

Comparison of the Effects of Cucurbitaceae Seeds Oils and Refined Palm Oil in the Prevention of Cardiovascular Disease Risk

Howele Ouattara^{1*}, Donatien Albert Atsamo², Bazoumana Ouattara¹, Vazoumana Kone³ and Seraphin Kati Coulibaly⁴

Received: 2 June, 2021; Accepted: 23 July, 2021; Published: 7 February, 2022

*Corresponding author: Howele Ouattara, Training and Research Unity of Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Cote d'Ivoire, Tel: 225-0754-4664; E-mail: ouattarahowe@gmail.com

Abstract

Palm oil, the most oil consumed in the world, because of its high saturated fatty acid content, is nowadays recognized to enhance Cardiovascular disease risk (CVD). In this study, we sought to propose Cucurbitaceae seeds oils (CSO) as reducing CVD risk and a good source of income. Then, physicochemical parameters, composition and nutritional value of *Citrullus lanatus* seeds oil (HCL), *Lagenaria siceraria* seeds oil (HLS), *Cucumeropsis mannii* seeds oil (HCM) were compared to that of Refined palm oil (RPO) and among them. Four diets, conformed to that proposed by AIN-93G but differing to the oil, were formulated by using one of the CSO extracted or RPO. These diets served to feed four homogeneous groups of young rats (six per group) during 45 days. Diets consumption and efficacy were evaluated. Blood sample, collected at the end of the experimentation, were used to measure out hematological parameters, glycaemia, urea, creatinine, lipids parameters, Aspartate Aminotransferase, Alanine Aminine Transferase, sodium and calcium. Peroxide index and specific gravity were in accordance to the norm. Free fatty acid content of RPO and HLS were in the norm but acid values of the fourth oils were lower than the safe limit for consumption. Grading of most unsaturated fatty acid content was HCL, HCM, RPO and HLS respectively. Plasma parameters, organ weight and the coefficients calculated were not different ($p > 0.05$) to each other, but according to atherogenicity index, the grading of high inducing cardiovascular disease was HCM, RPO, HCL and HLS respectively. Without no technological treatment CSO compete with RPO. There are possibility that refined CSO reduced CDV than RPO.

Keywords: *Citrullus lanatus* seeds oil ; Refined palm oil ; Physicochemical parameters ; Composition ; Nutritional value ; Cardiovascular disease risk; Rat

Abbreviations

CVD: Cardiovascular Disease Risk ; CSO: Cucurbitaceae Seeds Oils; RPO: Refined Palm Oil; HDI: Refined palm oil « Dinor »; HLS: *Lagenaria siceraria* seeds oil ; HCL: *Citrullus lanatus* seeds oil; HCM: *Cucumeropsis mannii* seeds oil; RHDI: Diet in which oil used is refined palm oil; RHCL: Diet in which oil used is *Citrullus lanatus* oil; RHLS: Diet in which oil used is *Lagenaria siceraria* oil; RHCM: Diet in which oil used is *Cucumeropsis mannii* oil; RHDI: Diet in which oil used is refined palm oil; RHCL: Diet in which oil used is *Citrullus lanatus* oil; RHLS: Diet in which oil used is *Lagenaria siceraria* oil; RHCM: Diet in which oil used is *Cucumeropsis mannii* oil; ASAT: Aspartate Aminotransferase; ALAT: Alanine Aminine Transferase

Introduction

Five species of Cucurbitaceae, improperly called «pistaches» in French or «pistachio» in English, are found in Cote d'Ivoire. These species are *Lagenaria siceraria* (Molina), *Citrullus lanatus* (Thunb), *Cucubita moschata* (Duchesne), *Cucumeropsis mannii* (Naudin) and *Curcumis melo* (Subsp) [1]. Among them, the most important, because of its economic role are *Citrullus lanatus* (Thunb), *Lagenaria siceraria* (Molina) and *Cucumeropsis mannii* (Naudin). The names of these Cucurbitaceae, in Senoufo the local language in the North of Cote d'Ivoire where they are most cultivated are «Taho or Pkelegue » for *Lagenaria siceraria*, « Woilai » for *Citrullus lanatus* and « Fonguele » for *Cucumeropsis mannii*. According to Zoro Bi, et al. [2], the cost of Cucurbitaceae seeds was 1500 FCFA per kilogram of seeds in the year 2006. The price is at present 2000F CFA per kilogram of seeds. The seeds are consumed after being prepared in sauce in several regions of Africa especially during celebrations. They are sources of

energy, proteins, vitamins, trace element and particularly they are high sources of lipids [3-8] which value can be increased of. Unfortunately, Cucurbitaceae seeds oils (CSO) are neglected in favour of palm oil, soya been oil, colza oil, sunflower oil, palm seed oil, cottonseed oil, groundnut oil, coconut oil and olive oil which degree of consumption in the world are 33%, 27%, 16%, 10%, 4%, 3%, 3%, 2% and 2% respectively [9]. In Cote d'Ivoire, vegetables oils used in human diet are produced from shea seed, cotton seed, groundnut and palm tree. According to the quantity of palm oil production in a year, Cote d'Ivoire held the 10th place in the world and the second place after Nigeria in Africa [10]. Palm oil is the most oil produced in the world [11,12]. It is also the first oil produced and used in Cote d'Ivoire. Nowadays, the consumption of palm oil is often questioned for various reasons : on the one hand, its consumption has effects on cardiovascular health [13-15] because of its high saturated fatty acid contents which are palmitic fatty acid and stearic fatty acid. On the other hand, it threatens food security due to the possible accrual of

pollutants during refining; and finally, there is the debate on the sustainability of its cultivation in the countries of origin [16]. The food and nutrition sectors are increasingly adopting a more holistic view, which encompasses not only health, but also environmental protection and traceability. And yet, all ecologists are in agreement to say that industry of palm oil production destroyed forests and enhanced carbon dioxide [17]. Considering the castigation of palm oil, it appeared important to look for other sources of oils available which do not enhance environment pollution and which contain few saturated fatty acids in particular few palmitic acids. CSO can be an alternative solution, in Cote d'Ivoire, if the comparison of the effects of these oils give satisfactory results concerning the decrease of the different diseases for which palm oil is incriminated. Again, this solution can develop farming in the North and the North-Centre of Cote d'Ivoire, where Cucurbitaceae are more cultivated and then its cultivation can become an additional source of income. Moreover, in the literature, there is no study talking about the nutritional properties of the three CSO found in Cote d'Ivoire.

This study aims at comparing the physicochemical properties, the composition and the nutritional properties of the three CSO to each other and with that of refined palm oil (RPO).

Materials and Methods

Plant Material Obtention

Plant material consisted of seeds of the main pistachio consumed which are seeds of *Lagenaria siceraria*, seeds of *Citrullus lanatus* and seeds of *Cucumeropsis mannii*. These seeds, already hull, were brought from Katiola and Korhogo two regional capitals located respectively in the North and the North-Center of Cote d'Ivoire. The purpose of collecting or obtaining seeds from these two separate localities was to obtain some results which are in accordance to the reality of the area where Cucurbitaceae's are grown in the North-Center of Cote d'Ivoire seeing that the composition is influenced. They were brought from the local markets from Katiola and Korhogo in the months of January, February and March which is the moment where they are more available in these regions.

Botanical Identification

The specimens of seeds of *Lagenaria siceraria*, *Citrullus lanatus* and *Cucumeropsis mannii* were identified by botanical specialists of the National centre of Flora located inside the University of Felix Houphouët-Boigny-Abidjan, Cote d'Ivoire.

Some specimens are deposited at the herbarium of the National centre of Flora. The herbarium numbers are (Herbarium No. UCJ004413), (Herbarium No. UCJ004346), (Herbarium No. UCJ004373) respectively for *Lagenaria siceraria*, *Citrullus lanatus* and *Cucumeropsis mannii*.

