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Preservation Potentials of Essential Oils of *Ocimum basilicum* and *Ocimum gratissimum* from Two Agro-Ecological Zones on Freshwater Smoke-Dried *Oreochromis niloticus* Fish Sold in Some Local Markets in Cameroon

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Abstract

Dried fish are susceptible to bacteria and fungi attack and are liable to chemical changes which cause losses in quality and reduction of shelf-life. It is important therefore to maintain the quality of fish because continuous consumption of contaminated fish and their products may predispose consumers to health hazards. Maintenance of high quality fish therefore calls for adequate and effective preservation techniques. The study examined the effectiveness of essential oils of *Ocimum basilicum* and *Ocimum gratissimum* from two Agro-ecological zones of Cameroon in limiting the microbial proliferation and preserving the quality of smoke-dried *Oreochromis niloticus* fish stored at 25˚C for two months. The plant materials were harvested from the Western Highlands and Monomodal Humid Forest agroecological zones of Cameroon. Extraction of the essential oil from the plants was done by hydro-distillation. The fish species (*Oreochromis niloticus*) used in this study was chosen based on a survey study on the most consumed species of freshwater smoke-dried fish in the Western Highlands and Monomodal Humid Forest Agro-ecological zones of Cameroon. Heterotrophic bacteria counts, fungi counts and *Enterobacteriaceae* counts were used to assess the level heterotrophic bacteria, fungi and *Enterobacteriaceae* respectively in the fish samples during storage and were done by culture techniques using total plate count agar, potato dextrose agar and violet red bile glucose agar respectively.

Total volatile basic nitrogen, peroxide value, and thiobarbituric acid reactive substance assays were used as spoilage indices to assess the nutritional quality of the fish during storage. From the survey study, *Oreochromis niloticus* was the most consumed smoke-dried fish in the Western Highlands (35.45%) and Monomodal Humid Forest (34.55%) agroecological zones. All the EOs caused a significant reduction in the microbial loads, total volatile basic nitrogen, peroxide value, and thiobarbituric acid reactive substance of smoke-dried *Oreochromis niloticus* as storage progressed. However, the reduction in these values was more pronounced in samples treated with essential oils of *O. gratissimum* from the Western Highlands, with heterotrophic bacteria, fungi and *Enterobacteriaceae* counts being 5.89, 6.97 and 4.59 $\log_{10}$ cfu/g respectively at the end of the storage period. This was followed by essential oils of *O. gratissimum* from the Monomodal Humid Forest with heterotrophic bacteria, fungi and *Enterobacteriaceae* counts being 6.11, 7.79 and 4.86 $\log_{10}$ cfu/g respectively at the end of the storage period. Also, essential oils of *O. gratissimum* from the Western Highlands was more effective in preserving the fish quality as lowest total volatile basic nitrogen (12.29 mg/100g), peroxide value (2.79 mEq $O_2 \cdot Kg^{-1}$) and thiobabituric reactive substance (1.695 mg MDA/Kg) values were observed for fish samples treated with this extract at the end of the storage period. This was followed by essential oils of *O. gratissimum* from the Monomodal Humid Forest with total volatile basic nitrogen (14.95 mgN/100g), Peroxide value (3.23 mEq $O_2 \cdot Kg^{-1}$) and thiobabituric reactive substance (2.354 mg MDA/Kg) at the end of the storage period. From the results obtained, essential oils from *O. gratissimum* were more effective than that from *O. basilicum* in the two agroecological zones and should be considered as natural alternative to chemical preservatives for further application in food preservation.

Keywords

Oreochromis niloticus, Essential Oils, Fish Quality, *Ocimum basilicum*, *Ocimum gratissimum*, Agro-Ecological Zone

1. Introduction

Providing adequate food especially seafood for a rapidly increasing human population is one of the greatest challenges this day in the world [1]. In Cameroon, an annual requirement of 400,000 tons per year of fish is needed to meet the population demands as the consumption of fish per inhabitant is estimated at 20 kg/year [2] [3]. However, production is still insufficient to meet this demand, because 55% of this tonnage is imported mainly in the form of frozen fish [2]. Thus there is need for increasing production of fish and Nile tilapia (*Oreochromis niloticus*) has been reported to be one of the most produred [4] [5] and most smoked [6] fish species amongst the pervasive freshwater fishes in Cameroon as a result of high nutritional quality. It is also one of the world's most important food fishes, and the fourth most important species in global aquaculture produc-

tion by weight, accounting for about 8% of total global aquaculture production in 2016 [7]. World production of this fish grew from an estimated 1,034,000 tonnes in the year 2001 to nearly 4.6 million tonnes in the year 2019 [7].

Nile tilapia as other fishes serves as an important source of nutrients such as essential fatty acids, minerals, vitamins and animal proteins around the world, especially in the Africa [8] [9]. It is a highly perishable food commodity due to elevated water and nutrient contents that favour the growth of microoganism leading to rapid deterioration of the quality of the fish, therefore making it unfit for consumption [10]. Consumers nowadays are becoming more conscious of fish and fish product with high nutritional and organoleptic quality after processing [11]. Maintenance of high quality fish therefore calls for adequate preservation and processing techniques to delay or inhibit microbial growth and enhance it nutritional and organoleptic quality during storage [8]. In order to prevent fish spoilage, retained its nutritional and organoleptic quality, and increase its shelf life, various preservation techniques such chilling, boiling, freezing, use of chemical reagent, radiation, salting, drying and smoking have been used [12].

