

Effect of packaging on the chemical composition of stored citrus essential oil-preserved smoked clupeids (*Ethmalosa fimbriata* and *Sardinella maderensis*)

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ABSTRACT: The study examined the preservative potentials of lemon peel oil for smoked Clupeids, *Ethmalosa fimbriata* and *Sardinella maderensis* in combination with packaging materials on the chemical composition. Four hundred samples (400: 200 each of both fish samples) were divided into two: one part was coated with lemon peel oil (packaged and unpackaged in sterile polyethylene bags), while the uncoated part (packaged and unpackaged) served as control. Triplicate samples of fish were aseptically stored in cartons and evaluated weekly for their nutritional components during a 12-week storage period. All data were statistically analysed using Analysis of Variance. Packaged preserved fish samples had significantly ($p < 0.05$) better and higher nutritional components than the unpackaged samples. The study concluded that packaging materials in combination with essential oil from lemon peel are efficient in preserving smoked Clupeids as well as their nutrients during storage time. It was also found from this study that polyethylene bags in combination with cartons are better packaging material for smoked Clupeids.

Keywords: Clupeids, lemon peel oil, polyethylene bags, preserved sample, shelf-life.

INTRODUCTION

Fish constitutes a very important component of the diet for many people and often provides the much-needed nutrients for healthy living. Fish is a great source of protein, vitamins, minerals and omega-3 fatty acids, a key nutrient for brain development (Idakwo *et al.*, 2016; Belitz *et al.*, 2009). It serves as a principal source of dietary protein which is expensive in relation to other protein foods. The perishable nature of fish and shellfish makes them highly prone to vast variations in quality due to differences in species, environmental habitats, feeding habits and action

of autolytic enzymes as well as hydrolytic enzymes of microorganisms on the fish muscle (Idakwo *et al.*, 2016). Fish also contain significant amounts of all essential amino acids, particularly lysine in which cereals are relatively poor. It is also among the high-lysine foods listed by Whitbread (2024) including lean beef, chicken, pork, fish, shellfish, tofu, cheese, milk, beans, lentils, and peas. Consumption of fish has been associated with improved cardiovascular health and other health conditions thereby constituting an important component of diet for many

people (Ayeloja, 2016). Fish spoilage occurs as a result of the action of enzymes and bacteria present in fish and also the chemical oxidation of fat which causes rancidity (Braun and Sutherland 2006). Fish preservation involves the various methods used in the prevention of fish spoilage and lengthening of its shelf life. Smoking is one of the four chief ways to preserve fish for a longer time. Fish is smoked and roasted, hot-smoked or cold-smoked. Smoking simply means a heating process that dries the fish to preserve it from spoilage. Clupeids belong to the family *Clupeidae*; and 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 characteristically small (<50cm), schooling fish with silvery bellies and sides and greenish grey backs (Dewey, 2014). They have no spines in their fins but possess one short dorsal fin, deeply forked tails and ventral fins on their abdomens far behind the pectorals, deep bodies and large scales that slip off at a touch. The flesh of clupeids is oily, a feature that adds greatly to their flavour and provides valuable oil for the fisheries industry. *Ethmalosa fimbriata* (Bonga shad (English) and Agbodo/Shawa in Yoruba language, Ibat in Ibibio) belongs to the family *Clupeidae* and order *clupeiformes* (Froese and Pauly, 2011a). It is a coastal and estuarine clupeid found along the numerous estuaries of the Niger Delta Region. *Sardinella maderensis* is an oceanodromous (living and migrating the whole seas) pelagic filter-feeding clupeid (Froese and Pauly, 2011b). It is usually found in "schools" at either the surface or the middle of the water body (Idodo-Umeh, 2003). It is a silvery fish that is similar to the round *Sardinella* (*Sardinella aurita*) but differs with its grey caudal fins having black tips. The lemon tree's ellipsoidal yellow fruit is used for culinary and non-culinary purposes throughout the world, primarily for its juice which has both culinary and cleaning uses (Djenane, 2015). Lemons are a rich source of vitamin C and also they contain numerous phytochemicals including polyphenols and terpenes (Rauf *et al.*, 2014). Essential oils are present in the peels of *Citrus species* and have very powerful antimicrobial properties that destroy pathogenic bacteria, viruses and fungi. Fish and fish products are highly perishable; as such require immediate preservation in appropriate packaging for handling, distribution, and export (Sharmila *et al.*, 2020). Plastic materials are the first stop point when packaging of food is under consideration. This is because plastics are durable, cheap, strong, a good barrier to moisture, and light, and can be processed into different shapes. A very high percentage (70%) of plastic waste is generated from food packaging (Vibha *et al.*, 2021). This study is therefore aimed to determine the effect of packaging materials on the chemical composition of smoked clupeids (*E. fimbriata* and *S. maderensis*) preserved with citrus essential oil during storage.