Action on Cucurbitaceae Seeds Brought

Seeds brought were dried on the sun for four days in order to end the sun-dried process started by Cucurbitaceae-farmers. The

species were kept in each region and put on different plastic bags and sent at our Laboratory, located in University Peleforo Gon Coulibaly, for various investigations. At the laboratory, the same quantity from Katiola and from Korhogo of each species were mixed. Moisture content of the sun-dried seeds mixed was determined by heating 2 g of samples to a constant weight in crucible placed in an oven (MMM Medcenter GmbH (D-82152, Munich, Germany) maintained at 105 °C for 4 hours. These values calculated were $6.92 \pm 1.13\%$, 6.83 ± 1.17 , 7.08 ± 1.02 after three determinations respectively for *Citrullus lanatus* seeds, *Lagenaria siceraria* seeds and *Cucumeropsis mannii* seeds.

Oils Extraction

Oils were extracted in the mixture of each seed species using an electric grinder. The powder of each seed species was put in an expanded polystyrene bag and squeezed with a mechanical press for four hours. Oil which flowed out was caught in a can with a help of a funnel. At the end, the different oils obtained were filtered three times using thin sieves. These oils, shown on Figure 1, were utilized for different studies.

Physicochemical Parameters Determination

The acid and peroxide values were determined by the method of Devine and Williams [18]. The iodine value was obtained by the method of Strong and Koch [19]. Specific gravity was determined by the ratio of the mass of equal volume of Cucurbitaceae oil and water both kept at a temperature of 20 °C using a pycnometer according to the formula : $(m_2 - m_0) / (m_1 - m_0)$ with m_0 : mass of empty pycnometer, m_2 : mass of pycnometer full of oil and m_1 : mass of pycnometer full of water.

Characterization of CSO Using High Performance Liquid Chromatography Method

After each CSO was extracted, total fat acid and sterol composition were determined by molecular characterization. The characterization of total fatty acid was made in two steps. Firstly, compounds were separated using a High Performance Liquid Chromatography (ThermoFisher, France) and secondly the molecules separated were analyzed using also High Performance Liquid Chromatography (ThermoFisher, France) as part of apparatus. Sterol compounds were determined in three stages. Compounds which cannot be saponified were, in the first time, extracted. In the second time, they were isolated using thin coat silica gel chromatography method. At last, they were measured out using gaseous phase chromatography (chromatograph, GC8000).

Animal Breeding

Animals were bred, at 25 °C \pm 2 °C, in the animal house of the University Peleforo Gon Coulibaly of Korhogo (Cote d'Ivoire). During the breeding, rats were fed with food made by a company called "IVOGRAIN" which is specialized in mass production of livestock food. This food is produced industrially with the mixture of crude protein matter (15%), crude fat matter (3.5%), cellulose matter (12%), mineral matter (9%), calcium (1%), phosphorus

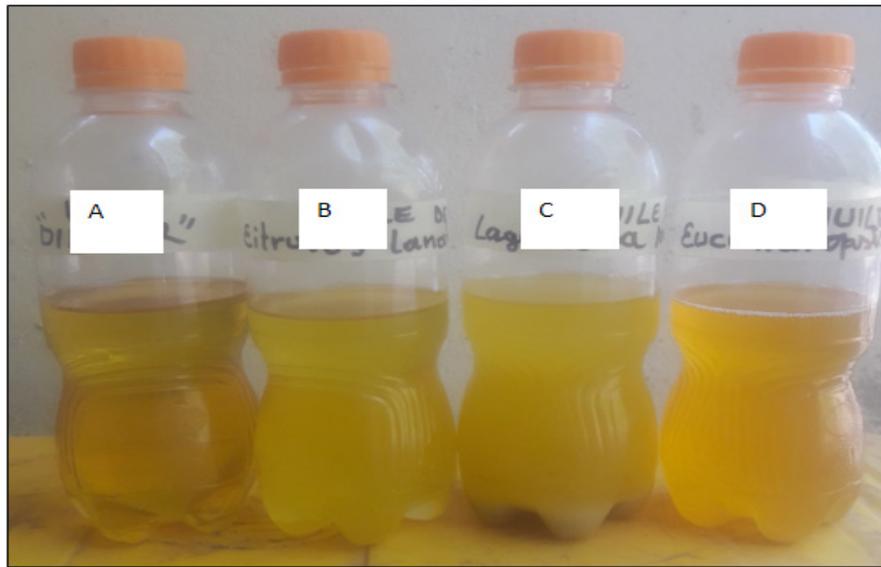


Figure 1 : Photo of refined palm oil and the cucurbitaceae seeds oils extracted
A : Refined palm oil extracted from palm fruit which is called in Cote d'Ivoire « Dinor »;
B : Oil extracted from *Citrullus lanatus* seeds;
C : Oil extracted from *Cucumeropsis mannii* seeds;
D : Oil extracted from *Lagenaria siceraria* seeds.

(0.9%), sodium (0.3%), vitamin A (15000 UI/kg), vitamin D3 (3000 UI/kg) and vitamin E (10 mg/kg).

Formulation of Diets used in the Experimentation

Four diets differing by the kind of oil, Refined palm oil (RPO) or *Citrullus lanatus* seeds oil (HCL) or *Lagenaria siceraria* seeds oil (HLS) or *Cucumeropsis mannii* seeds oil (HCM), were prepared. Each diet was composed by the mixture of 45.9% of maize starch, 10% of sugar cane, 5% of agar agar used as dietary fibre, 18% of lipids which were RPO or HCL or HLS or HCM, 13% of casein used as proteins sources, 3.5% of mineral mixture and 1% of vitamins mixture. All the diets were made as recommended by the American Institute of Nutrition according to Reeves, et al. [20]. In Table 1, it is shown the different percentages of ingredients used.

Preliminary Work before the Beginning of the Experimentation

The animal experiments were performed in compliance with the Ethical methodology approval used in Cote d'Ivoire University and the work has been reported in accordance with the ARRIVE guidelines (Animals in Research: Reporting In Vivo Experiments) [21]. Twenty four young *albino Wistar* rats with a mean weight of 51.5 ± 6.6 g were used. Animals age were between two and three weeks old at the beginning of the experimentation. At the beginning of the experimentation, animals were teamed in six homogeneous young groups of rats containing three males and three females. They were put individually in cages and maintained under standard laboratory conditions (temperature 25 ± 2 °C) with dark and light cycle (12h / 12h). In the cages, rats

were acclimatized to this condition and fed with the diet which will be utilized as experimentation diet during the duration of the experimentation and the five days before the beginning of the real experimentation.

Food Procedure during the Experimentation

After that, each group of rats constituted was fed *ad libitum* with one of the different diets formulated which are « diet RHDI » for RPO oil, « diet RHCL » in which CSO oil was used, « diet RHLS » that included HLS oil and « diet RHCM » for HCM oil for a 45 days diet. The food served was made in paste by adding to it a quantity of water in order to minimize the waste.

Dry Matter Measured

Every day, during the experimentation, when it was time to feed the rats, each kind of food which are, « diet RHDI », « diet RHCL », « diet RHLS » and « diet RHCM », was made in paste by adding to it a quantity of water clearly determined in order to minimize the waste. After that, a quantity of each mashed food made, was weighted and was given to each animal according to the group. Little mashed of the different food was, every day, weighted after being dried during 4 hours in an oven (MMM Medcenter GmbH (D-82152, Munich, Germany) at 105 °C and the weight obtained was written in a notebook. Then, with this sample the dry matter in each food given to animals can be calculated. The following day, before distributing the diets, the rests of food given the day before were separately collected and were weighted after being dried during 4 hours in an oven at 105 °C. The different weights obtained were also written in a notebook. This methodology permitted us to determine the total dry matter consumed every

Table 1: Percentages of nutriments used to formulate the different diets

Nutriments	Percentage (%)
Maizestarch	49.5
Sugar cane	10
Agar-Agar (dietary fibre)	5
Lipids	18
Proteins	13
Mineral mixture	3.5
Vitamins mixture	1

day by each animal which is the difference between the dry matter of food given the day before and the rest collected and dry the following day. Then, the total dry matter of food consumed by each group during the time of the experiment (45 days) is obtained by the summation of the dry matter consumed per day by each rat of the group during the 45 days. The mean Dry Matter Ingested every day (DMI/d) by each animal is obtained by the difference between Total Dry Matter of food consumed divided by 45.

