of Kogi state call it *uno-ba*. The virgin coconut oil is colorless and clear with the aroma of fresh coconut. Various researches have revealed that the factors such as location, age of nut, time of nut harvested and the age of copra affect yield. Coconut oil contains medium chain fatty acids of about 63%, which does not increase the cholesterol level in the blood. Medium chain fatty acids (MCFA) are transported directly to the liver to immediately provide energy and not deposited as fat [11-13]. The MCFA is gotten coconut oil, which makes it unique and different from other fat and oil, other oil contains Long Chain Fatty Acids (LCFA) which are most times not friendly to the human body. It has been used in the clinical area of enteral and parenteral nutrition in diverse medical conditions for the treatment of patients that suffer from fat mal-absorption [14-16]. Coconut has been used traditionally for treating the skin and hair growth since centuries ago. The quality characteristics of coconut can be identified through its physical and chemical properties such as iodine value, acid value, and peroxide value.

Therefore, this research is aimed at investigating the physiochemical properties, antioxidant activities, proximate and nutritional values of virgin coconut oil (VCO).

2. Materials and methods

Materials

The material used for this research is coconut (*Cocos nucifera)* of the family Arecaceae (palm family).

Reagents use

The reagent used includes: Ethanol, Chloroform, Acetic acid, Phenophitalin, Wijs reagent, Starch reagent, Potassium hydroxide, Sodium thiosuphate, Hydrochloride, Carbon tetrachloride, 1,1-diphenyl-

2-picrylhydrazyl (DPPH), methanol, distilled water, Selenium catalyst, Sulphuric acids, Boric acids indicator, Sodium hydroxide, n-hexane, Petroleum etherand Nitric acid.

Photo 1 : coconut (*Cocos nucifera)* from Nigeria

Apparatus used

The apparatus used includes: Conical flask**,** crucible, macro-kjeldahl digestion flask, Beaker, Retard stand, Burette, Pipette, Water bath, Hot plate, Sieve material, Bucket, Digestion block, Volumetric flask, Soxhlet, Filter paper, Cotton wool, Buchner funnel, Oven, Desiccator, Muffle furnace, Atomic absorption spectrophotometer (AAS) and Flame photometer.

Method

Collection of sample

The coconut was gotten from Anyigba market, Dekina Local Government Area of Kogi State, Nigeria.

Preparation of sample

Virgin coconut oil (VCO) was separated from the coconut as follows. The fresh coconut meat was shredded and then grated to make coconut milk. The coconut milk was mixed with warm water and then filtered with sieve material to separate the coconut milk from the chaff; this process was done repeatedly until the milk was fully recovered. The protein presents the coconut milk creates an emulsion of oil and water; the milk was left in a bowl been covered for 24 hrs at room temperature. Then form a layer, the upper layer that contains the oil and chaff was discarded. The sample gotten was heated for about 1hr at a temperature of 40˚C, this leaves behind the crude oil and some residues. It is separated out by filtering using sieve material. The virgin coconut oil was stored in air tight container for further investigations [17].

Determination of Percentage Yield

The yield, in percent, was calculated from the weighed mass of sample and the weighed mass of oil extracted, using the formula below:

$$yield\ (\%) = \frac{Mass\ of\ oil\ extracted}{Mass\ of\ sample\ used} \times\ 100 \qquad (1)$$

Determination of Free Radical Scavenging Activities

The free radical scavenging activity of oil samples against by 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Various concentration of the sample were prepared in methanol (25, 50, 100, 200, 400 µg mL^{-1}). 1 mL of oil was added to 1.0 ml methanolic solution of the DPPH (1 mM). The mixture was shaken vigorously an allowed to stand at room temperature in dark for 20 minutes. The absorbance was read against reagent blank at 517nm. Inhibition of free radical by DPPH in percent (I, %) was calculated as follows:

$$\text{I}\ (\%) = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times\ 100 \qquad (2)$$

