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Antioxidant Effect of Plants Aqueous Extract on Lipid Stability of *Oreochromis niloticus* during Traditional Sun and Smoke Drying in Far-North Cameroon

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Abstract

Fishes are suitable for sun drying and smoking. But their high contains of unsaturated fatty acids make them very prone to lipid oxidation. However, some plants are rich in natural antioxidants and have been shown to be potent inhibitors of lipid oxidation during processing of meat. The effect of three aqueous plants extracts on lipid stability of *Oreochromis niloticus* collected in Far-North Cameroon during processing was evaluated. Results show the high nutritious of these fish with high levels of proteins, lipid and ash (71.02%, 13.71% and 10.65%, respectively). It has been observed that oleic acid (C18:1), followed by palmitic acid (C16:0) and linoleic acid (C18:2) are the most dominant fatty acids present in *Oreochromis niloticus* lipids. Their lipid also showed a much higher content of omega-3 polyunsaturated fatty acids (PUFA) compared to omega 6 PUFA. The total phenolic contents (TPC) of the three plants ranged from 12,150 to 16,050 mg/100 g. The leaves of *Moringa oleifera* exhibited the higher content of TPC. The results of iodine, peroxide value and thiobarbituric acids test were revealed that the aqueous extracts of the tree plants had antioxidant properties. These plants extracts inhibit lipid oxidation of *Oreochromis niloticus* during processing. Increasing of the concentration of plant extracts enhanced their antioxidant activities and the highest oxidation inhibitor was obtained at 30 g/l. *Moringa oleifera* leaves inhibit highly the lipid oxidation of *Oreochromis niloticus*.

Keywords

Oreochromis niloticus, *Moringa oleifera*, *Solanum melongena*, *Zingiber*

officinalis, Lipid Stability, Smoke and Sun Dry

1. Introduction

The number of hungry people has been increasing in the world during the last two years. According to the FAO, the growth was 1.5% in 2018. Sub-Saharan African countries are the most food insecure. The Far North region statistically in Cameroon is the most affected concerning food insecurity, with 35% of population suffering from malnutrition [1]. The nutrition situations of population in this region are critical. However, in this part of Cameroon, several food sources exist and can be used to remediate undernutrition. Fish present in this region like many regions in the world are a good source of animal protein essential for a balanced diet. They are also sources of minerals and lipid. Due to their richness in essential amino acids such as methionine, cystein and lysine, fish protein can complete the lack of amino acids in diet based on cereal [2]. Consumption of fish rich in unsaturated fatty acids particularly omega-3 had a beneficial effect on human health. Polyunsaturated fatty acids like eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and arachidonic acid are involved in regulating biosynthesis of prostaglandin and promoting healing of wounds. EPA, DHA and arachidonic acid can also protect human against several problems such as artherosclerosis, diabetes, cardiovascular diseases and mood disorder [3].

However, fishes and marine products are more perishable due to their high content of water (75% - 80% wet by weight) and unsaturated fatty acids. Water contains in fish and fish products are a favourable environment for the development of microorganisms, which causes alteration of these products [4]. In the other hand, unsaturated fatty acids present in marine product are unstable and susceptible to oxidation. Lipid oxidation is the principal cause of food deterioration. Oxygen, light and high temperature are the factors which accelerated oxidation of lipid [5]. During lipid oxidation, off-flavour compounds are produces and some toxic compounds, which decreases the nutritional value of food. These toxic products react with DNA of consumers and causes cancer [6]. Many technics like salting, solar drying, smoke drying, freezing, refrigeration fermentation are used to limit deterioration of fresh fishes in the world. Among these various processing techniques, in developing countries like Cameroon, solar and smoke drying are the most used. These techniques prolong the shelf life of fish and increase certain nutrient availability [7]. Some researchers found that smoking and solar drying affect the nutritive value of fish. During processing, several reactions take place, particularly lipid hydrolysis and lipid oxidation. Lipid deterioration produces some toxics components like peroxides, aldehydes, ketones and others [7].

Then, to limit lipid oxidation and maintain the food quality during processing, butylhdroxyanisole (BHA), butylhydroxytoluene (BHT) and propyl

gallate (PG) which are synthetic antioxidants have been commonly used in food industry. However, many researchers reveal their implication in many health problems such as cancers, cardiovascular diseases and the toxicity effects. Their used is becoming restricted in many developing countries. Due to their potential health benefit, today, industrials have an increasing interest in natural antioxidants. Natural plants extract may play an important role for inhibition of oxidation processes in food and foodstuff [8].

Many plants can be sources of natural antioxidant. The polyphenols or phenolic compounds present in plant are the much important natural antioxidants. Phenolic extracts of vegetables, cereals grains, fruits, and spices are showed its positive effects on prevention of lipid oxidation. The antioxidant activity of the phenolic compound is linked on their structure, hydrogen-donating potential, ability to chelate metal ions [9]. *Moringa Oleifera* leaves, *Solanum melongena* fruits and *Zingiber officinalis* roots are natural sources of antioxidants present in abundance in Far-North region. These plants contain many compounds like vitamins C and E, carotenoids and polyphenols, which can be, are involved to oxidative stability of oils [10]. They can be use to preserve the quality of fish during processing.

