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Antimicrobial potency of *Xylopia aethiopica* Essential oil on Stored Smoked *Clarias gariepinus (*Burchell, 1822)

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Abstract

Smoked fish has a relatively short shelf life. The shelf life of smoked fish can be extended with different preservative methods including use of bio-preservatives. African pepper Essential Oil (ApEO) extracted using hydrodistillation method exhibits antibacterial and antifungal properties. This study assessed the potential of ApEO as an antimicrobial agent in limiting the activities of spoilage microorganisms in smoked catfish during storage at both refrigerated and ambient temperatures. Smoked catfish samples were preserved with synthetic antioxidants and African pepper essential oil (ApEO) as bio-preservative and kept in high density polyethylene bags and aluminum foil pouches. Samples treated with ApEO were sprayed with 1.0% (v/w). The total aerobic bacteria, total coliform and fungal counts of preserved samples were determined using pour plate method. Samples were evaluated at 14 days interval for a period of 84 days for refrigerated (6 ± 2 °C)samples except for the samples stored at ambient temperature (28 ± 2 °C) which was evaluated after 1 week. The initial microbial count of freshly smoked catfish (with or without treatment) was 0.00 log cfu g^{-1}. At the end of refrigerated storage, the total bacterial, coliform and fungal counts for preserved smoked catfish ranged between 0.00 - 7.88 log cfu g^{-1}, 0.00 – 6.94 log cfu g^{-1} and 0.00 – 7.47 log cfu g^{-1}, respectively in the two packaging materials used. A combination of 1% (v/w) ApEO with 5% (w/w) salt was effective in limiting bacterial and fungal growth in smoked catfish kept in high density polyethylene bag beyond 10 weeks at refrigerated temperature. The study concluded that the shelf life of smoked catfish was extended by ApEO by limiting the rate of microbial proliferation during storage at refrigerated temperature for 10 weeks.

Keywords: Extended shelf life, Antimicrobial, African pepper Essential Oil, Preservative, Refrigeration

Introduction

Spices are known to contain active compounds which possess antimicrobial activity. Essential oils (EOs) are components of spices. These oils have been established to possess antimicrobial properties and are Generally Regarded As Safe (GRAS) for consumption and environmental-friendly. Essential oils from spices and herbs which are popularly used have been established to show antimicrobial activity against most common food spoilage bacteria and fungi (Tovide *et al*., 2016). Some of these species include: *Listeria* spp., *Staphylococcus* spp., *Salmonella* spp., *Escherichia* spp., *Pseudomonas* spp., *Aspergillus* spp., *Cladosporium* spp. and many others. Essential oils have been obtained from spices and herbs which include: ginger, garlic, allspice, nutmeg, mustard, cinnamon, cumin, clove, bay, thyme, pepper, basil, oregano, sage, rosemary and so on (Ozogul *et al*., 2004). The oil is named after the plant from which it is extracted (Somesh *et al*., 2015).

Fish is susceptible to decomposition, development of rancidity and microbial spoilage immediately after harvest because it is a rich source of nutrient for microbial growth and activities (Cheng *et al*., 2015). Therefore, there is a great need to process and preserve the fish with the aim of increasing its shelf life (Subramanian, 2007). Some of the processing and preservative techniques are salting, drying, smoking, canning, freezing, pickling and irradiation among others. Smoked fish have been reported to be heavily contaminated with many species of bacteria including members of the genera *Escherichia* and *Staphylococcus* due to the greater unhygienic conditions of the environment in which they are processed and/ being sold (Adelaja *et al*., 2013). The public health concern of smoked fish is therefore, due to the poor handling and processing either by the processors, marketers or the consumers. This results in a short shelf life of locally processed smoked fish and which in no doubt affect the commercialization (Adelaja *et al*., 2013).

And many studies have reported the antimicrobial activities of several plant extracts, including black sesame (Shittu *et al.*, 2007), garlic (Agatemor, 2009), olives, chardonnay grapes, black raspberries and orange essential oils (George *et al*., 2010). The antimicrobial activity of the essential oil of African pepper has been evaluated on some Gram positive and Gram negative food-borne pathogens by Fleischer *et al*. (2008). It was established that essential oils obtained from different parts of African pepper (the fresh fruit, dried fruits, leaf, stem bark and root bark) showed varied activity on the test organisms except on *Escherichia coli*. Several studies have documented the antimicrobial potential of extracts and essential oil from African pepper in the preservation of foods (Adeniran *et al*., 2015). Although, its extracts and essential oil have antimicrobial activity, there is no documentation on the preservative effect of its essential oil on smoked catfish. Hence, this study investigated the antimicrobial potential of African pepper essential oil on smoked

Clarias gariepinus in two different packaging materials stored at refrigerated and ambient temperatures for duration of 12 weeks and one week, respectively.

Materials and Methods

Plant identification, fish sample preparation and treatment

African pepper (*Xylopia aethiopica* A. Rich.) was obtained from Odo-ori Market, Iwo, Osun-State, Nigeria. It was identified and authenticated at Herbarium unit, the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Catfish (*Clarias gariepinus* B.) was obtained from the Wet Laboratory, Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

Catfish weighing 600 – 800 grams was used for this study. The live catfish was stunned and then beheaded. The head, fins and tail of the catfish were removed. The fish was then cut into chunks (thickness 1.5 cm) using a manual calibrated slicer, to obtain uniformity. The chunks of the catfish were washed with clean water until the sliminess of the catfish was properly removed and cleaned. The chunks were then allowed to be air-dried and subjected to smoking in a smoking kiln **(**ARCOS, France**)**. Catfish was smoked at 90 ± 5 ℃ for 6 h.