The spoilage of food particularly seafood can be caused by enzymatic autolysis, oxidation and microbial growth [13] [14]. Proteolytic enzymes present in fish muscle contribute to post mortem degradation of fish proteins during storage and processing [13]. Fish is rich in lipids and after death, fish lipids are subjected to lipolysis and auto-oxidation [15]. The main reactants in these processes are atmospheric oxygen and fish lipids but the reactions are initiated and accelerated by heat and light. Although fish contains lipids that undergo oxidative degradation, it also contains large amounts of protein and non protein nitrogeneous (NPN) compounds that favour microbial growth [15]. Microbiological spoilage has been noted as the major cause of fish deterioration, followed by oxidative rancidity and enzymatic denaturation of protein [15]. The action of microorganisms on protein and non protein nitrogeneous compounds in fish results in the formation of biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones which are responsible for the unpleasant off-flavors in fish [16] [17]. Also, bacteria such as *Enterobacteriacceae*, *Shewanella putrifaciens*, and *P. phosphoreum* obtain their energy by reducing trimethyl amine oxide (TMAO) present in fish to trimethylamine (TMA), an ammonia-like compound that may be toxic to consumers [16]. All these actions decrease the shelf life and nutritional quality of fish and other seafoods. Total volatile basic nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARS) are used universally as spoilage indices to determine the level of microbial deterioration of fish protein [13] and oxidation of fish lipid [15] respectively.

There are four basic methods used for preserving fish that helps to prevent fish from spoilage and degradation [18]. These include heating, freezing, controlling water activity (smoking and drying) and irradiating [18]. Smoking and Sun drying are traditional preservation methods common in most parts of Africa

[10]. These preservation methods are based on principle of making fish unfavorable for the growth of spoilage organisms through the reduction of the moisture content. Although smoking improves the organoleptic profile (colour, texture, taste and flavor) of the fish, it also contaminate the fish by depositing toxic and carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, heterocyclic aromatic amines, and β-carbolines which are dangerous to health [19]. These toxic carcinogenic compounds contamination from smoked fish can be significantly decreased by improving the smoking process by not placing the fish directly in contact with thesmoke source [19]

In Sub-Saharan African countries including Cameroon and Nigeria, where electricity is unstable in the rural areas, frozen storage of fish is difficult and freshwater fish operators in these localities are increasingly using smoking and sun drying [20] as preservation strategies to reduce the moisture content and quantity of microbial flora that invade and colonize fish [10] [20]. Although smoking and sun drying are used in extending the shelf-life of fish, hygienic conditions during smoking and storage are questionable [21] and the final product is often sold in open markets where the fishes are displayed uncovered on tables which further exposes the products to contamination by bacteria, fungi, insects and to chemical or oxidative changes [8] [21]. To minimize such contaminants and chemical process, it is necessary to use effective natural antimicrobial, antioxidant and insect repellent agents having little or no toxicological effects in addition to smoking since chemical or synthetic preservatives have been reported to be capable of causing adverse reactions that are dangerous to health [22].

Plants of the Lamiaceae family including *Ocimum basilicum* and *Ocimum gratissimum* are widely distributed around the globe [23] and have been largely used in different places including Cameroon as spices and for the treatment of headaches, respiratory problems, influenza, stomach pain, inflammations of the throat, diarrhea, worms, and kidney malfunction with little or no toxicological effect reported [24]. These plants produce large amount of secondary metabolites with antimicrobial and antioxidant properties [23] [25], that protect them against predators, microbial attack and possible diseases. Such properties could be transferred to food to provide protection against microbial contaminants and oxidative spoilage. Essential oils are example of such products and have been reported to have remarkable antibacterial, antioxidant and antifungal properties [22] [26] that make them potential candidates in food preservation [23]. Given the documented information about the activity of these plants in fighting certain ailments caused by pathogentic micro-organism [24], there is still scarcity of scientific information about the use of this plant or its essential oil as biological preservative in fish preservation against pathogenic spoilage micro-organisms. It is in this respect that this work aimed at exploring the potential of hydrodistilled essential oils from these plants in extending the shelf life and preserving the quality of freshwater smoke-dried *Oreochromis niloticus* fish.

2. Materials and Methods

2.1. Determination of Most Consumed Species of Freshwater Smoke-Dried Fish in the Western Highlands and Monomodal Humid Forest Agro-Ecological Zones of Cameroon

Representative localities from the Western Highlands (Mezam, Boyo, Ngo-Ketunjia, and Bui) and Monomodal Humid Forest (Meme, Ndian, Fako, and Manyu) agro-ecological zones of Cameroon were randomly selected. Four thousand (4000) open and closed-ended questionnaires were administered to fish consumers in the two agroecological zones (2000 for each zones) through face-to-face interviews with fish consumers. Purposive sampling of the fish consumers was done such that the questions were administered only to fish consumers who came to buy fish in the markets. Initially, the fish consumers or respondents were asked for their consent before answering the questions.