According to our knowledge, there are no studies about evaluation of the antioxidant activities of water extracts of plant on lipid stability of fishes during processing in Cameroon, especially in Far North Region. Therefore, the aim of this study was to determine the effect of used of plant extract addition derived from *Moringa Oleifera* leaves, *Solanum melongena* fruits and *Zingiber officinalis* roots on lipid stability of *Oreochromis niloticus* consumed in Far-North Cameroon during smoke and sun drying processing.

2. Materials and Methods

2.1. Materials and Study Site

The leaves of *Moringa oleifera*, the fruits of *Solanum melongena* and the roots of *Zingiber officinale* used in this study are plants commonly consumed in Cameroon. These plants were harvested and bought from Maroua local market in January 2018. This area is located between latitude 10˚ and 13˚ North and between longitude 13˚ and 16˚ East [11]. After collection and transport to the laboratory, the fruits of *Solanum melongena* and the roots of *Zingiber officinale* were washed with tap water, left to drain and cut in small parts. All theses and the fresh leaves of *Moringa oleifera* were dried at 50˚C for 48 h in an electric air-dried oven. The dried samples were grounded using electric food (Panasonic, Kyoto, Japan) to obtain fine powders which can pass through 1 mm sieve. These powders were kept in the desiccators for further use.

Samples of fresh *Oreochromis niloticus* used in this work were bought in March 2018 from local Maga market in Far-North Region of Cameroon. They were transported immediately once collected in iceboxes to the Laboratory. In the laboratory, they were washed to remove external dirt, dried with disposable

towel paper. The average weight of each fish was 200 g with lengths ranging from 20 to 30 Cm. The fish samples after eviscerated were washed with distilled water and left to drain. They were divided in tree lots. The first was use as a control, the second and the third lots were prepared respectively for the sun drying and smoking process.

2.2. Preparation of Plant Extracts

To prepare a plant extract, specific quantities (10, 20, 30 g) of each plant powder were added separatory to 1 L of distilled water containing in a round bottom flask. 50 g of salt were also added to separate 1L of distilled water. The mixtures were then heated to boil and reflux for 10 minutes. They were filtered hot trough a clean Wathman paper N˚ 4. The filtrate was then cooled to room temperature (~24˚C).

Salt was used in this experiment because in many part of world, it is a common preservative for dried product.

The concentration of extract was estimated using the formula:

$$\text{Concentration} = \text{Weight of additive (g)}/\text{volume of water (L)}$$

2.3. Pre-Treatment of Fish

The samples of fish prepared above were randomdly assigned to five antioxydants treatments. The treatments were the control (0%), 1%, 2% and 3% of each plant powder extract (*Moringa oleifera* leaves, fruits of *Solanum melongena* and roots of *Zingiber officinale*) and 5% of brine.

Each treatment was replicated with 3 fish per replicate. The fish were soaked in the plant extract for 10 minutes. After that, the fish were drained and divided in 3 lots. The first was use as a control, the second and the third lot were prepared respectively for the sun drying and smoking process. A control sample was also sun and smoke dried after soaking in distilled water. One part of control (raw fish) was used to the determination of proximate composition and fatty acid profile of raw fish.

2.4. Drying Methods

2.4.1. Sun Drying

For sun drying process, the pretreated *Oreochromis niloticus* were cut into two equal halves. Cut was making along longitudinal axis of the fish body from mouth to tail but the two halves of the body remain attached in the tail fin region. The sun drying was proceed by exposing of the fish (*Oreochromis niloticus*) to ambient sunlight at day time (8 a.m to 5 p.m) for 3 days due to the climatic conditions (in drying period, moisture content of air was comparatively less). At the same time, temperature was recorded (25˚C - 47˚C). During sun drying, the fishes were covered with mosquito net to prevent insects and others pests. The fishes during the sun dry processing were turned over from time to time to ensure homogenous drying.

2.4.2. Smoking

For smoking process, the pre-treated *Oreochromis niloticus* were used. These fresh fish were spread out on smoking trays. The trays were then stacked on smoking oven fired with hard wood, and marked at temperatures greater than 70˚C. To obtain a dried smoked fish, the process took 7 hours. Fishes samples during processing were turned at intervals to ensure homogenous drying.

The dried samples of *Oreochromis niloticus* were packaged with plastic bag and stored at 4˚C for further analysis.

2.5. Lipid Extraction

After smoking and sun drying treatments, oils were extracted from raw and processing fish according to the Bligh and Dyer [12] methods. The extracted oil was stored at 4˚C for further analysis.

2.6. Analytical Methods

2.6.1. Proximate Composition of Fish

Moisture, ash, protein and lipid in the fish samples were determined using standard analytical methods described by AOAC procedures [13]. Moisture content was determined by drying fish in oven at 103˚C until a constant weigh was achieved according to the AOAC procedure 925.40 [13]. Ash content was determined by incineration fish sample for 12 h at 550˚C in furnace according to the AOAC procedure 942.05 [13]. Nitrogen (N) content was determined using Kjeldahl method, according to AOAC procedures 984.13 [13]. The protein content was calculated as 6 × 6.25. Lipid content was determined using Soxhlet apparatus with hexane, following AOAC procedures 963.15 [13]. The carbohydrate content was calculated by difference. All samples were analysed in triplicate.

