Scientific Research Publishing

Food and Nutrition Sciences, 2024, 15, 1201-1217
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Evaluation of the Influence of Thermooxidation of an Unconventional *Scyphocephalium ochocoa* Oil on Its Quality and on the Biological Parameters of Wistar Rats

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How to cite this paper: Elenga, M., Ibara Beah Inianga, T., Wossolo Lingomo, S., Miantoko Zebita, G.R., Moulengo Massamba, S., Itoua Okouango, Y.S. and Mananga, V. (2024) Evaluation of the Influence of Thermooxidation of an Unconventional *Scyphocephalium ochocoa* Oil on Its Quality and on the Biological Parameters of Wistar Rats *Food and Nutrition Sciences*, **15**, 1201-1217.
https://doi.org/10.4236/fns.2024.1512076

Received: June 27, 2024
Accepted: December 8, 2024
Published: December 11, 2024

Abstract

The aim of this study was to evaluate the effects of thermooxidation of *S. ochocoa* oil on its quality and on the biochemical and anthropometric parameters of Wistar rats. *S. ochocoa* seeds from the Lekoumou department were used for oil extraction at the press. The parameters measured were oil content and chemical indices, determined for samples of oil unheated and heated to 220˚C as a function of the number of heatings and the environment (open air, freezer). Two diets were created: the control diet and the treated diet, in which 10% oil was substituted for the control diet. These diets were fed to the rats for 28 days. Biochemical, anthropometric and atherogenicity parameters were measured. The results show that *S. ochocoa* oil has a saturated character and a blackish color, and it solidifies or coagulates rapidly. Unheated oil samples placed in a freezer and unheated samples placed in a simple freezer have relatively lower acid values (0.860 and 0.703 mg KOH/g respectively) than other samples. Acid index values for heated samples increase progressively with the number of heatings (1.063 ± 0.005 for 1st heating, 1.290 ± 0.010 for 2nd heating

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and 1.890 ± 0.006 for 3rd heating). The oil's peroxide value increased with temperature and oxidation (65.6 ± 0.03 for unheated oil vs. 97.9 ± 003 for oil heated to 220˚C). The diet supplemented with this oil resulted in a significant increase in plasma cholesterol levels, and a significant decrease in triglyceride levels. The plasma atherogenicity index reveals the risk of cardiovascular disease associated with this food.

Keywords

Thermooxidation, Oil, *S. ochocoa*, Chemical Indices, Biological Parameters, Wistar Rats, Plasma Atherogenicity Index, Congo

1. Introduction

Vegetable oils play a very important role in the human diet. They contribute to improving the organoleptic quality of food, to the proper functioning of the body and represent a source of energy, vitamins (ADEK) and essential fatty acids [1] [2]. These oils are sometimes consumed after frying at a temperature above the boiling point of water [2] [3]. When food is fried, the fatty acids present in the oil undergo significant chemical and physical alterations, resulting in a change in organoleptic characteristics, a reduction in nutritional value and the formation of products harmful to health, such as free radicals and aldehydes [4] [5]. These compounds are capable of disrupting membrane structures and cellular functions, which can lead to the onset of degenerative diseases such as cancer, the aging process and cardiovascular disease, the leading cause of morbidity and mortality worldwide [6] [7] and the most common cause of death in the United States, more than all cancers combined [8]. The most significant chemical alterations are due to oxidation reactions which, depending on their extent, can reduce nutritional value, alter taste, and modify the texture and appearance of the foodstuff [9]. The phenomenon of fatty acid oxidation leads to organoleptic degradation, with the appearance of a characteristic "rancid" flavor that alters product quality [10].

Lipid thermo-oxidation generally leads to undesirable consequences, damaging organoleptic qualities and nutritional value, and toxic substances may be formed.

The populations of the Lékoumou department in Congo-Brazzaville in the heart of the Congo basin [11], to improve the organoleptic qualities of edible dishes, particularly the cassava leaf dish (saka saka), use a condiment from spontaneous edible flora called the powder of *S. ochocoa* fruits. It is possible to extract oil from this powder. This oil could undergo several chemical transformations leading to its alteration during cooking. According to Hayes and Koshla [12] myristic acid may be the dietary saturated fatty acid that induces the greatest increase in plasma cholesterol. The aim of our work is therefore to assess the effects of thermooxidation of *Scyphocephalium ochocoa* oil on its quality and on the biological parameters of Wistar rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

The plant material used in our study consisted of *Scyphocephalium ochocoa* seeds (**Figure 1**), which were used for oil extraction.