2.2. Assessment of the Effectiveness of Essential Oils of *Ocimum basilicum* and *Ocimum gratissimum* in Limiting the Microbial Loads and Maintaining the Quality of Smoke-Dried Nile Tilapia during Storage

2.2.1. Collection of Plant Material and Extraction of Essential Oils

Leaves of *Ocimum basilicum* and *Ocimum gratissimum* were harvested from Western Highlands and Monomodal Humid Forest Agro-ecological zones of Cameroon. Samples were identified and authenticated at the National Herbarium of Cameroon (HNC) under registration numbers 29880HNC (*O. gratissimum*) and 42742/HNC (*O. basilicum*). The essential oils from the plant's leaves were extracted by hydrodistillation using Clevenger-type apparatus following procedure describe by [27], with some modification. A mass of 1000 g of dried plant material was put in a pressure pot containing 4 L of water and heated for six hours. The residual concentrated solution was dried with $MgSO_4$ put in an air tide brown screw cap glass vials and stored at 4˚C until use for further experiments.

2.2.2. Preparation of Fish Samples for Analysis

Assessment of the effects of these oils on the storage stability of the fish was done following the procedure described by [22] with some modifications. Briefly, 3 kg of fresh water smoked dried Nile tilapia was purchased at Bamenda food market and taken to the laboratory. In the laboratory, the fish was processed and divided into 100 small sizes, each weighing 15 g, and treated as follows: Twenty (20) of the fish fillets serve as the control. 40 fish fillets were treated by adding 2 mL of essentials oils of *Ocimum gratissimum* from the Western Highlands to 20 of the fish fillets and 2 mL of essentials oils of *Ocimum gratissimum* from the Monomodal Humid Forest to the other 20 fillets. The remaining 40 fish fillets were treated by adding 2 ml of essentials oils of *Ocimum basilicum* from the Western Highlands to 20 of the fish fillets and 2 ml of essentials oils of *Ocimum basilicum* from the Monomodal Humid Forest to the other 20 fillets. The control and treated samples were put in separate dishes and stored at 25˚C after which

microbiological and biochemical analyses of the fish were carried out every two weeks for 2 months.

2.2.3. Antimicrobial Assessment of the Essential Oils

This was done by counting the whole plate to determine the number of bacterial and fungal growth and using the number obtained to multiply by the dilution factor. The total number of heterotrophic bacteria, fungi, and *Enterobacteriaceae* were determined using total plate count agar (TPCA), potato dextrose agar (PDA), and violet red bile glucose Agar (VRBGA) respectively following the procedure described by [22] with some modifications. After the first fourteen days of storage, 10 g of the experimental samples were measured and blended with 30 ml of distilled water. From the obtained inoculums, four 10-fold serial dilutions were made and 1 ml of each dilution was pipetted and spread separately on petri dishes containing 15 mL of TPCA, PDA, and VRBGA. The Petri dishes containing TPCA and VRBGA were incubated at 37˚C for 48 hours while the plates containing PDA were incubated at 28˚C for 4 days. After each incubation period, total bacteria and fungi colonies were counted and recorded as colony-forming units per gram (cfu/g) and further converted to $\log_{10}$ base values ($\log_{10}$ cfu/g).

2.2.4. Assessment of the Effect of the Essential Oil on Fish Quality

Total volatile basic nitrogen (TVB-N), peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were used as quality indices to assess the effectiveness of the oils on stored fish samples and were determined following procedure described by [22] [28] with some modifications.

For TVB-N determination, 10 g of each fish sample were blended with 50 ml trichloroacetic acid (10% TCA) for 5 minutes. The homogenates were divided into two equal parts to obtain duplicate samples. Each parts was then centrifuged at 500 rpm for 3 minutes, and the resulting supernatant filtered through a Buchner funnel using Whatman N˚ 1 filter paper to obtain a clear extract. Distillation of the filtrate was done in tubes containing 2 g of MgO, 1 drop of silicon antifoaming agent, and 100 ml distilled water. The resulting distillate was collected in flask containing 25 ml of 3% aqueous boric acid solution and 0.04 ml of methyl red indicator for the titration of the volatile bases. The distilled TVBN was titrated against an aqueous 0.1N HCl solution and the quantity of TVBN in mg of nitrogen per 100 g of fish sample was determined using the equation:

$$\mathrm{TVBN}\left(\mathrm{mgN}/100\mathrm{g}\right)=\frac{V\times C\times 14}{\text{weight of sample}}\times 100$$

where V is the volume of HCl added and it concentration (C), 14 represent the molecular weight of nitrogen.

For PV determination, 10 g of each fish sample was measured and homogenized with 70 ml mixture of chloroform/methanol/distilled water (in the ratio 20:30:20) for 2 minutes. A further 20 ml of chloroform and 10 ml of distilled water added, homogenized and filtered using a Whatman No.1 filter paper. The filtrate was stored in an air-tied container and the remaining defatted cake un-

dergoes the same process as above. The final filtrate was added to the previously obtained filtrate and centrifuged at 3000 rpm for 10 minutes and the resulting chloroform lipid mixture collected into tube containing 2 g of anhydrous sodium sulphate. The mixture was homogenized and filtered using Whatman No.1 filter paper and the solvent evaporated at 25˚C using a rotatory evaporator. The obtained oil was stored in an air-tied container at −20˚C until further use. The PV in the obtained oil was quantified by weighing 20 mg of the oil and put into test tube containing chloroform/methanol (2:3). 50 µl Ammonium thiocyanate (0.3 g/ml) was added to the test tube and vortexed for 10 seconds after which, 50 µl of ferrous chloride (Fe^{2+}) solution was added and vortexed for 10 seconds. The sample was allowed in the dark for 5 min, then 1 ml in duplicate transferred to quartz cuvettes, and the absorbance of each solution measured at 510 nm using the UV/Vis spectrophotometer. A blank solution containing all the reagents as above except the sample was prepared and the resulting absorbance value was subtracted from that of the sample. Using a calibration plot, the PV was calculated using the equation:

$$\mathrm{PV}\left(\text{mequiv peroxide/Kg}\right)=\frac{\left(As-Ab\right)\times\ m}{55.84\times 2\times\ mo}$$

where *As* is the absorbance of the sample at 510 nm; *Ab* is the absorbance of the blank (control) at 510 nm; *m* is the slope of the Fe (III) calibration plot; 55.84 is the atomic weight of Fe; 2 is the factor to convert milliequivalents (mequiv) of oxygen to mequiv of peroxide, *mo* is the sample weight in grams.