Calculation of the Mean Weight Gain

The mean weight gain of each rat of each group during the time of the experimentation was calculated. The weight gain of each rat is obtained by the difference between the final body weight of a rat and the initial body weight of the same rat. Because there are six rats in a group and because the time of the experimentation is 45 days, the Mean Body Weight per group (MBW) is the summation of the difference between Final body weight (FBW) and Initial body weight (IBW) of the six rats of the group divided by 6 and by 45. Then, the Mean body weight (MBW) of each animal per group was obtained using the next formula: $MBW = \frac{\sum(FBW - IBW)}{6 \times 45} \pm SEM$

Calculation of the Mean Alimentary Efficacy Coefficient

The Alimentary Efficacy Coefficient (AEC) expresses the efficiency with which the diet has being ingested. This value was obtained by dividing the body weight gain per day of each rat of the group during the time of the experimentation by the Dry Matter Ingested (DMI) every day by each rat. Seeing that there are six rats per group, the Mean alimentary efficacy coefficient (MEAC) per group was obtained by summation the AEC of each rat in the group which value obtained was divided by the number of rats in the group (6). The following formula was used : $MAEC = \frac{\sum(AEC)}{6} \pm SEM$

Blood Sample Collected and Biochemical Parameters Analysis

At the end of the experimentation, blood sample was collected, after animal fast, at the vena cava level of all animal and put in individually vacuum valve. No anesthesia was used when we collected the blood sample. The method consist in using for each

animal a “pipette Pasteur” which we cut the tije and put the point on the vena cava located under the eye and the blood which flow out is colletcted inside the pipette Pasteur. When a specimen of blood is collected a surgical spirit is used to desinfect the vena and the animal still alive is put in a cage and is nourished during the duration of it life.

The blood samples were centrifuged at 8000 r.p.m for 15 min to harvest the plasma which was used for the various analysis. Serum samples were used for analysis of glyceamia, urea, creatinine, cholesterol parameters (triglycerides, total cholesterol, LDL cholesterol, HDL cholesterol and atherogenicity index calculation), aspartate aminotransferase (ASAT or TGO), alanine aminotransferase (ALAT or TGP), plasmatic sodium, plasmatic chlorine and plasmatic calcium using appropriate kits and an automatic chemistry analyzer (Hitachi model 902, Roche). All reagents and chemicals were purchased from Sigma-Aldrich.

Hematological Parameters

Also, at the end of the experimentation, blood sample was collected at the vena cava level of all animal and put in individually tube containing Ethylene diamine tetra acetic (EDTA). These blood samples were used for full blood count. Then, hematological parameters including hemoglobin content, total count of Red blood cells (RBC) and White blood cells (WBC), differential count of leukocytes such as granulocyte (%), lymphocyte (%), monocyte (%), Hematocrit (Hct), Mean cell volume (MCV), Mean corpuscular hemoglobin concentration (MCHC) and platelet count were measured using an automatic hematological analyzer (Symex-kx-21N).

Statistical Analysis

The experimental results were expressed mean and standard error (mean ± standard error). Data were assessed by the method of analysis of ANOVA and Newman-Keuls test thanks to STATISTICA 6.0 Software. The level $p \leq 0.05$ was considered as the cut-off value for significance. Curves were drawn using Microsoft Execl 2010.

Results

Physico-chemical Parameters of the Different Oils

The acid index of the three Cucurbitaceae oils are significantly higher ($p \leq 0.01$) than that of RPO. But, there is significant difference ($p \leq 0.05$) among the acid index of the three Cucurbitaceae oils. In fact, HLS has an acid index significantly lower ($p \leq 0.05$) than that of HCL and that of HCM and HCL acid index is significantly lower ($p \leq 0.05$) than that of HCM.

Iodine value of HCL is significantly higher ($p \leq 0.01$) than that of HCM which in turn is significantly higher ($p \leq 0.01$) than that of HLS. Iodine value of RPO value is significantly higher ($p \leq 0.01$) than that of HLS but it is significantly lower ($p \leq 0.05$) than that of HCL and it is slightly lower ($p > 0.05$) than that of HCM.

Peroxide index of HCL and HCM are significantly lower ($p \leq 0.01$) than that of RPO and HLS. That of HCL is also very lower ($p \leq 0.01$) than that of HCM. On the other hand, that of RPO is higher ($p \leq 0.05$) than that of HLS.

Specific gravity of HCM is almost the same with that of RPO. The Specific gravity of HCM and RPO are higher ($p \leq 0.05$) than that of HCL and lower than ($p \leq 0.05$) that of HLS [Table 2].

Molecular Characterization of the Different Oils

The results of the characterization are shown that *Citrullus lanatus seeds oil* (HCL) and *Lagenaria siceraria seeds oil* (HLS) contain high quantity of lauric acid. The percentage of lauric acid found in these oils is up above 15 % of the total component. The quantity of lauric acid found in *Cucumeropsis mannii seeds oils* (HCM) is the half of that found in *Citrullus lanatus seeds oil* and *Lagenaria siceraria seeds oil* when lauric acid found in refined palm oil do not reach 1% of it total component.

Among the experiment oils, refined palm oil is the most saturated followed respectively by *Cucumeropsis mannii seeds oil*, *Lagenaria siceraria seeds oil* and *Citrullus lanatus seeds oil*. Refined palm oil is also the most rich in palmitic acid and stearic acid among the four oils. *Citrullus lanatus seeds oil* contain more polyunsaturated fatty acids than the others oils and refined oil is the most lower in polyunsaturated fatty acids. All these results are shown in Table 3.

Growth Performance of Rats Fed with the Experimental Diets

Growth performance of rats which are consumed the different diets differing by the kind of oil used are shown in Figure 2. On this figure, it is shown a rapid growth of rats whatever the kind of oil consumed. However, this increase is higher in case of *Lagenaria siceraria seeds oil* consumed than the other oils which have practically the same influence on rats growth.

Evaluation of Parameters in Relation with Diets Consumption

Dry matter ingested by rats fed with diet RHDI is significantly low ($p < 0.05$) than that of rats fed with diet RHCL and high significantly low ($p < 0.01$) than that of rats fed with diet RHLS. On the other hand, dry matter calculated in case of diet RHDI

consumed is high than that of rats fed with diet RHCM. Dry matter ingested by rats fed with diet RHCL is virtually the same with that of rats fed with diet RHLS.

Body weight of rats fed with the different diets are shown in table 3. There is no significant difference ($p > 0.05$) between these values obtained on rats fed with diet RHDI, diet RHCL and diet RHCM. However, body weight of rats fed with diet RHLS is higher ($p < 0.05$) than body weight of rats fed with diet RHDI, diet RHCL and diet RHCM.

All the mean alimentary efficacy coefficient calculated whatever the diet consumed are not significantly different ($p > 0.05$) to each other. Table 4 is shown the parameters in relation with diet consumed.