2.6.2. Fatty Acid Composition of Fish Oil

Oil extracted from fish samples were used for the determination of fatty acid profile. Fatty acids compositions of fish oil were investigated after conversion of their Fatty Acid Methyl Esters (FAME) using boron trifluoride-methanol method. The lipids were saponified and esterified for fatty acid analysis by the method of Metcafe *et al.* [14]. The FAMEs were anlyszd on a Hewlett Packard 5880 gas chromatography (GC) equipped with a Flame Ionisation Detector (FID). The esters were separated on a 50 m × 0.20 mm id Wall-coated open tubular fused silicia capillary columm coasted with Carbowax 20M. Columm injector and detector temperature were 200˚C and 300˚C, respectively. The carrier gas was helium and the split ratio was 100/1. Identification and quantification of fatty acids were performed by comparison of their peak with the relevant peak areas of the corresponding standard fatty acids. Each fatty acid was then expressed as a percentage of the total fatty acids quantified. All experiments were carried out in triplicate.

2.6.3. Total Phenolic Content

Phenolic compounds were extracted using extraction method according to Wo-

meni *et al.* [15]. Twenty grams (20 g) of each plant powder (*Moringa oleifera* leaves, fruits of *Solanum melongena* and roots of *Zingiber officinale*) were extracted with 400 mL of methanol for 48 h at room temperature. The mixture wasregularly subjected to shaking during extraction. The extract was filtered with Whatman No. 1 filter paper, and residue was again extracted with 200 mL of methanol to ensure maximum extraction of phenolic compounds. The combined filtrates were subjected to rotary evaporation at 40˚C under reduced pressure for removal of the solvent.

2.6.4. Effect of Treatment on Lipid Oxidation Parameters

1) Iodine value

The iodine value (IV) of fish oil samples was determined using the Wijs method, as described by O'Keefe and Pike [16]. The IV was expressed as gram of iodine absorbed per 100 g samples.

2) Peroxide value

The peroxide value (PV) was determined using method according by Santha and Decker [17].

fish oil samples (0.0101 - 0.025 g) were mixed in a disposable glass tube with 9.8 ml chloroform-methanol (7:3 v/v) on a vortex mixer for 2 - 4 s. Ammonium thiocyanate solution 30% (50 µl) was added and the sample was mixed on a vortex mixer for 2 - 4 s. Then, 50 µl iron II solution was added to the sample and mixed using a vortex mixer for 2 - 4 s. after 5 min incubation at room temperature, the absorbance of the sample was determined at 500 nm against a blank that contained all the reagents except the sample using a spectrophotometer (Perkin Elmer, Norwalk CT, USA). The entire procedure was conducted in subdued light and completed within 10 min.

To construct a standard curve of Fe (III) concentration vs absorbance, a serial of dilutions of standard solution of iron (III) chloride was prepared and treated exactly as before, except that standard was added instead of the sample, then concentration vs absorbance was plotted. The peroxide value, expressed as milliequivalents of O2/kg sample, was calculated using the following formula:

$$\text{Peroxide value} = \frac{(\text{As} - \text{Ab}) \times \text{m}}{55.84 \times \text{m}_0 \times 2}$$

where As = absorbance of the sample; Ab = absorbance of the blank; m = slope, obtained from the calibration curve (in this experiment 38.40); m_0 = mass in grams of the sample; 55.84 = atomic weight of iron.

3) Thiobarbituric acid measurement (TrBars)

Secondary oxidation products were evaluated using thiobarbituric acid value as described by Dapper and Hadley [18]. The results were expressed as mg of malondialdehyde (MDA) per kg of sample.

4) Measurement of free fatty acid (FFA)

FFA content was determined according to the method of AFNOR [19]. The fish oil sample (1 g) was dissolved in 100 mL of ethanol and some drops of phe-

nophtalein were added as an indicator and swirled vigorously. The mixture was then titrated with potassium hydroxide (0.1M). The FFA was expressed as % oleic acid.

2.7. Statistical Analysis

Each determination was performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistics were performed using the Microsoft office excels program. Differences were evaluated by ANOVA using Statistical Package of Social Science (SPSS 16.0). Significance levels were set at $p < 0.05$.

