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Physical Composition, Proximate, Phytochemical and Impact of Coconut Oil on Lipid Profile of Albino Rats

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Abstract:
Background*: Coconut oil is a food supplement derived from the Cocos nucifera L of Cocoideae subfamily and Arecaceae family, the oil is obtained from coconut kernel and it is anatural functional oil. This study was undertaken to evaluate the physical, proximate, phytochemical composition and the effect of coconut oil on lipid profile indices of albino rats.*
Materials and Methods:*The proximate analysis was according to AOCS, phytochemical analysis was carried out with GC-FID.Thirty-five (35) albino rats were used for the lipid profile evaluation. Two phases of experiment with four (4) groups of male albino rats comprising of five (5) animals each, averagely weighing 180g-200g were employed. Phase 1 of the experiment was treated with coconut oil supplemented diet, where rodent chow was mixed with coconut oil while phase 2 experimental animals were treated with coconut oil by oral gavage. Group 1 served as the control for both phases while group 2, 3 and 4 were treated with 3ml/kg, 6ml/kg and 12ml/kg of coconut oil respectively for 4weeks.*
Results*: The physical examination of the samples showed that the appearance of hot press method of extraction of coconut oil is pale-yellow while the oil from cold press method of extraction is colourless. Oil yield for hot press method is higher than that of cold press method. The result of proximate composition showed high level of crude fat for the hot and cold press methods. Moisture content for hot press method is lower than the cold press method. Crude protein values were same for both hot and cold press methods. Carbohydrate content for hot press method is lower than that of cold press method. The phytochemical composition shows a significant amount of flavonoids, alkaloids, phenol, and tannins, while oxalate, sapogenin and phytate were in trace quantity. Treatment with coconut oil supplemented diet and oral administration of coconut oil showed significant increase ($p \leq 0.05$) in triglyceride and HDL levels but LDL levels showed a decrease when compared with the control.*
Conclusion:*It is observed that coconut oil does not pose any significant health challenge to the animals.*
Key Word*: Coconut oil; Proximate; Phytochemical; Lipid profile.*

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I. Introduction

*Cocos nucifera Linn*of cocoideae subfamily and Arecaceae family is universally called the ‘‘coconut tree’’ and is the most naturally wide-ranging fruit plant on earth [1]. Coconut is a fibrous drupe, made up of a thin rigid coating known as the exocarp, a heavier cover of tough mesocarp, the firm endocarp, white endosperm termed kernel and a big cavity full with liquid [1]. Coconuts are commonly categorized into two broad kinds namely tall and dwarf coconut [2]. All through history, humans have used medicinal plants for management of disease condition. The component (alkaloids and flavonoids) of *Cocos nucifera*oil has some biotic effects, such as anti-inflammatory, antioxidant, antifungal, antimicrobial, and antitumor activities [3]. One of the principal natural yieldsfrom coconut fruit is the coconut oil which has beenused in earliest time as a functional food and pharmaceuticals, it is often called “miracle oil” [4]. The coconut is a functional foodbecause it offers health profits further than its nutritional content [5]. Coconut kernel products such as coconut milk and coconut oil are eaten primarily for the superb sense of taste that the fats in coconut transport to food. Coconut oil is a vegetable oil extracted from coconut kernel [6]. It is a nutritional supplement resulting from the fruit of *Cocos nucifera Linn;* it is a colourless to pale yellow oil [7] and composed largely of medium-chain fatty acids that don’t take part in the biosynthesis and conveyance of cholesterol [8]. The existing health situation categorized by the utmost occurrence of cardiac ailments stresses the intake of fats and oils that can lessen lipid levels in serum and tissues to conserve human health [9].

II. Material And Methods

Coconut collection

De-husked matured coconuts with the shell were purchased at the Mbiama market in Ahoada West Local Government Area of Rivers State. The coconuts were identified and authenticated by Dr. Stanley Dimkpa as *Cocos nucifera* (dwarf specie) at Crop Science Department, Rivers State University, Port Harcourt.

