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CHEMICAL COMPOSITION OF STORED SMOKED CLUPEIDS, *ETHMALOSA FIMBRIATA* AND *SARDINELLA MADERENSIS* AFTER PRESERVATIVE TREATMENT WITH CITRUS ESSENTIAL OIL

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Abstract: *The preference for smoked fish by Nigerians has made it imperative that the quality of smoked fish product be assessed during storage to reduce the loss associated with storage by bacteria which has a direct effect on the nutrient content of the fish. This study aimed to examine the preservative potentials of lemon peel oil on smoked Clupeids, Ethmalosa fimbriata and Sardinella maderensis and its resultant effect on the chemical components. Two hundred samples, of which 100 each of Bonga and Sardine; were divided into two and a part coated with the essential oil while the other part was not (both bonga and sardine). Triplicate samples of Bonga and Sardine were aseptically stored in cartons and evaluated weekly for its chemical components during a 12-week storage period. There was significant ($p<0.05$) increase in peroxide values in preserved samples (Bonga: 3.10−8.75 meq/kg, Sardine: 3.35−9.75 meq/kg) compared to unpreserved samples (Bonga: 2.15−5.20 meq/kg, Sardine: 2.60−0.05 meq/kg). This study provides information on lemon essential oil has the ability to improve the shelf life and chemical composition of clupeids during storage.*

Keywords: *Ethmalosa fimbriata, lemon peel oil, preservative, Sardinella maderensis*

1. Introduction

Fish is the only protein source that contains significant amount of all the essential amino acids, particularly lysine in which cereals are relatively poor. Fish protein can be used therefore to complement the amino acids pattern and improve the overall protein quality of mixed diet [1]. Consumption of fish has been associated with improved cardiovascular health and other health conditions thereby constituting an important component of diet for many people [2]. Fish is a food of excellent nutritional value, providing high-quality protein rich in essential amino acids, and a wide variety of minerals, including phosphorus, magnesium, iron, zinc, and iodine in marine fish [3]. Fish is an easily perishable commodity and deterioration in quality is due to changes taking place to the various constituents like proteins, lipids etc. Therefore, knowledge of the chemical components will help determine the optimum processing and storage conditions, in order to preserve the quality. Clupeids belong to the family *Clupeidae*; and

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include many of the most important food fishes (shads, herrings, sardines etc) commonly caught for the production of fish oil and fish meal. Clupeids are the most valuable family food fishes in the world in the order *Clupeiformes*. They are the group of fish with a form most like the original form of the bony fishes (Teleostei) from which all other bony fishes evolved. Clupeids (*Ethmalosa fimbriata* and *Sardinella species*) and Scombroid (jacks, barracuda and tuna) fisheries are the main components of the Nigerian pelagic fishery. *Ethmalosa fimbriata* (Bonga shad (English) and Agbodo in Yoruba language) belongs to the family *Clupeidae* and order clupeiformes [4]. It is a coastal and estuarine clupeid found along the numerous estuaries of the Niger Delta Region. *Sardinella maderensis* is an oceano dromous pelagic filter-feeding clupeid. It is usually found in "schools" at either the surface or middle of the water body [5]. It is a silvery fish that is similar to the round Sardinella (*Sardinella aurita*) but differs with its grey caudal fins having black tips. Citrus species are small to medium-size shrubs or trees cultivated throughout the tropics and subtropics [6]. Lemons are a rich source of vitamin C and also, they contain numerous phytochemicals including polyphenols and terpenes [7]. The use of natural preservation methods has been on a low level due to the advent of synthetic preservative methods; however synthetic preservatives have been discovered to have negative impact on human health [8]. Essential oil is generally found in raw plant materials such as flowers, leaves, wood, bark, roots, seeds, and peels (peels and seeds that would otherwise be referred to as wastes) [9]; and is a resource that has not been fully exploited. Thus, this study hopes to determine the chemical composition of citrus essential oil-preserved smoked clupeids (*E. fimbriata* and *S. maderensis*) during the storage.

2. Material and methods

Description of Study Area

The study was carried out in the Water Recirculatory System Unit of the Federal College of Freshwater Fisheries Technology, New Bussa. Niger State.

Description of Experimental Site

The experimental site is one of the stores in the Water Recirculatory System on which the cartons carrying the fish were placed.

Experimental Set-up

Freshly smoked samples (200 nos: 100 each of both fish) of both bonga (*Ethmalosa fimbriata*) and sardine (*Sardinella maderensis*) were bought from Makun-omi market, in Ijebu-ode, Ogun State. The lemon (Citrus limon) essential oil (300 mL) was poured in a small bowl. The lemon essential oil was dabbed with a clean cotton wool and used to coat the fish that were to be coated with lemon essential oil (Plate 2). There were two parts of fish- a part coated with the essential oils and the other part that was not coated with lemon essential oil (for both bonga (Plate 1) and sardine - Plates 3 and 4). This makes the experimental set-up to be four. Bonga – samples coated with essential oil and uncoated samples. Sardine – samples coated with essential oil and uncoated samples. The coated samples were put in white polyethylene bags and placed in cartons and the uncoated samples packed in carton without polyethylene bags. These were then stored at ambient temperature (±25 °C) for twelve weeks.

