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Effect of packaging on the proximate composition of stored citrus essential oil-preserved smoked clupeids (*E. fimbriata* and *S. 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 proximate composition. Two hundred samples (400 nos: 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 in sterile polyethylene bags) served as control. Triplicate samples of fish were aseptically stored in cartons and evaluated weekly for its nutritional components during a 12-week storage period. All data were 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 stored smoked fish and the nutrients for which consumers go for it during storage time.

Keywords: *Ethmalosa fimbriata,* lemon essential oil, packaging materials, proximate composition, *Sardinella maderensis*, storage period.

INTRODUCTION

Fish serves as a source of protein, vitamins and minerals, and contains some essential nutrients required in small quantities for supplementing infants' and adults' diets. In Nigeria, fish is served fresh or smoked and forms as much cherished delicacy that cuts across socio-economic, age, religious and educational barriers (Abolagba and Uwagbai, 2021). Fish serves as one of the cheapest protein in human diets as it provides 55% of the dietary intake of animal protein to the average Nigerian (Adekoya and Miller, 2004). After death, fish provides a favourable medium for microbial growth because it is highly perishable. In Nigeria, approximately 40% of total fish landings are lost to postharvest losses (Abdullahi *et al*., 2019). There are a number of factors that contribute to fish spoilage in Nigeria,as reported by Abdullahi *et al.* (2019), and these are high ambient temperatures, considerable distances from landing sites to the points of utilisation and inadequate infrastructure for postharvest processing and handling. Therefore, it is imperative to process and preserve some of the fish caught in the period of abundance, to ensure an all-year-round supply. This will invariably reduce postharvest losses, increase the shelf-life of fish, and guarantee a sustainable supply of fish all year round. Fish processing serves as one of the methods

of giving the fish product a form that is attractive to consumers and also extends the storage life of the fish. The organoleptic and flavour characteristics of processed fish to be stored should ensure full healthy safety of the product, proper sanitary conditions, as well as rendering it impossible for the development of harmful micro-organisms and toxins (Adeyeye and Adubiaro, 2018). *Ethmalosa fimbriata* (Bonga shad (English) and Agbodo in the Yoruba language) belongs to the family *Clupeidae* and order *clupeiformes* (Froese and Pauly, 2011a,b). It is pelagic, anadromous and occurs in inshore waters, lagoons and more than 300km up rivers. It is euryhaline (i.e. has the ability to tolerate varying salt concentrations) and feeds by filtering phytoplankton (Abowei, 2009). It is usually marketed in the dried form to all parts of the country (Idodo-Umeh, 2003). *Sardinella maderensis* is an oceanodromous pelagic filter-feeding clupeid. It is usually found in "schools" at either the surface or middle of the water body (Idodo-Umeh, 2003). *S. maderensis*has the ability to tolerate very low salinities when they travel into estuaries and lagoons, where they are found mostly near the surface of the water (Tous *et al.,* 2015). Lemons are a rich source of vitamin C, and they also 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. Essential oils are made up of many different volatile compounds, which often vary between species (Olonisakin, 2014). The present study aims to combine the preservative ability of lemon essential oil and polyethylene bags in extending the shelf life of stored smoked Bonga, *Ethmalosa fimbriata* and Sardine, *Sardinella maderensis* over a storage period.

MATERIALS AND METHODS

Sample collection and identification

Lemon fruits were bought from a local market in Ibadan, Oyo State, and the identification was done at the Pure and Applied Botany Department of the Federal University of Agriculture, Abeokuta (FUNAAB). Four hundred (400), 200 each of sardine and Bonga, freshly smoked samples of both Bonga (*Ethmalosa fimbriata*) and sardine (*Sardinella maderensis*) were bought from a local market in Ijebu-ode, Ogun State.

Preparation of fish samples for shelf-life studies

The Bonga 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-quoted 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 were packed singly in white polyethylene bags and placed in cartons, while the second 50 were packed in cartons. The same procedure was followed for the preparation of sardine samples. These were then stored at ambient temperature (±25°C) for twelve weeks. The samples were afterwards taken in triplicate weekly to the laboratory for proximate analysis.

Proximate Analysis of stored smokedclupeids

This was done weekly according to Ayeloja (2016), and the parameters determined include:

Moisture content

The moisture content of each sample was estimated using the oven drying method (AOAC, 1995). Five grams of a homogenous mixture were placed in weighed crucibles maintained at 105°C in an oven until constant weight was obtained. The samples were then transferred to a desiccator to cool at ambient temperature and weighed again. The difference in weight of samples before and after oven drying (weight loss) indicated the moisture content expressed in percentage.

Protein content

The total nitrogen (crude protein) was determined by the Kjeldahl method (AOAC, 1995). A known weight (0.5 g) of prepared fish sample was weighed on a nitrogen-free paper. The paper was wrapped around the sample and dropped at the bottom of the Kjeldahl digestion flask together with 6 glass beads and 4 spatula-full granular mixtures of $CuSO_4$ and K_2SO_4 as a catalyst. Concentrated H_2SO_4 (20 ml) was carefully added. The flask was gently heated on a heating mantle in an inclined position in a fume cupboard until full digestion (when the liquid changed from brown to colourless). The content of the flask was transferred to a clean 100 ml volumetric flask and made up to volume. Twenty-fivealiquotswere used for distillation, and total nitrogen was determined colourimetrically.

Lipid content

Lipid determination was carried out using the Soxhlet method with petroleum ether as solvent (AOAC, 1995).