MATERIALS AND METHODS

Sample collection and identification

Lemon (*Citrus limon*) fruits were bought from a local market in Ibadan, Oyo State and the identification was done by the Pure and Applied Botany Department of the Federal University of Agriculture, Abeokuta (FUNAAB). Two hundred (200) freshly smoked samples of both bonga (*Ethmalosa fimbriata*) and sardine (*Sardinella maderensis*) were bought from a local market, in Ijebu-ode, Ogun State. They were transported in cartons from the market to the laboratory according to Oladosu-Ajayi (2020).

Preparation of fish samples for shelf life studies

The fish samples were divided into two equal parts of 100 samples each. The first 100 samples were all coated with the lemon essential oils. These were then further divided into two equal parts of 50 samples each. The first essential oil-coated 50 samples were put singly in white polyethylene bags and placed in cartons while the second part was placed in the carton singly without white polyethylene bags. The second 100 samples were not coated with lemon essential oil. These were also further divided into two equal parts of 50 samples each. The first 50 are packed singly in white polyethylene bags and placed in cartons, while the second 50 are packed in cartons both bonga and sardine. These were then stored at ambient temperature ($\pm 25^{\circ}\text{C}$) for twelve weeks.

Chemical components analysis

Twenty grams each of smoked clupeids were collected weekly from the various packaging materials for chemical analysis.

Total volatile basic nitrogen (TVB-N)

TVB-N was calculated according to the method of AOAC (1995). Ten grams of stored smoked clupeid was minced and weighed. Thereafter, 200ml of 7.5 % aqueous trichloroacetic was added, mixed for 1 minute in a Marlex blender, and then filtrated to make an extract. 25 ml of filtrate was transferred into a 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 the solution turned pink.

$$\text{TVB} - \text{N} (\text{mgN}(100 \text{ g})^{-1}) = \frac{14\text{mg}(\text{mol})^{-1} \times a \times b \times 300}{25 \text{ ml}}$$

Where a = ml of Sulphuric acid; b = Normality of Sulphuric acid.

Trimethylamine nitrogen (TMA-N) content

TMA was determined by the micro diffusion method (AOAC, 1995). A 10 g sample was homogenized with 20 ml of 20 % Trichloroacetic acid (TCA). The homogenate was filtered through filter paper No. 4 into 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 the 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 0.5 ml saturated potassium carbonate were taken in the outer chamber of the unit. The unit was sealed with glass with a lid and gently swirled, then kept overnight undisturbed. The amount of unreacted acid in the chamber was determined by titration against standard 0.01 NaOH using Tashiro's indicator. A blank was run simultaneously and prepared with 1 ml of 20% TCA solution. TMA-N was calculated as mg/100 g of muscle as follows:

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

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 (V_1) and analyzed for hypoxanthine content. A known volume of aliquot of the perchloric acid extract (V_3) was neutralized with KOH-buffer (V_2) solution, then the neutralized extract (V_4) 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 AOAC (1995) formula:

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

Peroxide value

Ten grams of sample was added to 1 g powdered potassium iodide (KI) and 20 cm³ solvent mixtures (2vol glacial acetic acid + 1 vol chloroform) was placed in boiling water for 30 seconds. The content was then poured into a flask containing 20 cm³ of KI solution and titrated with 0.002 n sodium thiosulphate using starch as an indicator. Peroxide was calculated and expressed as milliequivalent (meq) peroxide per kilogram of sample (AOAC, 1995);

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

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

pH determination

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

Statistical analysis

Data obtained at each stage of the study was statistically analyzed using Analysis of Variance (ANOVA) and the means were separated using the Duncan Multiple Range Test according to Sanders (1990). The statistical package used for this was SPSS 17.