For thiobarbituric acid reactive substances (TBARS) determination, 10 g of samples at every two weeks intervals of storage was blended with 30 ml trichloroacetic acid (10% TCA) for 5 minutes. 5 ml of ethanolic solution of butylated hydroxytoluene (BHT, 1 g/l) was added to the homogenate. This was followed by centrifugation at 500 rpm for 20 minutes and filtration using Whatman No 1 filter paper. 1 ml filtrate were heated at 90˚C for 20 minutes in tubes each containing 1 ml of 0.02 M TBA and 0.1 mL BHT (1 g/l), cooled to room temperature and the absorbance measured at 532 nm. The absorbance values of the TBA-MDA adduct in the samples were subtracted from the blank which contain 1 ml distilled water, 1 ml TBA (0.02 M), and 0.1 ml BHT (1 g/l). Using a standard curve, the concentrations of MDA in the samples were determined using the formula:

$$\text{mg MDA/Kg of fish}=\frac{\begin{array}{c}\text{Absorbance of sample}\times\text{Molar weight of MDA}\times\\ \text{Volume of extraction}\times\text{Dilution of extract added to TBA}\times 1000\end{array}}{\text{Weight of sample}\times\text{slope of standard curve}\times 1000}$$

3. Results and Discussion

3.1. Determination of Most Consumed Species of Fresh Water Smoke-Dried Fish in Two Agroecological Zones of Cameroon

3.1.1. Socio-Demographic Characteristics of Participants

Out of the 2000 respondents from the Western Highlands 30.35% and 69.65% were male and female respectively. 27.90% of the respondents were single while

72.10% were married. Similarly, out of the 2000 respondents from the Monomodal Humid Forest, 20.25% and 79.75% were respectively male and female. 44.50% of the respondents were single while 55.50% were married. Table 1 summarizes the gender, marital status, and level of education of the respondents.

3.1.2. Smoke-Dried Fish Predominantly Consumed in the Western Highlands and Monomodal Humid Forest Agroecological Zones of Cameroon

Consumer's responses on the species of freshwater smoke-dried fish consumed were presented in Table 2. Nile tilapia (*Oreochromis niloticus*) was the most predominant species consumed in the Western Highlands (35.45%) and Monomodal Humid Forest (34.55%) due to their availability as well as low cost. African catfish (*Clarias gariepinus*) and African bony tongue or Kanga (*Heterotis niloticus*) were less consumed due to high prices and seasonal unavailability. Smoke-dried fishes were generally consumed and appreciated in the Western Highlands and Monomodal Humid Forest Agro-ecological zones of Cameroon. The frequency of fish consumption by respondent varied from one species to another. Some respondents preferred consuming more than one species of smoke-dried fish because of their good taste. [4] had noted that Nile tilapia is the most interesting fish species farmed in Cameroon. In terms of fish consumption preference, our study showed that Nile tilapia was the most consumed fish compared to other fishes in the Western Highlands (35.45%) and Monomodal Humid Forest (34.55%) agroecological zones of Cameroon. This was probably because of its low cost and seasonal availability. This was followed by Common carp (*Cyprinus carpio*), African catfish (*Clarias gariepinus*), African bony tongue

Table 1. Gender, marital status and level of education of the respondents (n = 4000).

	Western Highlands (n = 2000)		Monomodal Humid Forest (n = 2000)	
	Frequency of respondents	Percentages (%)	Frequency of respondents	Percentages (%)
Gender				
Male	607	30.35	405	20.25
Female	1393	69.65	1595	79.75
Marital Status				
Single	558	27.90	890	44.50
Married	1442	72.10	1110	55.50
Level of education				
No formal education	28	1.4	31	1.55
Primary	458	22.90	657	32.85
Secondary	1015	50.75	1010	50.50
University	499	24.95	302	15.10

Table 2. Fish species predominantly consumed.

Species name	Western Highlands (n = 2000)		Monomodal Humid Forest (n = 2000)	
	Frequency of respondents	Percentages (%)	Frequency of respondents	Percentages (%)
Nile tilapia (*Oreochromis niloticus*)	709	35.45	691	34.55
African bony tongue/Kanga (*Heterotis niloticus*)	256	12.80	296	14.80
African catfish (*Clarias gariepinus*)	498	24.90	393	19.65
Common carp (*Cyprinus carpio*)	519	25.95	490	24.50
Banded fish (*Hemichromis fasciatus*)	10	0.50	118	5.90
Others	8	0.40	12	0.60
Total	**2000**	**100**	**2000**	**100**

(*Heterotis niloticus*) and Banded fish (*Hemichromis fasciatus*). These results are not in agreement with those obtained by [29] in the bimodal humid forest agroecological zone (Centre Region) of Cameroon. In their study, African catfish followed by Common carp were the most consumed fishes. This diviation in results could be due to differences in sampling places, time of sampling and the sampled population. According to [30], geographical factors affect preferences and consumption patterns of some food products.