3. Results and Discussion

3.1. Proximate Composition

The proximate composition of raw *Oreochromis niloticus* is shown in **Table 1**. Raw whole fish had 84.67% ± 1.15%, 15.65% ± 0.82%, 8.71% ± 0.36%, 71.02% ± 1.012% and 4.02% ± 0.74%, respectively for mean moisture (wet weight), ash, crude lipid, crude protein and carbohydrate (dry weight). Moisture content of raw fish noted in this work agrees with the result obtained by Tenyang *et al.* [20] from some fresh fish collected in Maga Lake (~78% wet weight). The result of ash content of raw *Oreochromis niloticus* obtained in this study was higher than that of three Pakistan freshwater fish species (7% - 12% dry weight) [21]. The high ash content noted in this work indicated that this fish is a good source of minerals hence nutritious [22]. The lipid content reported in this study was higher than those reported by Tiwo *et al.* [23] (4.54% dry weight) in the same specie collected in West region of Cameroon. Huss [22] reported that lipid content of fresh fish is influenced by species, geographical localisations, ages and diet. Fish species with more than 10% lipid content are considered as a fat fish [24]. *Oreochromis niloticus* used in this study can be classified as a medium fat fish due to their lipid content. Increase consumption of these fish in human diets is important because provides energy in the body. Consumption of fish lipid is also important for normal functioning of brain, which is made up of nearly 60% of lipid [25]. Polyunsaturated fatty acids presents in fish can helps in boosting

Table 1. Proximate composition of *Oreochromis niloticus.*

Components	Composition (means ± SD)
Moisture (g/100 g wet weight)	84.67 ± 1.15
Ash (g/100 g dry weight)	15.65 ± 0.82
Lipid(g/100 g dry weight)	8.71 ± 0.36
Proteins (g/100 g dry weight)	71.02 ± 1.12
Carbohydrate (g/100 g dry weight)	4.02 ± 0.74

SD, standard deviation, n = 3.

the immune system [26]. The recorded protein content of raw *Oreochromis niloticus* in this work is higher than those reported by Onyeike *et al.* [27] of 50% to 55% (dry weight) for another fish species. In the present investigation, crude protein obtained was higher compared to the value noted by Farhat and Abdul [21] in three freshwater fish. Consumption of *Oreochromis niloticus* in developing countries could help to prevent some problem due to proteins deficiencies in the population diet.

3.2. Fatty Acid Composition

The fatty acid composition of *Oreochromis niloticus* given in % of total fatty acids is presented in **Table 2**. In the present investigation, myristic acid (C14:0), palmitic acid (C16:0) and stearic acid (C18:0) were the predominant saturated fatty acids (SFAs). Palmitic acid was the highest in percentage in that fish species. The SFAs percentage (34.85 ± 0.21) of *Oreochromis niloticus* obtained in this study was lower than those of other four freshwater fishes (36.70% - 43.52%) collected in the same lake [20]. Monounsaturated fatty acids (MUFAs) content of *Oreochromis niloticus* was 36.30% total fatty acids, where palmitoleic acids (C16:1) and oleic acids (C18:1n-9) were the dominating MUFAs (**Table 2**). Oleic acid compared to palmitoleic acid had the higher percentage. The value of C18:1n-9 reported in this study was lower than 27.39 noted to *Clupea harengus* by others authors [28]. This fatty acid has exogenous origin and usually reflects the diet consumed by fish. Andrade *et al.* [29] showed that in South Brazil, C16:0 and C18:0 were the most dominating SFAs noted in freshwater fish, whereas, C16:1 and C18:1 were common among MUFAs. The highest quality of PUFAs in all fish species was associated with n-3 PUFAs compounds, including eicosapentaenoic acide (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), as the major components. In this study, *Oreochromis niloticus* contained these two PUFAs. The DHA content was higher compared to the EPA content. EPA and DHA have been reported to have preventive effects on many diseases [3]. Significant levels of EPA and DHA in *Oreochromis niloticus* indicated that these fish species can be used in human diet to supplement essential fatty acids. The most abundant n-6 PUFA was linoleic acid (C18:2n-6). These fish contain arachidonic acid (C20:4), which is a precursor of prostaglandin and thromboxan. These two molecules influence blood clot formation and attachment to endothelial tissue during wound healing, and also play an important role in growth [30]. The n-3/n-6 PUFA ratio has been suggested to be useful indicator for comparing relative nutritional values of fish lipids. The fish lipid of *Oreochromis niloticus* used in this study was characterized by low level of n-3 PUFA compared to n-6 PUFA, with low n-3/n-6 PUFA ratio. These results are in accordance with the finding of Farhat and Abdul [21].

3.3. Total Phenolic Contents (TPC) of the Three Plant Species

Phenolic compound which are antioxidant, have much influence on the stability

Table 2. Fatty acid composition of *Oreochromis niloticus* (%).

Fatty acid	% of total fatty acid
C14:0	2.80 ± 0.14
C15:0	1.10 ± 0.01
C16:0	21.55 ± 0.07
C17:0	1.70 ± 0.00
C18:0	4.80 ± 0.01
C20:0	0.60 ± 0.01
C22:0	1.40 ± 0.01
C24:0	0.90 ± 0.01
ΣSFA	**34.85 ± 0.23**
C16:1(n-9)	0.90 ± 0.01
C16:1(n-7)	5.65 ± 0.07
C17:1(n-7)	0.60 ± 0.01
C18:1(n-9)	22.60 ± 0.01
C18:1(n-7)	3.85 ± 0.07
C20:1(n-9)	1.25 ± 0.07
C20:1	1.15 ± 0.07
C24:1	0.10 ± 0.01
ΣMUFA	**36.30 ± 0.32**
C18:2(n-6)	10.00 ± 0.01
C18:3(n-3)	2.55 ± 0.07
C20:2(n-6)	0.60 ± 0.01
C20:3(n-3)	1.35 ± 0.07
C20:4(n-6)	0.60 ± 0.01
C20:5(n-3)EPA	1.45 ± 0.07
C22:2(n-6)	0.20 ± 0.01
C22:5(n-3)	1.15 ± 0.07
C22:6(n-3)DHA	3.60 ± 0.14
ΣPUFA	**22.30 ± 0.30**
Σn-3	**10.10 ± 0.28**
Σn-6	**12.20 ± 0.04**
n-3/n-6	**0.83**
PUFA/SFA	**0.64**