Data Collection

The samples were assessed weekly for its chemical composition in a 3-month shelflife study (to see the effect of essential oil on the chemical components of the stored smoked clupeids). This was done by taking 20 g each in triplicates from the cartons, put in sample bowls and taken for chemical analysis according to [2].

Total Volatile Basic Nitrogen (TVB-N)

TVB-N was calculated according to the method of [10]. Ten grams of stored smoked clupeid was minced and weighed. Thereafter, 200 mL of 7.5 % aqueous tricholoroacetic was added, mixed for 1minute in a Marlex blender, and then filtrated to make an extract. Twenty-five ml of filtrate was transferred into distillation flask, and then 6 mL of 10% NaOH was added, and distilled for 4 minutes. The distillate was gathered into 10 mL of 4 % of boric acid with methyl red and bromocresol green, and then titrated with 0.025N H_2SO_4 until solution turned oink.

$$TVB-N\ (mgN/100g) = \frac{(14mg/mol) \times a \times b \times 300}{25ml}$$

Where a = mL of Sulphuric acid,
b = Normality of Sulphuric acid

Trimethylamine nitrogen (TMA-N) content

TMA was determined by the micro diffusion method [10]. Ten grams sample was homogenized with 20 mL of 20% Trichloroacetic acid (TCA). The homogenate was filtered through filter paper No. 4 to a 100 mL standard flask. The residue was triturated with 80 mL of 5 % TCA and made up to the volume. The filtrate was then used for further analysis. Grease was applied on the edges of micro diffusion unit and 1 mL of 0.01N standard sulphuric acid was taken in the inner chamber, while 1 mL 20% TCA extract, 0.5 mL neutralized formaldehyde and 0.5mL saturated potassium carbonate were taken in the outer chamber of the unit. The unit sealed with glass with lid and gently swirled, then kept overnight undisturbed. The amount of un-reacted acid in the chamber was determined by titration against standard 0.01 NaOH using Tashiro's indicator. A blank was run simultaneously prepared with 1mL of 20% TCA solution. TMA-N was calculated as mg/100 g of muscle as follows:

$$TMA-N\ (mg\ \%) = \frac{(A-B) \times 0.14 \times \text{Volume of extract}}{\text{Volume of sample taken} \times \text{sample weight}} \times 100$$

Where: A = volume of 0.01N NaOH used for titration of the blank
B = volume of ml of 0.01N NaOH used for titration for the sample

Hypoxanthine content

Five grams of fish sample was blended with a known volume of perchloric acid (V1) and analyzed for hypoxanthine content. A known volume of aliquot of the perchloric acid extract (V3) was neutralized with KOH-buffer (V2) solution, then the neutralized extract (V4) was added to a test tube, analyzed and gave the hypoxanthine content (H) from the standard curve. The moisture content (M) was obtained after which hypoxanthine content was then calculated using the [10] formula:

$$H = \frac{H \times [V_1 + (0.01 \times M \times W)]}{V_4 \times W} \times \frac{(V_2 + V_3)}{V_3} \times \frac{1}{G}$$

V1 = Volume of perchloric acid
V2 = Volume neutralized with KOH-buffer
V3 = Volume of aliquot of the perchloric acid
V4 = Neutralized extract
M = Moisture content
H = Hypoxanthine content

Peroxide value

Ten grams of sample was added to 1 g powdered potassium iodide (KI) and 20 cm^3 solvent mixtures (2vol glacial acetic acid + 1 vol chloroform) were placed in boiling water for 30 seconds. The content was then poured into a flask containing 20 cm^3 of KI solution and titrated with 0.002N sodium thiosulphate using starch as indicator. Peroxide was calculated and expressed as milliequivalent (meq) peroxide per kilogram of sample [10].

$$\text{Peroxide value (meq/kg)} = \frac{(S - B)\,(N)\,1000}{\text{Unit of sample}}$$

Where B = Titration of blank, S = Sample titration, N = Normality of Sodium thiosulphate

Determination of pH

Five grams each of the smoked clupeids was blended with 30ml distilled water for 1minute. The homogenate was allowed to stay for 2 minutes after which the pH was measured by digital pH meter Jenway 3310 (Electronic Instruments Ltd., England)

Statistical analysis

Weekly data was taken in triplicate for each parameter determined. Data obtained at each stage of the study was statistically analyzed using Analysis of Variance (ANOVA) and the means separated using the Duncan Multiple Range Test according to [11]. The statistical package used for this was SPSS 17.