3.2. Effect of the Essential Oils on the Microbial Load and Spoilage of the Fish during Storage

3.2.1. Microbiological Analyses of Stored Fish Samples

The number of viable heterotrophic bacteria fungi as well as *Enterobacteriaceae* on each petri plate was counted every two weeks and the total number of colonies per gram was calculated. These values were further converted to $\log_{10}$ base values ($\log_{10}$ cfu/g). The heterotrophic bacteria count ($\log_{10}$ cfu/g) for the control sample were 2.32 ± 0.04 and 8.76 ± 0.08 $\log_{10}$ cfu/g during the beginning and 8th week of storage respectively. By using WH(OG)EO, these values decreased from 2.32 ± 0.07 to 5.89 ± 0.04 $\log_{10}$cfu/g from the start to the 8th week of storage. Similarly, by using MHF (OG)EO, the values decreased from 2.37 to 6.11 $\log_{10}$ cfu/g from the start to the 8th week of storage. The fungi and *Enterobacteriaceae* counts also showed a similar trend (**Table 3**). In general, there was a gradual increase in heterotrophic bacteria, fungi and *Enterobacteriaceae* counts in all samples during storage. The slight increase could be due to the hydrolysis of proteins and fats by natural fish enzymes in the fish samples and the subsequent release of nitrogenous compounds and fatty acids leading to suitable conditions for

Table 3. Heterotrophic bacteria, fungi, and *Enterobacteriaceae* count (log_{10} cfu/g) of smoke-dried *Oreochromis niloticus* treated with essential oils of *O. gratissimum* (OG) and *O. basilicum* (OB) from the Western Highlands (WH) and Monomodal Humid Forest (MHF) agroecological zones of Cameroon.

Treatments		Heterotrophic bacteria, fungi and *Enterobacteriaceae* counts (log_{10}cfu/g) per two weeks				
		Week 0	Week 2	Week 4	Week 6	Week 8
Viable heterotrophic bacteria	Control	2.32 ± 0.04^{Aa}	5.67 ± 0.04^{Ab}	6.22 ± 0.09^{Ac}	6.69 ± 0.21^{Ac}	8.76 ± 0.08^{Ad}
	WH(OG)-EO	2.32 ± 0.07^{Aa}	2.91 ± 0.03^{Ba}	3.33 ± 0.04^{Bb}	3.49 ± 0.04^{Bb}	5.89 ± 0.04^{Bc}
	MHF(OG)-EO	2.37 ± 0.03^{Aa}	2.16 ± 0.15^{Ba}	3.82 ± 0.19^{Bb}	4.96 ± 0.01^{Cc}	6.11 ± 0.09^{Cd}
	WH(OB)-EO	2.37 ± 0.03^{Aa}	4.82 ± 0.19^{Cb}	4.49 ± 0.04^{Cb}	4.89 ± 0.09^{Cb}	6.71 ± 0.02^{Cc}
	MHF(OB)-EO	2.36 ± 0.03^{Aa}	4.79 ± 0.01^{Cb}	4.87 ± 0.04^{Cb}	5.92 ± 0.04^{Dc}	7.21 ± 0.24^{De}
Fungi	Control	3.48 ± 0.01^{Aa}	6.59 ± 0.00^{Ab}	6.66 ± 0.05^{Ab}	8.63 ± 0.01^{Ac}	9.96 ± 0.04^{Ad}
	WH(OG)-EO	3.33 ± 0.01^{Aa}	4.64 ± 0.50^{Bb}	5.60 ± 0.45^{Bc}	6.85 ± 0.09^{Bd}	6.97 ± 0.03^{Bd}
	MHF(OG)-EO	3.35 ± 0.04^{Aa}	5.63 ± 0.19^{Cb}	5.99 ± 0.01^{Bb}	7.05 ± 0.08^{Cc}	7.79 ± 0.21^{Cc}
	WH(OB)-EO	3.41 ± 0.02^{Aa}	5.94 ± 0.03^{Cb}	6.24 ± 0.21^{Ac}	7.68 ± 0.05^{Cd}	8.80 ± 0.08^{De}
	MHF(OB)-EO	3.44 ± 0.01^{Aa}	6.32 ± 0.09^{Ab}	6.42 ± 0.03^{Ab}	8.07 ± 0.09^{Ac}	8.97 ± 0.11^{Dc}
Enterobacteriaceae	Control	2.53 ± 0.04^{Aa}	3.59 ± 0.05^{Ab}	5.31 ± 0.04^{Ac}	5.97 ± 0.02^{Ac}	6.58 ± 0.02^{Ad}
	WH(OG)-EO	1.53 ± 0.02^{Ba}	2.43 ± 0.04^{Bb}	2.95 ± 0.03^{Bb}	4.20 ± 0.03^{Bc}	4.59 ± 0.04^{Bc}
	MHF(OG)-EO	1.70 ± 0.03^{Ba}	2.69 ± 0.11^{Bb}	3.89 ± 0.09^{Cc}	4.63 ± 0.02^{Bd}	4.86 ± 0.06^{Bd}
	WH(OB)-EO	2.28 ± 0.09^{Aa}	2.53 ± 0.03^{Ba}	4.50 ± 0.07^{Db}	4.34 ± 0.03^{Bb}	5.64 ± 0.04^{Cc}
	MHF(OB)-EO	2.22 ± 0.05^{Aa}	3.49 ± 0.15^{Ab}	3.90 ± 0.09^{Cb}	5.48 ± 0.15^{Ac}	6.49 ± 0.02^{Ad}