Coconut oil Extraction

Two separate techniques were applied in the extraction of coconut oil (hot and cold press methods). Fresh matured coconut kernel were shelled to obtain the endosperm using a paired knife, the endosperm was thoroughly washed, weighed and later blend with little water using a food processor to obtain coconut milk. The coconut milk was filtered from the blend coconut with a cheese cloth over a wide-mouth jar and divided into two portions. The first portion of the coconut milk was refrigerated for 24hours. After 24hours, the milk was scooped and subjected to mild heating (hot press method) using an electric hot plate cooker at 60^oC for 30minutes until the water has completely evaporated then the protein coagulates to release the oil and the residue filtered through a cheese cloth to obtain pure coconut oil [10].The second portion of the coconut milk (cold press method) was transferred into an airtight jar and left unattended to for about 18hrs. The coconut milk will separate and form three layers, a layer of curds will appear at the top of the jar, the oil at the middle and water at the bottom of the jar. The curd is scooped out with a spoon and discarded; the oil is also scooped out and filtered severally to obtain pure coconut oil.

Determination of percentage yield of oil

The nuts were weighed before extraction using a weighing balance and its weight was recorded as actual weight of coconut, the extracted oil was weighed using same weighing balance and its weight was recorded as actual weight of oil [11]. This procedure was carried out for the hot press and cold press method of extraction and the percentage oil yield was calculated using the formulae below:

$$\% \text{ Oil yield} = \frac{\text{Actual weight of oil}}{\text{Actual weight of coconut}} \times 100$$

Proximate Analysis

Moisture Content Determination: The AOCS, 2004 Method was used to determine moisture content of the coconut oil samples [12, 13]. 2.0g of the coconut oil sample was placed into a pre-heated andpre-weighed crucible with lid, and then oven dried at 105^oC for 1hour. The crucible containing the sample was then placed in a desiccator and allowed tocool to room temperature for about 15minutes before re-weighing to obtain the moisture loss of the sample. The percentage moisture content was calculated using the formula below;

$$\text{Moisture loss} = \text{initial weight} - \text{final weight}$$

$$\% \text{ Moisture content} = \frac{\text{Moisture loss}}{\text{Sample weight}} \times 100$$

Ash Content Determination: Pre-weighedempty crucibles were placed in a muffle furnace for about 20 minutes, and then removed and placed in a desiccator for 30 minutes to cool off and then re-weighed. Portions of each sample was weighed into different crucibles, the crucibles were placed on a hot plate in a fume cupboard and the temperature gradually increased until smoking stops and the sample thoroughly charred. The crucibles are then placed in the muffle furnace for about 2hrs for ashing. As a grey colour of ash appears, the ashed crucibles were then placed in a desiccator and allowed to cool before re-weighing to obtain ash content. The percentage ash content was calculated using the formula below:

$$\text{Weight of ash} = \text{weight of ashed crucible} - \text{weight of empty crucible}$$

$$\% \text{ Ash content} = \frac{\text{Weight of ash}}{\text{Sample weight}} \times 100$$

Carbohydrate Content Determination: 1 g of the samples was weighed into a beaker and 10ml of perchloric acid ($HClO_4$) was added to each sample and shaked thoroughly for 20minutes. The mixture is then made up to 100ml with distilled water and filtered using a 110mm filter paper to obtain the filtrate which was then made up to 250ml (distilled water) using a 250ml conical flask. 100ml of distilled water was added to10ml of the solution above were 1ml is put in separate test tubes and 0.5ml glucose standard added to all test tubes alongside 2.5ml

of anthrone reagent which was boiled for 12minutes using a hot plate and allow to cool to room temperature. The solution was then transferredto a 1cm cuvettes in a UV-VIS Spectrophotometer and the absorbance of the samples and dilute standard recorded at a wavelength of 630nm against the reagent blank. Glucose standard served as the reagent blank. The percentage carbohydrate content was calculated using the formula below:

$$\% \text{ Carbohydrate} = \frac{25 \times B}{A \times W}$$

Where A = absorbance of dilute glucose standard
B = absorbance of dilute sample
W = weight of sample

Crude Protein Determination: Protein content determination was done using three stages namely:
Digestion
Distillation
Titration

Digestion stage: Digestion is done using the digestion flask. Samples (0.5g) were weighed into the digestion flask, 0.3g of copper sulphate and 3g of sodium tetraoxosulphate(vi) were transferred into the digestion flask containing the sample, 12ml of concentrated sulphuric acid was added into each of the sample in the same digestion flask and then transferred to the fume cupboard for digestion at 450^{o}C for 1hour. After digestion, it is then allowed to cool and made up to 100ml with distilled water.