3. Results

The chemical components of the smoked Bonga, *Ethmalosa fimbriata* preserved with lemon essential oil and packaged in polyethylene bags presented in table 1 showed the trimethylamine (TMA), total volatile basic nitrogen (TVBN) and peroxide values of the fish increasing significantly (p<0.05) with storage time while the pH (though not very significant (p<0.05)) and hypoxanthine values were decreasing.

Fig. 1 Plate 1 - Uncoated Bonga (*Ethmalosa fimbriata*) sample

Fig. 2 Plate 2 - Coated Bonga (*Ethmalosa fimbriata*) sample.

Source : Field Survey, 2018.

Fig. 3 Plate 3 - Uncoated Sardine (*Sardinella maderensis*).
Source : Field Survey, 2018

Fig. 4 Plate 4. Coated Sardine (*Sardinella maderensis*).
Source : Field Survey, 2018

The pH and hypoxanthine ranged from 6.25±0.35 at week 12 to 6.75±0.35 at week 1 and 2.40±0.14 to 10.50±0.71 respectively. The TMA, TVBN and peroxide value increased with increase in storage time and ranged from 13.00±1.41 to 23.30±0.28, 9.25±0.35 to 19.35±0.21 and 3.10±0.14 to 8.75±0.21 respectively.

Table 1.
Chemical analysis of preserved stored smoked bonga (*Ethmalosa fimbriata*) during storage.

Weeks	pH	TMA (mg/100g)	TVBN (mg/100g)	Peroxide (meq/kg)	Hypoxanthine
1	6.75±0.35^{abc}	13.00±1.41^{ab}	9.25 ±0.35^{a}	3.10 ±0.14^{a}	10.50 ±0.71^{h}
2	7.00±0.28^{c}	14.10±1.56^{abc}	12.50±0.71^{b}	4.00±0.28^{b}	8.10±0.14^{g}
3	6.60±0.14^{abc}	15.05±0.78^{abc}	13.30±0.14^{c}	4.95±0.78^{c}	7.25±0.35^{a}
4	6.70±0.14^{abc}	16.00±0.28^{abc}	13.60±0.14^{c}	6.00±0.28^{d}	6.35±0.21^{c}
5	6.70±0.14^{abc}	16.60±0.28^{abc}	14.50±0.42^{d}	6.65±0.21^{de}	5.40±0.28^{d}
6	6.59±0.41^{abc}	17.05±0.21^{abc}	15.50±0.14^{e}	7.10±0.14^{e}	5.10±0.14^{d}
7	7.00±0.28 ^{c}	18.15±0.50^{abc}	16.85±0.50^{f}	8.00±0.28^{f}	4.10±0.14^{c}
8	6.90±0.14^{bc}	18.855±0.07^{a}	18.25±0.35^{g}	8.20±0.28^{fg}	4.10±0.14^{c}
9	6.55±0.21^{abc}	17.65±0.21^{bc}	19.25±0.35^{hg}	8.75±0.21^{h}	3.65±0.21^{bc}
10	6.35±0.21^{ab}	20.70±0.14^{bc}	20.65±0.21^{hi}	9.10±0.14^{h}	3.55±0.07^{bc}
11	6.45±0.21^{abc}	21.75±0.21^{c}	18.70±0.28^{gh}	8.45±0.07^{fgh}	3.10±0.1^{b}
12	6.25±0.35^{a}	23.30±0.28^{c}	19.35±0.21^{h}	8.75±0.21^{gh}	2.40±0.41^{a}

Values denoted by different superscripts in the column differ significantly [$p<0.05$].
Key: TMA= TrimethylAmine; TVBN= Total Volatile Basic Nitrogen.

In the unpreserved smoked Bonga, *Ethmalosa fimbriata* packaged in polyethylene bags, there was steady and significant ($p<0.05$) decrease in pH and TMA

from week 1 (7.50±0.14 and 8.25±0.35) to week 9 (5.10±0.14 and 3.15±0.07) respectively (Table 2).
Hypoxanthine increase in the samples was significant (p<0.05) from week 1 (16.00±1.41) to week 10 (25.25±0.35). TVBN significantly (p<0.05) decreased with increase in storage period from week 1 (7.10±0.14) to week 11 (1.80±0.28) while the peroxide value ranged from 2.15±0.21 at week 1 to 5.20±0.21 at week 12.