RESULTS

The chemical components of the smoked bonga, *Ethmalosa fimbriata* preserved with lemon essential oil and packaged in polyethylene bags shown in Table 1 showed that trimethylamine (TMA), total volatile basic nitrogen (TVBN) and peroxide values of the fish increased significantly ($p < 0.05$) with storage time while the pH (though not significantly ($p < 0.05$)) and hypoxanthine values were decreasing. The pH and hypoxanthine ranged from 6.25 at week 12 to 6.75 at week 1 and 2.40 to 10.50 respectively. The TMA, TVBN and peroxide values increased with increase in storage time and ranged from 13.00 to 23.30, 9.25 to 19.35 and 3.10 to 8.75 respectively.

In the unpreserved smoked Bonga, *Ethmalosa fimbriata* packaged in polyethylene bags, there was a steady and significant ($p < 0.05$) decrease in observed in the pH and TMA from week 1 (7.50 and 8.25) to week 9 (5.10 and 3.15) respectively (Table 2). Hypoxanthine increase in the samples was significant ($p < 0.05$) from week 1 (16.00) to week 10 (25.25). TVBN significantly ($p < 0.05$) decreased with increase in storage period from week 1 (7.10) to week 11 (1.80 ± 0.28) while the peroxide value ranged from 2.15 at week 1 to 5.20 at week 12.

Table 3 shows the chemical components of the unpreserved smoked Sardine, *Sardinella maderensis* packaged in polyethylene bags. The decrease in TMA during the storage period was not significant ($p < 0.05$) and it ranged from 2.15 at week 12 to 8.25 at week 1. Peroxide value, TVBN and hypoxanthine decreased significantly (p

Table 1. Chemical Analysis of preserved stored smoked Bonga, *Ethmalosa fimbriata* packaged in polyethylene bags 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}	18.85±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 ^{gh}	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

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

Table 2. Chemical analysis of unpreserved stored smoked Bonga, *Ethmalosa fimbriata* packaged in polyethylene bags 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

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

Table 3. Chemical analysis of unpreserved stored smoked Sardine, *Sardinella maderensis* packaged in polyethylene bags 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

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

Table 4. Chemical analysis of preserved stored smoked Sardine, *Sardinella maderensis* packaged in polyethylene bags 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 ^{gh}
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

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

Table 5. Chemical analysis of unpreserved smoked Bonga, *Ethmalosa fimbriata* not packaged in Polyethylene during storage