Values are means ± standard deviation (n = 3). SFA: Saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

and may prevent deterioration through quenching of radical reactions responsible for lipid oxidation [31]. The results given in **Table 3** indicate the total phenolic contents of *Moringa oleifera* leaves, fruits of *Solanum melongena* and roots of *Zingiber officinalis*. As shown in **Table 3**, the TPC ranged from 12,150 to 16,050 mg/100 g. The leaves of *Moringa oleifera* exhibited higher content of TPC than the respective roots of *Zingiber officinalis* and fruits of *Solanum melongena*. TPC of fruits of *Solanum melongena* and roots of *Zingiber officinalis* were not significant different ($P > 0.05$). The difference noted in TPC of *M. oleifera* and others plants can be attributed to the environment of plant, the type of plants or/and the type of soil [32]. Various researchers have worked about content of phenolic compounds in *M. oleifera* and *Zingiber officinalis* [33] [34]. Comparison with the results obtained in the present study is hardly possible because of differing analytical methodologies, and the differences in the samples material and origin. Leonce *et al.* [35] determined the total phenolic content in *M. Oleifera* leaves growth in Haiti and found to be 2545 mg/100 on dry weight basis. Results also shown that the TPC of the *M. oleifera* leaves noted in this work were found to be higher than those determined in Southwestern Algeria (3552 mg/100 g on dry weight) [35]. *M. oleifera* leaves compared to *Solanum melongena* fruits and *Zingiber officinalis* roots presented a high antioxidant capacity due to their high content of total phenol.

3.4. Effect of Plant Extracts on Lipid Oxidation of Processing Fish

3.4.1. Effect on Iodine Value (IV)

Lipid oxidation is a major problem occurred in fish and fat food during processing. These reaction reduced shelf-life of fish and altering the quality and the nutritional value of food. Lipid with high content of unsaturation is most exposed to autooxidation. To know the relative unsaturation of lipid, iodine number is useful. IV is a parameter used to measure the average number of double bonds present in fat and oils. It is directly proportional to the amount of unsaturated fatty acid present in lipid [15]. **Table 4** illustrate the effect of salt and plant extracts on IV of the lipid of *Oreochromis niloticus* during smoking and sun drying. Based on results presented in this table, IV of control (sun and smoke dried tilapia without pretreated with salt or plant extract) were lower than

Table 3. Total Phenolic content of *Moringa oleifera* leaves, *Solanum melongena* fruits and *Zingiber officinalis* roots.

Plant material	Concentration (mg gallic acid/100 g dry weight)
Moringaoleiféra leaves	16050 ± 0.63^{a}
Zingiber officinale roots	12710 ± 0.95^{b}
Solanum melongena fruits	12150 ± 1.74^{b}

Values are means ± standard deviation (n = 3). Mean values in the same columm with different superscript letters are significantly different ($P < 0.05$).

Table 4. Changes in iodine value of *Orochromis niloticus* during processing.

Solution Concentration (g/l)	Iodine value (g I2/100 g of oil) of smoke dried *Orochromis niloticus*			Iodine value (g I_2/100 g of oil) of sun dried *Orochromis niloticus*		
	TMOL	TSMF	TZOR	TMOL	TSMF	TZOR
Control	39.65 ± 0.67^{l}	39.65 ± 0.67^{l}	39.65 ± 0.67^{l}	20.06 ± 0.69^{n}	20.06 ± 0.69^{n}	20.06 ± 0.69^{n}
NaCl 50 g/l	79.80 ± 0.64^{f}	79.80 ± 0.64^{f}	79.80 ± 0.64^{f}	31.02 ± 1.20^{m}	31.02 ± 1.20^{m}	31.02 ± 1.20^{m}
10	91.23 ± 0.47^{d}	80.41 ± 0.22^{f}	41.02 ± 0.82^{l}	67.98 ± 0.52^{h}	40.83 ± 1.00^{l}	30.01 ± 0.16^{m}
20	160.69 ± 0.72^{b}	80.80 ± 0.78^{f}	55.89 ± 0.76^{j}	80.09 ± 0.23^{f}	60.26 ± 0.12^{i}	39.67 ± 0.64^{l}
30	180.99 ± 0.53^{a}	83.03 ± 1.20^{e}	60.82 ± 0.23^{i}	100.47 ± 0.01^{c}	70.30 ± 0.01^{g}	44.70 ± 0.60^{k}