Distillation stage: About 10ml of boric acid indicator was transferred into a conical flask using a pipette with a filler and 20ml of 40% sodium hydroxide solution was added into a volumetric flask and made up to 100ml with distilled water which is connected to a distillation flask were 20ml of the digest was added to the same distillation flask and distilled immediately with the boric acid indicator in the conical flask for 5minutes.

Titration stage: The final stage of protein content determination where0.1M HCl was used to titrate the distilled sample. Percentage crude protein was calculated using the formula below:

% Crude protein = % Nitrogen × Protein Factor

Protein factor = 6.25

$$\% \text{ Nitrogen} = \frac{\text{Sample Titre} - \text{Blank Titre} \times \text{Normality of Acid} \times 1.4}{\text{Sample Weight (in 20ml)}}$$

Fat Content Determination: Fat content was determined by difference after adding up all the concentrations from various parameters then the difference is the fat content of the samples.

Phytochemical Analysis

Phytochemical Analysis was according to Kelly and Nelson [14].

Extraction of Phytochemicals: Portions ofthe samples were weighed using an analytical weighing balance in a crucible and transferred into a test tube then 15ml ethanol and 10ml of 50% m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60^{o}C for 60mins. After reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The test tube was rinsed with 20ml ethanol, 10ml of cold water, and 10ml of hot water and 3ml of hexane which was all transferred into the separatory funnel. These extracts were combined and washed three times with 10ml of 10% v/v ethanol aqueous solution. The solution is dried with anhydrous sodium and the solvent was evaporated. The sample was solubilized in 1000µl of pyridine of which 200µl was transferred to vials for analysis

Quantification by GC-FID: The analysis of free steroids was performed on a BUCK M910 Gas Chromatography equipped with a flame ionization detector. A RESTEK 15 meter MXT-1 column (15m × 250µm × 0.15µm) was used. The injector temperature was 280^{o}C with split less injection of 2µl of sample and a linear velocity of 30cms^{-1}, Helium was the carrier gas with a flow rate of 40ml/min. The oven operated initially at 200^{o}C, it was then heated to 330^{o}C at a rate of 3^{o}C min^{-1} and was kept at this temperature for 5min. The detector operated at a temperature of 320^{o}C. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals expressed in µg/ml [14].

Experimental Design

Thirty-five male albino rats weighing about 180-200g were purchased from the University of Port Harcourt, Department of Biochemistry animal house. Following acclimatization for one week, the animals were divided into four groups of five animals each for two phases of treatment that lasted for four weeks. The groups were labeled 1, 2, 3, and 4.

Group 1 animals served as control for both phases of treatment, they were given rodent chow and water only.

Groups 2, 3 & 4of phase 1 animals were given coconut oil supplemented diet (COSD). Rodent chow was mixed with coconut oil at 3ml/kg body weight which resulted in an average dose of 0.5ml of coconut oil to 10g of rodent chow per animal, 6ml/kg to an average dose of 1.0ml of coconut oil to 10g of rodent chow & 12ml/kg to an average dose of 2.0ml of coconut oil to 10g of rodent chow respectively.
Group 2, 3 & 4of phase 2 animals were given coconut oil by oral gavage at 3ml/kg to an average dose of 0.5ml, 6ml/kg to an average dose of 1.0ml & 12ml/kg to an average dose of 2.0ml respectively [9].

Blood Sample Collection
Animals were anaesthetized with chloroform at the end of the experiment and sacrificed by decapitation [15]. Blood samples were collected from each of the animal in an EDTA anticoagulant sample bottle for lipid profile analysis using a Spectrophotometer AD23.

Statistical Data Analysis:
Results were expressed as mean ± standard deviation of mean, one-way analysis of variance (ANOVA) was carried out using SPSS Version 21.0. Significant diff erences between control and treatment groups were considered using the Duncan's post hoc test at value$p \leq 0.05$.