Biochemical Parameters of Rats Fed with the Different Diets

Parameter of carbohydrate metabolism: Glycaemia

Glycaemia of rats fed with diet RHCL, diet RHCM and diet RHLS are not significantly different ($p > 0.05$) to that of diet RHDI. The values of glycaemia measured in blood sample of rats fed with diet RHCL and diet RHCM are not significantly different ($p > 0.05$) to each other. On the other hand, glycaemia of rats fed with diet RHCL is significantly low ($p > 0.05$) compared to that of rats fed with diet RHLS [Table 5].

Parameters of Nitrogen metabolism: Uremia and Creatinine

Uremia of rats fed with the different diets are not significantly different ($p > 0.05$) to each other. Rats fed with diets RHDI, RHLS and RHCL have quantity of blood creatinine which are not significantly different ($p > 0.05$) to each other. On the other hand, rats which consumed diets RHDI and RHLS have blood creatinine which are significantly high ($p \leq 0.05$) than that of rats which are consumed diet RHCM [Table 5].

Parameters of lipid metabolism

Blood total cholesterol

Total cholesterol measured on blood sample of rats fed with diets RHCM and RHLS are not significantly different ($p > 0.05$) to each other. On the other hand, these blood total cholesterol are significantly high ($p \leq 0.05$) than that measured on rats fed with diet RHCL. But, blood sample measured on rats fed with diet RHCL is significantly low ($p \leq 0.05$) than that measured on rats fed with diet RHCM [Table 5].

LDL Cholesterol

LDL Cholesterol level observed on blood sample of rats fed with diets RHCL, RHCM and RHCL are not significantly different ($p > 0.05$) to each other. On the other hand, these LDL Cholesterol level are significantly low ($p \leq 0.01$) compared to that of rats fed with diet RHCM [Table 5].

HDL cholesterol

HDL Cholesterol level observed on blood sample of rats fed with diets RHDI, RHLS and RHLS are not significantly different ($p >$

Alimentary diets	Acid index (mgNaOH/g of oil)	Iodine value (ml/g)	Peroxide index	Specificgravity
RPO	1.40±0.15 ^{a*}	52.67±1.34 ^{a*}	5.07±0.14 ^{a*}	0.92±0 ^{a*}
HCL	7.57±0.19 ^{aaa/bb/cc}	67.04±1.50 ^{aa/bbb/cc}	0.57±0.06 ^{aaa/bbb/cc}	0.90±0 ^{aa/bbb/cc}
HLS	6.61±0.43 ^{aaa/b*}	41.61±0.73 ^{aaa/b*}	4.13±0.23 ^{aa/b*}	0.94±0 ^{aa/b*}
HCM	8.39±0.23 ^{aaa/bb/c*}	54.92±1.27 ^{a/bbb/c*}	1.06±0.06 ^{aaa/bbb/c*}	0.92±0 ^{a/bbb/c*}

Values are means ± SE for three determinations

-HDI : Refined palm oil « Dinor » ;

-HLS : *Lagenaria siceraria* seeds oil ;

-HCL : *Citrullus lanatus* seeds oil ;

-HCM : *Cucumeropsis mannii* seeds oil

*This sign mean that comparisonis made according to this oil

a; b; c = no significant difference because $p > 0.05$.

aa;bb; cc =significantdifferencebecause $p \leq 0.05$.

aaa;bbb; ccc = high significant difference because $p \leq 0.01$.

aaaa;bbbb; cccc= very high significant difference because $p \leq 0.001$.

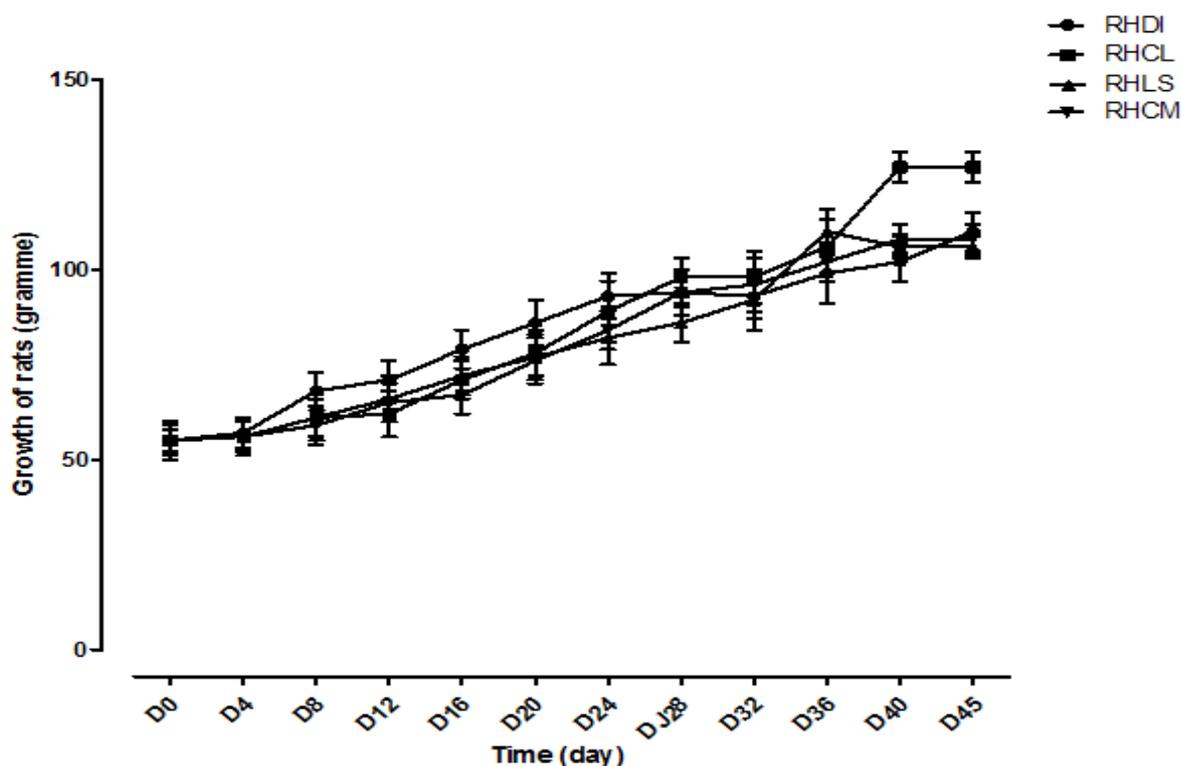


Figure 2 : Growth performance of rats fed with the experimental diets. What ever, the oil, We noticed a rapid growth of rats. This increase is greater at the end of the experimentation in the case of *Lagenaria siceraria* seed oil consumed than in other oils, but it was'nt significative ($p > 0.05$).

RHDI : Diet in which oil used is refined palm oil

RHCL : Diet in which oil used is *Citrullus lanatus* oil

RHLS : Diet in which oil used is *Lagenaria siceraria* oil

RHCM : Diet in which oil used is *Cucumeropsis mannii* oil

Table 3 : Fattyacid found in the different oils used in the experimentation

Lipids (oils)	Number of carbon and omega bond position	Refined palm « DINOR »(%)	<i>Citrulluslanatus</i> (%)	<i>Lagenariasiceraria</i> (%)	<i>Cucumeropsismannii</i> (%)
Laurica cid	C12 : 0n-0	0.87	16.08	16.90	7.51
Palmitic acid	C16 : 0n-0	22.34	---	11.21	5.35
Stearic acid	C18 : 0n-0	20.46	6.08	8.01	2.93
Arachidic acid	C20 : 0n-0	4.75	2.51	1.41	22.90
Total SFA	---	48.42	24.67	37.53	38.69
Oleic acid	C18: 1n-9	41.79	4.72	19.05	22.69
Linoleic acid	C18: 2n-6	0.59	44.12	15.56	11.59
Linolenic acid	C18: 3n-3	9.2	22.05	10.81	12.52
Total PUFA	---	9.79	66.17	26.37	24.11
---	---	---	1.64	14.47	14.09
---	---	---	1.58	2.57	0.41
---	---	---	1.22	---	---
Total fatty acids indeterminate	---	---	4.44	17.04	14.50