Table 2.
Chemical analysis of unpreserved stored smoked bonga (*Ethmalosa fimbriata*) during storage

Weeks	pH	TMA (mg/100g)	TVBN (mg/100g)	Peroxide (meq/kg)	Hypoxanthine
1	7.50±0.14 ^{e}	8.25±0.35^{i}	7.10±0.14 ^{g}	2.15±0.21 ^{e}	16.00±1.41 ^{a}
2	7.10±0.14 ^{d}	7.30±0.14 ^{h}	7.70±0.28^{i}	2.15±0.21 ^{e}	17.25±0.35 ^{b}
3	6.94±0.08 ^{d}	6.65±0.21 ^{g}	7.40±0.28 ^{hi}	2.65±0.21 ^{f}	17.50±0.42^{bc}
4	6.40±0.14 ^{c}	5.65±0.21 ^{f}	6.64±0.04^{g}	2.10±0.14^{e}	17.65±0.21^{bc}
5	6.40±0.14 ^{c}	4.35±0.21^{e}	5.80±0.28^{f}	1.65±0.21^{d}	18.55±0.50 ^{c}
6	6.20±0.28 ^{c}	4.10±0.14 ^{de}	5.30±0.14 ^{f}	1.50±0.14 ^{cd}	21.00±0.28 ^{d}
7	5.65±0.21 ^{b}	3.65±0.21^{bc}	4.50±0.14 ^{e}	5.65±0.21^{bc}	21.65±0.21^{d}
8	5.30±0.14 ^{ab}	3.30±0.14 ^{ab}	3.90±0.14 ^{d}	5.30±0.14^{bcd}	22.85±0.50 ^{e}
9	5.10±0.14 ^{a}	13.15±0.07 ^{a}	3.35±0.21 ^{c}	5.10±0.14^{bc}	24.15±0.50 ^{f}
10	5.20±0.14 ^{a}	2.90±0.14 ^{a}	2.75±0.35 ^{b}	5.20±0.14 ^{b}	25.25±0.35^{gh}
11	5.35±0.21 ^{ab}	3.90±0.14 ^{cd}	1.80±0.28 ^{a}	5.35±0.21^{bc}	25.59±0.41 ^{h}
12	5.20±0.28 ^{a}	3.35±0.21 ^{ab}	1.45±0.07 ^{a}	5.20±0.28 ^{a}	25.95±0.21 ^{h}

Values denoted by different superscripts in the column differ significantly [p<0.05].
Key: TMA= TrimethylAmine; TVBN= Total Volatile Basic Nitrogen.

There was an insignificant decrease in TMA (p<0.05) of unpreserved smoked Sardine, *Sardinella maderensis* packaged in polyethylene bags during the storage period and it ranged from 2.15±0.07 at week 12 to 8.25±0.35 at week 1 (Table 3). Peroxide value, TVBN and hypoxanthine decreased significantly (p<0.05) from week 1 (2.60±0.14, 8.25±0.35 and 10.50±0.71) to week 11 (0.54±0.65, 2.55±0.21 and 3.55±0.07) respectively.The results of the chemical composition of preserved smoked Sardine, *Sardinella maderensis* packaged in polyethylene bags presented in Table 4 showed that there was no significance (p<0.05) in the decrease in pH and increase in TMA, TVBN and peroxide values with storage time, while hypoxanthine increased significantly (p<0.05). TMA ranged from 13.00±1.41 at week 1 to 21.80±0.14 at week 12. TVBN and peroxide value ranged from 10.60±0.84 to 22.35±0.21 and 3.35±0.21 to 9.75±0.07 respectively.

4. Discussion

pH

The ability of Lemon essential oil to preserve food is mainly due to its contents of terpenes. Terpenes act as preservatives in food by disrupting the membranes of microorganisms thereby, making them inactive. The pH and hypoxanthine content of preserved bonga decreased significantly (p<0.05) with storage period while TMA, TVBN and peroxide value increased unlike its unpreserved counterpart whose hypoxanthine and peroxide increased

Table 3.
Chemical analysis of unpreserved stored smoked sardine (*Sardinella maderensis*) during storage