Five grams of homogenous sample mixture were put in the thimble of the Soxhlet extractor, and the solvent was poured into the distillation flask. Then, the condenser was connected to the top of the extractor and distillation flask containing the solvent at its bottom. The distillation flask was then put on a heating mantle from where the heat which boiled and vaporised the solvent was produced. The solvent penetrated the homogenised fish flesh and resulted in the extraction of oil, which was left in the flask, while the vapourized solvent continued for six hours for total oil extraction to be achieved.

Ash content

Ash content of fish samples was determined by incineration in a Carbolite Sheffield muffle furnace at 500°C (AOAC, 1995). The difference in weight of the fish samples before and after incineration was taken as the ash content and calculated thus:

$$\text{Ash content} = \frac{\text{A} - \text{B}}{\text{C}} x100\ \%$$

Where: A = weight of burned sample + dish, B = weight of dish, and C = weight of sample

Crude fibre content

Two grams of smoked fish were weighed into a round-bottomed flask, and 200 mL of pre-heated H_2SO_4 solution was added. The solution was gently boiled for about 30 minutes while maintaining a constant volume of acid by the addition of hot water. A Buckner flask funnel fitted with a Whatman filter was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was then filtered hot through the funnel under sufficient suction. The residue was washed several times with boiling water (until the residue was neutral to litmus paper) and transferred back into a beaker. Then 200 ml of NaOH was heated to the boiling point and kept at boiling temperature under a reflux condenser until used. Residue was washed back into the flask with 200 mL of the boiling NaOH solution. The flask was connected with a condenser and immediately filtered through the Gooch crucible. After thorough washing with boiling water, it was washed with 15ml of 95 % ethanol, and then the crucible was dried at 110°C to constant weight, cooled in a desiccator and weighed. The weight loss, which gives the crude fibre content (AOAC, 1995) and was calculated as:

$$\%\text{Crude fiber} = \frac{\text{A} - \text{B}}{\text{C}} x100$$

Where: A= weight of dry crucible and sample, B= weight of incinerated crucible and ash, C= sample weight.

Statistical Analysis

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

RESULTS

The proximate composition of the stored Sardine and Bonga varied considerably with storage time, preservative and packaging materials. The proximate composition (%) of unpreserved smoked Sardine, *Sardinella maderensis,* packaged in polyethylene bags storage is presented in Table 1. The moisture content ranged from 2.10+0.14 at week 12 to 7.65+0.21 at week 1. The moisture content decreased with an increase in storage period from the first week to the tenth week, though the decrease in week 10 was not significantly different from the 11th and 12th weeks. The crude protein, lipid, and crude fibre ranged from 14.65+0.21 to 40.25+0.35, 1.45+0.07 to 6.35+0.12 and 2.15+0.07 to 5.40+0.28 at week 12 and week 1, respectively. The ash also ranged from 2.05+0.07 to 6.65+0.21, while the nitrogen-free extract (NFE) increased weekly, ranging from 38.43+6.8 at week 1 to 77.50+0.14 at week 12.

The moisture content and crude fibre of smoked Sardine, *Sardinella maderensis* preserved with lemon essential oil and packaged in polyethylene bags during the storage period ranged from 4.40±0.14 to 7.35±0.07 and 6.30±0.14 to 7.75±0.35 at week 12 and week 1, respectively. The crude protein, lipid, ash and NFE all increased during the storage period with values ranging from 42.75±0.35 to 44.55±0.07, 8.40±0.14 to 9.50±0.42, 6.90±0.14 to 7.10±0.14 and 26.75±1.06 to 28.15±0.64 at week 1 and week 12, respectively (Table 2). All the increases and decreases were not significantly ($p<0.05$) different from one another except for that of moisture content.

There was no significant difference ($p<0.05$) in the increase in proximate composition (%) of smoked Bonga, *Ethmalosa fimbriata* preserved with lemon essential oil and packaged in polyethylene bags, as presented in Table 3. The moisture content ranged from 3.90±0.14 at week 12 to 7.10±0.14 at week 1. The crude protein, lipid, crude fibre, ash and NFE also ranged from 43.75±1.06 to 44.25±0.35, 8.85±0.21 to 9.60±0.14, 6.85±0.50 to 7.10±0.42, 6.75±0.35 to 26.70±1.56 to 27.60±1.13 at week 1 and week 12, respectively.

The unpreserved smoked Bonga, *Ethmalosa fimbriata,* packaged in polyethylene bags had all its proximate components except the NFE significantly ($p<0.05$) decreasing weekly throughout the storage period, as shown in Table 4. All its proximate components decreased

Table 1. Proximate composition (%) of unpreserved smoked sardine, *Sardinella maderensis* packaged in polyethylene bags during storage.