Figure 1. Photograph showing *Scyphocephalium ochocoa* seeds.

2.1.2. Animal Material

The animal material consisted of 10 male rats of the Rattus norvegicus species, of Wistar strain, aged between eight and twelve weeks and weighing between 97 and 153 g. These 10 animals were separated into two batches (control and treated) of 5 rats each (**Figure 2**). The rats were placed in chip-lined bottom cages, which were renewed every third day of the week. The experimental protocol and animal handling procedures were carried out in accordance with Good Laboratory Practice [13].

The animals were separated into two batches (control and treated) of 5 rats each.

(a) (b)

Figure 2. Photograph of two batches of wistar rats.

2.2. Methods

2.2.1. Description of the Various Extraction Stages for *S. ochocoa* Oil

S. ochocoa seeds from the Lekoumou department were shelled in order to separate the kernel from the shell, reduce friction and wear in the presses, improve the quality of the oil and cake [14] and increase the yield of the pressing stage [15]. The resulting kernels were cleaned to remove impurities. After cleaning, the almonds were then grated to facilitate drying and, above all, to separate the pulp from the kernel. The grated pulp was dried in a solar dryer for 2 to 3 days to eliminate

free water. The dried pulp was crushed using a grinder to facilitate oil extraction and increase yield. The powder obtained was then pressed to extract the oil. After extraction, the collected oil was packaged in a hermetically sealed breakable bottle. The oil production diagram is shown in Figure 3.

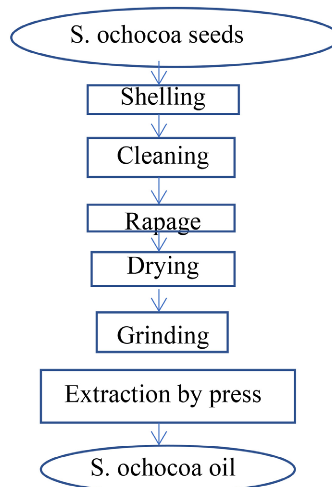


Figure 3. Extraction diagram for *S. ochocoa* oil.

The extracted oil content was calculated using the following formula:

$$T_H = \frac{M1}{M} \times 100$$

M = sample mass before extraction.

$M1$ = mass of oil obtained after press extraction.

2.2.2. Determining the Smoking Point

After extracting the oil, we proceeded to determine the smoke point in order to deduce the temperature at which the oil could be heated.

2.2.3. Determining the Smoking Point

After extracting the oil, we proceeded to determine the smoke point in order to deduce the temperature at which the oil could be heated.

2.2.4. Determination of Chemical Indices

Peroxide value

1 g of oil was introduced into an Erlen Meyer to which 10 mL of chloroform and 15 mL of acetic acid were added, then 1 mL of potassium iodide solution (1 mL distilled water →0.5 mL KI) was added. Once the Meyer Erlen was immediately stoppered, the resulting mixture was stirred for one minute and then left in the dark; then 75 mL of distilled water and a few drops of starch were added. The liberated iodine was titrated with the 0.01 N sodium thiosulfate solution until complete decolorization. Let V be the volume. Let V0 be the volume of 0.01 N sodium thiosulfate.

The peroxide value was calculated using the following formula:

$$Ip(\text{meq of } O_2/\text{Kg}) = \frac{(V1 - V0).N.10^3}{P}$$

With:

Ip: Peroxide value expressed in milliequivalents of active oxygen per kg of sample (meq O_2/kg oil).

$V0$: Volume in ml of sodium thiosulfate solution used for the blank test.

$V1$: Volume in ml of sodium thiosulfate solution used for the sample.

N: Normality of the sodium thiosulfate solution used (0.01 N).