Weeks	pH	TMA (mg/100g)	TVBN (mg/100g)	Peroxide (meq/kg)	Hypoxanthine
1	7.10±0.14 ^h	7.10±0.14 ^h	7.30±0.14 ^h	2.45±0.21 ^f	8.00±0.71 ⁱ
2	6.45±0.07 ^g	6.45±0.07 ^g	6.65±0.21 ^g	2.15±0.07 ^{ef}	7.54±0.48 ^{hi}
3	6.10±0.14 ^f	6.10±0.14 ^f	6.50±0.14 ^g	1.95±0.07 ^{de}	7.20±0.28 ^{fg}
4	5.60±0.14 ^e	5.60±0.14 ^e	6.10±0.14 ^f	1.70±0.14 ^{cde}	6.60±0.28 ^{ef}
5	5.30±0.14 ^d	5.30±0.14 ^d	5.70±0.14 ^e	1.60±0.14 ^{bcd}	6.10±0.14 ^{de}
6	4.90±0.14 ^c	4.90±0.14 ^c	5.30±0.14 ^d	1.35±0.07 ^{bc}	5.65±0.07 ^{cd}
7	4.55±0.07 ^b	4.55±0.07 ^b	5.10±0.14 ^d	1.10±0.14 ^b	5.25±0.07 ^c
8	4.55±0.07 ^b	4.55±0.07 ^b	5.10±0.14 ^d	1.10±0.14 ^b	5.25±0.07 ^a
9	4.30±0.14 ^{ab}	4.30±0.14 ^{ab}	2.85±0.21 ^c	0.54±0.65 ^a	4.10±0.14 ^b
10	4.05±0.07 ^a	4.05±0.07 ^a	2.40±0.14 ^b	0.07±0.01 ^a	3.75±0.07 ^b
11	4.05±0.07 ^a	4.05±0.07 ^a	2.40±0.14 ^b	0.07±0.01 ^a	3.75±0.07 ^b
12	4.25±0.07 ^a	4.25±0.07 ^a	1.90±0.14 ^a	0.04±0.01 ^a	3.05±0.07 ^a

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

< 0.05) from week 1 (2.60, 8.25 and 10.50) to week 11 (0.54, 2.55 and 3.55) respectively.

The result of the chemical composition of preserved smoked Sardine, *Sardinella maderensis* packaged in polyethylene bags is presented in Table 4. 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 at week 1 to 21.80 at week 12. TVBN and peroxide values ranged from 10.60 to 22.35 and 3.35 to 9.75, respectively.

The chemical analysis of unpreserved smoked Bonga, *Ethmalosa fimbriata* not packaged in Polyethylene bags is shown in Table 5. The result showed a significant (p<0.05) difference in the chemical components (TMA, TVBN, peroxide, hypoxanthine and pH) as they decreased weekly

even though there was no significant (p<0.05) difference in the decrease for weeks 9–11 for all the components. The pH decreased from 7.10 in week 1 to 4.25 in week 12, TMA 7.10 to 4.25, TVBN 7.30 to 1.90, peroxide 2.45 to 0.04, and hypoxanthine 8.00 to 3.05.

The pH of the preserved smoked Bonga, *Ethmalosa fimbriata* not packaged in Polyethylene bags remained neutral throughout the storage period while the TMA, TVBN and peroxide increased though not significantly (p<0.05) with decreasing hypoxanthine (Table 6). There was a gradual reduction in TMA, TVBN, peroxide value and hypoxanthine content which was not significantly (p<0.05) different throughout the storage period.

The pH of unpreserved smoked Sardine, *Sardinella maderensis* not packaged in polyethylene bags ranged

Table 6. Chemical analysis of preserved smoked Bonga, *Ethmalosa fimbriata* not packaged in Polyethylene during storage.

Weeks	pH	TMA (mg/100g)	TVBN (mg/100g)	Peroxide (meq/kg)	Hypoxanthine
1	7.20±0.14 ^{ab}	7.20±0.14 ^{abc}	5.25±6.01 ^a	3.40±0.42 ^a	13.50±0.71 ^{ab}
2	6.95±0.07 ^a	6.95±0.07 ^a	6.35±6.58 ^{ab}	3.10±0.14 ^a	14.75±0.35 ^c
3	7.10±0.14 ^{ab}	7.10±0.14 ^{abc}	11.25±0.35 ^{ab}	3.45±0.07 ^a	14.95±0.21 ^c
4	7.45±0.07 ^b	7.45±0.07 ^{bc}	11.70±0.42 ^b	3.35±0.35 ^a	14.65±0.21 ^c
5	7.30±0.14 ^{ab}	7.30±0.14 ^{abc}	12.35±0.21 ^b	3.55±0.50 ^a	14.60±0.57 ^c
6	7.10±0.14 ^{ab}	7.10±0.14 ^{abc}	12.12±0.21 ^b	3.20±0.28 ^a	14.65±0.21 ^c
7	7.50±0.14 ^b	7.50±0.14 ^c	12.25±0.35 ^b	3.35±0.21 ^a	14.50±0.42 ^{bc}
8	7.10±0.14 ^{ab}	7.10±0.14 ^{abc}	11.75±0.35 ^b	3.20±0.28 ^a	14.25±0.35 ^{bc}
9	7.04±0.23 ^{ab}	7.00±0.28 ^{ab}	11.40±0.57 ^{ab}	3.00±0.28 ^a	14.50±0.42 ^{bc}
10	6.85±0.50 ^a	6.85±0.50 ^a	10.20±0.28 ^{ab}	2.75±0.35 ^a	13.85±0.50 ^{bc}
11	7.45±0.07 ^b	7.45±0.07 ^{bc}	9.35±0.21 ^{ab}	5.00±0.71 ^b	13.50±0.71 ^{ab}
12	7.30±0.14 ^{ab}	7.30±0.14 ^{abc}	9.20±0.28 ^{ab}	4.80±1.13 ^b	12.55±0.35 ^a