Week	Moisture	Crude protein	Lipid	Crude fibre	Ash	NFE
1	7.65±0.21 ^{a}	40.25±0.35 ^{a}	6.35±0.12 ^{ab}	5.40±0.28 ^{a}	6.65±0.21 ^{a}	38.43±6.82^{fg}
2	7.20±0.28 ^{a}	39.50±0.71 ^{a}	6.65±0.21 ^{a}	5.35±0.21 ^{a}	6.10±0.14 ^{b}	35.20±0.57 ^{g}
3	6.25±0.35 ^{b}	35.50±0.71 ^{b}	5.85±0.21 ^{b}	4.90±0.14 ^{a}	5.20±0.28 ^{c}	42.30±0.28 ^{f}
4	5.25±0.35 ^{c}	30.70±0.99 ^{c}	4.75±0.35 ^{c}	4.25±0.35 ^{b}	4.60±0.14 ^{d}	50.55±0.64 ^{e}
5	5.00±0.28 ^{c}	26.00±1.41 ^{d}	4.10±0.14 ^{d}	4.10±0.14 ^{b}	4.10±0.14 ^{e}	56.70±1.56 ^{d}
6	4.75±0.35 ^{c}	23.55±0.64 ^{e}	3.65±0.21 ^{d}	3.80±0.28^{bc}	3.60±0.28 ^{f}	56.70±1.56 ^{d}
7	3.90±0.14 ^{d}	21.50±0.71 ^{f}	3.00±0.28 ^{e}	4.00±0.28 ^{b}	3.15±0.07 ^{g}	64.45±0.49 ^{c}
8	3.45±0.07^{de}	20.50±0.71 ^{f}	2.70±0.42^{ef}	3.75±0.35^{bc}	2.75±0.35^{gh}	66.85±0.21^{bc}
9	3.10±0.14^{ef}	18.75±0.35 ^{g}	2.45±0.35^{ef}	3.30±0.14 ^{cd}	2.90±0.14^{gh}	69.50±1.13 ^{b}
10	2.75±0.07^{fg}	20.15±0.94^{fg}	2.15±0.21^{fg}	2.90±0.14 ^{de}	2.95±0.07^{gh}	69.10±0.71^{bc}
11	2.40±0.14^{gh}	15.60±0.57 ^{h}	1.70±0.14^{gh}	2.55±0.07^{ef}	2.65±0.21 ^{h}	75.10±0.28 ^{a}
12	2.10±0.14 ^{h}	14.65±0.21 ^{h}	1.45±0.07 ^{h}	2.15±0.07 ^{f}	2.05±0.07^{i}	77.50±0.14 ^{d}

NFE = Nitrogen free extract. Values denoted by different superscripts in the column differ significantly [p < 0.05].

Table 2. Proximate composition (%) of smoked *Sardinella maderensis* preserved with Lemon (*Citrus limon*) oil and packaged in polyethylene bags during storage.

Week	Moisture	Crude Protein	Lipid	Crude Fibre	Ash	NFE
1	7.35±0.07 ^{a}	42.75±0.35 ^{b}	8.40±0.14 ^{cd}	7.75±0.35 ^{ad}	6.90±0.14 ^{b}	26.75±1.06^{abc}
2	6.90±0.14 ^{ab}	42.80±0.28 ^{b}	8.90±0.14^{abc}	7.90±0.14 ^{a}	7.45±0.07 ^{a}	26.05±0.07^{abc}
3	6.70±0.14^{bc}	43.50±0.71^{ab}	8.60±0.14^{bcd}	7.65±0.21^{abc}	7.30±0.14 ^{ab}	26.30±0.00^{abc}
4	6.70±0.14^{bc}	43.60±0.57^{ab}	8.45±0.35 ^{cd}	7.55±0.49^{abc}	7.20±0.28 ^{ab}	25.05±0.07 ^{c}
5	6.35±0.21 ^{c}	42.80±0.42 ^{b}	8.70±0.28^{bcd}	7.45±0.07^{abc}	7.50±0.14 ^{a}	26.50±0.14^{abc}
6	6.30±0.14 ^{c}	43.20±0.28^{ab}	8.10±0.14 ^{d}	3.80±0.28^{bc}	3.60±0.28 ^{f}	56.70±1.56 ^{d}
7	5.65±0.21 ^{d}	45.35±3.04 ^{a}	8.65±0.21^{bcd}	7.40±0.28 ^{abc}	7.15±0.21 ^{ad}	27.80±0.14 ^{ad}
8	5.55±0.21^{d}	43.80±0.42^{ad}	8.75±0.21^{bcd}	7.20±0.28^{bc}	7.15±0.21 ^{ad}	27.55±1.34^{ad}
9	5.35±0.21 ^{d}	44.65±0.21^{ad}	8.75±0.35^{bcd}	7.10±0.14 ^{cd}	7.10±0.14 ^{ad}	27.05±0.64^{abc}
10	5.25±0.21 ^{de}	44.55±0.07 ^{a}	8.90±0.42^{abc}	6.55±0.07 ^{de}	6.90±0.14 ^{b}	25.35±2.76^{bc}
11	4.85±0.94^{ef}	44.75±0.07 ^{a}	9.25±0.35 ^{ad}	6.55±0.21 ^{de}	7.10±0.14 ^{ad}	27.50±0.85^{abc}
12	4.40±0.14 ^{f}	44.55±0.07 ^{a}	9.50±0.42 ^{ad}	6.30±.14 ^{e}	7.10±0.14 ^{ab}	28.15±0.64 ^{a}

NFE = Nitrogen free extract.Values denoted by different superscripts in the column differ significantly [p < 0.05].

as the storage period progressed from week 1 to week 12. Moisture content (2.40±0.14 at week 12 to 8.10±0.14 at week 1), crude protein (13.50±0.71 at week 12 to 38.50±0.71 at week 1), lipid (1.85±0.21 at week 12 to 5.85±0.21 at week 1), crude fibre(1.40±0.14 at week 12 to 7.13±0.04 at week 1) and ash (2.35±0.21 at week 12 to 6.35±0.21 at week 1).