Acid value

In an Erlen Meyer 1; 25 mL + 0.5 mL phenolphthalein was brought to the boil ,at the elevated temperature, this product was carefully neutralized while stirring the Erlen Meyer with the 0.1 mol/l KOH solution until a persistent pink coloration appeared for at least 10 seconds. In an Erlen Meyer 2, 1 g of oil was weighed, and then neutralized ethanol (contents of Erlen Meyer 1) was added and mixed thoroughly. The contents were brought to the boil and then titrated with the KOH solution, shaking the contents vigorously during titration. The titration is stopped when the pink coloration persists for at least 10 seconds, then the burette (KOH volume) is dropped.

Acidity is given by the following formula

$$I_A = \frac{V \cdot c \cdot M}{10m}$$

With:

V: volume in ml of KOH solution used for titration.

c: exact concentration in mol/L of KOH solution.

m: mass of test sample in grams.

M: molecular weight of oleic acid (282 g/mol).

2.2.5. Effects of Thermooxidized Oil on Anthropometric and Biochemical Parameters

Diet formulation

Two diets were established.

-A control diet (standard rat diet from the laboratory of the Faculty of Science and Technology of the Université Marien Ngouabi).

-A treated diet in which 10% of *S. ochocoa* oil was substituted for the control diet on a mass basis, instead of palm oil.

The composition of the diet is shown in Table 1.

Table 1. Preparation of diets for the two batches used in the experiment.

Ingredients (g)	Indicator (g)	10% treated
Corn flour	45	45
Wheat flour	45	45
Soy flour	45	45

Continued

Milk	30	30
Peanut	30	30
Spinach	20	20
Bone meal	4	4
Palm oil	21.96	-
S. ochocoa oil	-	21.96
Table salt	0.6	0,6
Sub total	241.56	
Tap water (mL)	100	100
Total	341.56	341. 56

2.2.6. Conduct of the Experiment

Meals were distributed every 2 days in freeze-dried form. Water was provided ad libitum and renewed at two-day intervals. The animals were weighed at the start of the experiment and then every two days. The last weighing took place at the end of the experiment. The difference between the quantities of feed served and the rest, relative to the dry matter, is used to determine the quantity consumed.

2.3. Evaluation of Biochemical Parameters

2.3.1. Determination of Parameter Concentrations

The blood of each rat was centrifuged at 3000 rpm for fifteen minutes (15 min) using a DEESK TD4A-WS centrifuge to obtain the serum, which was analyzed by a brand-name spectrophotometer (Bio Mate 3S, USA). The reagents used in this report to determine the concentrations of these parameters were supplied by Cypress diagnostics (Finecare, Belgium), and the protocol described for each kit was followed.

2.3.2. Determination of Alanine Aminotransferase (ALAT)

In a 113 µL volume of reaction medium composed of 59 µL of working reagent 1 diluted in 10 µL of distilled water, 18 µL of working reagent 2, 17 µL of working reagent 3 diluted in 9 µL of distilled water preheated for 2 to 3 min at 37˚C were added 11 µL of serum previously diluted in 8 µL of distilled water, thus constituting a total reaction medium of 132 µL at pH 7.8. After shaking, the optical densities (OD) obtained every minute for 4 min on the integrated spectrophotometer at a wavelength equals 340 nm, were used to determine serum ALAT enzyme activity according to the following formula.

$$\text{Activity of ALAT}\left(\text{UI/L}\right) = \frac{-\Delta\text{DO}}{\Delta\text{temp}a} \times \text{ALAT}$$

2.3.3. Determination of Asparate Aminotransferase (ASAT)

In a heparinized EDTA tube containing 500 µL of reagent (total volume) pre-incubated at 37˚C for 2 - 3 minutes, 50 µL of the sample to be assayed was added.

After shaking for 1 minute, optical densities were read with a spectrophotometer at 340 nm to determine serum ASAT enzyme activity. This activity is calculated from λ (340 nm) and the ASAT factor (FASAT).

Creatinine assay

In three tubes (standard, blank and test) each containing 500 µL of reaction medium, 100 µL of creatinine standard, 100 µL of distilled water and 100 µL of sample were added respectively. After shaking, the first absorbance A1 of each tube was read, after 30 seconds, at 505 nm against the blank containing the reaction medium.