Means followed by different **Capital letters** along the columns and different **lowercase letters** along the rows are significantly different according to Tukeys Honest Significance Difference at 5% level. **WH(OG)-EO:** Essential oil of *Ocimum gratissimum* from the Western Highlands, **MHF(OG)-EO**: Essential oil of *Ocimum gratissimum* from the Monomodal Humid Forest, **WH(OB)-EO:** Essential oil of *Ocimum basilicum* from the Western Highlands, **MHF(OB)-EO**: Essential oil of *Ocimum basilicum* from the Monomodal Humid Forest.

microbial growth [22]. Statistically, essential oils (EO) of *Ocimum gratissimum* from the Western Highands, (WH(OG)-EO) and Monomodal Humid Forest (MHF(OG)-EO) and that of *Ocimum basilicum* from the Western Highlands (WH(OB)-EO) and Monomodal (MHF(OB)-EO) showed significantly ($P < 0.05$) lower counts when compared with the control samples, and the difference was more pronounced with WH(OG)-EO and MHF(OG)-EO. A similar effect had been reported by [22], who found that EO from *O. gratissimum* decreased significantly the level of microbial growth in *Scomber scombrus* fillets. In all, the fungi counts were generally higher than the heterotrophic bacteria and *Enterobacteriaceae* counts throughout the storage period. This could be due to the low water content in smoked-dried fish that favours the continuous growth of fungi over their bacteria counterparts whose growth rate is increase at a higher water activity.

3.2.2. Quality Indicators

1) Total Volatile Basic Nitrogen (TVB-N)

The TVBN content in smoke-dried *Oreochromis niloticus* gradually increases during storage in all the treatments. The TVBN values for samples treated with essential oils of *O. gratissimum* (OG) from the Western Highlands (WH) and Monomodal Humid Forest (MHF) agro-ecological zones of Cameroon gradually increases throughout the storage period from 2.01 ± 0.02 and 3.65 ± 0.31 mgN/100g to 12.29 ± 0.23 and 14.95 ± 0.08 mgN/100g respectively. Similarly, the TVBN contents for fish samples treated with essential oils of *O. basilicum* (OB) from the Western Highlands and Monomodal Humid Forest agro-ecological zones of Cameroon increases throughout the storage period from 3.58 ± 0.05 and 3.88 ± 0.01 mgN/100g to 19.12 ± 0.02 and 21.14 ± 0.022 mgN/100g respectively (Table 4). The increase may be as a result of the breakdown of free amino acids and proteins, degradation of nucleotides and oxidation of amines by autolytic enzymes and microbial activity [31]. It was observed that, compared with the control groups, TVB-N values for samples treated with WH(OG)-EO, MHF(OG)-EO, WH(OB)-EO, and MHF(OB)-EO were significantly ($P < 0.05$) lower throughout the storage period, except in the 2nd week when no significant change was noticed for MHF(OB)-EO treated samples. However, the TVBN value for samples treated with WH(OG)-EO and MHF(OG)-EO were comparatively lower than WH(OB)-EO and MHF(OB)-EO treated samples throughout storage. These results indicate that EOs of *O. gratissimium* from the two agroecological zones were more effective in delaying the rate of formation of TVB-N during storage. This may be attributed to the role of such oils on microbial populations and bacterial growth as antimicrobial agents [22].

2) Peroxide Value (PV)

The PV for the control samples and fish samples treated with all the EOs increase from the beginning up to the 6th week of storage. From the start till the 6th week of storage, the peroxide value (PV) for fish samples treated with WH(OG)EO

Table 4. Total Volatile Basic Nitrogen (mgN/100g) values of smoke-dried *Oreochromis niloticus* treated with essential oils of *O. gratissimum* (OG) and *O. basilicum* (OB) from the Western Highlands (WH) and Monomodal Humid Forest (MHF) agroecological zones of Cameroon.

Treatments	Total volatile basic nitogen (mgN/100g) per two weeks				
	Week 0	Week 2	Week 4	Week 6	Week 8
Control	3.99 ± 0.02^{Aa}	8.48 ± 0.17^{Ab}	16.31 ± 0.54^{Ac}	21.61 ± 0.57^{Ad}	24.17 ± 0.36^{Ae}
WH(OG)-EO	2.01 ± 0.02^{Ba}	2.44 ± 0.06^{Ba}	5.84 ± 0.16^{Bb}	9.88 ± 0.51^{Bc}	12.29 ± 0.23^{Bd}
MHF(OG)-EO	3.65 ± 0.31^{Aa}	5.72 ± 0.15^{Cb}	10.73 ± 0.29^{Cc}	14.49 ± 0.76^{Cd}	14.95 ± 0.08^{Cd}
WH(OB)-EO	3.58 ± 0.05^{Aa}	7.02 ± 0.23^{Db}	13.13 ± 0.45^{Dc}	16.62 ± 0.52^{Dd}	19.12 ± 0.02^{De}
MHF(OB)-EO	3.88 ± 0.01^{Aa}	8.13 ± 0.13^{Ab}	13.56 ± 0.45^{Dc}	18.60 ± 0.17^{Bd}	21.14 ± 0.22^{Be}