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

Table 7. Chemical analysis of unpreserved smoked Sardine, *Sardinella maderensis* not packaged in Polyethylene during storage.

Week	pH	TMA (mg/100g)	TVBN (mg/100g)	Peroxide (meq/kg)	Hypoxanthine
1	7.10±0.14 ^b	7.80±0.28 ^h	7.10±0.14 ^h	2.25±0.21 ^{cd}	8.75±0.21 ^j
2	6.35±0.21 ^a	7.10±0.14 ^g	6.55±0.21 ^g	2.10±0.14 ^{cd}	8.30±0.14 ^{ij}
3	6.20±0.28 ^a	6.45±0.07 ^{fg}	6.35±0.21 ^g	2.05±0.07 ^{cd}	7.80±0.28 ^{hi}
4	6.35±0.21 ^a	6.10±0.14 ^{ef}	6.10±0.14 ^{fg}	2.40±0.14 ^d	7.65±0.21 ^{gh}
5	6.35±0.21 ^a	5.50±0.70 ^{de}	5.80±0.28 ^f	2.05±0.07 ^{cd}	7.10±0.14 ^{fg}
6	6.65±0.21 ^{ab}	5.15±0.50 ^d	5.20±0.28 ^{de}	2.40±0.28 ^d	6.75±0.35 ^f
7	6.60±0.28 ^{ab}	5.10±0.14 ^d	5.30±0.14 ^e	2.25±0.21 ^{cd}	6.10±0.14 ^e
8	6.35±0.21 ^a	4.35±0.21 ^c	4.75±0.35 ^d	2.25±0.35 ^{cd}	5.20±0.28 ^d
9	6.65±0.21 ^{ab}	4.05±0.07 ^{bc}	4.10±0.14 ^c	1.90±0.14 ^{bc}	4.35±0.21 ^c
10	6.30±0.14 ^a	3.40±0.28 ^b	3.60±0.28 ^b	1.50±0.14 ^{ab}	3.70±0.42 ^b
11	6.25±0.35 ^a	2.65±0.21 ^a	3.25±0.21 ^{ab}	1.30±0.14 ^a	3.25±0.35 ^{ab}
12	7.00±0.28 ^b	2.10±0.14 ^a	2.90±0.14 ^a	1.10±0.14 ^a	3.00±0.28 ^a

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

from 6.20 to 7.10 throughout the storage period while the other chemical components (TMA, TVBN, peroxide and hypoxanthine) significantly ($p < 0.05$) decreased weekly (Table 7). There was no significant ($p < 0.05$) difference in the change in pH throughout the storage period which infers that it was stable from week 1 to week 12. TMA and TVBN gradually and significantly ($p < 0.05$) reduced from 7.80 and 7.10 at week 1 to 2.65 and 3.25 at week 11 respectively. Peroxide value and hypoxanthine content also reduced steadily and significantly ($p < 0.05$) during the storage period. Peroxide value ranged from 1.10 at week 12 to 2.25 at week 1 while hypoxanthine ranged from 3.00 at week 12 to 8.75.