III. Result

Table no 1: Physical composition of coconut oil

Parameter	**Hot press**	**Cold press**
Appearance	Pale-yellow	Colourless
Odour	Coconut smell	Coconut smell
Weight of coconut (g)	5040	7840
Weight of oil (g)	1880	1920
% Yield of oil	37.30	24.49

Table no 2: Proximate composition of coconut oil

Parameter (%)	**Hot press**	**Cold press**
Moisture	0.60 ± 0.01	0.71 ± 0.07
Ash	0.60 ± 0.00	0.57 ± 0.00
Protein	0.53 ± 0.00	0.53 ± 0.00
Fat	97.80 ± 0.13	97.30 ± 0.03
Carbohydrate	0.49 ± 0.14	0.91 ± 0.09

Values are mean ± standard deviation

Table no 3: Phytochemical composition of coconut oil

Component	**Sub-class**	**Concentration(µg/ml) Hot press**	**Concentration(µg/ml) Cold press**
Alkaloids	Spartein	8.1921	6.2817
	Ribalinidine	4.0663	1.7571
	Lunamarine	0.0436	0.4082
	Quinine	2.4256	2.4200
	Ephedrine	1.2405	1.0237
Total		15.9681	11.8907
Flavonoids	Proanthocyanin	0.0385	0.0143
	Flavone	5.8993	5.9499
	Anthocyanin	4.0042	1.9545
	Flavonones	9.1791	8.5421
	Kaempferol	4.0042	2.5297
	Catechin	6.8636	5.6369
	Epicatechin	9.8846	9.7993
	Naringenin	1.3920	1.3863
	Resveratrol	4.2495	3.2785
Total		45.6039	39.0915
Tannins		15.7741	4.6478
Saponins	Sapogenin	2.1814	1.8649
Phenol	Phenol	12.3371	11.4369
Oxalate		3.1351	2.6008

Phytate		0.4604	0.3769

Table no 4: Impact of Coconut Oil on Lipid Profile Indices

		Coconut Oil Supplemented Diet				**Oral Administration**			
Groups	**Treatment (ml/kg)**	**T.C (mmol/L)**	**T.G (mmol/L)**	**HDL (mmol/L)**	**LDL (mmol/L)**	**T.C (mmol/L)**	**T.G (mmol/L)**	**HDL (mmol/L)**	**LDL (mmol/L)**
1	Control	$2.30\pm0.02^{d}_{f}$	$0.63\pm0.04^{bd}_{f}$	$1.24\pm0.04^{bd}_{f}$	$0.78\pm0.05^{b}_{d}$	$2.30\pm0.03^{bd}_{f}$	$0.63\pm0.04^{bd}_{f}$	1.24 ± 0.04^{df}	$0.78\pm0.05^{b}_{df}$
2	3	$2.33\pm0.03^{a}_{df}$	$0.86\pm0.01^{*a}_{df}$	$1.33\pm0.02^{*a}_{f}$	$0.61\pm0.04^{*}_{af}$	$2.35\pm0.02^{*a}_{df}$	$1.08\pm0.04^{*a}_{df}$	$1.22\pm0.02^{ad}_{f}$	$0.63\pm0.04^{*}_{a}$
3	6	$2.36\pm0.02^{*}_{bc}$	$0.89\pm0.01^{*b}_{c}$	$1.32\pm0.02^{*c}_{f}$	$0.63\pm0.02^{*}_{cf}$	$2.46\pm0.03^{*b}_{cf}$	$1.13\pm0.03^{*b}_{cf}$	$1.31\pm0.01^{*b}_{cf}$	$0.63\pm0.03^{*}_{c}$
4	12	$2.37\pm0.01^{*}_{be}$	$0.89\pm0.01^{*b}_{e}$	$1.18\pm0.02^{*b}_{de}$	$0.78\pm0.03^{b}_{de}$	$2.58\pm0.04^{*b}_{de}$	$1.20\pm0.03^{*b}_{de}$	$1.37\pm0.03^{*b}_{de}$	$0.67\pm0.05^{*}_{e}$

Values are expressed as mean ± standard deviation of mean (SEM) for n=5. Values with different superscript letter *, a,b; c,d; e,f;) in the same rolls are significantly different at the 0.05 level ($p\leq0.05$).

* differ significantly when comparing group 1 with other group.

a,b differ significantly when comparing group 2 with other group.

c,d differs significantly when comparing group 3 with other group.

e,f differ significantly when comparing group 4 with other group.