Week	pH	TMA (mg/100g)	TVBN (mg/100g)	Peroxide (meq/kg)	Hypoxanthine
1	7.50 ± 0.14^{f}	8.25 ± 0.35^{c}	8.25 ± 0.35^{g}	2.60 ± 0.14^{d}	10.50 ± 0.71^{i}
2	7.10 ± 0.14^{e}	7.30 ± 0.14^{a}	7.70 ± 0.28^{fg}	2.15 ± 0.21^{cd}	9.85 ± 0.50^{hi}
3	6.90 ± 0.14^{e}	6.65 ± 0.21^{a}	7.40 ± 0.28^{f}	2.65 ± 0.21^{d}	9.35 ± 0.21^{h}
4	6.35 ± 0.21^{a}	5.65 ± 0.22^{b}	5.75 ± 0.35^{e}	2.25 ± 0.35^{d}	8.25 ± 0.35^{g}
5	6.40 ± 0.14^{d}	4.35 ± 0.21^{a}	5.80 ± 0.28^{e}	1.65 ± 0.21^{d}	8.25 ± 0.35^{g}
6	6.20 ± 0.28^{d}	4.10 ± 0.14^{a}	5.30 ± 0.14^{e}	1.50 ± 1.14^{bc}	7.30 ± 0.14^{f}
7	5.65 ± 0.21^{c}	3.65 ± 0.21^{a}	4.50 ± 0.14^{d}	1.25 ± 0.07^{b}	5.67 ± 0.50^{e}
8	5.30 ± 0.14^{bc}	3.30 ± 0.14^{a}	3.90 ± 0.14^{c}	1.30 ± 0.14^{b}	5.10 ± 0.14^{de}
9	5.10 ± 0.14^{b}	3.15 ± 0.07^{a}	3.50 ± 0.21^{c}	1.25 ± 0.07^{b}	4.65 ± 0.21^{cd}
10	5.20 ± 0.28^{bc}	2.90 ± 0.14^{a}	2.75 ± 0.35^{b}	1.10 ± 0.14^{b}	4.10 ± 0.14^{bc}
11	5.20 ± 0.28^{bc}	2.15 ± 0.07^{bc}	2.55 ± 0.21^{ab}	0.54 ± 0.65^{a}	3.55 ± 0.07^{ab}
12	4.60 ± 0.28^{a}	2.15 ± 0.07^{a}	2.15 ± 0.07^{a}	0.05 ± 0.01^{a}	3.30 ± 0.14^{a}

Values denoted by different superscripts in the column differ significantly [$p<0.05$].
Key:TMA= TrimethylAmine; TVBN= Total Volatile Basic Nitrogen.

Table 4.
Chemical analysis of preserved stored smoked sardine, *Sardinella maderensis* during storage

Week	pH	TMA (mg/100g)	TVBN (mg/100g)	Peroxide (meq/kg)	Hypoxanthine
1	6.93 ± 0.11^{bc}	13.00 ± 1.41^{a}	10.60 ± 0.84^{a}	3.35 ± 0.21^{a}	16.00 ± 1.41^{a}
2	7.00 ± 0.28^{c}	14.10 ± 1.56^{a}	12.50 ± 0.71^{a}	4.00 ± 0.28^{a}	17.25 ± 1.77^{ab}
3	6.60 ± 0.14^{abc}	15.05 ± 0.78^{a}	13.30 ± 0.14^{a}	5.00 ± 0.71^{a}	17.85 ± 0.50^{bc}
4	6.25 ± 0.07^{a}	15.95 ± 0.64^{a}	10.20 ± 0.28^{a}	5.60 ± 0.28^{a}	18.20 ± 0.28^{bc}
5	6.70 ± 0.14^{abc}	16.64 ± 0.34^{a}	14.50 ± 0.42^{a}	6.50 ± 0.21^{a}	19.35 ± 0.21^{cd}
6	6.40 ± 0.14^{ab}	17.05 ± 0.21^{a}	15.50 ± 0.14^{a}	7.10 ± 0.14^{a}	20.44 ± 0.79^{d}
7	7.00 ± 0.28^{c}	17.05 ± 0.21^{a}	15.50 ± 0.14^{a}	7.10 ± 0.14^{a}	22.25 ± 0.35^{e}
8	6.75 ± 0.35^{abc}	18.39 ± 0.12^{a}	18.25 ± 0.35^{b}	8.20 ± 0.28^{a}	23.65 ± 0.21^{ef}
9	6.55 ± 0.21^{abc}	19.65 ± 0.21^{a}	19.25 ± 0.35^{a}	8.75 ± 0.21^{b}	24.35 ± 0.21^{fg}
10	6.35 ± 0.21^{a}	20.74 ± 0.20^{a}	20.65 ± 0.21^{a}	9.10 ± 0.14^{a}	25.20 ± 0.2^{fgh}
11	6.60 ± 0.28^{abc}	21.30 ± 0.28^{a}	21.25 ± 0.35^{a}	9.45 ± 0.07^{a}	25.60 ± 0.14^{gh}
12	6.70 ± 0.28^{abc}	21.80 ± 0.14^{a}	22.35 ± 0.21^{a}	9.75 ± 0.07^{a}	26.65 ± 0.21^{h}

Values denoted by different superscripts in the column differ significantly [$p<0.05$].
Key: TMA= TrimethylAmine; TVBN= Total Volatile Basic Nitrogen.

with the others decreasing. pH is a reliable indicator of the degree of freshness or spoilage, because it is used as an indicator of the extent of microbial spoilage in fish [12]. pH of preserved bonga in this study dropped from 6.75 to 6.25 and this agreed with [12] who said that the normal pH of fish muscle is 7 however; this value post mortem varies from 6.0−7.1. There was no significant (*p*<0.05) difference in the pH values of the preserved bonga throughout the storage period. The pH in 1st–9th week was not significantly (*p*<0.05) different from one another which also was not significantly (*p*<0.05) different from the 10th–12th week. This showed that lemon essential oil has the ability to maintain a neutral post mortem pH in bonga during storage. Despite the fact that the pH of the unpreserved bonga fell in the favorable range, its quality depreciated weekly and this was evident in the protein degradation (reduction in TMA).