Experimental design

Smoked fish was allowed to cool before treatments were added. Treatments include: addition of BHT, salt, African pepper essential oil (ApEO) and a combination of salt and ApEO. Smoked fish was treated by spraying the outer surface of the fish muscle with ApEO inside an enclosed box. Six experimental groups were designed as follows for Catfish;

Group A: Control (No treatment)
Group B: 0.01% (w/w) Butylated hydroxyltoluene (BHT)
Group C: 5% (w/w) Salt
Group D: 1% (v/w) African pepper Essential Oil (ApEO)
Group E: 1% (v/w) African pepper Essential Oil (ApEO) with 3% (w/w) salt
Group F: 1% (v/w) African pepper Essential Oil (ApEO) with 5% (w/w) salt

Twenty-one (21) pieces of chunks for each experimental group were then packed in two different packaging materials; High density polyethylene bag and Aluminum foil pouch. These were then stored at refrigerated temperature (6 ± 2 ℃) for a period of 84 days. Samples were taken and analyzed at 14 days interval.

Nine (9) pieces of the fish chunks were packed in two different packaging materials; High density polyethylene bag and Aluminum foil pouch for each experimental group. These were then stored at ambient temperature (28 ± 2 ℃) for a period of 14 days. Samples were taken and analyzed at 7 days interval.

Microbial Analyses

The microbial analyses, Total aerobic bacterial count (TABC), Total coliform count (TCC) and total fungal count, were carried out on the freshly prepared smoked catfish and also throughout the storage period.

Total aerobic bacterial count

The Total aerobic bacterial count of stored smoked catfish sample was determined by pour plate method using Maximum Recovery Diluent (MRD) for serial dilution and plated on Nutrient agar (NA Lab M) as the culture medium (Harrigan and McCance, 1976; Harrigan, 1998). Counts of bacterial colonies were determined from smoked fish samples stored at both ambient and refrigeration temperatures. The plates were incubated inverted at 37 ℃ for 48 hours after which colonies formed were counted and expressed as colony forming units per gram (cfu g^{-1}).

Total coliform count

Dilutions obtained for each treatment were poured into petri-dish and about 20 ml of molten MacConkey agar was poured on each plate. The plates were incubated at 37 ℃ for 24 h. Colonies were counted and the number of colonies was multiplied by the reciprocal of the dilution factor (Harrigan and McCance, 1976; Harrigan, 1998).

Mould and yeast count

The total mould and yeast count for the stored fish samples was carried out according to the method of Harrigan and McCance (1976) and Harrigan (1998). The samples were cultured on Potato Dextrose Agar (PDA) medium during the period of storage. The inoculated plates were incubated for 48 - 72 hours at 30 ℃. The fungal colonies on each plate were enumerated and calculated as colony forming units (cfu) per g of sample cfu g^{-1} = (Number of colonies x Dilution factor).

Statistical Analysis

The data obtained from microbiological analyses were subjected to inferential and descriptive statistics. A statistical package for Social Sciences (SPSS, version 23) was used to calculate the means, standard error of means and Analysis of Variance (ANOVA) for all triplicate readings. Statistically significant difference was detected by comparing experimental values using one-way ANOVA. The means were separated by Duncan's multiple range tests. The significance level was determined at 5% level.

Results and Discussion

Changes in Total Aerobic Bacterial Count of preserved smoked catfish during

refrigeration

The Total aerobic bacterial count (TABC) observed during the storage of both the untreated and preserved smoked catfish samples kept in HDPE bags and foil pouch stored in a refrigerator for twelve weeks are shown in Tables 1a and 1b respectively. The initial mean value for total aerobic bacteria count was observed to be 0.00 log cfu g^{-1} in freshly smoked catfish samples in both the untreated sample (control sample) and the preserved samples (ApEO- and salt- treated samples). The insignificant microbial count in the freshly smoked catfish samples could be because of the observation of Good Manufacturing Practices (GMP) during and after processing of the smoked catfish. Likewise, the effectiveness of the smoking temperature and time (90 °C for 6 hours) is sufficient to eliminate vegetative microbial cells. Also, the initial microbial density (low value) of the raw material could have accounted for this observation. Findings by Oyelese, (2006) and Dutta *et al*. (2018) also reported insignificant microbial count in freshly hot-smoked *Tenualosa ilisha*, *Oreochromis mossambicus* and *Pangasius hypophthalmus* which was similar to the observation in this study. Egbal *et al*. (2013) also reported a similar finding in freshly smoked catfish samples.