TMOL: Treatment with *Moringa oleifeara* leaves extract; **TSMF**: Treatment with *Solanum melongena* fruit extract; **TZOR**: treatment with *Zingiber officinale* roots extract. Values are means ± standard deviation (n = 3). Mean values with different superscript letters are significantly different ($P < 0.05$).

those for treated samples. Sun dried tilapia (control) compared to smoked tilapia (control) had the lower value of IV. The difference observed may be due to processing applied. Rorvik [36] revealed that smoking compared to sun drying increases mostly the shelf-life of fish as a result of the combined effect of dehydratation, antimicrobial and antioxidant activity of several of the smoke constituents. As seems in Table 4, IV of treated fish samples compared to controls increases with increasing of the plant extract concentration and samples treated with plant extract at 30 g/l had the higher IV. It is noted that at the same plant extract concentration, smoke dried fish compared to sun dried fish had the higher IV. Low IV in fish controls compared to treated fish showing that plant extract in all concentration reduced alteration of fish lipid during processing by delay oxidation of unsaturated fatty acids. The antioxidant compounds present in all plants used can be responsible of the activity noted. The NaCl used in this work also limit lipid oxidation of *Orochromis niloticus*, however the degree of prevention is lower compared to use of plant extract. The leaves of *Moringa oleifera* used in this study had a higher total antioxidant capacity, consequently to high total polyphenol content (Table 3). The polyphenol present in all plant extract gives hydrogen atoms from their hydroxyl groups thus reducing the formation of primary oxidation compounds, which are hydroperoxides [37]. Similar results have been obtained by Kiin-kabari *et al.* [9] when study the effect of extracts from three indigenous species on the chemical stability of smoke dried catfish during storage in Nigeria. Simat *et al.* [38] also showed that plant extract had the preventive effect on lipid oxidation of fish oil. The results of IV observed in this work were in agreement with the peroxide value presented in Table 5.

3.4.2. Effect on Peroxide Value (PV)

Hydroperoxide is a primary oxidation product obtained during reaction between unsaturated fatty acids and oxygen molecular. The total content of hydroperoxide can be used as a measure of the extent of oxidation in the early stages. The

Table 5. Change in peroxide value of *Orochromis niloticus* during processing.

Solutions concentration (g/l)	Peroxide value (meq d'O_2/Kg of oil) of smoke dried *Orochromis niloticus*			Peroxide value (meq d'O_2/Kg of oil) of sun dried *Orochromis niloticus*		
	TMOL	TSMF	TZOR	TMOL	TSMF	TZOR
Control	11.88 ± 0.88^{cde}	11.88 ± 0.88^{cde}	11.88 ± 0.88^{cde}	20.63 ± 0.88^{a}	20.63 ± 0.88^{a}	20.63 ± 0.88^{a}
NaCl 50 g/l	7.50 ± 0.00^{i}	7.50 ± 0.01^{i}	7.50 ± 0.01^{i}	17.09 ± 0.59^{b}	17.09 ± 0.59^{b}	17.09 ± 0.59^{b}
10	3.13 ± 0.88^{lmn}	9.38 ± 0.89^{gh}	10.00 ± 0.01^{fg}	11.88 ± 0.83^{cde}	12.25 ± 0.35^{cd}	13.13 ± 0.58^{c}
20	2.50 ± 0.01^{mn}	5.40 ± 0.29^{k}	7.30 ± 0.12^{i}	6.88 ± 0.80^{ij}	10.63 ± 0.88^{efg}	11.09 ± 0.30^{def}
30	1.88 ± 0.78^{n}	3.40 ± 0.42^{lm}	4.38 ± 0.85^{kl}	5.63 ± 0.82^{jk}	7.92 ± 0.59^{i}	8.35 ± 0.07^{hi}

TMOL: Treatment with Moringa oleifeara leaves extract; **TSMF**: Treatment with *Solanum melongena* fruit extract; **TZOR**: treatment with *Zingiber officinale* roots extract. Values are means ± standard deviation (n = 3). Mean values with different superscript letters are significantly different ($P < 0.05$).

formation of hydroperoxides is measured by the test namely peroxide test. The peroxide value of fish control (pre-treated without NaCl or aqueous plant extract before drying) and treated fish samples are presented in Table 5. A significant decrease ($P < 0.05$) was noted in almost all the processed samples compared to the controls. Concerning the control samples, sun dried fish compared to smoked dried fish had the higher value of peroxide (20.63 meq·O_2/kg of oil). The lower value of peroxide obtained in smoked sample may be due to the processing. These are in line with the reporter of Doe [39] who demonstrated that phenols, carboxylic acids are the constituents of smoke. These components have antioxidant activity and prevent *Orochromis niloticus* against lipid peroxidation during smoking. The PV of the controls were significantly higher ($P < 0.05$) than that of fish pre-treated with NaCl and aqueous plant extract at different concentration. This means that these plant extract and NaCl were able to slow down the production of hydroperoxides during smoking and sun drying. The PV of all pre-treated samples decreased significantly ($P < 0.05$). Those pre-treated with NaCl (50 g/l) compared to that pre-treated with plant extract have the higher value of peroxide. Within the fish pre-treated with plant extract, the PV decreased significantly ($P < 0.05$) with increased of the concentration of plant extract and fish pre-treated with plant extract at 30 g/l concentration had the lower value. These results are in line with the finding of Kumulo-Johnson *et al.* [40] who noted the lower value of peroxide in hot smoked catfish treated with fresh garlic compared to that of untreated sample. The PV obtained in this study during processing are lower than those noted by Kiin-Kabari *et al.* [9] when evaluated the effect of extracts from *piper guinensis* on the chemical stability of smoked dried catfish during one day of storage. The higher stability exhibit by all plants treated samples in this work during smoking could be link to the synergic effect of the antioxidant compounds liberated during smoking and the antioxidant compoundspresent in plant extracts. Gubben and Denton [41] reported that *Moringa oleifera* leaves contain high amount of vitamin C and E and phenols which are potent antioxidant. The aqueous extract of *Moringa Oleifera*