Ninety (90) seconds after the first reading, a second absorbance A2 was read at 505 nm on the spectrophotometer [16]. Creatinine concentration is calculated as follows:

$$\text{Créatinine (mg/L)} = \frac{(A2 - A1)\text{sample}}{(A2 - A1)\text{standard}} \times \text{Standard concentration}$$

HDL Cholesterol assay

HDL cholesterols are lipoproteins that capture cholesterol molecules deposited in tissues, including arteries, and transport them to the liver for elimination via the digestive tract via bile.

To a reaction medium consisting of 150 µL of working reagent 1 and 50 µL of working reagent 2, were added 2.5 µL of product to be determined (standard and sample). After shaking and automatic incubation at 16 - 25˚C for 5 minutes, the absorbance of the test material compared with the standard is obtained by reading the optical density at 600 nm. HDL concentration was calculated using the following formula:

$$\text{HDL−C (mmol/L)} = \frac{(\text{Sample OD})}{\text{Standard OD}} \times \text{Standard concentration}$$

Determination of total cholesterol

In a reaction medium consisting of 47 µL of working reagent diluted in 93 µL of distilled water, 2 µL of test material (standard and sample) was added. After stirring and automatic incubation at 16 - 25˚C for 5 minutes, the absorbance (Abs) of the test material was measured and compared with the standard using an integrated spectrophotometer at a wavelength of λ = 505 nm. Total cholesterol concentration is calculated as follows:

$$\left(\text{Total cholesterol}\left(\text{mmol/L}\right)\right) = \frac{\text{Sample OD}}{\text{Standard OD}} \times \text{Concentration}$$

Triglyceride assay

For a volume of 2 µL of product to be assayed (standard or sample), a reaction medium consisting of 120 µL of working reagent diluted in 28 µL of distilled water was added. After stirring and automatic incubation at room temperature for 2 minutes, the absorbances (Abs) of the sample were measured with a spectrophotometer at a wavelength of 505 nm in comparison with the standard. Concentration is determined as follows:

$$\text{Total Cholestérol (mmol/L)} = \frac{\text{Sample OD}}{\text{Standard concentration}} \times \text{Standard DO}$$

Glucose assay

To a series of three tubes (blank, calibrator and sample) containing one milliliter of the glucose enzyme solution were added 10 µL of distilled water, 10 µL of calibrator and 10 µL of test serum. The mixture was incubated at 37˚C for 5 min. Optical density is measured at 500 nm against the enzymatic solution blank. Glucose concentration is determined using the formula below:

$$\text{Glucose}\left(\text{g/L}\right) = \frac{\text{Sample OD}}{\text{Standard concentration}} \times \text{Standard DO}$$

LDL cholesterol

LDL cholesterol was calculated using the formula of Friedewald and *et al.* [17].

$$\text{LDL}\left(\text{mmol/L}\right) = \text{CT} - \text{HDL} - \frac{\text{TG}}{5}$$

Plasma atherogenicity index

Atherogenic risk was assessed by the following ratio:

$$\text{IAP} = \frac{\text{CT}}{\text{HDL}}$$

Statistical analysis

Data processing, data entry and the production of raw tables and figures were carried out using Word 2019 and Excel (Office 2019), using Student's t-test, the software used for statistical processing of results at the 5% threshold. The values reported throughout this document are presented as mean ± Standard Error (SE). The significance threshold is $p < 0.05$.

3. Results and Discussion

3.1. Results

3.1.1. Oil Collected after Extraction by Mechanical Press

Figure 4. Photograph showing *Scyphocephalium ochocoa* oil heated to 220˚C.

Figure 5. Photograph showing cooled *Scyphocephalium ochocoa* oil.

Figures 4-5 show heated and cooled *S. ochocoa* oil respectively. *S. ochocoa* oil has a characteristic color and odor, and is highly saturated. The oil solidifies as it cools, becoming hard within a few hours of heating. The content of this oil obtained by press extraction is 52.61%.