Table 5 shows the proximate composition of unpreserved smoked Bonga, *Ethmalosa fimbriata* not packaged in polyethylene bags. The moisture content, crude protein, lipid, crude fibre and ash decreased steadily and significantly ($p < 0.05$) with storage time, while the NFE increased. NFE ranged from 40.15±1.63 at week 1 to 84.75±1.06 at week 12. The moisture content, crude protein, lipid, crude fibre and ash decreased significantly ($p<0.05$) and steadily with storage period. The moisture content, crude protein, lipid, crude fibre and ash ranged from 1.65±0.21 to 8.50±0.70, 10.50±0.71 to 34.00±1.41, 1.10±0.14 to 6.85±0.21, 1.15±0.07 to 4.75±0.35 and 1.35±0.21 to 5.75±0.35, respectively.

The proximate composition of preserved smoked Bonga, *Ethmalosa fimbriata,* not packaged in polyethylene bags, is presented in Table 6. There was a decrease in proximate components of the fish samples, but the rate at which this happened was not high but more significant ($p<0.05$) than in the unpreserved samples. The moisture content ranged from 2.50±0.21 at week 12 to 7.35±0.07 at week 1. Crude protein, crude fibre and NFE ranged from 35.50±0.71 to 40.50±0.35, 5.80±0.07 to 7.45±0.35 and 29.95±1.63 to 42.60±1.06, respectively.

Table 3. Proximate composition (%) of smoked Bonga, *Ethmalosa fimbriata* preserved with lemon (*Citrus limon*) oil and packaged in polyethylene bags during storage.

Week	Moisture	Crude protein	Lipid	Crude fibre	Ash	NFE
1	7.10±0.14^{a}	43.75±1.06^{ab}	8.85±0.21^{abc}	6.85±0.50^{a}	6.75±0.35^{a}	26.70±1.56^{a}
2	7.05±0.14^{a}	44.00±0.71^{ab}	9.60±0.14^{e}	7.15±0.50^{ab}	6.94±0.09^{a}	26.65±0.35^{a}
3	6.70±0.14^{b}	43.50±0.71^{ab}	8.60±0.14^{a}	7.65±0.21^{b}	7.30±0.14^{ab}	26.30±0.00^{a}
4	6.60±0.14^{bc}	43.05±0.78^{ab}	9.35±0.07^{de}	6.85±0.50^{a}	6.85±0.50^{a}	26.30±0.14^{a}
5	6.30±0.14^{cd}	42.75±0.35^{a}	9.35±0.21^{de}	7.20±0.28^{ab}	6.80±0.85^{a}	26.60±0.28^{a}
6	6.10±0.14^{d}	43.05±0.35^{ab}	9.20±0.28^{bcde}	7.50±0.14^{ab}	7.50±0.42^{b}	26.80±0.57^{a}
7	5.55±0.07^{e}	43.65±0.21^{ab}	8.90±0.14^{abcd}	7.65±0.21^{ab}	7.45±0.07^{ab}	26.55±0.21^{a}
8	5.35±0.21^{e}	44.30±0.28^{b}	8.65±0.21^{a}	7.40±0.28^{ab}	7.75±0.0.21^{ab}	26.55±0.21^{a}
9	5.30±0.14^{e}	44.25±0.07^{b}	8.80±0.28^{ab}	7.30±0.14^{ab}	7.50±0.14^{ab}	26.85±0.50^{a}
10	5.20±0.28^{e}	44.25±0.35^{b}	9.00±0.28^{abcd}	7.15±0.07^{ab}	7.30±0.14^{ab}	27.10±0.14^{a}
11	4.10±0.14^{f}	44.20±0.14^{ab}	9.30±0.14^{cde}	7.25±0.35^{ab}	7.35±0.07^{ab}	27.80±0.42^{a}
12	3.90±0.14^{f}	44.25±0.35^{b}	9.60±0.14^{e}	7.10±0.42^{ab}	7.55±0.07^{ab}	27.60±1.13^{a}

NFE = Nitrogen free extract. Values denoted by different superscripts in the column differ significantly [$p < 0.05$].

Table 4. Proximate composition (%) of unpreserved smoked Bonga, *Ethmalosa fimbriata*packaged in polyethylene bags during storage.

Week	Moisture	Crude protein	Lipid	Crude fibre	Ash	NFE
1	8.10±0.14^{l}	38.50±0.71^{j}	5.85±0.21^{f}	7.13±0.04^{l}	6.35±0.21^{g}	34.10±0.65^{a}
2	7.70±0.14^{h}	35.75±1.06^{l}	5.50±0.42^{f}	7.05±0.07^{l}	6.20±0.28^{g}	37.80±1.98^{b}
3	6.25±0.35^{g}	32.60±0.71^{l}	5.85±0.21^{f}	4.90±0. 14^{h}	5.2±0.28^{ef}	42.30±0.28^{c}
4	6.30±0.14^{g}	32.40±0.57^{h}	4.45±0.35^{e}	4.90±0. 14^{h}	5.35±0.21^{f}	46.60±0.71^{d}
5	6.10±0. 14^{g}	29.00±0.14^{g}	3.95±0.21^{de}	4.35±0.21^{g}	4.70±0.42^{de}	53.10±0.14^{e}
6	5.30±0. 14^{f}	25.50±0.71^{f}	3.80±0.28^{d}	3.60±0.57^{f}	4.60±0. 14^{d}	53.10±0.14^{e}
7	4.90±0. 14^{e}	22.50±0.71^{e}	3.65±0.21^{d}	2.85±0.21^{e}	4.35±0.12^{cd}	61.75±1.06^{f}
8	4.35±0.21^{d}	20.75±0.35^{d}	3.10±0.14^{c}	2.65±0.21^{de}	3.90±0.14^{c}	65.25±0.64^{g}
9	3.90±0. 14^{c}	19.25±0.35^{cd}	2.90±0.14^{bc}	2.30±0. 14^{cd}	3.35±0.35^{b}	68.20±0.14^{h}
10	3.10±0. 14^{b}	17.7±0.35^{c}	2.65±0.21^{ab}	2.05±0.07^{bc}	3.10±0.14^{b}	71.35±0.35^{l}
11	2.80±0. 14^{b}	15.50±0.71^{b}	2.25±0.21^{ab}	1.74±0.20^{ab}	2.85±0.07^{ab}	74.90±0.99^{j}
12	2.40±0. 14^{a}	13.50±0.71^{a}	1.85±0.21^{a}	1.40±0.14^{a}	2.35±0.21^{a}	78.50±1.41^{k}