-SFA : Sarurated Fatty acids
-PUFA : Poly unsaturated Fatty acids
-UFA:Unsaturated Fatty acids

Table 4 : Parameters in relation with diet consumption

Diets	Dry matter ingested (g/d)	Mean body weight (g/d)	Mean alimentary efficacy coefficient
RHDI	0.78 ± 0.05 ^{a*}	1.01 ± 0.16 ^{a*}	1.31 ± 0.28 ^{a*}
RHCL	1.04 ± 0.16 ^{aa/b/ccc}	1.09 ± 0.15 ^{a/bbb/c}	1.10 ± 0.31 ^{a/ b/c}
RHLS	1.18 ± 0.03 ^{aaa/b*}	1.54 ± 0.09 ^{aaa/b*}	1.42 ± 0.31 ^{a/b*}
RHCM	0.6 ± 0.16 ^{aa/bbb/c*}	1.14 ± 0.39 ^{a/bb/c*}	2.00 ± 0.90 ^{a/b c*}

The values are shown as means± SE.

RHDI: Diet in which oil used is refined palm oil

RHCL: Diet in which oil used is *Citrulluslanatus* oil

RHLS: Diet in which oil used is *Lagenariasiceraria* oil

RHCM: Diet in which oil used is *Cucumeropsismannii* oil

*Mean the comparisonis made using this diet as reference

a; b; c = when there is no significant difference ($p > 0.05$) ;

aa;bb; cc = significant difference ($p \leq 0.05$) ;

aaa;bbb; ccc = high significant difference ($p \leq 0.01$) ;

aaaa;bbbb; cccc= very high significant difference($p \leq 0.001$).

0.05) to each other. On the other hand, HDL Cholesterol level observed on blood sample of rats fed with diets RHCL and RHCM are significantly high ($p \leq 0.05$) compared to that measured on blood sample of rats fed with diet RHDI. Concerning HDL Cholesterol measured on rats fed with diet RHCL, it is significantly high ($p \leq 0.01$) compared to that of rats fed with diet RHDI [Table 5].

Atherogenicity index

Calculation of atherogenicity index of rats fed with diets RHLS and RHCL are not significantly different to each other. On the other hand, these values of atherogenicity index calculated are very low compared to that of rats fed with diets RHDI and RHCM. However, atherogenicity index of rats fed with diet RHDI is significantly low ($p \leq 0.05$) compared to that fed with diet RHCM [Table 5].

Measurement of blood triglyceride

Blood triglyceride of rats fed with diets RHCL and RHLS are not significantly different ($p \geq 0.05$) to each other but they are very significantly low ($p \leq 0.001$) compared to that obtained on rats fed with diet RHDI and die RHCM. On the other hand, blood triglyceride measured on rats fed with diets RHDI are significantly low ($p \leq 0.05$) compared to that measured on rats fed with diets RHDI [Table 5].

Parameters in relation with liver functioning: TGO and TGP

TGO level on blood sample of rats fed with diets RHLS and RHCM are not significantly different ($p \geq 0.05$) to each other but these blood sample level are significantly low ($p \leq 0.05$) compared to that fed with diet RHCM. TGO level on blood sample of rats fed with diets RHCL is not significantly different to that measured on rats fed with diet RHDI but it is very significantly high ($p \leq 0.001$) compared to TGO level of rats fed with diet RHLS (Table 5).

TGP level on blood sample of rats fed with diets RHDI and RHCL are not significantly different ($p \geq 0.05$) to each other. Again, TGP level on blood sample of rats fed with diets RHLS and RHCM are not significantly different ($p \geq 0.05$) to each other but these blood sample level are significantly high ($p \leq 0.05$) compared to that fed with diet RHDI [Table 5].

Parameter of Mineral Metabolism: Blood sodium and Blood calcium

Sodium level in blood sample of rats fed with diets RHCL, RHLS and RHCM are significantly high ($p \leq 0.01$) compared with that of rats fed with diet RHDI. Sodium level in blood sample of rats fed with diets RHCM is significantly high ($p \leq 0.05$) than that of rats fed with diet RHLS. This sodium level in blood sample of rats fed with diets RHCM is significantly high ($p \leq 0.01$) than that of rats fed with diet RHCL. There is no significant difference ($p > 0.05$) of sodium level in blood sample between rats fed with diet RHCL and diet RHLS [Table 5].

Values of calcium measured on blood sample of rats fed with the different diets are not significantlt differents ($p > 0.05$) to each other [Table 5].

Evaluation of Haematological Parameters Measured on Blood Sample

Erythrocytic bloodline parameters

Red blood cells (RBC)

Red blood count on blood sample of rats fed with diets RHDI, RHLS and RHCM are not significantly different ($p > 0.05$) to each other. On the other hand, there are significantly high ($p \leq 0.05$) compared to that of rats fed with diet RHCL [Table 6].

Hemoglobin

The quantity of hemoglobin in red blood cells of rats fed with diets RHDI, RHLS and RHCM are not significantly different ($p > 0.05$) to each other. On the other hand, there are significantly high ($p \leq 0.01$) compared to that of rats fed with diet RHCL [Table 6].

Hematocrit

The percentage of Red blood cells in blood sample of rats fed with diets RHDI, RHLS and RHCM are not significantly different ($p > 0.05$) to each other. On the other hand, there are significantly high ($p \leq 0.01$) compared to that of rats fed with diet RHCL [Table 6].

Mean corpuscular volume (MCV)

Mean Corpuscular Volume of rats fed with diets RHDI, RHLS, RHCM and RHCL are not significantly different ($p > 0.05$) to each other [Table 6].

Mean corpuscular hemoglobin (MCH)

Mean Corpuscular Hemoglobin of rats fed with diets RHDI, RHLS, RHCM and RHCL are not significantly different ($p > 0.05$) to each other [Table 6].

Mean corpuscular hemoglobin concentration (MCHC)

Mean Corpuscular Hemoglobin Concentration of rats fed with diets RHDI, RHLS, RHCM and RHCL are not significantly different ($p > 0.05$) to each other (Table 6).

Leucocytic bloodline parameters

White Blood Cells (WBC)

White blood cells count on blood sample of rats fed with diets RHDI, RHLS, RHCL and RHCM are not significantly different ($p > 0.05$) to each other (Table 6).

Neutrophil

The number of neutrophil in blood sample of rats fed with diets RHDI, RHLS, RHCL and RHCM are not significantly different ($p > 0.05$) to each other [Table 6].

Eosinophil

The number of neutrophil in blood sample of rats fed with diets RHDI, RHLS and RHCL are not significantly different ($p > 0.05$) to each other. On the other hand, there are significantly high ($p \leq 0.01$) compared to that of rats fed with diet RHCM [Table 6].

Table 5: Biochemical parameters of rats fed with the different diets E.