3.1.2. Effects of Thermooxidation on the Chemical Parameters of *S. ochocoa* Oil

Acid values of *Scyphocephalium ochocoa* oils Table 2 shows the acid index values as a function of the treatment *Scyphocephalium ochocoa* oil underwent. The table shows that the more the *Scyphocephalium ochocoa* oil was heated, the higher the percentages of free fatty acids represented by numbers 3, 4 and 5. Exposing the oil to the open air for two weeks caused the degree of acidity to increase (sample no. 7). Samples of unheated *S. ochocoa* placed in the freezer and not heated had lower acid values than all the other samples.

Table 2. Variation in acid value of *S. ochocoa* oil as a function of temperature.

N	Oil samples	Acid values
1	Unheated	0.703 ± 0.008
2	Unheated and then frozen	0.860 ± 0.008
3		1.063 ± 0.005 (1st heating)
4	Heated to 220˚C	1.290 ± 0.010 (2nd heating)
5		1.890 ± 0.006 (3rd heating)
6	Heated to 220˚C then frozen	1.923 ± 0.006
7	Heated to 220˚C then exposed to air	5.74 ± 0.030

Values are expressed as mean ± mean standard error (ESM).

3.1.3. Peroxide Value of *Scyphocephalium Ochocoa* Oil

Table 3. Variation in peroxide values as a function of temperature and oxidation.

N	Oil samples	Peroxide values
1	Unheated	65.6 ± 0.03
2	Unheated and then frozen	67.3 ± 0.15
3		97.9 ± 0.03 (1st heating)
4	Heated to 220˚C	103.9 ± 0.03 (2nd heating)
5		107.5 ± 0.26 (3rd heating)
6	Heated to 220˚C then frozen	115.9 ± 0.16
7	Heated to 220˚C then exposed to air	174.3 ± 0.16

Values are expressed as mean ± mean standard error (ESM).

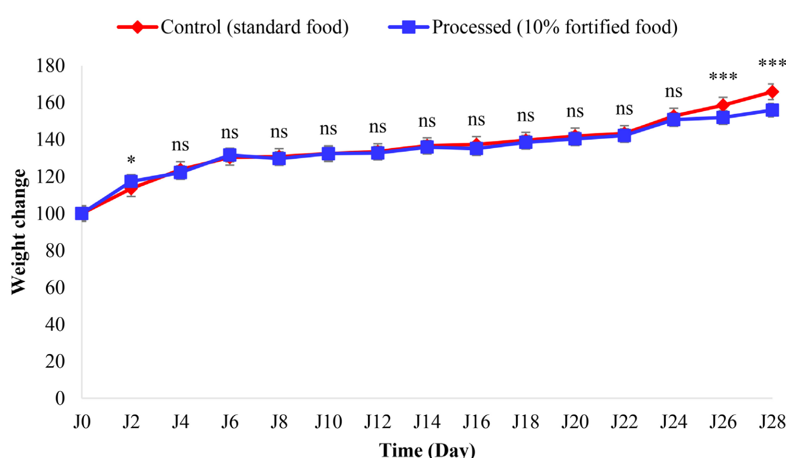
The results concerning the effect of temperature and oxidation on the peroxide value of *S. ochocoa* oil (Table 3) showed that the peroxide value increased with temperature. Indeed, the peroxide value of sample 1 (unheated oil) is lower than

the peroxide values of the other samples. On the other hand, exposure to open air for around two weeks significantly altered the quality of the oil: Ip = 174.3 meq/kg O_2, as opposed to 115.9 meq/kg O_2 for the sample heated 3 times.

3.1.4. Effects of Consumption of Thermooxidized *S. ochocoa* Oil on Biological Parameters in Wistar Rats

Weight change, quantity consumed, total dry matter ingested and weight gain.

Weighing of rats fed the standard diet and rats fed the *S. ochocoa* oil-enriched diet was monitored over the 28-day period. Figure 6 shows the weight evolution of the rats as a function of time (day). Compared with rats fed the standard diet, there was a non-significant increase ($p < 0.05$ from D2 to D24) for the batch of rats fed the diet enriched with *S. ochocoa* oil, and a significant decrease ($p < 0.05$ from D26) for the batch of rats fed the *S. ochocoa* oil (10%).



Results are expressed as mean ± standard error on the mean n = 5 rats per batch, ***$p < 0.05$ significant difference from standard diet rats; ns: not significant.

Figure 6. Weight evolution of rats over 28 days.