Means followed by different **Capital letters** along the columns and different **lowercase letters** along the rows are significantly different according to Tukeys Honest Significance Difference at 5% level. **WH(OG)-EO:** Essential oil of *Ocimum gratissimum* from the Western Highlands, **MHF(OG)-EO**: Essential oil of *Ocimum gratissimum* from the Monomodal Humid Forest, **WH(OB)-EO:** Essential oil of *Ocimum basilicum* from the Western Highlands, **MHF(OB)-EO**: Essential oil of *Ocimum basilicum* from the Monomodal Humid Forest.

and MHF (OG)EO increases from 1.21 ± 0.01 to 4.41 ± 0.16, and 1.10 ± 0.02 to 4.50 ± 0.02 mEq $O_2 \cdot kg^{-1}$ of fish oil respectively. Similarly, the peroxide value (PV) for fish samples treated WH(OB)EO and MHF (OB)EO increases from 0.47 ± 0.09 to 6.99 ± 0.10, and 1.33 ± 0.02 to 6.87 ± 0.08 mEq $O_2 \cdot kg^{-1}$ of fish oil respectively (Table 5). However, the PV values for all samples after reaching a maximum in the 6th week decrease to 2.79 ± 0.11, 3.23 ± 0.28, 4.12 ± 0.15, and 5.91 ± 0.11 mEq $O_2 \cdot kg^{-1}$ for WH(OG)EO, MHF (OG)EO, WH(OB)EO and MHF (OB)EO respectively at the 8th week of storage. The increase in PV value could be attributed to high degree of unsaturation of lipids [32]. A decrease in PV during the 8th week of storage could be due to the decomposition of hydroperoxide, yielding a wide variety of stable compounds including aldehydes. These results were similar to those reported by [33], who noted a significant increase in PV in fish oil only after the 2nd to the 8th week of storage, and then a slight dropped in the value after the 12th week of storage. The difference in the biological activity of the EOs of *O. gratissimum* and *O. basilicum* from the two agroecological zones was noticed. Compared with WH(OB)-EO and MHF(OB)-EO treated samples, the PV values for samples treated with WH(OG)-EO and MHF(OG)-EO were significantly lower ($P < 0.5$) during storage, with WH(OG)-EO showing a higher antioxidant activity. Such findings may be attributed to the high antioxidant effect of essential oils of *O. gratissimum* which is related to the scavenger nature of their flavonoids and phenolic contents [25]. The high antiradical activity of essential oils of *Ocimum gratissimum* could be due to the fact that they contain large amount of highly active phenolic compound eugenol as reported by [34]. It was also noted that the ability of the essential oils in limiting the formation of hydroperoxide during storage varied within the two geographical locations. WH(OG)-EO and WH(OB)-EO showed significant reduction in PV compared to that MHF(OG)-EO and MHF(OB)-EO respectively. These results were

Table 5. Peroxide values (milliequivalents oxygen/Kg) of smoke-dried *Oreochromis niloticus* treated with essential oils of *O. gratissimum* (OG) and *O. basilicum* (OB) from the Western Highlands (WH) and Monomodal Humid Forest (MHF) agroecological zones of Cameroon.

Treatments	Peroxide Value (milliequivalents oxygen/Kg) per two weeks				
	Week 0	Week 2	Week 4	Week 6	Week 8
Control	1.30 ± 0.02^{Aa}	4.92 ± 0.09^{Ab}	7.57 ± 0.09^{Ac}	8.45 ± 0.20^{Ad}	7.29 ± 0.23^{Ac}
WH(OG)-EO	1.21 ± 0.01^{Aa}	2.44 ± 0.02^{Bb}	2.81 ± 0.03^{Bb}	4.41 ± 0.16^{Bc}	2.79 ± 0.11^{Bb}
MHF(OG)-EO	1.10 ± 0.02^{Aa}	1.48 ± 0.09^{Ca}	4.21 ± 0.12^{Cb}	4.50 ± 0.02^{Bb}	3.23 ± 0.28^{Cd}
WH(OB)-EO	0.47 ± 0.09^{Ba}	2.58 ± 0.03^{Bb}	5.77 ± 0.09^{Dc}	6.99 ± 0.10^{Cd}	4.12 ± 0.15^{De}
MHF(OB)-EO	1.33 ± 0.02^{Aa}	4.57 ± 0.08^{Ab}	5.82 ± 0.148^{Dc}	6.87 ± 0.08^{Cd}	5.91 ± 0.11^{Ec}

Means followed by different **Capital letters** along the columns and different **lowercase letters** along the rows are significantly different according to Tukeys Honest Significance Difference at 5% level. **WH(OG)-EO:** Essential oil of *Ocimum gratissimum* from the Western Highlands, **MHF(OG)-EO**: Essential oil of *Ocimum gratissimum* from the Monomodal Humid Forest, **WH(OB)-EO:** Essential oil of *Ocimum basilicum* from the Western Highlands, **MHF(OB)-EO**: Essential oil of *Ocimum basilicum* from the Monomodal Humid Forest.

supported by [26], who noted that the scavenging nature of EOs of *O. gratissimum* (OG) varies significantly ($P < 0.05$) from two geographical locations that is, Western highland (Dchang) and Bimodal Humid Forest (Yaounde) agroecological zones of Cameroon, with that from Dchang having the highest activity.