NFE = Nitrogen free extract. Values denoted by different superscripts in the column differ significantly [$p < 0.05$].

Table 5. Proximate Composition (%) of unpreserved smoked Bonga, *Ethmalosa fimbriata* not packaged in Polyethylene bags during storage.

Week	Moisture	Crude Protein	Lipid	Crude Fibre	Ash	NFE
1	8.50±0.70^{a}	34.00±1.41^{a}	6.85±0.21^{a}	4.75±0.35^{a}	5.75.±0.35^{a}	40.15±1.63^{a}
2	7.10±0.14^{b}	31.50±0.70^{b}	6.65±0.21^{b}	4.25±0.07^{b}	5.25±0.07^{b}	45.25±0.49^{b}
3	6.70±0.14^{bc}	30.20±0.28^{b}	6.10±0.14^{b}	4.05±0.07^{bc}	4.90±0.14^{c}	48.05±0.07^{fg}
4	6.30±0.14^{bc}	28.0±1.41^{c}	5.90±0.14^{b}	3.70±0.14^{bc}	4.55±0.07^{d}	14.60±12.73^{g}
5	5.90±0.14^{de}	25.50±0.71^{d}	5.55±0.07^{c}	3.45±0.07^{de}	4.10±0.14^{c}	55.60±0.84^{ef}
6	5.60±0.14^{ef}	22.50±0.71^{e}	5.30±0.14^{cd}	3.25±0.07^{de}	3.90±0.14^{e}	59.456±0.49^{edf}
7	5.30±0.14^{f}	20.50±0.71^{f}	5.05±0.07^{d}	3.10±0.14^{de}	3.35±0.07^{f}	62.50±0.28^{edf}
8	5.05±0.07^{fg}	18.25±0.35^{g}	4.60±0.14^{e}	3.25±0.63^{ef}	3.30±0.14^{f}	65.55±0.63^{ed}
9	4.70±0.14^{g}	17.25±0.35^{gh}	4.10±0.14^{f}	3.10±0.14^{ef}	2.55±0.07^{g}	69.30±0.28^{b}
10	2.35±0.21^{h}	15.50±0.07^{h}	2.05±0.07^{g}	2.65±0.21^{fg}	2.05±0.07^{h}	75.40±1.13^{b}
11	2.05±0.71^{hi}	13.00±1.41^{i}	1.40±0.14^{h}	2.15±0.07^{g}	2.05±0.07^{h}	56.85±1.13^{de}
12	1.65±0.21^{i}	10.50±0.71^{j}	1.10±0.14^{h}	1.15±0.07^{h}	1.35±0.21^{i}	84.75±1.06^{a}

NFE = Nitrogen free extract. Values denoted by different superscripts in the column differ significantly [$p < 0.05$].

Table 6. Proximate composition (%) of preserved smoked Bonga, *Ethmalosa fimbriata* not packaged in Polyethylene bags during storage.

Week	Moisture	Crude Protein	Lipid	Crude Fibre	Ash	NFE
1	7.35±0.07^{a}	40.50±0.35^{a}	8.20±0.14^{a}	7.45±0.35^{a}	6.65.±0.35^{a}	29.95±1.63^{a}
2	7.15±0.14^{b}	41.50±0.71^{b}	8.20±0.14^{b}	7.65±0.07^{b}	6.90±0.07^{b}	28.60±0.49^{b}
3	7.00±0.14^{bc}	41.70±0.71^{b}	8.55±0.35^{b}	7.50±0.07^{bc}	6.85±0.14^{c}	28.40±0.07^{fg}
4	7.10±0.14^{bc}	41.75±1.06^{c}	8.30±0.14^{b}	7.35±0.14^{bc}	6.50±0.07^{d}	29.00±12.73^{g}
5	6.25±0.35^{de}	41.40±0.35^{d}	8.75±0.21^{c}	7.70±0.07^{de}	6.85±0.14^{c}	29.05±0.84^{ef}
6	5.90±0.21^{ef}	40.80±0.35^{e}	8.90±0.28^{cd}	7.50±0.07^{de}	6.70±0.14^{e}	30.02±0.49^{edf}
7	6.25±0.35^{f}	41.50±0.35^{f}	8.90±0.14^{d}	7.35±0.14^{de}	7.35±0.07^{f}	28.65±0.28^{edf}
8	6.15±0.14^{fg}	41.55±0.71^{g}	8.75±0.35^{e}	7.20±0.63^{ef}	6.65±0.14^{f}	29.70±0.63^{ed}
9	5.35±0.21^{g}	41.25±0.71^{gh}	9.20±0.35^{f}	7.10±0.14^{ef}	6.20±0.07^{g}	30.90±0.28^{b}
10	5.20±0.21^{h}	40.10±0.35^{h}	9.05±0.28^{g}	6.90±0.21^{fg}	5.90±0.07^{h}	32.85±1.13^{b}
11	3.25±0.21^{hi}	38.50±0.71^{i}	8.65±0.21^{h}	6.45±0.07^{g}	5.75±0.07^{h}	37.50±1.13^{de}
12	2.50±0.21^{i}	35.50±0.71^{j}	8.30±0.14^{h}	5.80±0.07^{h}	5.30±0.21^{i}	42.60±1.06^{a}

NFE = Nitrogen free extract. Values denoted by different superscripts in the column differ significantly [p < 0.05].