Table 1a: Total aerobic bacteria count (log cfu g^{-1}) of preserved smoked catfish stored in high density polyethylene at refrigeration temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aG}	0.00 ± 0.00^{aG}	0.00 ± 0.00^{aD}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aA}
2	0.00 ± 0.00^{cF}	2.89 ± 0.02^{aE}	2.23 ± 0.02^{bF}	0.00 ± 0.00^{cD}	0.00 ± 0.00^{cE}	0.00 ± 0.00^{cA}
4	5.51 ± 0.03^{aD}	2.75 ± 0.03^{cF}	4.57 ± 0.01^{bE}	0.00 ± 0.00^{dD}	0.00 ± 0.00^{dE}	0.00 ± 0.00^{dA}
6	7.37 ± 0.04^{aC}	5.45 ± 0.03^{bD}	4.65 ± 0.04^{cD}	5.46 ± 0.01^{bC}	4.28 ± 0.09^{dC}	0.00 ± 0.00^{eA}
8	7.46 ± 0.01^{aB}	5.57 ± 0.02^{bC}	4.85 ± 0.06^{dC}	5.44 ± 0.01^{cC}	4.06 ± 0.01^{eD}	0.00 ± 0.00^{fA}
10	7.68 ± 0.02^{aA}	5.96 ± 0.04^{bB}	5.49 ± 0.01^{dB}	5.62 ± 0.01^{cB}	4.55 ± 0.04^{eB}	0.00 ± 0.00^{fA}
12	5.04 ± 0.03^{eE}	6.48 ± 0.01^{aA}	6.21 ± 0.01^{bA}	5.85 ± 0.01^{cA}	5.53 ± 0.01^{dA}	0.00 ± 0.00^{fA}

Table 1b: Total aerobic bacteria count (log cfu g^{-1}) of preserved smoked catfish stored in aluminum foil pouch at refrigeration temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aG}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aD}
2	0.00 ± 0.00^{bE}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bD}
4	0.00 ± 0.00^{bE}	2.78 ± 0.02^{aE}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bD}
6	5.56 ± 0.02^{aD}	3.69 ± 0.03^{cD}	2.42 ± 0.01^{dD}	5.44 ± 0.02^{bC}	0.00 ± 0.00^{eE}	0.00 ± 0.00^{eD}
8	7.59 ± 0.04^{aA}	5.11 ± 0.04^{cC}	2.90 ± 0.01^{eC}	5.31 ± 0.02^{bD}	3.10 ± 0.02^{dC}	2.18 ± 0.03^{fC}
10	7.23 ± 0.02^{aB}	5.98 ± 0.02^{cB}	3.12 ± 0.03^{eB}	6.34 ± 0.02^{bB}	5.82 ± 0.02^{dB}	2.56 ± 0.04^{fB}
12	6.07 ± 0.01^{dC}	6.73 ± 0.04^{cA}	3.59 ± 0.03^{fA}	7.88 ± 0.02^{aA}	7.28 ± 0.01^{bA}	3.72 ± 0.02^{eA}

Means with the same small alphabet superscript across rows are not significantly different at $p< 0.05$
Means with the same capital alphabet superscript along column are not significantly different at $p < 0.05$

*****Samples with counts lesser than 25 colonies at 10-fold dilution of stock sample were reported as 0.00 log cfu g^{-1}

Key: CA: Smoked catfish without preservative; CB: Smoked catfish with BHT; CC: Smoked catfish with 5% NaCl; CD: Smoked catfish with 1% African pepper dried fruit essential oil; CE: Smoked catfish with 1% African pepper dried fruit essential oil + 3% NaCl; essential oil + 3% NaCl; and CF: Smoked catfish with 1% African pepper dried fruit essential oil + 5% NaCl;

Nevertheless, microbial load was observed to increase from 0.00 to 5.00 log cfu g^{-1} in the control sample (without preservative) after two weeks of storage in high density polyethylene bag at 6 ± 2 ℃. Smoked catfish samples preserved with 0.01 % (w/w) BHT (CB) and 5% (w/w) salt (CC) both had an increase of 2.89 and 2.23 log cfu g^{-1} respectively after two weeks of refrigerated storage (HDPE). The increase in microbial count for the control sample, samples CB and CC could be due to germination of initially inhibited vegetative (stressed) cells or provision of suitable growth conditions for other microorganisms (psychrophiles) (Couvert *et al*., 2010). Smoked catfish preserved with 1.0% (v/w) ApEO (CD) had limited bacterial count until the sixth week of refrigerated storage. Smoked catfish sample treated with 1.0% (v/w) ApEO showed similar trend as the sample treated with the combination of 1.0% (v/w) ApEO with 3% (w/w) NaCl (CE). These samples (CD and CE) had limited bacterial count until the sixth week of refrigerated storage. Smoked catfish treated with a combination of 1.0% (v/w) ApEO with 5% (w/w) NaCl had an insignificant bacterial count for 12 weeks of refrigerated storage in HDPE bag.

Comparing the treatments in terms of antibacterial activities, treatment with 1.0% (v/w) ApEO limited bacterial growth in smoked catfish for a period of 28 days, thereby extending the shelf life of smoked catfish at refrigerated storage by 14 days. However, addition of 5%

(w/w) NaCl to 1% (v/w) ApEO inhibited bacterial growth in smoked catfish for 12 weeks. Hence, the addition of 5% (w/w) NaCl to smoked catfish enhanced the effectiveness of 1.0% (v/w) ApEO as an antibacterial in extending the shelf life of smoked catfish beyond 10 weeks at refrigeration temperature using HDPE bag as packaging material.

The acceptable limit for presence of total aerobic bacteria in smoked fish products is not expected to exceed 5 log cfu g^{-1} of fish sample while the maximum limit is between 6 and 7 log cfu g^{-1} (Health Protection Agency, 2009). All the samples treated with ApEO did not reach the minimum limit (5 log cfu g^{-1}) during the twelve weeks of refrigerated storage. The control sample (CA) exceeded the acceptable limit for TABC by the 6^{th} week (7.37 log cfu g^{-1}). This is an indication that by the end of the sixth week of refrigerated storage, the control sample (without preservative) was not safe for human consumption. While the samples treated with synthetic antioxidant (BHT) and salt only (5% w/w) exhibited similar pattern. These samples (CB and CC) exceeded the acceptable limit for TABC count in smoked fish after the 10^{th} week of storage at 6 ± 2 °C in HDPE package (6.48 and 6.21 log cfu g^{-1} respectively). Thus, the addition of salt and ApEO to the smoked fish samples limited bacterial growth either through a bactericidal or bacteriostatic effect.