3) Thiobarbituric acid reactive substances (TBARS)

The TBARS values for all samples showed an increasing trend throughout the storage period. TBARS values for the control samples were 0.019, 0.201, 0.940, 1.957, and 3.170 mgMDA/kg sample at the start, 2nd, 4th, 6th, and 8th weeks of storage respectively. By using WH(OG)EO, TBARS values were 0.017, 0.082, 0.284, 0.973, and 1.695 mgMDA/kg at the start, 2nd, 4th, 6th, and 8th weeks of storage. For MHF(OG)EO, TBARS values were 0.017, 0.045, 0.295, 0.137, and 2.154 mgMDA/kg at the start, 2nd, 4th, 6th, and 8th weeks of storage respectively. Similarly, the TBARS values increase throughout the storage period when WH(OB)EO and MHF(OB)EO were used (Table 6). The highest TBARS value (3.170 mgMDA/kg) was recorded for the control sample while the lowest TBARS value (1.695 mgMDA/kg) was recorded for fish samples treated with WH(OG)EO at the end of the storage period. The increase in TBARS in all samples throughout the storage period is likely due to the oxidation of lipids. A marked increase in this index in all samples was noticed from the 6th week to the 8th week of storage. This marked increase in TBAR was coincidental with the decrease in PV, probably due to the decomposition of hydroperoxides (primary products of lipid oxidation) into more stable secondary products. It is worth noting that, although the TBARS increase in all fish samples treated with WH(OG)-EO, MHF(OG)-EO, WH(OB)-EO, and MHF(OB)-EO, the increase was significantly ($P < 0.05$) lower when compared to the control. This was probably due to the strong antioxidant potential of the EOs. The ability of EOs to act as free radical scavengers was probably due to their phenolic hydroxyl groups that donate H-atoms to peroxyl radicals (ROO), thereby, slowing down the peroxidation of unsaturated lipids [25]. The

Table 6. Thiobarbituric acid reactive substances (TBARS) values of smoke-dried *Oreochromis niloticus* treated with essential oils of *O. gratissimum* (OG) and *O. basilicum* (OB) from the Western Highlands (WH) and Monomodal Humid Forest (MHF) agroecological zones of Cameroon.

Treatments	Thiobarbituric acid reactive substances (mg MDA/Kg) per two weeks				
	Week 0	Week 2	Week 4	Week 6	Week 8
Control	0.019 ± 0.002^{Aa}	0.201 ± 0.022^{Ab}	0.940 ± 0.093^{Ac}	1.957 ± 0.015^{Ad}	3.170 ± 0.156^{Ae}
WH(OG)-EO	0.016 ± 0.002^{Aa}	0.082 ± 0.008^{Ba}	0.284 ± 0.031^{Bb}	0.973 ± 0.046^{Bc}	1.695 ± 0.077^{Bd}
MHF(OG)-EO	0.017 ± 0.001^{Aa}	0.045 ± 0.005^{Ba}	0.295 ± 0.046^{Bb}	1.137 ± 0.031^{Bc}	2.354 ± 0.046^{Cd}
WH(OB)-EO	0.019 ± 0.001^{Aa}	0.119 ± 0.009^{Cb}	0.514 ± 0.046^{Cc}	1.596 ± 0.061^{Cd}	2.351 ± 0.077^{Ce}
MHF(OB)-EO	0.018 ± 0.000^{Aa}	0.156 ± 0.002^{Cb}	0.667 ± 0.046^{Cc}	1.433 ± 0.077^{Cd}	2.415 ± 0.155^{Ce}

Means followed by different **Capital letters** along the columns and different **lowercase letters** along the rows are significantly different according to Tukeys Honest Significance Difference at 5% level. **WH(OG)-EO:** Essential oil of *Ocimum gratissimum* from the Western Highlands, **MHF(OG)-EO**: Essential oil of *Ocimum gratissimum* from the Monomodal Humid Forest, **WH(OB)-EO:** Essential oil of *Ocimum basilicum* from the Western Highlands, **MHF(OB)-EO**: Essential oil of *Ocimum basilicum* from the Monomodal Humid Forest.

Lowest values of TBARS were observed for the samples treated with WH(OG)-EO indicating that WH(OG)-EO exerts a stronger antiradical activity. The relatively high antioxidant activity of WH(OG)-EO could be due to its high phenolic content. These results are in agreement with that carried out by [22], who noted that the ability of EOs of *O. gratissimum* from Mbonge in limiting lipid oxidation in *Scomber scombrus* was significantly higher than that of essential oils of *O. gratissimum* from Bambili.

4. Conclusion

This work had the main objective to valorize the essential oils of *O. basilicum* and *O. gratissimum* from two geographical locations of Cameroon in the preservation freshwater smoke-dried fish. *Oreochromis niloticus* was the most consumed species of smoke-dried fish in the Western Highlands and Monomodal Humid Forest agro-ecological zones of Cameroon. All the EOs showed significant reduction in the heterotrophic bacteria fungi and *Enterobacteriaceae* counts compared to the control samples but reduction in these values was more pronounced with EOs of *O. gratissimum* from the two geographical locations than *O. basilicum*. Similarly, all the EOs showed reduction in TVBN, PV, and TBARS in smoke-dried *Oreochromis niloticus* compared to the control samples as storage progressed. However, the reduction was more pronounced for samples treated with EOs of *O. gratissimum* from the Western Highlands followed by that from the Monomodal Humid Forest. The study therefore provides a strong baseline for consideration of essential oils of *O. gratissimum* as natural alternative to chemical preservatives for further application in food preservation.

Conflicts of Interest

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

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