Hypoxanthine

The weekly increase in hypoxanthine in unpreserved bonga also further buttressed that preservative ability of lemon essential oil because this reduced from 10.50 to 2.40 mg/100g in the preserved samples compared to the increase observed in its unpreserved counterparts. This increase was as a result of bacteria or spoilage organisms' infestation of the fish during storage. [13] stated that the accumulation of hypoxanthine in fish tissues determines the initial phases of autolytic bacteria spoilage as storage time increases. This also corroborates the findings of [14] who opined that increase in hypoxanthine production with time is related to increase in autolytic enzymes. This result also aligns with [15] who found out that hypoxanthine increases when Iosine Monophosphate (IMP) contents in fish begin to decrease.

Trimethylamine

Trimethylamine (TMA) in the unpreserved bonga decreased significantly (*p*<0.05) (from 8. 25 to 3.35 mg/100 g) while it increased in the preserved samples (13.00 to 23.30 mgN/100 g). The significant (*p*<0.05) decrease in the unpreserved samples went below the recommended level for human consumption (10-15 mgN/100 g) found by [16] while that of the preserved samples fell within (from week 1 to 3) even though there was no significant (*p*<0.05) difference in the TMA from weeks 2 to 8. The TMA values for weeks 9 to 12 were also not significantly (*p*<0.05) different from weeks 2 to 7. This thus infers that smoked bonga preserved with lemon essential oil can be stored for three months and still be fit for human consumption with the protein content still intact. TMA is used to determine fish quality and its values are usually used as advanced spoilage indicators. [17] attributed increase in TMA in fish muscles to putrefaction by spoilage bacteria. The quality of fish can be estimated by sensory tests, microbial methods or by chemical methods such as measuring volatile compounds, lipids oxidation, determination of Adenosine triphosphate (ATP) breakdown products and the formation of biogenic amines [18].

Total Volatile Basic Nitrogen

Total Volatile Basic Nitrogen (TVBN) is an important characteristic for the assessment of quality in seafood products and appears as the most common chemical indicators of marine fish spoilage [19]. It usually corroboratively checks fish freshness if its organoleptic assessment is in doubt [20]. TVBN is a group of biogenic amines formed in non-fermented food products during storage [21].

TVBN in preserved bonga significantly (*p*<0.05) increased with increase in TMA weekly unlike the unpreserved samples (Table 11). This increase was in line with the findings of [18] who discovered that TVBN and TMA are directly related to storage time in frozen fish. Furthermore, the results of this study showed that the quality of bonga in storage improved as the TVBN values from the beginning to the end of the study fell

within the range of values meant for high quality fish (≤ 25mg/100g) [19], [18], [22].
There was significant (p<0.05) weekly decrease in pH of unpreserved sardine (*Sardinella maderensis*) from almost neutral (7.50±0.141) in week 1 to acidic (4.60±0.283) while the preserved samples ranged from the slightly acidic to alkaline (6.93±0.107 to 6.70±0.283). The pH was only slightly acidic in week 1 which progressed gradually to neutrality along the weeks to week 7 which was not significantly (p<0.05) different from weeks 8 to 12. The results indicated that extent of spoilage in the preserved samples was gradual as the weekly pH changes were not significantly (p<0.05) different. TVBN of the sardine samples preserved with lemon essential oil increased weekly though the increase was not significantly (p<0.05) different. The TVBN values for week 1 to 7 were not significantly (p<0.05) different from those of week 9 to 12. This suffices to say that the keeping quality of preserved sardine with lemon essential oil improves gradually up till the 8th week after which the quality changes will not differ from the previous weeks. This increase could be attributed to gradual degradation of the initial protein to more volatile products [23]. The results of this study were also in line with [24] who discovered variations in TVBN in dried Tuna samples treated with plant extracts. The preserved samples had their TMA increasing throughout the twelve weeks even though the increase was not significantly (p<0.05) different from one another. This means that TMA in the preserved sardine samples was maintained while it was lost gradually in the unpreserved samples. [25] stated that TMA is used to determine the quality of products and the maximum allowed as acceptable for human consumption is 10-15mgN/100g. With respect to the results obtained in this study, the TMA ranged from 13.00±1.414 to 21.80±0.141 (preserved samples) and since the values were not significantly different from one another, the preservative effect of lemon essential oil for fish preservation was evident.