compared to those of *Zingiber officinalis* roots and *Solanum melongena* fruits prevent mostly the production of hydroperoxide in fish during processing. This result confirms the total phenolic content obtained in the three plants species. Apart control sample (sun dried sample), those who were pre-treated with NaCl (50 g/l) and those pre-treated with plant extract (10 g/l) who exhibited a PV higher than 10 meq·O_2/kg of oil, which is a recommended value [42], the others samples have Peroxide in the accepted value (<10 meq·O_2/kg of oil). Oil becomes rancid generally when the PV rise 20 meq·O_2/kg of oil [43]. Except sundried fish control, in all the fish samples, PV did not rise 20 meq·O_2/kg of oil.

All these three plant can be recommended as potent source of antioxidant to limit lipid oxidation of *Orochromis niloticus* during processing. However, *Moringa oleifera* at 30 g/l of concentration will be most indicated.

3.4.3. Effect on TBars

Aldehydes, ketones, epoxides, hydroxyl compounds and polymers are the secondary oxidation products occurred in food lipid when they were exposed to further oxidation conditions. Malondialdehyde is one of the most abundant aldehyde generated during secondary lipid oxidation. They most commonly used as oxidation marker [44]. To measure lipid peroxidation products in cells, tissues of animals and vegetal food products and oils, Thiobarbituric acid reactives substances (TBARS) are mostly used. Change in TBA of *Orochromis niloticus* samples pretreated with aqueous plant extracts, NaCl solution and fish control are presented in **Table 6**. The control samples were found to contain the highest TBA value, follow by samples pretreated with NaCl solution and that pretreated with aqueous plant extracts. The sun dried fish compared to smoke dried fish had the higher TBA value. The TBA value as seen in **Table 6** decreased with increasing of the aqueous plant extracts concentration and samples pretreated with aqueous plant extracts at 30 g/l concentration had the lowest TBA. The lower TBA value in pretreated fish samples compared to control is due to the activity of phenolics compounds present in plant extract and antioxidant compound

Table 6. The thiobarbituric acid reactive substances (TBARS) value of *Orochromis niloticus* during processing.

Solutions concentration (g/l)	Sr-TBA value (mg MDA/of oil) of smoke dried *Orochromis niloticus*			Sr-TBA value (mg MDA/of oil) of sun dried *Orochromis niloticus*		
	TFMO	TFSM	TRZO	TFMO	TFSM	TRZO
Control	12.46 ± 0.40^{c}	12.46 ± 0.40^{c}	12.46 ± 0.40^{c}	13.73 ± 0.18^{b}	13.73 ± 0.18^{b}	13.73 ± 0.18^{b}
NaCl 50 g/l	8.39 ± 0.02^{hj}	8.39 ± 0.02^{hi}	8.39 ± 0.02^{hi}	10.12 ± 0.04^{d}	10.12 ± 0.04^{d}	10.12 ± 0.04^{d}
10	8.52 ± 0.04^{h}	9.48 ± 0.25^{e}	10.06 ± 0.06^{d}	9.60 ± 0.06^{e}	12.29 ± 0.03^{c}	14.07 ± 0.05^{a}
20	6.29 ± 0.04^{k}	8.17 ± 0.05^{i}	9.12 ± 0.28^{fg}	9.19 ± 0.02^{fg}	9.36 ± 0.06^{ef}	13.48 ± 0.02^{b}
30	5.12 ± 0.05^{l}	7.53 ± 0.11^{j}	7.79 ± 0.10^{j}	8.90 ± 0.04^{g}	8.46 ± 0.08^{h}	12.32 ± 0.0^{c}

TMOL: Treatment with Moringa oleifeara leaves extract; **TSMF**: Treatment with *Solanum melongena* fruit extract; **TZOR**: treatment with *Zingiber officinale* roots extract. Values are means ± standard deviation (n = 3). Mean values with different superscript letters are significantly different ($P < 0.05$).

present in smoke. The aqueous extract of *M. oleifera* leaves as shown in Table 5, are able to limit mostly the formation of secondary oxidation products in fish lipid during processing compared to others NaCl solution and aqueous extract of *Zingiber officinalis* roots and *Solanum melongena* fruits. As previously noted with iodine and peroxide values, the activity of aqueous plant extract was concentration-dependent. The present results were in accordance with those noted by Kiin-Kabari *et al.* [9] when evaluated the effect of three indigenous species extract on the chemical stability of smoke dried catfish during storage. The malondialdehyde in high concentration is dangerous because they are able to alter/cross-link a variety of biological macromolecule and contribute to its toxicity and its mutagenic properties. Covalent modification of lipoproteins with MDA may play a pathogenic role in atherosclerosis.