Table 8 shows the chemical analysis of preserved smoked Sardine, *Sardinella maderensis* not packaged in polyethylene bags. The pH remained neutral throughout

the storage period as it had no significant ($p < 0.05$) difference weekly. TMA, TVBN, peroxide and hypoxanthine were significantly ($p < 0.05$) increasing weekly. TMA, TVBN and hypoxanthine gradually and significantly ($p < 0.05$) decreased during storage (11.50, 8.40 and 13.50 at week 1 which dropped to 12.50, 7.35 and 12.30 at week 11, respectively).

DISCUSSION

The chemical composition of the unpreserved bonga (*Ethmalosa fimbriata*) not packaged in polyethylene bags as shown in Table 5 showed its pH going from neutral (7.10 in week 1) to highly acidic (4.25 in week 12). The significant ($p < 0.05$) decrease in pH observed throughout

Table 8. Chemical analysis of preserved smoked Sardine, *Sardinella maderensis* not packaged in Polyethylene during storage.

Week	pH	TMA (mg/100g)	TVBN (mg/100g)	Peroxide (meq/kg)	Hypoxanthine
1	7.35±0.21 ^a	11.05±0.78 ^a	8.40±0.57 ^{cd}	2.90±0.14 ^{ab}	13.50±0.78 ^{bc}
2	7.20±0.28 ^a	12.50±0.71 ^{bc}	8.35±0.21 ^c	2.55±0.07 ^a	13.35±0.21 ^{bc}
3	7.35±0.21 ^a	12.65±0.21 ^{bc}	8.20±0.28 ^{bc}	2.65±0.21 ^a	13.20±0.28 ^{bc}
4	7.40±0.57 ^a	12.60±0.57 ^{bc}	8.85±0.21 ^{de}	2.75±0.35 ^a	13.65±0.21 ^c
5	7.35±0.21 ^a	13.75±0.35 ^{bcd}	8.95±0.64 ^{de}	2.70±0.42 ^a	13.80±0.28 ^c
6	7.50±0.14 ^a	14.30±0.71 ^d	9.65±0.21 ^e	2.80±0.28 ^a	13.85±0.78 ^c
7	7.40±0.14 ^a	13.85±0.50 ^{cd}	9.35±0.21 ^e	2.60±0.57 ^a	13.80±0.28 ^c
8	7.30±0.14 ^a	13.75±1.06 ^{bcd}	9.05±0.78 ^{de}	2.65±0.21 ^a	13.30±0.28 ^{bc}
9	7.50±0.14 ^a	13.10±0.14 ^{bcd}	8.35±0.21 ^c	2.75±0.07 ^a	13.20±0.28 ^{bc}
10	7.30±0.14 ^a	12.75±0.35 ^{bc}	7.80±0.28 ^{bc}	3.75±0.35 ^c	13.85±0.50 ^{bc}
11	7.10±0.14 ^a	12.50±0.71 ^{bc}	7.35±0.21 ^{ab}	3.60±0.28 ^{bc}	12.30±0.42 ^{ab}
12	7.00±0.28 ^a	12.35±0.21 ^b	6.80±0.28 ^a	3.15±0.05 ^b	11.50±0.71 ^a