Table 7. Proximate composition (%) of unpreserved smoked sardine, *Sardinella maderensis* not packaged in polyethylene bags during storage.

Week	Moisture	Crude Protein	Lipid	Crude Fibre	Ash	NFE
1	8.75±0.35^{c}	33.00±1.41^{h}	7.10±0.14^{f}	4.25±0.07^{f}	5.10.±0.14^{e}	41.80±1.27^{a}
2	8.25±0.35^{c}	30.50±0.71^{g}	6.95±0.07^{f}	4.05±0.07^{f}	4.90±0.14^{e}	45.35±0.07^{b}
3	8.25±0.35^{c}	30.50±0.71^{g}	6.95±0.07^{f}	4.05±0.07^{f}	4.90±0.14^{e}	45.35±0.07^{b}
4	8.55±0.21^{c}	28.50±0.70^{f}	6.60±0.14^{f}	3.35±0.21^{e}	4.35±0.21^{d}	48.65±1.07^{c}
5	4.75± 6.01^{abc}	25.50±0.71^{e}	5.50±0.71^{e}	3.10±0.14^{de}	4.20±0.28^{d}	52.95±0.78^{d}
6	8.25±0.35^{c}	24.25±0.35^{e}	5.65±0.21^{e}	3.25±0.21^{e}	4.35±0.07^{d}	53.25±0.07^{d}
7	7.50±0.14^{c}	21.60±0.85^{d}	5.10±0.14^{e}	2.75±0.35^{cd}	4.10±0.14^{d}	58.95±0.78^{e}
8	7.10±0.14^{bc}	21.25±0.35^{d}	4.30±0.28^{d}	2.55±0.35^{bc}	3.10±0.14^{c}	61.70±0.28^{f}
9	5.80±0.28^{abc}	17.60±0.57^{c}	3.65±0.21^{c}	2.20±0.28^{b}	2.35±0.50^{g}	68.40±0.14^{g}
10	5.10±0.14^{abc}	14.65±0.50^{b}	3.10±0.14^{bc}	1.55±0.07^{a}	2.00±0.28^{b}	73.60±0.14^{h}
11	3.25±0.35^{ab}	13.50±0.71^{ab}	2.75±0.35^{b}	1.30±0.14^{a}	1.50±0.14^{a}	77.70±0.99^{i}
12	2.65±0.21^{a}	12.25±0.35^{a}	2.10±0.14^{a}	1.15±0.21^{a}	1.10±0.14^{i}	80.75±0.49^{j}

NFE = Nitrogen free extract. Values denoted by different superscripts in the column differ significantly [p < 0.05].

Table 7 shows the proximate composition of unpreserved smoked Sardine, *Sardinella maderensis* not packaged in polyethylene bags. The result shows the crude protein, lipid, moisture content, crude fibre and ash significantly ($p < 0.05$) decreasing weekly with increasing NFE. Moisture content ranged from 2.65±0.21 at week 12 to 8.75±0.35. Crude protein, lipid, crude fibre and ash ranged from 12.25±0.35 to 33.00±12.25, 2.10±0.14 to 7.10±0.14, 1.15±0.21 to 4.25±0.07, 1.10±0.14 to 5.10±0.14, respectively. NFE increased from 41.80±1.27 at week 1 to 80.75±0.49 at week 12.

Preserved smoked Sardine, *Sardinella maderensis,* not packaged in polyethylene bags, had its crude protein, lipid, crude fibre and ash decreasing with increasing NFE, though not significantly ($p<0.05$) different from one another (Table 8). The moisture content decreased significantly ($p<0.05$) from 7.65±0.21 at week 1 to 2.35±0.21 at week 12. Lipid decreased, though not significantly ($p<0.05$), from 7.75±0.21 at week 1 to 8.80±0.85 at week 12. Crude protein, crude fibre and ash ranged from 34.65±0.21 to 41.50±0.71, 5.80±0.21 to 6.30±0.42 and 5.30±0.14 to 5.60±0.28, respectively.

DISCUSSION

Effect of lemon essential oil on the proximate composition of smoked clupeids

All nutrients except Nitrogen Free Extract (NFE) in

Table 8. Proximate composition (%) of preserved sardine, *Sardinella maderensis* not packaged in polyethylene bags during storage.