Where: A_{blank}= Absorbance of blank & A_{sample}= Absorbance of sample

Determination of moisture content

The moisture content was determined by standard method. 2 g of the sample was placed in the crucible and heated at 105ºC, until a constant weight was attained. The moisture content of the sample was calculated as loss in weight of the original sample and express as percentage moisture content.

$$\text{Moisture}\ (\%)\ = \frac{\text{W2} - \text{W3}}{\text{W2} - \text{W1}} \times 100 \qquad (3)$$

Where:

W1= initial weight of empty crucible

W2 = weight of crucible +sample before drying

W3 = Final weight of crucible + sample after drying.

Determination of crude protein

The crude protein was determined using standard method. 2g of the sample was weighed along with 20cm^3 of distilled water into a macro-kjeldahl digestion flask. It was shaken and allowed to stand for so times. One tablet of selenium catalyst was then added followed by the addition of 20cm^3 concentrated sulphuric acids. The flask was then heated on the digestion block at 100^0C for 4 hours, until the digest became clear. The flask was then removing from the block and allowed to cool. The content was transferred into 50cm^3 volumetric flask and diluted to the mark with water. An aliquot of the digest (10cm^3) was transferred into another micro- kjeldahl flask and placed in the distilling outlet of the micro-kjeldahl distillation unit. Conical flasks containing 5cm^3 of boric acids indicator was placed under the

condenser outlet. Sodium hydroxide solution (10cm^3, 40%) was added to the contents in the kjeldahl flask by opening the funnel stopcock. The distillation starts and the heat supplied to avoid sucking back. When all the available distillate was collected in 5cm^3 of boric acids, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.01M of H_2SO_4. The end point was obtained when the colour of the distillate changed from green pink. The percentage Nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein.

$$\textbf{Nitrogen}\ (\%) = \frac{\text{Vs} - \text{Vb} \times \text{N} \times 0.01401}{W} \times\ 100 \qquad (4)$$

Where Vs = Titer value of the sample ; Vb = Volume of acid required to titrate and N = Normality of acid and W = Weight of sample in grams

Determination of crude lipid

Crude lipid was determined using the soxhlet extraction method. 10g of the sample were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column which was connected to the condenser. 200ml of n-hexane was used to extract the lipid.

$$\textbf{fat}(\%) = \frac{\text{W2} - \text{W3}}{\text{W1}} \times\ 100 \qquad (5)$$

Where:

W_2 = weight (g) of filter paper and sample before extraction

W_3 = weight (g) of filter paper and sample after extraction

W_1 = weight of sample (g).

Determination of crude Fibre

Crude fibre was determined using standard method. 5g of the sample was weighed and mixed with 1.25% H_2SO_4 were heated for 30 minutes and was filtered with a Buchner funnel. The residue was washed with distilled water until it was acid free. 200ml of 1.25% NaOH was used to boil the residue for 30 minutes; it was filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCl and twice with ethanol. Finally, it was rinsed with petroleum ether three times. The residue was then put in crucible and dried at 105^{o}C in an oven over night. After cooling in desiccators, it was ignited in a muffle furnace at 550^{o}C for 90 minutes, to obtain the weight of the ash:

$$\textbf{CrudeFibre}\ (\%) = \frac{\text{W2} - \text{W3}}{\text{W1}} \times\ 100 \qquad (6)$$

Where:

W_1 = Weight (g) of the sample ; W_2 = Weight (g) of the sample + crucible before drying

W_3 = Weight (g) of the sample + crucible after drying

Determination of crude of ash content

The total ash content of substance is the percentage of the inorganic residue remaining after the organic matter has been ignited. 2g of the sample was weighed and placed in a crucible and ignited in a muffle furnace at 550^{o}C for 6 hours. It was then cooled in a desiccator and weighed at room temperature to get the weight of the, using the formula below:

$$\textbf{Ashcontent}\ (\%) = \frac{W2}{W2} \times 100 \quad (7)$$

Where: W_1 = weight of the original food&W_2 = weight of the ash

Carbohydrate determination

The carbohydrate content was determined by subtracting the summed up percentage composition of moisture, protein, lipid, fiber and ash contents from 100

$$\textbf{Carbohydrates}\ (\%) = 100 - (\%\text{moisture} + \%\text{protein} + \%\text{lipid} + \%\text{fibre} + \%\text{ash} \quad (8)$$