Peroxide Value

Peroxide value is a primary indicator of oxidation of fat (rancidity) [26]. The findings from this study revealed that there was a significant (p<0.05) decrease in the peroxide value of the unpreserved sardine while it increased in the samples preserved with lemon essential oil, though not significantly (p<0.05) different from one another. There was no significant (p<0.05) difference in the peroxide values of preserved sardine in week 1 to 8 as well as in week 10 to 12. This shows that the quality of sardine preserved with lemon essential oil can only improve weekly up to the 9th week after which it may not be different. stated that peroxide value is indirectly related to rancidity, this thus showed that rancidity in the unpreserved fish samples increased with storage time while it decreased in the preserved samples. The findings of [27] could also be further buttressed as, the higher the peroxide values of fish, the better the quality the quality, which further proved the efficiency of lemon essential oil as a preservative for fish. This finding thus corroborates [28] who found ginger to be an effective agent of rancidity reduction. [29] also related peroxide value of fish to its moisture content and opined that they are directly related to each other. This study is also in line with this opinion as the moisture content of the preserved samples decreased from 7.35±0.07 to 4.40±0.14 while that of the unpreserved samples went from 7.65±0.21 to 2.10±0.14. Thus, it can be concluded that the values from this study are still within acceptable limit of spoilage since their peroxide values still fall within the acceptable range of below 10-20 meq/kg [16].

5. Conclusion

Conclusively, it can be inferred from the results of this study that Lemon essential oil had a pronounced effect on the chemical composition of smoked Clupeids (*Ethmalosa fimbriata*, Bonga and *Sardinella maderensis*, Sardine) during storage. This is because it was able to keep the chemical indices within the nutritionally recommended limits during storage using natural preservatives that are healthy to consumers of fish. The study also found out that fish consumers can be assured of good quality smoked fish and the environment in good state, whether they eat the fish instantly or keep it on the shelf for future consumption. Therefore; the following are recommended from this study;

1. Other citrus essential oils (sweet orange (Citrus sinensis), tangerine (Citrus reticulata), lime (Citrus aurantifolia) can be experimented to see if similar effects will be produced.
2. Lemon essential oil could be infused into thin permeable materials which can then be used wrap the fish to see the preservative effect on the fish.
3. Preservation of fish can be done naturally at reduced costs: The problem of naturally preserving Bonga and Sardine can be done at low cost without any inherent health hazard.

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7. References

[1]. OSIBONA, A.O.; KUSEMIJU, K. AND AKANDE, G.R. (2009). Proximate Composition and Fatty Acids Profiles of the African Catfish (Clarias gariepinus). Research Gate.

[2]. AYELOJA, A.A. (2016). Evaluation of the Effects of Natural Preservatives and Packaging Materials on the Shelf life of Smoked African Mud Catfish (Clarias gariepnus, Burchel 1822). Ph.D Thesis submitted the Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta. Ogun State.

[3]. ARIÑO, A., BELTRÁN, J.A., HERRERA, A., RONCALÉS, P. (2013): Fish and seafood: Nutritional Value-Encyclopedia of Human Nutrition (Third Edition), Pp: 254-261.

[4]. FROESE, R. AND PAULY, D. eds. (2011). “Ethmalosa fimbriata” in Fish Base. June 2011 version Idodo-Umeh, 2003

[5]. IDODO-UMEH, G. (2003). Freshwater Fishes of Nigeria (Taxonomy, Ecological notes, Diets and Utilization). ISBN 978-8052—01-0. Idodo-Umeh Publishers Ltd.

[6]. DARJAZI, B.B. (2014). Comparison of Peel Components of Pummelo (Citrus grandis) obtained using Cold-Pressing and Hydrodistillation Method. J. Life Sci. Biomed. 4(2): 71-77

[7]. RAUF, A.; UDDIN, G. AND ALI, J. (2014). Phytochemical Analysis and Radical Scavenging Profile of Juices of Citrus sinensis, Citrus aurantiofolia and Citrus limon. Organic and Medicinal Chemistry Letters.https://doi. Org/10.1186/2191-2858-4-5

[8]. TOVIHO, O.A. (2014). Efficacy of Grape (Citrus paradisa) Essential Oil in the Preservation of od Smoked-dried Illisha Africana. Bachelor of Aquaculture and Fisheries Management project submitted to the Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta. Ogun State.

[9]. JOKERS, D. (2012). The Healing Properties of Essential Oil. Health Rangers FREE email newsletter.

[10]. AOAC, (1995). Official Methods of Analysis. 16th Ed. Association of Official Analytical Chemists. Washington DC, USA

[11]. SANDERS, D.H. (1990). Statistics. A First Course, 5th Ed, New York. McGraw-Hill.