Diets	Glycemia (g/l)	Uremia (g/l)	Creatinine (mg/l)	T.Chol(g/l)	LDL (g/l)	HDL (g/l)	LA (LDL/HDL)	Trigly (g/l)	TGO (UI/l)	TGP (UI/l)	Sodium (mmol/l)	Calcium (mmol/l)
RHDI	0.78±0.31 ^{a*}	0.17±0.02 ^{a*}	7.3±1.1 ^{a*}	0.96±0.12 ^{a*}	0.57±0.07 ^{a*}	0.12±0.02 ^{a*}	4.75±3.5 ^{a*}	1.41±0.05 ^{a*}	8±3.27 ^{a*}	2.7±1.49 ^{a*}	93.8±4 ^{a*}	8.43±0.03 ^{a*}
RHCL	0.60±0.39 ^{a/b/c}	0.17±0.06 ^{a/b/c}	5.8±1.6 ^{a/b/c}	0.86±0.30 ^{a/b/cc}	0.61±0.14 ^{a/b/cc}	0.19±0.01 ^{aa/b/c}	3.21±14.0 ^{aaa/b/c}	1.09±0.43 ^{a/b/c}	7.8±4.8 ^{a/bb/c}	2.8±1.95 ^{a/bb/cc}	109±6 ^{aaa/b/cc}	7.05±0.25 ^{a/b/c}
RHLS	0.99±0.12 ^{a/b*}	0.18±0.05 ^{a/b*}	6.7±0.5 ^{a/b*}	1.14±0.13 ^{aa/b*}	0.60±0.05 ^{a/b*}	0.19±0.04 ^{aa/b*}	3.15±1.25 ^{aaa/b*}	1.50±0.14 ^{a/b*}	3.01±1.4 ^{aa/b*}	1.03±0.5 ^{aa/b*}	116±8 ^{aaa/b*}	8.58±0.03 ^{a/b*}
RHCM	0.75±0.27 ^{a/b/c*}	0.18±0.01 ^{a/b/c*}	5.5±0.9 ^{a/bb/*}	1.22±0.03 ^{aa/b/*}	0.84±0.02 ^{aaa/bb/bc*}	0.16±0.24 ^{aa/b/c*}	5.25±0.08 ^{aaa/bbb/c*}	1.26±0.15 ^{a/bb/c*}	3.45±0.2 ^{aa/b/c*}	1.13±0.04 ^{aa/b/c*}	128±2 ^{aaa/bb/c*}	8.45±0.03 ^{a/b/c*}

The values are shown as means ± SE.

RHDI: Diet in which oil used is refined palm oil

RHCL: Diet in which oil used is *Citrullus lanatus* oil

RHLS: Diet in which oil used is *Lagenariasiceraria* oil

RHCM: Diet in which oil used is *Cucumeropsismannii* oil

*Mean the comparison is made using this diet as reference

a; b; c = when there is no significant difference ($p > 0.05$);

aa; bb; cc = significant difference ($p \leq 0.05$);

aaa; bbb; ccc = high significant difference ($p \leq 0.01$);

aaaa; bbbb; cccc = very high significant difference ($p \leq 0.001$)

Table 6: Hematological parameters of rats fed the various experimental diets				
Parameters	Diet	Diet	Diet	Diet
	RHDI	RHCL	RHLS	RHCM
Erythrocytic bloodline parameters				
RBC ($10^6/mm^3$)	1.67±0.05 ^{a*}	1.28±0.4 ^{aa/bb/cc}	1.73±0.09 ^{a/b*}	1.67±0.05 ^{a/b/c*}
Hemoglobin (g/100ml)	5.51±0.44 ^{a*}	4.35±1 ^{aa/bb/cc}	5.63±0.38 ^{a/b*}	5.9±0.59 ^{a/b/c*}
Hematocrit (%)	16.4±1.32 ^{a*}	13±3.21 ^{aa/bb/cc}	16.8±1.17 ^{a/b*}	17.4±1.68 ^{a/b/c*}
MCV (fl)	96.4±4.3 ^{a*}	103±8.7 ^{a/b/c}	97.2±5.3 ^{a/b*}	98.2±3 ^{a/b/c*}
MCH (pg)	32±1.25 ^{a*}	34.4±2.8 ^{a/b/c}	32.4±2.42 ^{a/b*}	33±0.9 ^{a/b/c*}
MCHC (g/100ml)	33.4±0.85 ^{a*}	33.5±0.1 ^{a/b/c}	33.5±0.33 ^{a/b*}	33.3±0.26 ^{a/b/c*}
Leucocytic bloodline parameters				
RBC ($10^3/mm^3$)	0.64±0.04 ^{a*}	0.68±0.07 ^{a/b/c}	0.68±0.07 ^{a/b*}	0.70±0.04 ^{a/b/c*}
Neutrophil ($10^3/mm^3$)	33.3±12 ^{a*}	32.2±15.3 ^{a/b/c}	27±6.3 ^{a/b*}	24.4±8.4 ^{a/b/c*}
Eosinophil ($10^3/mm^3$)	8±2 ^{a*}	8±0.2 ^{a/b/cc}	9±2.4 ^{a/b*}	16±2 ^{aaa/bbb/c*}
Monocytes ($10^3/mm^3$)	15.5±4.4 ^{a*}	8±0.21 ^{aa/bb/cc}	21.4±3.5 ^{a/b*}	29.6±2.2 ^{a/b/c*}
Lymphocytes ($10^3/mm^3$)	599±44 ^{a*}	643±59 ^{a/b/c}	635±67 ^{a/b*}	637±58 ^{a/b/c*}
Platelet				
Platelets ($10^3/mm^3$)	140±32 ^{a*}	134±38 ^{a/b/c}	139±24 ^{a/b*}	122.5±19 ^{a/b/c*}

The values are shown as means± SE of six determinations.

RHDI:Diet in which oil used is refined palm oil

RHCL:Diet in which oil used is *Citruslanatus* oil

RHLS:Diet in which oil used is *Lagenariasiceraria* oil

RHCM :Diet in which oil used is *Cucumeropsismannii* oil

*Mean the comparison is made using this diet as reference

a; b; c = when there is no significant difference ($p > 0.05$);

aa;bb; cc = significant difference ($p \leq 0.05$);

aaa;bbb; ccc = high significant difference ($p \leq 0.01$);

aaaa;bbbb; cccc= very high significant difference ($p \leq 0.001$).

Monocytes

The number of monocytes in blood sample of rats fed with diets RHDI, RHLS and RHCM are not significantly different ($p > 0.05$) to each other. On the other hand, there are significantly high ($p \leq 0.01$) compared to that of rats fed with diet [Table 6].

Lymphocytes

The number of neutrophil in blood sample of rats fed with diets RHDI, RHLS, RHCL and RHCM are not significantly different ($p > 0.05$) to each other [Table 6].

Platelet

The detailed account of platelet in blood sample of rats fed with the different diets are not significantly different ($p > 0.05$) to each other [Table 6].

Discussion

According to the acid index determined, it's appeared that RPO contain low free fatty acid than LSO which in turn contain low free fatty acid than HCL. CMO is the oil which contain the most quantity of free fatty acid among the different oils used in our study. Compare the quantity of soda used to neutralize 1 g of free fatty contain in the different oils to the standard norm of Codex alimentarius (Maximum of 6.6 mg of NaOH/g) and the International Olive Council (Maximum 4 mg of NaOH/g of oil), RPO and LSO are in the norm suggesting that these two oils can be consumed without any risk of damage on health but not the others. But, acid index value obtained are lower than the minimum safe limit (15%) mean for consumption. This suggests that these oils have low deteriorating rate and can therefore be stored for relatively long period [22].

According to the results obtained, the ranking of the different oils per low peroxide index value is HCL, CMO, LSO and RPO. That mean that the degree of the oxidation of unsaturated fatty acids contain in RPO is higher than that contain in LSO which is also higher than that contain in LSO and which in turn is lower than that contain in RPO [23,24]. Then, RPO is the oil in which unsaturated fatty acids are the most oxidized. That proves that, cucurbitaceae oils are good for consumption.

Norm of Codex alimentarius and the International Olive Council standard about peroxide index are fixed to a maximum level of 20 meq O_2 /Kg of oil [25]. All our study sample have a peroxide index

value which are in accordance with the commercial norm. This testify that the methodology we used to make extraction of the cucurbitaceae oils do not provoke major oxidation of these oils. Also, that mean that the time of storage do enhance the raise of rancidity [26].