3.1.5. Feed Intake

Table 4 shows the amount of feed consumed (g/d). The results showed a non-significant increase in the amount consumed on the 10% fortified diet compared with the standard diet.

The results concerning the quantity of food consumed (Table 4) showed that this increased non-significantly in the 10% fortified diet compared with the standard diet.

Table 4. Average food consumption during experimentation.

Diet	Quantity consumed (g/day)
Standard diet	197.665 ± 8.362
Diet enriched at 10%	198.076 ± 9.320

3.1.6. Weight Gain

Table 5 shows the body weight gained by the rats during the 4-week experiment.

The table shows that treated rats gained fewer grams than rats fed the standard diet. Body weight gain is the difference between the final and initial body weight of the rat.

Table 5. Animal body weight gain over 28 days.

Diet	Starting weight (g)	Weight after 28 J (g)	Weight gain (g)	Weight gain (g/day)
Standard diet	114.25 ± 1.27	190.62 ± 4.67	76.37 ± 5.45	+2.72 ± 0.33
Diet enriched at 10%	119.16 ± 1.15	183.57 ± 3.39	64.41 ± 4.60	+2.19 ± 0.24

Results are expressed as an average.

3.1.7. Organ Weight Changes During Experimentation

Table 6 shows the relative weights of organs harvested at the end of the diet enriched with 10% S.ochocoa oil. The table shows that the diet did not significantly alter the relative weight of organs such as kidneys and heart ($p > 0.05$) compared with the control batch. On the other hand, there was a significant difference in liver weights between the two diets ($p < 0.05$).

Table 6. Relative organ weights after 28 days.

Organs	Indicator	10% treated
Liver	2.583 ± 0.171	2.209 ± 0.136**
Heart	0.386 ± 0.0266	0.399 ± 0.0342 ns
Right kidney	0.261 ± 0.014	0.287 ± 0.011 ns
Left kidney	0.244 ± 0.010	0.247 ± 0.005 ns

Results are expressed as means ± Standard Error (SE), for n = 5, expressed ns = not significant ($P > 0.05$).

3.1.8. Hepato-Somatic Index

Table 7 shows the variation in hepato-somatic index (HSI) between diets. The table shows that the HSI of the standard diet was higher than that of the diet enriched with *S. ochocoa* oil.

Table 7. Variation in hepato-somatic index according to diets.

Diet	Poids du foie	Poids corporel	IHS
Standard diet	2.583 ± 0.171	190.620 ± 4.670	1.355 ± 0.270
Diet enriched at 10%	2.209 ± 0.136	183.570 ± 3.390	1.203 ± 0.240

Values are expressed as mean ± mean standard error.

3.1.9. Effects of *S. ochocoa* Oil on Biochemical Parameters

Results concerning biochemical parameters in rats (**Table 8**) show that the addition of 10% enriched *S. ochocoa* oil to the standard diet resulted in a significant $P > 0.05$ increase in levels of AST, glucose, total cholesterol, LDL and plasma athe-

rogenicity index. On the other hand, the diet resulted in a significant P < 0.05 decrease in Triglyceride, VLDL and HDL concentrations. There was also a non-significant decrease in creatinine and ALAT levels.

Table 8. Effect of thermooxidized oil consumption on biochemical parameters of laboratory rats.

Biochemical parameters	Control (standard feed)	Treated (feed enriched at 10%)
ASAT (UI/L)	108.662 ± 1.013	127.996 ± 0.997***
ALAT (UI/L)	61.366 ± 1.905	57.402 ± 2.206 ns
Créatinine (mg/dL)	0.0768 ± 0.017	0.050 ± 0.006 ns
Glucose (g/L)	0.803 ± 0.066	1.723 ± 0.0613***
CT (mg/dL)	83.924 ± 1.797	96.478 ± 1.773**
HDL (mg/dL)	42.802 ± 3.474	19.100 ± 1.003***
LDL (mg/dL)	20.366 ± 3.667	67.1392 ± 1.805**
VLDL(g/dL)	20.756 ± 0.337	10.238 ± 0 .354***
TG (g/dL)	103.780 ± 1.688	51.194 ± 1.771***
IAP	2.010 ± 0.161	5.112 ± 0.303***

Results are means ± Standard Error (ESM), for n = 5, expressed ns = not significant, *** = P > 0.05, (*) = significance compared to control: (*) = p < 0.05; (**) = p < 0.01; (***) = p < 0.001.