3.4.4. Effects on Free Fatty Acids

Hydrolysis of triglycerides in oil and fatty food produced free fatty acids. To measure the percentage of free fatty acids in given amount of oil, acid value (AV) is mostly used. The acid value of fish control (pre-treated without NaCl or aqueous plant extract before drying) and treated fish samples are presented in Table 7. The AVs of *Orochromis niloticus* pretreated with NaCl and aqueous plant extracts before smoking and sun drying were significantly lower ($P < 0.05$) than that of the control groups. After smoking and sun drying, AVs of fish samples pretreated with NaCl compared to that pretreated with aqueous plant extracts were found to be higher. Plants extracts used in the present study were more effective than NaCl in lowing the AV of fish lipid. The AVs of the all sun dried samples were as follows: control > Pretreated with NaCl > pretrated with aqueous plant extract at 10 g/l > pretrated with aqueous plant extract at 20 g/l > pretratedwith aqueous plant extract at 30 g/l. The AVs of fish pretreated with 20 and 30 g/l of aqueous plant extracts before smoking showed no significant ($P > 0.05$) difference after smoking. The result also shows that all the three plants are able to stabilize the *orochromis niloticus* lipid. Their AVs are lowers than that of

Table 7. Changes of acid value of *Orochromis niloticus* during processing.

Solution Concentration (g/l)	Acid value of smoke dried *Orochromis niloticus* (% oleic acid)			Acid value of sun dried *Orochromis niloticus* (% oleic acid)		
	TMOL	TSMF	TZOR	TMOL	TSMF	TZOR
Control	4.06 ± 0.25^{k}	4.06 ± 0.25^{k}	4.06 ± 0.25^{k}	45.43 ± 0.04^{a}	45.43 ± 0.04^{a}	45.43 ± 0.04^{a}
NaCl (50 g/l)	2.70 ± 0.35^{l}	2.70 ± 0.35^{l}	2.70 ± 0.35^{l}	40.89 ± 0.66^{b}	40.89 ± 0.66^{b}	40.89 ± 0.66^{b}
10	1.85 ± 0.13^{mn}	2.21 ± 0.06^{lm}	2.39 ± 0.05^{lm}	23.83 ± 0.29^{h}	29.29 ± 0.46^{e}	31.80 ± 0.15^{c}
20	1.41 ± 0.25^{no}	1.51 ± 0.36^{no}	2.17 ± 0.21^{lm}	22.39 ± 0.25^{i}	28.17 ± 0.04^{f}	30.32 ± 0.33^{d}
30	1.04 ± 0.23^{o}	1.41 ± 0.49^{no}	1.93 ± 0.23^{mn}	19.87 ± 0.18^{j}	25.38 ± 0.00^{g}	28.17 ± 0.21^{f}

TMOL: Treatment with Moringa oleifeara leaves extract; **TSMF**: Treatment with *Solanum melongena* fruit extract; **TZOR**: treatment with *Zingiber officinale* roots extract. Values are means ± standard deviation (n = 3). Mean values with different superscript letters are significantly different ($P < 0.05$).

fish pretreated with NaCl. The low AVs in pretreated fish with plants extracts might be due to higher antoxidant potential of these plants to control oxidation during processing. The AVs of all pretreated somoke dried fish in this work were higher than that of smoked catfish pretreated with three indigenous species that reported by Kiin-Kabari *et al.* [9] in Nigeria. These results are in disagreement with those presented by Guinores *et al.* [45] who showed that garlic extract has no significant effect on the free fatty acid or lipid autolysis in sardine oil extracted from smoked sardines.

4. Conclusion

The results of the present study showed that *Oreochromis niloticus* collected in Maga Lake in Far North, Cameroon are a good source of proteins, lipids and ash. These fish had a high nutritional quality due to their lipid richness in unsaturated fatty acids especially linoleic acid, EPA and DHA. Regular consumption of *Oreochromis niloticus* is therefore recommended for vulnerable groups of population to solve the malnutrition problems and contributed to recommended nutrient intakes. All the processing used in this work change oxidation parameters of the *Oreochromis niloticus*. The aqueous plants extracts of *Moringa oleifera* leaves, *Solanum melongena*fruits and *Zingiber officinale* roots were more effective than control and NaCl in inhibition of *Oreochromis niloticus* against lipid oxidation during sun and smoke dried. The use of aqueous extract of *Moringa oleifera* leaves at 30 g/l concentration provided the best antioxidant protection against lipid oxidation during sun and smoke dried. Smoke dried compared to sun dried can be recommended for drying of *Oreochromis niloticus* because appears to have the higher lipid quality.

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Conflicts of Interest

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

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