According to Specific gravity value obtained which are similar to the norm established by the Codex alimentarius and the International Olive Council (0.910-0.916), we can claim that the different oils used are in the pure state testifying again that the methodology we choose to make extraction is a very good method.

Iodine value of RPO obtained in our study is in extent between limits (45-58) found in the literature [27]. Because all the iodine value value obtained with the different oils used are inferior to 100 mg / 100 g, we give claim that they are non-drying [28]. According to these iodine value obtained, HCL is the oil which iodine value is the most high following by HCM, RPO and HLS respectively. Knowing that more an oil has a high iodine value more this oil contain double bond, we can suggest that the ranking of high content of double bond is HCL, HCM, RPO and HLS. The molecular characterization give the same order of unsaturated fatty acid content even if there is a percentage of component which are not being determined. Following the result of Fokou, et al. [29], we can say that HCM and HLS from North and Centre of Cote d'Ivoire are contained low unsaturated fatty than that of Cameroun (69% of unsaturated fatty acid found in HCM and 78% of unsaturated fatty acid found in HLS). Ziyada, et al. [30] are found a quantity of 71% of unsaturated fatty acid in HCM from Soudan. The different of composition can depend of the type of ground and the pluviometry. In fact, it is know that the factor of the variation in nutrient content in crops depend to the cultivation environment [31].

The quantity of dry matter ingested by rats which are consumed diet RHCL and diet RHLS were practically the same but were superior than that of rats which consumed diet RHDI and diet RHCM. Diet RHCL and diet RHLS may have the same appetite due probably to their good arome and flavour which may be better than that of diets RHDI and RHCM. In fact, according to several researchers [32,33], food consumption depend to several factor such as physiological state of the organism and also the characteristics of the food which are their arome, their flavour and their chemical composition. It is observed a low dry matter

ingested in our study than the result of the study made by Meite, et al. [34] when they utilized defatted cake of *Citrullus lanatus* to make bread which served to feed rats. That is due to the fact that bread make contain low quantity of lipids which are food constituent the most energetic.

Mean body weight are in the same way with the dry matter ingested. In any case, mean alimentary efficacy coefficient calculated were not significantly different ($p > 0,05$) because the differents food have the same constituent and group of animals constituted were in the same physiological state. The difference beetwen food was the kind of oil. Even if the different oil used do not have the same arome and flavour, these characteristics do not impact on the differents mean alimentary efficacy coefficient.

There is certainly some difference beetween glyceamia measured on blood sample of the different group of rats but all these glyceamia may be normal because according to Durimel, et al. [35] normal glycaemia on rat are between 0.7 g/l and 1.2 g/l and glyceamia measured in our study are between $0,60 \pm 0,39$ g/l and $0,99 \pm 0,12$ g/l. Then, these diets do not provoke mayor disruption on carbohydrate metabolism. Moreover, when we observed the standard deviation, these diets can be able to reduce glyceamia. The reduction of glycaemia could be a good thing for person who suffered from hypoglycaemia attack.

The fact that plasmatic calcium and phosphorus are not significantly different ($p > 0.05$) to each other explained similar bone metabolism whatever the diet consumed.

According to the plasma sodium level measured, we can believe that consumption of refined palm oil is associated to low risk of cardiovascular disease than cucurbitaceae oils. In any case, seeing that oils are used in our experimentation beyond the norm, lipidic parameters are the main factor to estimate the cardiovascular risk. Values of lipid parameters (triglycerides, total cholesterol, LDL-cholesterol and HDL-cholesterol) measured in blood sample are difficult to explain. On the other hand, atherogenicity index calculation give the better way to evaluate risk of cardiovascular disease and obesity [36-38]. Then, according to atherogenicity index calculation, the consumption of diet RHLS may lead to low risk of cardiovascular disease than diet RHCL consumption and diet RHCL consumption in turn may lead to low risk of cardiovascular disease than diets RHDI and RHCM. At finisf, the rank of inducing cardiovascular disease highly is RHCM, RHDI, RHCL and RHLS. So, RPO come in the fird position, only befor HLS in the risk of inducing cardiovascular disease. We know that one of the arguments underpinning the questioning of palm oil healthfulness is its saturated fatty acids content, and specifically the relationship of palmitic acid to cardiovascular health [39,40]. And in our work, the molecular characterization show that palmitic acid content in RPO is very higher than that found in all CSO. That is an argument which indicated that RPO may induce cardiovascular disease than all CSO used in our study.

Urea and creatinine measured on blood sample of the different group of rats do not show any significant difference ($p > 0.05$)

when compared to each other suggesting that the functioning of kidneys is good whatever the diet consumed [41,42]. These results are corroborated by the differents kidneys weight which are not significantly differents to each other whatever the diet consumed.

TGO and TGP level on blood sample of rats fed with diets RHDI and RHCL are significantly higher ($p \leq 0.05$) than that of rats fed with diets RHCM and RHLS. That can explain a high risk of liver disease due to consumption of diets RHDI and RHCL than the consumption of diets RHCM and RHLS. HCL was recognized in previous study made by Madhavi, et al. [43] for it protector effects on liver.

The fact that hematological parameters measured on rats fed with the different diets were not significantly different ($p > 0.05$) from each other imply that medullar production of erythrocyte, leucocyte and platelet was similar on bone marrow cells of the different rats whatever the diet consumed.

Conclusion

We can notice that, in spite of no technological treatment done on «pistachio» oils, are better than RPO in reducing cardiovascular disease risk. Therefore, Pistachio oils are constituted a good alternative oils to replace palm oil in human diet if their extraction is made with cold-pressed as we do it. If cucurbitaceae seeds is used in industry of oil for human diet, the sector of cucurbit seeds could be an important activity for agriculture in Côte d'Ivoire. Another studies can be made in order to determinate their insaponifiable (vitamins, antioxidant, carotin) content. Also, deodorization can permit to add some nutritioal value on these oils.

Authors' Contributions

Howele Ouattara wrote the experimental protocol, Atsamo Donatien Albert corrected and completed this protocol. Both the two first authors wrote the first draft of the manuscript. Bazoumana Ouattara conducted the staticall analysis of the data. Vazoumana Kone who is a student was a precious help in carrying out the experiment while Séraphin Kati-Coulibaly (the professor) revised the first draft and gave some recommandation. This work was financed by the three firts author's. At finished, all authors approved the final manuscript.

Ethics Approval and Consent to Participate : All experiments were approved by the regulation in force in Cote d'Ivoire University.