The smoked catfish without treatment (control sample) stored in aluminum foil pouch (AFP) had limited bacterial load until the fourth week of storage at 6 ± 2 °C. Similar trend was observed in the sample treated with 1.0% (v/w) ApEO only. At the end of the 4^{th} week of storage, all the samples (both control and treated samples) had limited bacterial load (0.00 log cfu g^{-1}) except sample CB (sample treated with BHT) which had an increase of 2 log cfu g^{-1}. The control sample was no longer fit for human consumption after the 6^{th} week of refrigerated storage. Only the samples preserved with 5% (w/w) salt (sample CC) and the combination of 1% (v/w) ApEO with 5% (w/w) salt (sample CF) did not reach the acceptable TABC limit by the end of the 12^{th} week of storage in the AFP package stored at 6 ± 2 °C.

The migration of water from the storage environment of the samples into the aluminium foil pouch could likely be responsible for the observed increase in the water content of the smoked catfish samples. Hence, creating a suitable environment for some bacteria to grow in the stored catfish samples. The packaging materials employed for this study were observed to have a noticeable effect on the total aerobic bacterial load of stored preserved smoked catfish samples. Comparing the HDPE bag and AFP packaging materials in terms of the total aerobic bacterial count, the HDPE bag was observed to be much effective in controlling bacterial load most especially with the samples treated with 1.0% (v/w) ApEO. The efficacy of the antimicrobial components present in African pepper essential oil could have been enhanced by the packaging condition created by the HDPE bag. Also, the synergistic effect due to the interactions between the packaging material- HDPE bag, the packaging condition and volatile compounds of African pepper essential oil on the bacterial load and biota could be responsible for this observation. This was similar to the findings of

Skandamis and Nychas (2002) with preservation of fresh meat with oregano essential oil and modified packaging condition.

The temperature at which hot smoking (90 ± 5 ℃) took place was effective in destroying most vegetative bacterial cells present in the fresh fish sample, though the heat generated might not be sufficient to kill the bacterial spores present. Appearance of bacteria over the storage time is likely due to the germination and multiplication of these bacterial spores, as well as activation of inactivated vegetative bacterial cells, thus causing spoilage of the smoked fish (Hwang *et al*., 2009; Dutta *et al*., 2018).

Also, the findings by Dutta *et al*. (2018) observed that as storage time increased, microbial density increased in the stored smoked fish samples at refrigerated temperature (6 ± 2 ℃). According to the report of Kykkidou *et al*. (2009), thyme–oregano oil treatment was effective in eliminating the growth of bacteria in modified atmosphere-packaged fish under refrigerated storage, which was in agreement with the findings in this study. Smoked catfish samples treated with 1.0% (v/w) ApEO had lower TABC compared with the samples treated with synthetic antioxidant and salt. This is an indication that ApEO, with or without salt, has a preservative potential as an antibacterial agent in extending the shelf life of smoked catfish.

Influence of preservative on Total Aerobic Bacterial Count of smoked catfish stored at ambient temperature

The initial Total aerobic bacterial count (TABC) for freshly smoked catfish was 0.00 log cfu g^{-1} (TABC < 1 x 10^{-1} per gram of catfish sample). The TABC of smoked catfish increased from a range of 0.00 to 4.61 - 7.50 log cfu g^{-1} for samples packed in HDPE bag and 2.02 – 6.83 log cfu g^{-1} for samples packed in foil pouch (Tables 2a and 2b). The smoked catfish sample treated with a combination of 1.0% (v/w) ApEO with 3% (w/w) salt (CE) kept in HDPE bag had the lowest TABC and had not reached the minimum acceptable limit (5 log cfu g^{-1}) for TABC in smoked foods after 7 days at ambient storage.

Samples treated with a combination of 1.0% (v/w) ApEO with either 3% or 5% (w/w) salt (samples CE and CF) kept in aluminum foil pouch had the least TABC (2.02 and 2.69 log cfu g^{-1} respectively) overall. This indicated that the combination of 1.0% (v/w) ApEO with either 3% or 5% (w/w) salt were more effective in foil pouch packaging material than in HDPE bag at ambient temperature (28 ± 2 ℃).