Mineral Analysis

The minerals present were determined using standard method 1g of the pulverized sample was placed in a crucible and was ignited in a muffle furnace at 550^{o}C for 6 hours. The resulting ash was dissolved in 10ml of 10% HNO_3 and heated slowly for 20 minutes. After then, it was filtered and the filtrate was used for the determination of the mineral content. Atomic absorption spectrophotometer (AAS) was used to determine Ca and Fe, while flame Photometer was used for the determination of Na, and K in the filtrate.

Physiochemical Properties of Coconut Oil

Peroxide value

Peroxide value of the VCO was determined using standard method. 1.0 g of oil extract was accurately weighed into a 250 mL conical flask. 1.0 g of powdered potassium iodide was added to the sample. 20 mL of acetic acid and chloroform was added each, swirling the flask and carefully place in a boiling water bath, the content was boiled until the sample was completely dissolved. 20 mL of 5% potassium iodide was added alongside with 50mL of distilled water and the mixture was swirled. 1 mL of starch solution was added as an indicator. The mixture was then titrated with 0.1 M sodium thiosulphate until the blue-gray color disappears in the aqueous layer. The peroxide value was then calculated using the formula below:

Peroxide Value = (S - B) × W × N (9)

Where: S = volume of the titrating ($Na_2S_2O_3$) sample ; B = volume of the titrating ($Na_2S_2O_3$) blank and M = morality of the titrating solution ($Na_2S_2O_3$) and W = weight of oil (g)

Acid value

Acid value was determined following the standard method. 1.0g of oil extract was accurately weighed into a 250 mL conical flask. The phenolphthalein (4 drops) was added as an indicator, the mixture was shaken vigorously. The mixture was titrated with 0.01 M potassium hydroxide until a pink color which persists for like 15 seconds was obtained. The acid value was calculated, using the formula below:

$$\textbf{Acid Value} = \frac{V \times M \times 56.11}{W} \quad (10)$$

Where: V = volume of the titrating solution (KOH) ; M = morality of the titrating solution (KOH)
W = weight of oil (g) and 56.11 = molecular mass of KOH

Saponification value

Saponification value is determined following the standard method. 2.0g of oil extract was accurately weighed into a 250 mL conical flask. 25 mL of equal volumes of ethanol and potassium hydroxide was added. The mixture was heated in a water bath for 30 minutes. 4 drops of phenolphthalein was added as an indicator. The mixture was then titrated with 0.5 M hydrochloric acid. A blank titration was also carried out in the same way. The saponification value was calculated using the formula below:

$$\textbf{Saponification value} = \frac{(V1 - V2) \times 28.05}{W} \quad (11)$$

Where: V_1 = Volume (mL) of 0.5 M HCl used (sample) ; V_2 = Volume (mL) of 0.5 M HCl used (blank)
W = weight of the oil used (g) and 28.05 = $^1/_2$Molecular weight of KOH (56.11)

Iodine value

Iodine value was determined using standard method. 1.0g of oil extract was accurately weighed into a 250 mL conical flask. 15 mL of carbon tetrachloride was added to dissolve the sample. 25 mL of Wijs reagent was added and the mixture was stirred and stored in a dark place for 30 minutes. 20 mL of 10% potassium iodide was added to the mixture alongside with 100mL of distilled water. The mixture was then titrated with 0.1 M sodium thiosulphate until the yellow color disappeared. 1 mL of starch solution was added as an indicator and the mixture was titrated further until the blue starch-iodine color disappeared. The Iodine value was calculated using the formula below:

$$\textbf{IodineValue} = \frac{2.69 \times M \times (V1 - V2)}{W} \quad (12)$$

Where:
V_1 = Volume (mL) of 0.1 M $Na_2S_2O_3$ used (sample); V_2 = Volume (mL) of 0.1 M $Na_2S_2O_3$ used (blank)
M = molarity of the $Na_2S_2O_3$; W = weight of the oil used (g) and 2.69 = equivalent weight of iodine.