[12]. ERKAN, N. AND OZDEN, O. (2008). Quality Assessment of Whole and Gutted Sardine (Sardina pilchardus) stored in Ice. Int. J. Food. Sci. Technol.43:1549-1559

[13]. AMOS, B. (2007). Analysis of Quality Deterioration at Critical Steps/Points in Fish Handling in Ugandan and Iceland: Suggestions for Improvement. United Nations University, Uganda. http//www.unuft.is/static/fellow/document/amos06prf.pdf

[14]. EL-DEEN, G. and El-Shamery, M.R. (2010). Studies on Contamination and Quality of Fresh Fish Meats during Storage. Egypt. Acad. J. Biol. Sci. 2:65-75

[15]. HAMADA-SATO, N.; USUI, K.; KOBAYASHI, T.; IMADA, C.; WATANABE, E. (2005). Quality Assurance of Raw Fish based on HACCP concept. Food Control 16(4): 301-307

[16]. CONNELL, J.J. (1995). Control of Fish Quality. 4th Edition, Wiley Blackwell Publihsers, London, England. ISBN-13-9780852382264. Pages: 256

[17]. IDAKWO, P.Y. NEGBENEBOR, C.A.; BADAU, M.H. AND GBENYI, D.I. (2016). Total Volatile Basic Nitrogen and Trimethylamine content of 'Bunyi youri' as influenced by the addition of Glucose and Clove during Storage. International Journal of Biotechnology and Food Science 4(5): 81-85. ISSN: 2384-7344

[18]. GULSUN, B.O.; ESMERAY, K.; ALI, S.O. AND OZOGUL, F. (2009). Sensory, Microbiological and Chemical Assessment of the Freshness of Red Mullet (Mullus barbatus) and gold band Goat fish (Upeneus moluccensis) during Storage in Ice. Food Chemistry 114(2): 505-570

[19]. AMEGOVU, A.K.; SSENINJOGI, M.L.; OGWOK, P.; MAKOKHA, V. (2012). Nucleotide Degradation Products, Total Volatile Basic Nitrogen, Sensory and Microbiological Quality of Nile Perch (Lates niloticus) fillets under Chilled Storage. Journal of Microbiology, Biotechnology and Food Sciences 2, 653-666

[20]. CASTRO, P.; PADRON, J.C.P.; CABALLERO, M.J.; SANJUAN, E. (2006). Total Volatile Basic Nitrogen and its use to Assess Freshness in European Sea bass Stored in Ice. Food Control 17(4): 245-248 doi:10. 1016/j. Foodcont.

[21]. HORSFALL, M; KINIGOMA, B.S. AND SPIFF, A.I. (2006). Evaluation of the levels of Total Volatile Bases and Trimethylamine formed in Fish stored at Low Temperature. Chemical Society of Ethiopia, 20, 155-159

[22]. EU/EC (2008). Amending Regulations (EC) No 2074/2005 as regards the Total Volatile Basic Nitrogen (TVBN) limits. In: COMMUNITIES, T.C.O.T.E (ed) 1022/2008. Official Journal of European Union

[23]. DARAMOLA, J.A.; FASAKIN, E.A.; ADEPARUSI, E.O. (2007). Changes in Physicochemical and Sensory Characteristics of Smoked-dried Fish Species stored at ambient Temperature. African Journal of Agriculture, Nutrition and Development Vol. 7(6). DOI. 10.18697/ajfand.17.1980

[24]. RAHMAN, M. S.; AL-AMRI, O. S. AND AL-BULUSHI, I. M. (2002). Pores and Physico-Chemical Characteristics of Dried Tuna Produced by Different methods of Drying. Journal of Food Engineering53(4);301-313. DOI: 10. 1016/50260-8774(01) 00169-8

[25]. GOULAS, A.E.; KONTOMINAS, M.G. (2005). Effect of Salting and Smoking Method on the keeping Quality of Chub Mackerel (*Scomber japonicus*): Biochemical and Sensory Attributes. Food Chemistry ISSN: 0308-8146

[26]. ADEYEMI, K.; OLORUNSANYA, O.; ABE, O. (2013). Effect of Citrus seed Extracts on Oxidative Stability of raw and cooked Chicken Meat. Iran. J. appl. Anim. Sci. 3, 195-199

[27]. IHEAGWARA, M.C. (2013). Effect of Ginger Extract on Stability and Sensorial Quality of Smoked Mackerel (*Scomber scombrus*) Fish. Journal of Nutrition and Food Sciences 3: 199. Doi: 10.4172. ISSN: 2155-9600

[28]. KUMOLU-JOHNSON, C.A.; NDIMELE, P.E. (2011). A Review on Post Harvest losses in Artisanal Fisheries of Some African Countries. Journal of Fisheries and Aquatic Sci 6: 365-378.

[29]. WAINDU, C.; JAMALA, G.Y. (2013). A Study of Rancidity of Fish sold in Mubi Markets, Adamawa State, Nigeria. IOSR Journal of Agriculture and Veterinary Science 6(2): 47-50.