Table 2a: Total aerobic bacterial count (log cfu g^{-1}) of preserved smoked catfish stored in high density polyethylene at ambient temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}
1	7.50 ± 0.02^{aA}	7.47 ± 0.00^{aA}	7.15 ± 0.06^{bA}	6.47 ± 0.01^{cA}	4.61 ± 0.02^{eA}	6.19 ± 0.04^{dA}

Table 2b: Total aerobic bacterial count (log cfu g^{-1}) of preserved smoked catfish stored in aluminum foil pouch at ambient temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}
1	6.83 ± 0.03^{aA}	5.03 ± 0.02^{cA}	5.00 ± 0.00^{cA}	6.12 ± 0.01^{bA}	2.02 ± 0.02^{eA}	2.69 ± 0.01^{dA}

Means with the same small alphabet superscript across rows are not significantly different at $p< 0.05$
Means with the same capital alphabet superscript along column are not significantly different at $p < 0.05$
*****Samples with counts lesser than 25 colonies at 10-fold dilution of stock sample were reported as 0.00 log cfu g^{-1}

Key: CA: Smoked catfish without preservative; CB: Smoked catfish with BHT; CC: Smoked catfish with 5% NaCl; CD: Smoked catfish with 1% African pepper dried fruit essential oil; CE: Smoked catfish with 1% African pepper dried fruit essential oil + 3% NaCl; essential oil + 3% NaCl; and CF: Smoked catfish with 1% African pepper dried fruit essential oil + 5% NaCl

Effect of preservative on the total coliform count of smoked catfish during refrigeration storage

The total coliform count (TCC) obtained for both the untreated and preserved smoked catfish during refrigerated storage (6 ± 2 ℃) for duration of twelve weeks is shown in Tables 3a and 3b. The initial total coliform count of both untreated and preserved freshly smoked catfish was observed to be 0.00 log cfu g^{-1}. Absence of coliform in freshly smoked catfish is an indication of Good Manufacturing Practices (GMP) during processing, which

Table 3a: Total coliform count (log cfu g^{-1}) of preserved smoked catfish stored in high density polyethylene at refrigeration temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aD}	0.00 ± 0.00^{aA}
2	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aD}	0.00 ± 0.00^{aA}
4	3.74 ± 0.03^{aE}	2.20 ± 0.04^{cE}	2.11 ± 0.04^{bE}	0.00 ± 0.00^{dE}	0.00 ± 0.00^{dD}	0.00 ± 0.00^{dA}
6	5.34 ± 0.05^{bC}	5.49 ± 0.01^{aA}	2.79 ± 0.01^{dD}	3.46 ± 0.01^{cD}	0.00 ± 0.00^{eD}	0.00 ± 0.00^{eA}
8	5.69 ± 0.03^{aA}	4.49 ± 0.01^{cD}	5.06 ± 0.01^{bC}	4.00 ± 0.01^{dC}	2.22 ± 0.04^{eC}	0.00 ± 0.00^{fA}
10	5.58 ± 0.02^{aB}	4.96 ± 0.01^{cC}	5.20 ± 0.02^{bB}	4.26 ± 0.01^{dB}	3.52 ± 0.02^{eB}	0.00 ± 0.00^{fA}
12	5.02 ± 0.02^{dD}	5.03 ± 0.02^{dB}	5.46 ± 0.01^{cA}	5.72 ± 0.03^{aA}	5.70 ± 0.01^{bA}	0.00 ± 0.00^{eA}

Table 3b: Total coliform count (log cfu g^{-1}) of preserved smoked catfish stored in aluminum foil pouch at refrigeration temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aD}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aD}	0.00 ± 0.00^{aC}
2	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aD}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aD}	0.00 ± 0.00^{aC}
4	0.00 ± 0.00^{bE}	2.04 ± 0.04^{aE}	0.00 ± 0.00^{bD}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bD}	0.00 ± 0.00^{bC}
6	4.43 ± 0.04^{aD}	3.13 ± 0.04^{bD}	0.00 ± 0.00^{dD}	2.45 ± 0.01^{cD}	0.00 ± 0.00^{dD}	0.00 ± 0.00^{dC}
8	7.30 ± 0.01^{aA}	4.66 ± 0.02^{bC}	2.86 ± 0.02^{dC}	3.10 ± 0.08^{cC}	2.32 ± 0.06^{eC}	0.00 ± 0.00^{fC}
10	7.06 ± 0.03^{aB}	5.74 ± 0.01^{bB}	3.04 ± 0.01^{eB}	5.14 ± 0.02^{cB}	3.56 ± 0.03^{dB}	2.16 ± 0.03^{fB}
12	5.75 ± 0.04^{dC}	6.47 ± 0.00^{bA}	3.31 ± 0.01^{fA}	6.94 ± 0.03^{aA}	$6.08\pm0.00c^{A}$	3.76 ± 0.05^{eA}

Means with the same small alphabet superscript across rows are not significantly different at $p< 0.05$
Means with the same capital alphabet superscript along column are not significantly different at $p < 0.05$
*****Samples with counts lesser than 25 colonies at 10-fold dilution of stock sample were reported as 0.00 log cfu g^{-1}

Key: CA: Smoked catfish without preservative; CB: Smoked catfish with BHT; CC: Smoked catfish with 5% NaCl; CD: Smoked catfish with 1% African pepper dried fruit essential oil; CE: Smoked catfish with 1%

African pepper dried fruit essential oil + 3% NaCl; essential oil + 3% NaCl; and CF: Smoked catfish with 1% African pepper dried fruit essential oil + 5% NaCl;

can help eliminate coliform, thus making the food safe for human consumption. Dutta *et al*. (2018) also reported the absence of coliform in the freshly prepared hot smoked *Tenualosa ilisha*, *Oreochromis mossambicus* and *Pangasius hypophthalmus* until 7 days after storage at 6 ± 2 °C. There was no coliform growth observed in the samples examined by Dutta *et al*. (2018), which agreed with the findings in this study.