Week	Moisture	Crude protein	Lipid	Crude fibre	Ash	NFE
1	7.65±0.21^{f}	41.50±0.71^{bc}	7.75±0.21^{a}	6.30±0.42^{ab}	5.60±0.28^{ab}	31.20±0.42^{bc}
2	7.10±0.14^{e}	41.75±1.06^{bc}	8.75±0.35^{ab}	6.75±0.07^{bc}	5.80±0.28^{abc}	29.85±1.20^{abc}
3	7.50±0.14^{ef}	41.69±0.27^{bc}	8.70±0.14^{ab}	6.75±0.35^{bc}	6.30±0.14^{abcd}	29.10±0.28^{ab}
4	7.65±0.21^{f}	41.75±1.06^{bc}	8.75±0.35^{ab}	7.35±0.35^{cde}	6.45±0.64^{bcd}	28.05±2.62^{ab}
5	7.10± 0.14^{e}	42.50±0.71^{c}	8.95±0.64^{ab}	7.70±0.42^{de}	6.90±0.64^{d}	26.80±2.26^{a}
6	7.10±0.42^{e}	41.75±1.06^{bc}	8.50±0.71^{ab}	7.65±0.21^{de}	6.60±0.57^{bcd}	28.50±2.12^{ab}
7	6.40±0.14^{d}	42.10±0.85^{d}	8.95±0.64^{ab}	7.90±0.42^{e}	6.75±0.35^{cd}	27.90±2.12^{ab}
8	6.30±0.14^{d}	41.25±0.35^{bc}	9.05±0.21^{b}	7.75±0.35^{de}	6.40±0.28^{bcd}	29.25±0.92^{ab}
9	6.10±0.14^{d}	40.75±0.35^{bc}	9.25±0.35^{b}	7.10±0.14^{cd}	6.75±0.35^{cd}	30.05±0.07^{abc}
10	4.65±0.21^{c}	40.25±0.35^{b}	8.95±0.64^{ab}	6.65±0.21^{bc}	6.50±0.71^{bcd}	33.00±2.12^{c}
11	3.35±0.21^{b}	34.25±1.77^{a}	8.10±0.14^{ab}	6.30±0.14^{ab}	5.75±0.35^{abc}	42.25±0.92^{d}
12	2.35±0.21^{a}	34.65±0.21^{a}	8.80±0.85^{ab}	5.80±0.28^{a}	5.30±0.14^{a}	43.10±0.71^{d}

NFE = Nitrogen free extract. Values denoted by different superscripts in the column differ significantly [$p < 0.05$].

unpreserved sardine decreased through the storage period (Table 1). This shows that spoilage has not only made the fish unpalatable to consumers, but it has also reduced its quality and, as such, affected its consumer acceptability. The consumer acceptability is reduced by spoilage, and consequently, the nutrients which consumers take from fish. Although the moisture content and crude fibre in the preserved samples reduced, the reduction was not as significant ($p<0.05$) as it was in the unpreserved samples (Moisture content-7.35 to 4.40 (preserved sample), 7.65 to 2.10 (unpreserved sample), Crude fibre- 7.75 to 6.30 (preserved sample), 5.40 to 2.15 (unpreserved sample) (Tables 1 and 2). The decrease in moisture content of the unpreserved sardine samples was in line with Ayeloja *et al* (2013), who reported the same in smoked catfish not preserved with spices and stored for three months at ambient temperature. The reduction in crude protein and lipid aligned with the findings of Taniya and Kannan (2016), who observed a decrease in crude protein (10.07 to 5.97%) and lipid (11.68 to 3.57%) of stored smoked Indian oil sardine. The crude protein content, lipid, ash and NFE increased significantly ($p<0.05$) throughout the storage period (Table 7). This result showed that lemon essential oil was not only capable of preserving the fish from spoilage but also the nutrients which consumers take from it. Crude protein and lipid of preserved sardine samples for week 10 to 12 were not significantly ($p<0.05$) different from one another, which therefore means that storing sardine for this long will still keep its nutrients intact, not only that, the same remarkable effect will be produced if it was stored for just 10weeks. The ability of lemon essential oil to preserve sardine from spoilage and conserve its nutrients was also corroborated by the reduction in moisture content (7.35 to 4.40), which is a substrate for spoilage organisms to thrive. Crude fibre in the preserved samples decreased with an increase in storage period, and this disagreed with Ayeloja (2016), who reported a decrease in crude protein of smoked catfish preserved with garlic. This increase in fibre was attributed to the high fibre content of the preservative used, which is low in lemon essential oil. The spices and herbs used by Taniya and Kannan (2016) also had a remarkable increase in fibre content of sardine, which reduced the crude protein.

Moisture content of bonga (*Ethmalosa fimbriata*) preserved with lemon essential oil significantly ($p<0.05$) decreased with increase in crude protein, lipid, crude fibre, ash and nitrogen-free extract, which were not significantly ($p<0.05$) different along the weeks throughout the storage period (Table 3). This reduced moisture content showed that the keeping quality of preserved bonga improved with storage time because the condition for rapid bacteria growth was worsened (Boran and Karacam, 2011). The increased crude protein throughout the storage period was in line with Aberoumad and Pourshafi (2010) who stated that the lower the moisture content, the higher the lipids and crude protein and the higher the energy density of the fish. All nutrients except NFE decreased significantly ($p<0.05$) in unpreserved bonga samples (Table 4). Lipids content decrease could be attributed to oxidation of the poly unsaturated fatty acids (PUFA) contained in the tissue of bonga to products such as peroxides, aldehydes, ketones and free fatty acids (Guéraud *et al.,* 2010). Daramola *et al* (2007) also reported reduction in lipid in five different smoked freshwater fish species- *Heterotis niloticus* (bony tongue), *Labeocoubie* (African carp), *Parachanna obscura* (Snake fish), *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African mud catfish) during storage at ambient temperature for eight weeks. The ash content, which is an indication of the mineral content of the fish (Adeleke and Odedeji, 2010), reduced significantly throughout the storage period compared to its preserved counterparts. Consequent to the decrease in other nutrients in the unpreserved bonga samples, the NFE increased considerably and significantly ($p<0.05$). This thus infers that the degradation of the protein and other nutrients during spoilage increased the non-protein nutrients.