IV. Discussion

The physical assessment of coconut oil for the hot press and cold press method of extraction as shown in table 1 showed that the appearance (colour) of coconut oil for the hot press method of extraction is pale yellow while that of the cold press method is colourless. The difference in colour for both extraction methods could be as a result of heat applied during extraction of the hot press coconut oil. This finding supports Gopala*et al*. [7] review on the colour of coconut oil. The percentage oil yield for hot press coconut oil is higher than that of the cold press coconut oil. The higher yield of oil obtained in hot press coconut oil may be due to the applied heat for extraction as heatiscapable of eliminating moisture from oil samples whichimproves the yield of oils [6].

The proximate composition of coconut oil as shown in table 2 givesthe overall nutritional composition of thecoconut oil for both the hot and cold press method of extraction. The result showed that the moisture content for the hot press method is lower than that of the cold press method which could be as a result of heat employed during extraction. Though the moisture content for both the hot and cold press methods is relatively low, it is suitable to maintain low moisture content in oil samples as it will improve the shelf life of the oil by averting rancidity and oxidation processes [16]. Oil from both extraction methods contains appreciable amount of crude fat, the highpercentage of fats in the hot and cold press coconut oil samples also makes coconut oil adiscrete potential for the oil manufacturing industry [17]. The protein content of the coconut oil samples (hot and cold press method of extraction) is similar and in trace amount, it indicates that the coconut oil contains very low amount of protein. The carbohydrate content for the hot press coconut oil is lower than that of the cold press coconut oil. The decrease in the content of carbohydrate for hot press coconut oil could be due to the heating method employed during extraction. This finding supports a report on the effect of cooking procedures on accessible and inaccessible carbohydrates of selected tropical plants [18].

The result of the phytochemical screening of coconut oil for the hot and cold press method of extraction as shown in table 3 reveals the presence of kaempferol, catechin, anthocyanin, resveratrol, flavonones, epicatechin, flavone which are flavonoids as the highest component of coconut oil followed by alkaloids (spartein, quinine, lunamarine, ribalinidine and ephedrine).The hot press coconut oil showed an increase in the presence of tannins compared to the cold press coconut oil which could be as a result of the heat applied during extraction. Lee *et al*. [19] reported that an increase in temperature with time increases tannin and catechin contents of non-astringent persimmon fruit juice. The concentration of tannins in the coconut oil may not be sufficient to prompt toxicity, thus it may be suitable for use in herbs or at best nutraceuticals [20].The coconut oil samples for the hot and cold press method of extraction also contains phytochemical components such as sapogenin, phenol, phytate, and oxalatewhich are beneficial to human health.

The result of the impact of coconut oil on lipid profile indices as shown in table 4 indicates a significant ($p\leq0.05$) increase in T.C, T.G and HDL levels for both treatment phases however HDL level for group 4 animals in the COSD treatment phase shows a significant decrease compared to the control group. It is observed that LDL levels significantly ($p\leq0.05$) decreased for both treatment phases compared to the control group except for the group 4 animals in the COSD phase of treatment where the difference is insignificant.A likely description for the elevated HDL levels obtained in this study may be as a result of the high percentage of

lauric acid and myristic acid present in CO [21]. Serum TC and TG levels for the oral administration treatment phase increased significantly compared to that of the COSD treatment phase. There is a progressive significant increase in HDL levels for the oral administration treatment phase while HDL levels for the COSD treatment phase decreased as the dose levels increased. All these may also be as a result of ingesting the coconut oil sample directly especially for the oral administration treated groups. These findings are similar to a study that VCO intake is potentially beneficial for elevating HDL levels [22], also the lipid profile parameter results in this study relates to the findings of Cardoso *et al*. [23] who assessed the effect of CO on lipid profiles amid patients with coronary artery disease. They noted that HDL levels were significantly elevated in participants consuming CO.

V. Conclusion

This study showed that coconut is a potential source of natural oil. The nutritional contents, phytochemicals and antioxidant properties present in coconut oil are sufficient to improve health conditions. Also the study revealed thatcoconut oil within the concentration used may not pose any significant health challenge to animals as it improves HDL and decreases LDL levels.

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