3.2. Discussion

3.2.1. *S. ochocoa* Oil Obtained after Extraction by Mechanical Pressing

The *S. ochocoa* oil obtained by pressing in our study is saturated, blackish in color and rapidly solidifies or coagulates.

The texture of *S. ochocoa* oil when hot and after cooling confirms the saturated nature of the fat contained in this seed belonging to the myristicaceae family, which contains a large quantity of saturated fatty acids, notably myristic acid.

The blackish color could be explained by the fact that the seed coat was pressed with the kernel at the same time. Solidification or coagulation could also be due to the chemical composition of the oil, which has a very low melting point. The results of our work corroborate those of Paul [18], who found that *Scyphocephalium ochocoa* oil is a concrete oil, melting at around 45˚C., pure white when obtained from carefully shelled kernels, but can take on a blackish color if all or part of the tegument has been extracted at the same time. The fatty acids in this oil consist almost entirely of myristic fatty acid, with very small amounts of oleic fatty acid [18].

The oil content in our study was 52.61%. Our results differ from those obtained by Paul, 1957 (18) (70%) using the Soxhlet method. This difference may be explained by the extraction method. According to Rosenthal and *et al*. [19], the Soxhlet method is the most efficient way of extracting oil from oilseeds, achieving extraction yields close to 100%.

3.2.2. Effect of Thermooxidation on the Chemical Parameters of *S. ochocoa* Oil

Effect of heating and oxidation on the acid value of *S. ochocoa* oil. The acid number gives an indication of the free fatty acid content resulting from the thermal hydrolysis of glycerides.

Our results show that exposing the oil to open air for two weeks causes an increase in the amount of acid. The relatively high acid value of the sample heated to 220˚C and then exposed to open air can be attributed to heating and oxidation for 15 days, which explains why there may have been an initiation of triglyceride degradation during storage [20].

The unheated oil samples placed in the freezer and the unheated oil samples showed relatively lower acidity values (0.860 and 0.703 mg KOH/g respectively) than the other samples. This indicates a low level of free fatty acids in these oils.

Our results show that the acid index values of the heated samples increase progressively with the number of heatings (1.063 ± 0.005 for 1st heating, 1.290 ± 0.010 for 2nd heating and 1.890 ± 0.006 for 3rd heating). This observation was underlined by several authors [21]-[23] who showed that the more the oil is heated, the higher the acidity.

3.2.3. Effect of Temperature and Oxidation on the Peroxide Value of *S. ochocoa* Oil

The increase in the peroxide value is linked to changes in the concentration of hydroperoxides formed during thermooxidation or autoxidation of AGIs.

Our results show that the peroxide value of the oil increases with temperature and oxidation (65.6 ± 0.03 for unheated oil versus 97.9 ± 003 for oil heated to 220˚C). The relatively high peroxide value of the heated sample exposed to the open air could be explained by heating and oxidation for 15 days, which promoted the release of free radicals. The same finding was made by several authors [21]-[23].

3.2.4. Evolution of Body Weight During Experimentation, Food Consumption and Weight Gain

Rats treated with a diet supplemented with 10% *S. ochocoa* oil showed an increase in body weight over the 24 days of the experiment, but there was a significant reduction in body weight from day 26 onwards, compared with rats fed the standard diet. This diet did not have a negative influence on the rats' growth, which was visible from the first week onwards. The significant reduction in body weight of the rats in the treated batch from day 26 could be explained by the fact that the wild rats had infiltrated the laboratory and eaten part of their meal. Our results differ from those of several authors [24] [25], who worked on the effect of the thermooxidation of vegetable oils and observed growth retardation in rats, which would result from toxicity induced by the presence of oxygenated derivatives, from a reduced lipid intake due to the degradation of oleic acid during oil oxidation, but also from a deficiency in essential fatty acids. This was also due to the fact that our oil was not highly oxidized and did not impair feeding behavior or

the availability of growth-promoting amino acids such as L-lysine and tryptophan.