3. Results and discussion

The coconut oil sample collected from Anyigba market, Dekina Local Government Area Kogi State Nigeria, was analyzed to determine its proximate properties, and the results are given in Table 1. The mineral contents presence in coconut oil was determined and the results are presented in Table 2. The coconut oil was analyzed for its physiochemical properties, and the results are given in Table 3. The free radical scavenging of coconut oil was tested and the results obtained are given in Table 4.

Table 1: Biochemical characteristics of coconut oil

Proximate analysis	Value (%)
Moisture content	14.28
CrudeProtein	9.26
CrudeLipid	39.72
Crude Fibre	7.22
Ash Content	1.075
Carbohydrate	28.45

Table 2: Minerals Present in coconut oil

Element	Amount present (mg/kg)
Ca	38.55
K	138.15
Na	58.6
Fe	1.82
P	0.79
C	0.015
Zn	5.35

Table 3: Physiochemical Properties of Coconut Oil

Type of test carried out	Value
Peroxide Value	1.5
Acid Value	10.55
Saponification Value	134.50
Iodine Value	0.5918

Table 4: Antioxidant Activity (DPPH) (%)

Concentration	coconut oil	vitamin C.
25	37.00	93.00
50	41.00	94.00
100	48.00	95.00
200	56.00	96.00
400	61.00	97.00

The IC_{50} of the coconut oil sample was determined from the plot of percentage inhibition of the coconut oil against concentration (Figure 1), and the IC_{50} of Vitamin C which serves as the standard antioxidant, was determined from the plot of percentage inhibition of the Vitamin C against concentration (Figure 2). Based on the value obtained, IC_{50} the VCO is 22.0µg/ml while that of vitamin C (standard) is 1.19 µg/ml and so it falls into a very strong antioxidant category based on the average mean of IC_{50} <50µg/ml. A compound is said to be very active antioxidant if it's IC_{50} <50µg/ml [18]. The mineral composition of coconut oil in mg/kg were analyzed and the results in (Figure 3) shown that coconut oil coconut oil contain high amount of potassium (K) and least amount of carbon.