Coliforms were not observed in the stored fish samples until after the second week of storage, regardless of the treatment given. However, coliform was observed to be present in the control sample (CA) (3.74 log cfu g^{-1}), sample preserved with BHT (CB) (2.20 log cfu g^{-1}) and the sample preserved with 5% (w/w) salt (2.11 log cfu g^{-1}) after two weeks of storage at refrigerated condition (6 ± 2 ℃) for samples kept in HDPE bag. Although, coliform was absent in the sample preserved with 1.0% (v/w) ApEO alone until after the fourth week of storage (3.46 log cfu g^{-1}) at refrigeration temperature (6 ± 2 ℃) in HDPE bag. There was no coliform in the sample preserved with a combination of 1.0% (v/w) ApEO with 5% (w/w) salt (CF) throughout the twelve weeks of refrigerated storage.

For the samples packed in foil pouch and kept at refrigeration temperature (6 ± 2 ℃), the total coliform count increased from 0.00 to 2.04 log cfu g^{-1} only in the sample preserved with BHT while the control sample had no coliform count until after the fourth week of storage. Although the combination of 1.0% (v/w) ApEO with 5% (w/w) salt was not as effective in the foil pouch as it was observed in the HDPE bag. The sample treated with the combination of 1.0% (v/w) ApEO with 5% (w/w) salt (CF) had 2.16 log cfu g^{-1} coliform count after the eighth week of storage at refrigerated temperature (6 ± 2 ℃) in the aluminum foil pouch- packaging material.

Fish with a good quality which is safe for human consumption is not expected to have a total coliform count that exceeds 2.00 log cfu g^{-1} of food sample. The untreated smoked catfish, samples preserved with BHT and 5% (w/w) salt (Samples CA, CB and CC) had exceeded this limit by the fourth week of refrigerated storage. This indicated that they were no longer safe for human consumption. While the sample treated with 1.0% (v/w) ApEO only became unacceptable after 28 days compared with the control sample (sample CA). The control sample, the sample treated with BHT (sample CB) and the sample treated with 5% (w/w) salt (sample CC) kept in foil pouch became unsafe for human consumption after 14 days. This is an indication of the antimicrobial potential of the ApEO in limiting growth of coliform in the preserved smoked catfish samples during storage at 6 ± 2 ºC.

Influence of preservative on Total coliform count of smoked catfish stored at ambient temperature

The Total coliform count (TCC) of preserved smoked catfish (with or without treatment) increased from 0.00 log cfu g^{-1} (week 0) to a range of 4.22 – 7.47 log cfu g^{-1} for samples kept in HDPE bag and 1.60 – 6.31 log cfu/g for samples kept in foil pouch between week 0 and week 1 (Tables 4a and 4b). The sample treated with a combination of 1.0% (v/w) ApEO with 3% (w/w) salt had the lowest TCC (4.22 log cfu g^{-1}) for samples stored in HDPE bag while samples treated with a combination of 1.0% (v/w) ApEO with either 3 or 5% (w/w) salt had the lowest TCC (1.60 and 2.43 log cfu g^{-1}) in foil pouch at ambient temperature (28 ± 2 °C) after storage for 7 days. Although, only the sample treated with a combination of 1.0% (v/w) ApEO with 3% (w/w) salt kept in foil pouch stored at ambient temperature was within the acceptable limit for TCC (2 log cfu g^{-1}) in smoked seafood safe for human consumption after storage for 7 days.

Table 4a: Total coliform count (log cfu g^{-1}) of preserved smoked catfish stored in high density polyethylene at ambient temperature

Storage Period (weeks)	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}
1	5.59 ± 0.01^{eA}	7.47 ± 0.00^{aA}	$6.98\pm0.0.01^{bA}$	6.43 ± 0.01^{cA}	4.22 ± 0.01^{fA}	6.17 ± 0.01^{dA}

Table 4b: Total coliform count (log cfu g^{-1}) of preserved smoked catfish stored in aluminum foil pouch at ambient temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}
1	6.31 ± 0.01^{aA}	4.27 ± 0.01^{cA}	4.99 ± 0.00^{bA}	6.08 ± 0.01^{aA}	1.60 ± 0.30^{eA}	2.43 ± 0.02^{dA}

Means with the same small alphabet superscript across rows are not significantly different at $p< 0.05$
Means with the same capital alphabet superscript along column are not significantly different at $p < 0.05$
*****Samples with counts lesser than 25 colonies at 10-fold dilution of stock sample were reported as 0.00 log cfu g^{-1}

Key: CA: Smoked catfish without preservative; CB: Smoked catfish with BHT; CC: Smoked catfish with 5% NaCl; CD: Smoked catfish with 1% African pepper dried fruit essential oil; CE: Smoked catfish with 1% African pepper dried fruit essential oil + 3% NaCl; essential oil + 3% NaCl; and CF: Smoked catfish with 1% African pepper dried fruit essential oil + 5% NaCl

Effect of preservative on mould and yeast count of smoked catfish stored at refrigeration temperature

The initial mould and yeast count in the freshly smoked (treated and untreated) catfish sample was 0.00 log cfu g^{-1} (Tables 5a and 5b). Absence of mould and yeast in the freshly prepared smoked catfish is an indication of Good Manufacturing Practices (GMP) during processing. Findings according to Dutta *et al.* (2018) also recorded absence of mould and yeast growth in freshly prepared hot smoked fish samples but increase in fungal growth occurred with increase in storage time at refrigeration temperature (6 ± 2 ℃). Fungal growth was observed after two weeks of storage in the sample preserved with BHT (CB) kept in HDPE bag and foil pouch (3.02 and 2.76 log cfu g^{-1} respectively). There was no fungal growth in the control sample (CA), sample preserved with 5% (w/w) salt (CC) and sample treated with 1% (v/w) ApEO alone (CD) until the sixth week in both HDPE bag and aluminum foil pouch. Although samples treated with 5% (w/w) salt did not reach the maximum acceptable limit for fungal load (4 log cfu/g) (Leroi *et al*., 2001) in smoked catfish in the two packaging materials used (HDPE bag and foil pouch) throughout the twelve weeks