References

1. Zoro Bi IA, Koffi KK, Dje Y, Malice M, Baudoin JP. Botanical and agronomic characterization of three species of cucurbits consumed in sauce in West Africa: *Citrullus sp. Cucumeropsis mannii Naudin* and *Lagenaria siceraria (Molina)* Stand. Biotechnology Agronomy Society and Environmen. 2003; 7: 189-199.
2. Zoro Bi IA, Koffi KK, Dje Y. Indigenous cucurbits of Cote d'Ivoire: a review of their genetic resources. Sciences et Nature. 2006; 3: 1-9.
3. Badifu GIO. Effect of processing on proximate composition, antinutritional and toxic contents of kernels from Cucurbitaceae species grown in Nigeria. Journal of Food Composition and Analysis. 2001; 14: 153-161.
4. United States Department of Agriculture (USDA). USDA nutrient database for standard reference. 2002; release 15.
5. United States Department of Agriculture (USDA). Nutrient Database. 2006; Sr-15, 11218-1206.
6. Omafuvbe BO, Falade OS, Osuntogun BA, Adewusi SRA. Chemical and biochemical changes in African locust bean (*Parkia biglobosa*) and melon (*Citrullus vulgaris*) seeds during fermentation to condiments. Pakistan Journal of Nutrition. 2004 ; 1(3) : 140-145. DOI: 10.3923/pjn.2004.140.145
7. Loukou AL, Gnakri D, Dje Y, Kippre AV, Malice M, Baudoin J P, Bi IA. Macronutrient composition of three cucurbit species cultivated for seed consumption in Cote d'Ivoire. African Journal of Biotechnology. 2007 ; 6(5) : 529-533.
8. Azhari S, Xu YS, Jiang QX, Xia WS. Chemical and nutritional properties of Seinat (*Cucumis melo* var. tibish) seeds. Journal of Academia and Industrial Research. 2014; 2(9): 495-499.
9. United States Department of Agriculture (USDA). Foreign Agricultural Service, Office of Global Analysis. 2017. 36p.
10. FAOSTATISTIC. 2014.
11. Jacquemard JC. The oil palm, Versailles, Quae editions, coll; (pref. Philippe Lhoste): « Tropical agriculture in your pocket», 2012. p.5.
12. United States Department of Agriculture (USDA). China Soybean Imports Lowered Imports down 16 Million Tons Since June, United States Department of Agriculture (USDA). Oilseeds: World Markets and Trade; United States Department of Agriculture : Foreign Agricultural Service: Washington, DC, USA. 2018
13. FAO. Fats and oils in human nutrition. Report of a joint expert consultation. 1996 ; 5-137.
14. D Bester, A J Esterhuysen, E J Truter, J van Rooyen. Cardiovascular effects of edible oils: A comparison between four popular edible oils. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2010; 23: 334-348. doi: 10.1017/S0954422410000223
15. Nathan A Berger. Obesity and cancer pathogenesis. Ann N Y Acad Sci. 2014; 1311: 57-76. doi: 10.1111/nyas.12416.
16. Eva Gesteiro, Luis Guijarro, Francisco J Sanchez Muniz, Maria Del Carmen Vidal Carou, Ana Troncoso, Lluís Venanci. Palm Oil on the Edge. Nutrients. 2019; 11: 2008. doi: 10.3990/nu11092008
17. Margono BA, Potapov PV, Turubanova S, Stolle F, Hansen MC. Primary forest cover loss in Indonesia over 2000-2012. Nature Climate Change. 2014; 4: 730-735.
18. Devine J, Williams PN. The Chemistry and technology of edible oils and fats. 1st ed. London: Pergamon Press; 1961.
19. Strong FM, Koch GH. Biochemistry laboratory manual. 2nd ed. Dubuque IA : I.M.C ; 1974.
20. Reeves PG, Nielsen FH, Fahey GCJ. AIN-Purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 1993; 123 (11): 1939-1951. doi: 10.1093/jn/123.11.1939
21. Carol Kilkenny, William J Browne, Innes C Cuthill, Michael Emerson, Douglas G Altman. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. PLoS Biol. 2010; 8(6): e1000412. doi: 10.1371/journal.pbio.1000412
22. Ekpa OD, Ekpa UJ. Comparison of the characteristic parameters and deterioration properties of oil from the Tenera and Dura variety of the oil palm. Nigerian J Chem Res. 1996; 1 : 26-33. DOI: 10.4314/njcr.v1i1.35613
23. Rahmani M. Chemical composition of "virgin" argan oil. Cahiers Agricultures. 2005; 14(5): 461-465.
24. Womeni HM, Ndjouenkeu R, Kapseu C, Parmentier M, Fanni J. Application of the drying-frying process to shea kernels: influence on the chemical quality indices and melting properties of butter. Oilseeds Fats Lipids. 2006 ; 13(4), 297-302. doi.org/10.1051/ocl.2006.0012
25. Connell JJ. Control of fish quality, Fishing news. 4th edition. England: Wiley ; 1995. ISBN: 978-0-852-38226-4. 256p.
26. Ouaouich et Chimi. Olive oil producer's guide. United Nations Industrial Development Organization. Vienne: 2007.
27. Alfred T. Ullmann's Encyclopedia of Industrial Chemistry. 6th Edition. Fats and Fatty Oils, Wiley-VCH Verlag GmbH & Co. 2002.
28. Codex alimentarius. Standards for named vegetable oils. CXS 210-1999. 1999.
29. Fokou E, Achu MB, Kansci G, Ponka R, Fotso C. Tchiegang et Tchouanguep FM. Chemical composition of some cucurbitaceae oils from Cameroon. Pakistan Journal of Nutrition. 2009; 8(9): 1325-1334.
30. Ziyada AK, Elhussien SA. Physical and chemical characteristics of *Citrullus lanatus* Var. *Colocynthis* Seed Oil in Journal of Physical Science. 2008; 19(2): 69-75.
31. Fageria NKVC, Baligar VC. Encyclopedia of soils in the environment. Daniel Hillel. 2005; ISBN: 978-0-12-348530-4. 2200p.
32. Martin R Yeomans, John E Blundell, Micah Leshem. Palatability: response to nutritional need or need-free stimulation of appetite ? Br J Nutr. 2004; 92: S3-14. doi: 10.1079/bjn20041134.
33. E Guichard. Interactions between flavor compounds and food ingredients and their influence on flavor perception. Food Reviews International. 2007; 18(1): 49-70. doi.org/10.1081/FRI-120003417
34. Meite A, Kouame KG, Katil Coulibaly S, Offoumou AM. Study of the nutritional value of normal bread and composite breads containing delipidated seed flour of *Citrullus lanatus* (Cucurbitaceae). Bulletin of the Royal Society of Sciences of Liege. 2008; 77: 80-103.
35. Durimel, Etienne, Mannoni, Souckchaine. Hormonal regulation of blood sugar in rats. Bachelor of Biology, University of Antilles Guyannes. 2002; 1-6.

36. Gaojun Cai, Ganwei Shi, Sheliang Xue, Wei Lu. The atherogenic index of plasma is a strong and independent predictor for coronary artery disease in the Chinese Han population. *Medicine (Baltimore)*. 2017; 96(37):e8058. doi: 10.1097/MD.00000000000008058
37. K Bora, M S Pathak, P Borah, Md I Hussain, D Das. Association of the Apolipoprotein A-I gene polymorphisms with cardiovascular disease risk factors and Atherogenic indices in patients from Assam, Northeast India. *Balkan J Med Genet*. 2017; 20(1): 59-70. doi: 10.1515/bjmg-2017-0002
38. Xiaowei Zhu, Lugang Yu, Hui Zhou, Qinhu Ma, Xiaohua Zhou, Ting Lei, et al. Atherogenic index of plasma is a novel and better biomarker associated with obesity: a population-based cross-sectional study in China., *Lipids in Health and Disease*. 2018; 17: 37 doi.org/10.1186/s12944-018-0686-8
39. Carlo Agostoni, Luis Moreno, Raanan Shamir. Palmitic acid and health: Introduction. *Crit Rev Food Sci Nutr*. 2016;b56(12): 1941-1942. doi: 10.1080/10408398.2015.1017435.
40. Lucci P, Borrero M, Ruiz A, Pacetti D, Frega NG, Diez O, et al. Palm oil and cardiovascular disease: A randomized trial of the effects of hybrid palm oil supplementation on human plasma lipid patterns. *Food Funct*. 2016; 7(1): 347-354. doi: 10.1039/c5fo01083g
41. Bankir L. Urea and the kidney, In: *The Kidney*. 5th ed. Brenner BM, Rector FC Jr. Philadelphia: WB Saunders; 1986. 571p-606p.
42. Seronie S, Vivien M, Galteau M, Carlier MC, Hadj A. Serum creatinine assay in 2003: analytical inventory and calibration standardization test. *Annals of clinical biology*. 2004; 62: 165-175.
43. Madhavi P, Rao M, Vakati K, Rahman H, Eswaraiiah CM. Evaluation of anti-inflammatory activity of *Citrullus lanatus* seed oil by In-vivo and Invitro models. *Int Res J Pharm App Sci*. 2012; 2(4): 104-108.