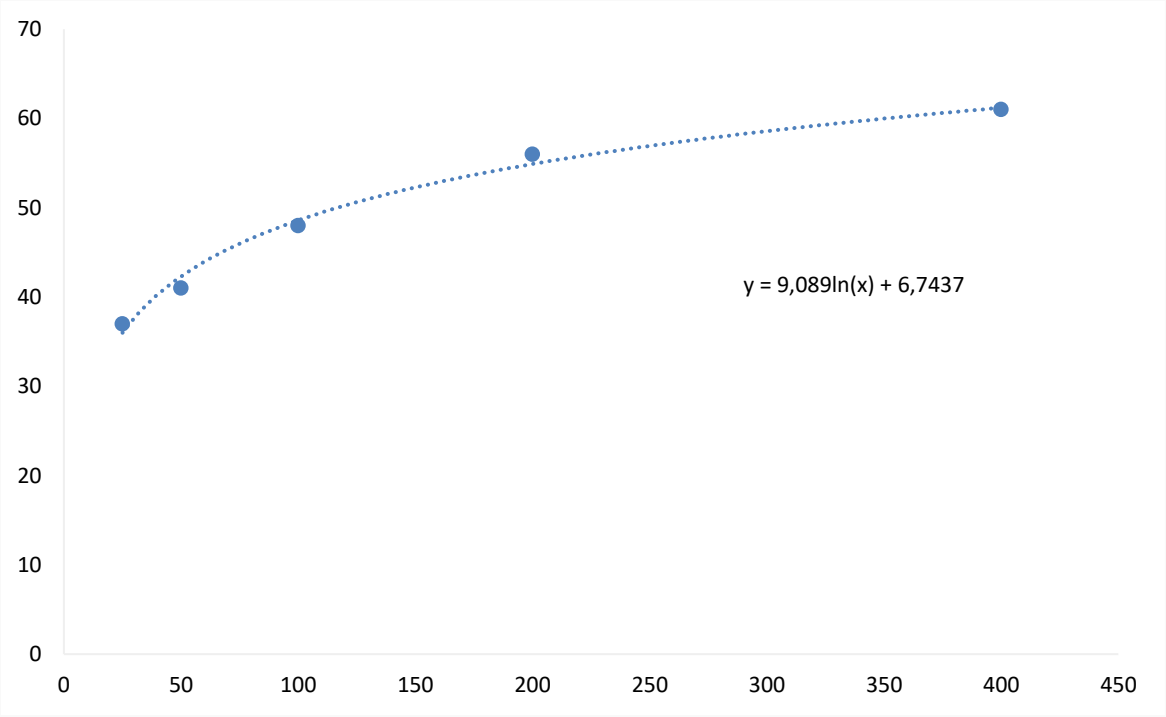

Figure 1: Plot of Percentage Inhibition of VCO against Concentration

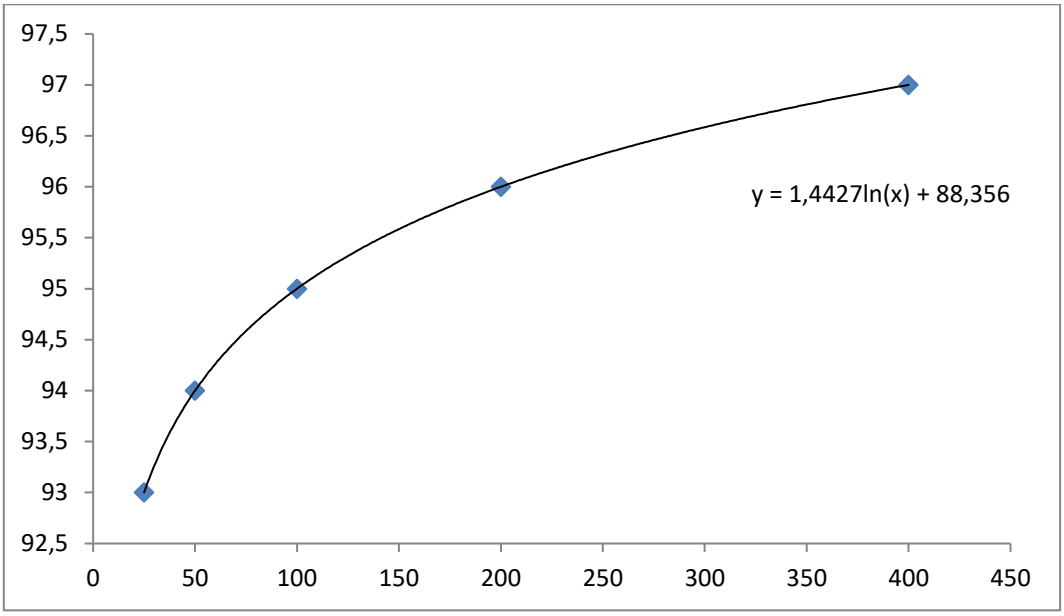

Figure 2: Plot of Percentage Inhibition of Vitamin C against Concentration

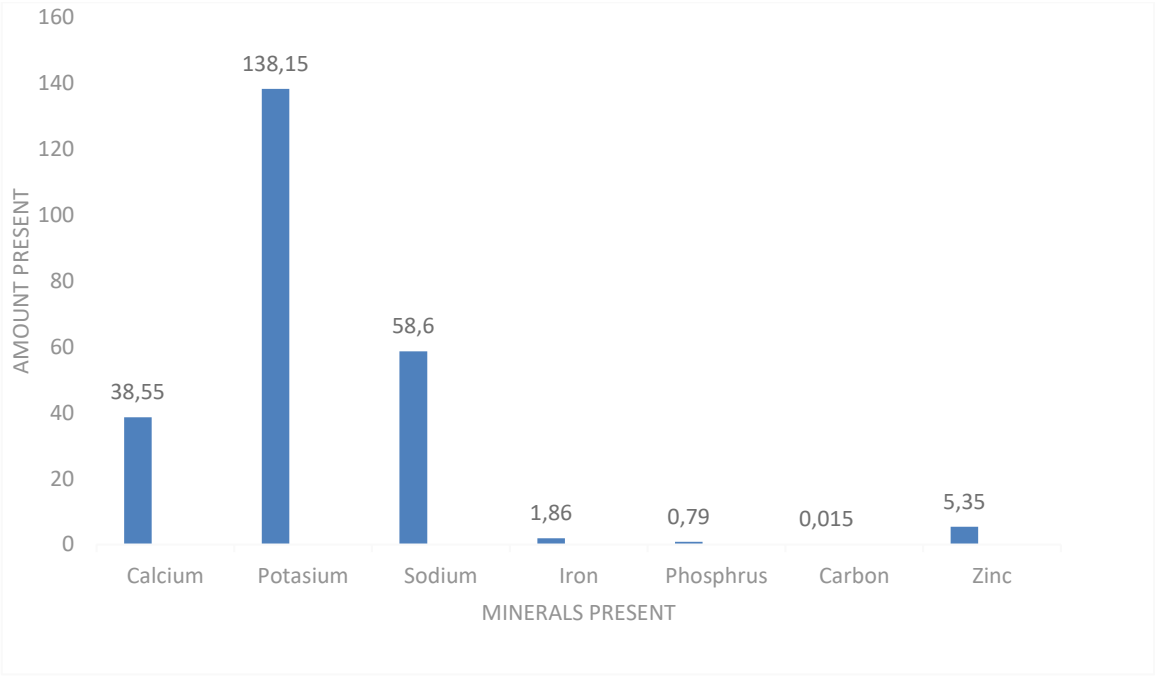

Figure 3: Mineral Composition of Coconut Oil

Discussion

Percentage Yield

The oil extract was liquid at room temperature. The yield of which was shown in Table 1, indicates that 918.1g of the sample yields 172.53 g of the oil, which is equivalent to 200 mL. The results showed that the extraction was in good yield. The values of proximate proprieties determined are similar to those obtained recently by Nigerian Coconut oil [19] but too higher than those evaluated Indonesian one [20].

Physiochemical Properties

The quality of coconut oil was analyzed by evaluating the physicochemical properties such as peroxide, acid, iodine and saponification values. Results are presented in (Table 3).The VCO has low iodine value and found to be 0.5918; the low iodine value of the VCO means the virgin coconut oil has high percentage of medium chain saturated fatty acid. The higher the iodine value, the more unsaturated fatty acid bonds are present in a fat [11]. Saponification value provides the information of the average chain length and hence the molecular weight of the fatty acid in the oil. The higher saponification numbers of the oil indicate higher solubility of Soap that can be made from it. The Saponification value obtained from the oil sample was 134.50 mg KOH/g which is in range with the value obtained by [20]. The high saponification value indicates that the oil can be a better choice for soap production. The high saponification number reflects the number of the fatty acid molecules [21].

Acid value (AV) is an important indicator of oil quality. AV is the number of milligrams of KOH (in milligrams) required to neutralize free fatty acids present in 1.0 g of oil sample. The acid value of VCO was found to be 0.673 which is in agreement with the acid value of 0.6 and 0.4 recommended by FDA/WHO. Peroxide value is used as a measure of the extent to which rancidity reactions occurred during storage. The quality and stability of fats and oils can be indicated by using the peroxide value. The virgin coconut oil showed low peroxide values of 1.5 meq/kg oil which indicates a relatively good quality of this oil. The higher the peroxide values the faster the oil rancidity due to relative higher oxidation in oils.