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

the storage period did not agree with Jeyasanta *et al* (2018) who discovered an increased pH of three seafoods during a six months cold storage trial. They also stated that increased pH is caused by enzymatic degradation of the fish muscles, which means that despite the unpreserved status of the fish, its muscles were not quickly degraded. TMAN and TVBN contents decreased significantly ($p < 0.05$) all through the storage though the TVBN of unpreserved samples decreased more significantly ($p < 0.05$) than that of the preserved samples (Table 6). Idakwo *et al.* (2016) found that the recommended limit of acceptability of TVBN for fish is 20 to 30 mg N/100 g. The TVBN values for both preserved and unpreserved samples were lower which thus infer that essential oil from lemon peel is a good preservative for smoked clupeids especially during storage. Suchitra and Sarojnani (2012) reported a gradual increase in TVBN in fish during storage room temperature and related it to the elevation of temperature and subsequent microbiological and biochemical changes in the fish muscle. The findings of this study therefore show that there was no form of change (whether microbiological or biochemical) in the stored clupeids during the storage. An increase in TVBN in fish during storage was also attributed by Idakwo *et al.* (2016) to an indication of the continuous production of volatile bases due to the breakdown of proteins by the action of microbes. The peroxide values decreased significantly ($p < 0.05$) in the unpreserved samples while they increased in the preserved samples, although the values for the first 10 weeks were not significantly ($p < 0.05$) different from one another but different from the 11th and 12th week. This showed that rancidity was increasing in the unpreserved samples while it decreased in the preserved samples which is in line with Kumolu-Johnson and Ndimele (2011) who found peroxide value to be inversely related to rancidity. Waindu and Jamala (2013) also attributed

reduced peroxide value to reduced moisture content, thus the unpreserved samples with the lowest moisture content had a low peroxide value which resulted from increased water absorption rate with an inverse relationship with thickness of packaging material (Olayemi, 2012). Therefore, the bonga preserved with lemon essential oil remained in good condition but the ones packaged in polyethylene bags and coated with lemon essential oil were better. Hypoxanthine reduced significantly ($p < 0.05$) in the unpreserved samples till the 12th week of storage while it increased from week 1 to week 6 when it started reducing from week 7 to week 12 even though there was no significant ($p < 0.05$) difference in the hypoxanthine contents of week 7-10 compared to week 2. Since hypoxanthine accumulation equals degradation by autolytic enzymes, it thus means spoilage activity began at the onset of storage but this was arrested by the lemon essential oil.

The pH of unpreserved sardine (*Sardinella maderensis*) not packaged in polyethylene bags was slightly acidic throughout the storage period (Table 7) while the preserved samples had neutral pH all through (Table 8). This stable pH showed the antioxidant ability of lemon essential oil to make the condition unfavourable for microorganisms to thrive. Rancidity in the unpreserved sardine significantly ($p < 0.05$) increased weekly while it decreased in the preserved samples. The reduction in the peroxide values of the unpreserved samples was in line with Iheagwara (2013) who observed a reduction in the peroxide value of *Scomber scombus* stored for twenty days. Lemon essential oil showed the same rancidity reduction ability like garlic as used by Kumolu-Johnson and Ndimele (2011). TMA and TVBN in the unpreserved samples significantly ($p < 0.05$) decreased with the storage period which infers that the degradation of proteins in the fish samples was reducing. This correlative decrease in

TMA and TVBN was not in agreement with Gulsun *et al.* (2009) who opined that they both increase with storage time. TMA and TVBN for the preserved sardine samples increased weekly till week eight although the various values for the remaining four weeks were not significantly different from one another and from the previous weeks. Their increase aligned with Gulsun *et al.* (2009) and the TVBN values fell within the high-quality grading of Amegovu *et al.* (2012) for fish. The effect of polyethylene bags in combination with cartons as packaging material was evident as the quality of the preserved samples not in polyethylene bags though good were surpassed by the samples packaged in them.

Conclusion and Recommendation

It can be concluded that Lemon essential oil in combination with polyethylene bags and cartons as packaging materials had a significant effect on the chemical composition of Clupeids (Bonga: (*Ethmalosa fimbriata*) and Sardine: *Sardinella maderensis*) during storage. This is because of its ability to conserve the chemical components throughout the storage period; as such it can be used as a preservative for it. The results of the study also revealed polyethylene bags in combination with cartons as a better packaging material for the smoked clupeids. Lemon peel essential oil is therefore recommended as a preservative for fish and also for enhancing its marketing because of the sheen it gives to the fish to make it more attractive. The findings of this study also recommend the storage of clupeids preserved with lemon essential oil in cartons after it is first packaged in polyethylene bags for as long as twelve weeks as done in this study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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