In terms of food consumption, rats on the enriched diet consumed more than those on the control diet, averaging 198.65 g every 2 days compared with 197.07 g. Our results differ from those of Raphaël *et al.* [26]. This difference may be explained by the fact that heating our food to 220˚C did not alter the taste of the meal, nor did it impair the rats' feeding behavior. The rats enjoyed the diet, confirming that *Scyphocephalium ochocoa* improves the organoleptic qualities of the dishes.

3.2.5. Biochemical Parameters

Cardiac risk factors such as serum lipids were therefore measured, and the plasma atherogenicity index was calculated to assess the indirect effect the extract might have on the heart. Serum lipids and the atherogenicity index are markers that have been successfully used as markers of major risk factors in the development of coronary heart disease such as ischemic heart disease, myocardial infarction and stroke [27]-[29]. After dosing, a significant reduction in blood triglyceride levels was observed. Lipoprotein lipases hydrolyze the triglycerides carried by the chylomicrons and release the fatty acids to the tissues, which is why the triglyceride concentration decreased. Our results corroborate those of Ajuwon and Spurlock [30], who studied the effects of palm oil on the lipid profile of rats and found that the drop in triglyceridemia could be attributed to its higher content of SFAs, considered to be pro-inflammatory. According to these authors, this inflammatory outcome of endotoxin metabolism is differentially modulated by the lipid composition of the diet. Indeed, chronic ingestion of a lipid-rich diet generates metabolic endotoxemia at plasma level. To explain, digestion of lipid-rich meals alters intestinal permeability and increases endotoxin absorption from lipopolysaccharides present in the gastrointestinal tract [31].

The increase in LDL concentration is due to the rise in cholesterol levels, since the role of LDL is to deliver cholesterol to peripheral tissues, which capture LDL via their receptors.

LDL through their receptors. LDL is the main pro-atherogenic lipoprotein. When LDL cholesterol levels exceed normal metabolic capacity, there is a risk of cholesterol accumulation in the arteries, followed by oxidation of these lipoproteins. High-density lipoproteins (HDL), unlike LDL, capture cholesterol esters and excess free cholesterol from the bloodstream and direct them to the liver, where they are broken down into bile acids. HDL plays an important role in reducing blood and peripheral cholesterol concentrations and inhibiting atherosclerotic plaque formation in the aorta [32]. On this basis, the results of this study suggest that the effect of *S. ochocoa* oil on lipid balance may derive from the capture and hepatic purification of atherogenic cholesterol. However, the values obtained are lower than those obtained in a study focusing on stroke and cardiovascular disease in women in the USA [33].

As far as blood glucose levels are concerned, an increase was observed after 28 days of consumption in all rats compared to their controls. In fact, during a high

energy load, increased glucose synthesis is favored from glycerol derived from the complete hydrolysis of triglycerides [34].

On the other hand, thermooxidized *S. ochocoa* oil induced a non-significant decrease in serum creatinine levels in rats, compared with the control batch. It should be noted that creatinine, derived essentially from creatine-phosphate catabolism, is completely filtered and eliminated by the glomeruli, but is neither reabsorbed nor excreted by the tubules. In view of these results, consumption of thermooxidized *S. ochocoa* oil for 28 days is safe in rats. This implies that the latter does not influence the renal mechanisms responsible for regulating this metabolite. As a result, it represents a parameter of choice for assessing glomerular filtration rate [35]. As Emelie [36] points out, knowledge of food is one of the ways to preserve health.

4. Conclusions

The aim of the present work was to evaluate the effects of thermooxidation on *Scyphocephalium ochocoa* oil and the impact on the health of laboratory rats.

The study showed that *S. ochocoa* seed is rich in oil. This oil has a compact structure at room temperature when cooled.

Acid and peroxide values show the alteration of this oil after heating to 220˚C, and increase with the number of heating cycles.

Incorporating 10% of the oxidized oil into the standard diet did not affect the growth of young rats. A diet enriched with this oil increases plasma cholesterol levels, as well as those of so-called "bad cholesterol" transporters. The plasma atherogenicity index was significantly elevated ($p < 0.05$) compared with that of the control batch, indicating the risk of cardiovascular disease.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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