Table 5a: Mould and yeast count (log cfu g^{-1}) of preserved smoked catfish stored in high density polyethylene at refrigeration temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aA}
2	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aA}
4	0.00 ± 0.00^{bE}	3.02 ± 0.04^{aD}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bA}
6	4.56 ± 0.02^{aC}	4.60 ± 0.10^{aC}	2.20 ± 0.03^{bB}	4.56 ± 0.07^{aC}	0.00 ± 0.00^{cC}	0.00 ± 0.00^{cA}
8	6.14 ± 0.43^{aA}	5.09 ± 0.04^{bA}	2.20 ± 0.06^{cB}	5.09 ± 0.04^{bA}	0.00 ± 0.00^{dC}	0.00 ± 0.00^{dA}
10	5.21 ± 0.04^{aB}	5.00 ± 0.02^{bAB}	2.29 ± 0.04^{dAB}	4.73 ± 0.01^{cB}	2.04 ± 0.03^{eB}	0.00 ± 0.00^{fA}
12	4.46 ± 0.01^{bD}	4.91 ± 0.01^{aB}	2.33 ± 0.03^{dA}	3.48 ± 0.01^{cD}	2.23 ± 0.04^{eA}	0.00 ± 0.00^{fA}

Table 5b: Mould and yeast count (log cfu g^{-1}) of preserved smoked catfish stored in aluminum foil pouch at refrigeration temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aB}
2	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aF}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aE}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aB}
4	0.00 ± 0.00^{bE}	2.76 ± 0.10^{aE}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bE}	0.00 ± 0.00^{bC}	0.00 ± 0.00^{bB}
6	4.62 ± 0.03^{aD}	4.41 ± 0.02^{bD}	2.39 ± 0.03^{cD}	4.64 ± 0.05^{aC}	0.00 ± 0.00^{dC}	0.00 ± 0.00^{dB}
8	5.92 ± 0.01^{aA}	4.71 ± 0.02^{bC}	2.96 ± 0.03^{dC}	4.04 ± 0.03^{cD}	0.00 ± 0.00^{eC}	0.00 ± 0.00^{eB}
10	5.88 ± 0.02^{aB}	4.98 ± 0.03^{cB}	3.09 ± 0.02^{dB}	5.72 ± 0.04^{bB}	2.23 ± 0.02^{eB}	0.00 ± 0.00^{fB}
12	5.70 ± 0.02^{bC}	5.37 ± 0.03^{cA}	3.21 ± 0.01^{eA}	7.47 ± 0.01^{aA}	4.81 ± 0.01^{dA}	3.00 ± 0.01^{fA}

Means with the same small alphabet superscript across rows are not significantly different at $p< 0.05$
Means with the same capital alphabet superscript along column are not significantly different at $p < 0.05$
*****Samples with counts lesser than 25 colonies at 10-fold dilution of stock sample were reported as 0.00 log cfu g^{-1}

Key: CA: Smoked catfish without preservative; CB: Smoked catfish with BHT; CC: Smoked catfish with 5% NaCl; CD: Smoked catfish with 1% African pepper dried fruit essential oil; CE: Smoked catfish with 1% African pepper dried fruit essential oil + 3% NaCl; essential oil + 3% NaCl; and CF: Smoked catfish with 1% African pepper dried fruit essential oil + 5% NaCl;

of refrigerated storage. Treatment of smoked catfish with a combination of 1.0% (v/w) ApEO with 5% (w/w) salt was observed to be more effective out of all the treatments given. The smoked catfish samples treated with a combination of 1.0% (v/w) ApEO with 5% (w/w) salt (CF) kept in HDPE bag had no fungal growth (0.00 log cfu g^{-1}) throughout the twelve weeks of storage at refrigeration temperature (6 ± 2 ℃). While a fungal count of 3.00 log cfu g^{-1} was noticed in similar sample (sample CF) kept in foil pouch after the tenth week of storage at 6 ± 2 °C.

According to FAO/APHCA (1989), smoked fish samples with moisture content beyond 12% is easily vulnerable to mould growth few days after processing, especially when not properly stored (Amponsah *et al*., 2018). The moisture content of smoked fish samples in this study were beyond 12% which could likely account for growth of mould in the treated packaged smoked catfish few weeks after storage. High environmental humidity played a key role in increase in the moisture content of the fish samples, thereby creating a favourable condition for mould outbreak in the stored smoked fish samples. Egbal *et al*. (2013) also reported profuse growth of mould in the smoked catfish (10% brined) sample at week 4 during refrigerated storage.