Effect of packaging materials on proximate composition of smoked clupeids

The nutritional composition of unpreserved bonga (*Ethmalosa fimbriata*) packaged in cartons only without polyethylene bags reduced significantly ($p<0.05$) with storage period, as shown in Table 5. The nutrients in the bonga not packaged in polyethylene bags before being put in the cartons dropped significantly ($p<0.05$) with storage time, unlike the ones packaged in it before being placed in the carton (Table 4). The moisture content reduced from 8.50±0.70 in week 1 to 1.65±0.21 in week 12, unlike the one in Table 4 (8.10±0.141 to 2.40±0.141). This loss of moisture could be attributed to the absence of the polyethylene bag and the low water absorption rate of the carton (Olayemi, 2012). The crude protein also decreased drastically and significantly ($p<0.05$) with storage period, though this did not agree with Agomuo *et al.* (2017), who ranked carton as second second-best packaging material in terms of nutritional composition. There was also a significant ($p<0.05$) reduction in fat content of the fish, and this agreed with Olayemi *et al.* (2011), although the polyethylene-paper-polyethylene package was the best packing material. The reduction in the crude protein of the samples weekly also led to a significant ($p<0.05$) increase in nitrogen-free extract (NFE), which was evidence of the protein breakdown leaving carbohydrate behind. The preservative ability of lemon essential oil was evident in the proximate composition of bonga (*Ethmalosa fimbriata*) that was preserved with lemon essential oil and packaged in cartons without polyethylene bags (Table 6). Moisture content reduction in these samples was not as drastic but was more significantly ($p<0.05$) different from the unpreserved samples. This was probably because of the oil film on the fish skin that reduced interaction between it and the atmosphere. Though the crude protein reduced from 40.50±0.35 in week 1 to 35.5±0.21 in week 12, the quantity could still be acceptable. The fat content increased significantly ($p<0.05$) but low compared to the ones that were both preserved with oil and packaged in polyethylene bags. The significant ($p<0.05$) weekly increase in fat was in line with Daramola *et al* (2007), who discovered that crude protein and fat relate inversely with each other. Generally, there was a significant ($p<0.05$) weekly increase in crude protein, fat, crude fibre and ash contents of the preserved samples that were not packaged in polyethylene bags from week 1 to 10, after which it dropped. This infers that storing fish preserved with lemon essential oil in cartons without polyethylene bags will still give good nutrient results till the 10th week. The overall assessment of these fish samples confirmed the ability of lemon essential oil to keep fish in storage and in good nutritional condition. In comparison, lemon essential oil will work better in extending the shelf life of fish when combined with polyethylene bag in a carton.

There was a more significant ($p<0.05$) difference in moisture content reduction in preserved sardine samples not packaged in polyethylene bags inside cartons than in the unpreserved samples (Tables 7 and 8). Moisture content ranged between 8.75±0.35 – 2.65±0.2 (unpreserved) and 7.65±0.21 – 2.35±0.21 (preserved). This moisture content reduction in sardine did not agree with Olayemi *et al*. (2011), who had 8.80 as the least moisture content in smoked dried catfish stored for six months and packaged in various modified polyethylene-paper packages. Also, the crude protein of the unpreserved sardine samples significantly ($p<0.05$) reduced while the preserved samples were more stable along the weeks because their reduction was not significantly ($p<0.05$) different from one another during the storage period, and this agrees with Olayemi *et al*. (2011). The fat content of the preserved samples also increased significantly ($p<0.05$) alongside the fibre and ash, unlike the unpreserved samples. This variation ascertains the use of lemon essential oil as a preservative for fish to conserve the nutrients in it. Fat content was reduced in all the packaging materials used for smoked dried catfish by Olayemi *et al*. (2011) in the six-month storage period. The results also further suggested that polyethylene as a packaging material was efficient in maintaining the protein content of the smoked fish.

Conclusion and Recommendations

The findings from this study have shown the remarkable effect of Lemon essential oil as a preservative for smoked clupeids during storage. It was also found that it had a more significant effect on the nutritionalcomposition of the stored Clupeids when combined with packaging materials. It can thus be inferred that the proximate contents of Bonga, *Ethmalosa fimbriata* and Sardine, *Sardinella maderensis,* can be kept intact while on the shelf using a combination of lemon essential oil and polyethylene bags during storage. These findings have thus made it expedient to recommend the following:

1. Lemon essential oil can be used as an effective natural preservative for Bonga, *Ethmalosa fimbriata* and Sardine, *Sardinella maderensis* during storage while the nutrients in it remain intact.
2. Bonga, *Ethmalosa fimbriata* and Sardine, *Sardinella maderensis,* can be stored for as long as 12 weeks when preserved with lemon essential oil and packaged in polyethylene bags before being put in cartons on the shelf.
3. The efficient preservative effect of lemon essential oil from this study has shown that even where polyethylene bags are not available, Bonga, *Ethmalosa fimbriata* and Sardine, *Sardinella maderensis* can still be stored for 10 weeks.
4. Fish can be stored sandwiched by peels of lemon to

see if the same effect would be produced as the oil to reduce the cost of production by removing the cost of extraction.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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