Proximate Analysis (Qualitative analysis of macromolecules in food) and nutritional value

The result of the proximate analysis of the coconut meat is shown in (Table 5). The data obtained shows that coconut meat compares favorably with other fast energy giving food stuffs. Hence, it can be added to some food content as an additive [22]. The moisture content of the VCO was found to be 14.28% while the crude protein and crude lipid were found to be 9.255 and 39.72 % respectively. The crude fibre contents of the coconut was 7.22%, this percentage is enough to keep the digestive system healthy as the major role of the crude fibe in the human system. Also, the ash contents and the carbohydrates content were found to 1.075 and 28. 45% respectively.

The minerals present in the oil were also were analyzed and the result obtained shown in (Table 4) shows that the oil contains almost all the minerals needed for the body in order to enhance proper functioning of the body system such as potassium (K) and sodium (Na) which is present in high amount of 138.15 and 58.6 respectively. Due to their importance in the body systems, as they are known to regulate fluid balance in the body, send nerves signals and regulate muscle and heart contractions, they also help to reduce blood pressure, help in protection against stroke. Hence, the regular intake of VCO

could help in the enhancement of these minerals. Calcium present was 38.55 which make the oil suitable in maintaining strong bones and tooth, it also helps the blood vessel to move blood throughout the body and help releases hormones and enzymes that affect almost every function in the human body. Other minerals such as Zn, Fe, P, and C were also present but in trace amounts.

Antioxidant activity

The antioxidant activity of the oil was analyzed and the results in (Table 4) shows that VCO has ability to fight against free radicals in human body as also documented in literature [23-25]. Free radicals are toxic by-products of oxygen metabolism that can cause significant damage to living cells and tissues, the process known as oxidative stress while antioxidant are the vitamins and minerals which the body uses to counteract oxidative stress. Survey of literature indicated that VCO is a nutritional and medicinal food in the traditional coconut growing areas [26]. It is an unrefined kernel oil obtained from fresh and mature coconut (*Cocos nucifera*) by mechanical or natural means, with or without the use of heat and without chemical bleaching and deodorizing [27-29]. It is being known for many beneficial health effects associated with its phenolic acids and flavonoid contents [30-32]. Phytochemical analysis found that p-coumaric and ferulic acids are the major potent phenolics in the VCO [32-34] as shown below:

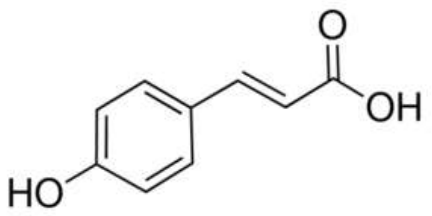

Figure 4: structure of p - coumaric acid

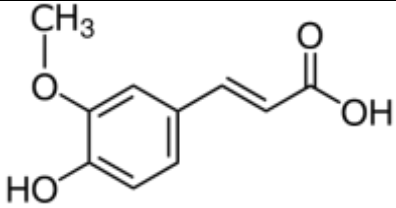

Figure 5: Structure of ferulic acid

[35]

It's clear that the difference in molecular structure between these compounds is the presence of methoxy group in Ferulic acid. Antidiabetic and cardiac potential of phenolic acids such as coumaric acid, ferulic acid, and gallic acid is less compared to caffeic acid and cinnamic acid [36-39].

Conclusion

Conclusively, from Physiochemical properties, proximate and nutritional composition, antioxidant properties study, the oil has a great chance to be considered as alternative oil for food as the saturated fatty acid is more of the medium chain fatty acid which does not store up in body but are directly converted into energy and take part in metabolism. It can therefore be used in the industries for soap and cosmetic production.

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