Influence of preservative on total mould and yeast count of smoked catfish stored at ambient temperature

The total mould and yeast count (MYC) of preserved smoked catfish stored at ambient temperature varied between 4.11 - 5.60 log cfu g^{-1} for samples kept in HDPE bag and 0.00 - 5.79 log cfu g^{-1} for samples kept in foil pouch as shown in Tables 6a and 6b. The smoked catfish sample preserved with a combination of 1.0% (v/w) ApEO with 5% (w/w) salt kept in foil pouch (sample CF) had no fungal growth after storage for 7 days at ambient temperature. Although, there was significant difference ($P < 0.05$) in the mould and yeast count of the control sample (sample CA) and the sample preserved with BHT (sample CB), 5% (w/w) salt (sample CC), 1.0% (v/w) ApEO alone (sample CD) and a combination of 1.0% (v/w) ApEO with 5% (w/w) salt (sample CF). Though the mould and yeast count of the sample treated with a combination of 1.0% (v/w) ApEO and 3% (w/w) salt (sample CE) (5.41 log cfu g^{-1}) was not significantly different ($P > 0.05$) from the untreated sample (sample CA) ((5.60 log cfu g^{-1}) kept in foil pouch at ambient temperature.

Table 6a: Mould and yeast count (log cfu g^{-1}) of preserved smoked catfish stored in high density polyethylene at ambient temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}
1	5.60 ± 0.02^{aA}	5.08 ± 0.01^{bA}	4.11 ± 0.00^{dA}	5.08 ± 0.30^{bA}	5.41 ± 0.01^{abA}	4.56 ± 0.01^{cA}

Table 6b: Mould and yeast count (log cfu g^{-1}) of preserved smoked catfish stored in aluminum foil pouch at ambient temperature

Storage weeks	CA	CB	CC	CD	CE	CF
0	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aA}
1	5.79 ± 0.02^{aA}	3.59 ± 0.02^{bA}	2.10 ± 0.00^{dA}	$5.74\pm0.0.02^{aA}$	3.28 ± 0.02^{cA}	0.00 ± 0.00^{eA}

Means with the same small alphabet superscript across rows are not significantly different at $p< 0.05$
Means with the same capital alphabet superscript along column are not significantly different at $p < 0.05$
*****Samples with counts lesser than 25 colonies at 10-fold dilution of stock sample were reported as 0.00 log cfu g^{-1}

Key: CA: Smoked catfish without preservative; CB: Smoked catfish with BHT; CC: Smoked catfish with 5% NaCl; CD: Smoked catfish with 1% African pepper dried fruit essential oil; CE: Smoked catfish with 1%

African pepper dried fruit essential oil + 3% NaCl; essential oil + 3% NaCl; and CF: Smoked catfish with 1% African pepper dried fruit essential oil + 5% NaCl

Findings of Idris *et al*. (2010) reported the absence of mould growth in 7.5% and 10% (w/w) ginger extract-treated smoke-dried catfish until after 4 weeks of storage at ambient condition. The observation in this study was however at variance with the submission by Idris *et al*. (2010). This may be due to the difference in the pre-treatment (brining and treatment with different concentrations of ginger extract) of the smoke-dried fish samples, which could also be responsible for the low moisture content of the smoke-dried fish samples compared with high moisture content of smoked fish samples used for this study. The treatment with the combination of 1.0% (v/w) ApEO with salt kept in aluminum foil pouch at ambient temperature preserved the smoked catfish samples such that the samples remained within the acceptable limit for fungal growth (4 log cfu g^{-1}) in smoked seafood. This finding is contrary when compared with those samples kept in HDPE bag which had exceeded the fungal growth limit including the control sample and the BHT treated sample (HDPE bag) after storage for 7 days. This is an indication that a combination of 1.0% (v/w) ApEO treatment with aluminum foil pouch packaging was much effective in limiting fungal growth in smoked catfish at ambient temperature during storage. Presence of air in a packaging material (Vermieren *et al*., 2003), ability of food to grasp oxygen (Smith *et al*., 1986), storage period and temperature (Matos *et al*., 2005) have been asserted to encourage the proliferation of mould and yeast growth in stored food. Foil pouch provided barrier against air in the packaged smoked catfish sample compared to the HDPE bag, thereby reducing the rate of proliferation of fungi in the stored smoked catfish samples.

Generally, the absence of microbial count observed after hot smoking of *Clarias gariepinus*, used for this study could be attributed to the effects of dehydration and antimicrobial activities of the smoke constituents besides the high temperature at which hot smoking was carried out (Ogbadu, 2014). Cold shock on microbes explained the elongated lag phase of the microorganisms during refrigerated storage. Gradual reduction in the intensity of the antimicrobial compounds (Smoke constituents + BHT, Smoke + 1.0% (v/w) ApEO, Smoke + 5% (w/w) salt, Smoke + 1.0% (v/w) ApEO + salt) present in both treated and untreated (smoke only) smoked fish samples, presence of oxygen, succession by psychrophiles, cold loving bacteria psychotrophs and fungi could have influenced the increase in microbial density observed as storage period extended.

Conclusions

The shelf life of smoked catfish was extended by the application of the essential oil from African pepper with salt. This additive inhibited microbial growth beyond 10 weeks at refrigerated temperature (6 ± 2 ℃) in smoked catfish, thus, indicating reduced microbial load and activity in the preserved samples. Hence, it is evident that smoked catfish (*Clarias gariepinus* Burchell, 1822) can be shelf-stable for 12 weeks at refrigerated temperature (6 ±

2 ℃) using African pepper essential oil in combination with other preservatives (Hurdle technology).

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Authors Contributions

OIO designed, conducted the research and wrote the manuscript while **HAA** supervised the research and corrected the manuscript. **OIO** and **HAA** processed the response for publication.

Conflicts of Interest

The authors do not have any potential conflict of interest to declare.

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