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Shelf-Life Determination of Smoked Fish Using Different Packaging Materials

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ABSTRACT

Twenty frozen fishes of different species which include sardine, mackerel, catfish and panla were purchased from four different markets in Awka, five each. The frozen fishes were taken to fishery department of Anambra State Polytechnic, Mgbakwu for smoking; the fishes were aseptically smoked using traditional kiln and oven drying method. The aseptically smoked fishes were packaged in five different packaging materials produced in the laboratory namely; Polyethylene (PP) it serves as control, PCP, PCDP, PFP and ATCP the samples were packaged in triplicate and stored at room temperature for 30days. Thickness, water and oil absorption rates of the packaging materials were determined using standard methods. The shelf life of the packaged samples were determined in which the microbial load, sensory evaluation and proximate analysis were determined at interval of 0day, 3rd day, 7th day,21day and 30th day of storage period. Sensory evaluation was evaluated by 10 panelists using 9 point hedonic scale. The isolated organisms are Bacillus spp, Enterobacter spp., Staphylococcus aureus, Klebsiella pneumonia, Micrococcus spp. the fungi isolated are Aspergillus fumigate, Aspergillus oryzae, Fusarium spp. Mucor, Rhizopus sp.and Saccharomyces cerevisiae. At 0day no organism was isolated from fish samples expect in smoked and oven dried panla fish. Their TVC ranges from $0.2x10^2$-$0.8x10^2$, on 3rd day the TVC ranges from $0.9x10^2$-$2.2x10^3$, on 7th day it ranges from $0.3x10^2$-$5.1x10^2$, at 14day it ranges from $2.5x10^2$-$8.5x10^2$, on 21st day ranges from $1.2.x10^6$-$8.2x10^6$ at 30th day the TVC/CFU/g ranges from $5.1x10^6$-9. Smoked mackerel and catfish have better taste than oven dried. Oven dried fish have better texture, colour, smell and general acceptability. The thickness, water absorption rate and oil absorption rate of packaging materials ranges from 0.4-3.7, 0.003-0.052 and 0.0001-8.799. PP and ATCP has the best sensory rating. Proper packaging of smoked fish can extend the keeping quality of the product.

Keywords: Aseptically, packaged, smoked, shelf-life, storage.

I. INTRODUCTION

Smoking is a century old method of food preservation. Fish smoking is one of the traditional fish processing methods aimed at preventing or reducing postharvest losses. Smoking involves heat application to remove water and it inhibits bacterial and enzymatic actions of fish (Kumolu Johnson et al., 2009, Abolagba and Melle,2008). Earlier authors (Olley et al., 1988, Clucas, and Ward 1996, Horner, 1997, Eyo, 2001, Sengor et al., 2004 and Olokor et al., 2007) also noted that apart from giving the product a desirable taste and odor, smoking provides a longer shelf-life through its anti-bacterial and oxidative effect, lowering of pH, imparting desirable colorations as well as accelerating the drying process and acting as antagonist to spoilage. Smoking is the most popular method of fish processing (Olley et al.,1988).

Eyo (2001) reported a 50% annual loss of the fish caught in Nigeria to post harvest spoilage irrespective of the preservation methods employed. The aim of food processing and preservation is to inhibit microbial growth, improve acceptability and above all extend the shelf-life of the products either by way of use preservatives, refrigerating or traditionally by either salt-curing or smoking. However, it is a suitable medium for growth of micro-organisms, if poorly processed (Oparaku and Mgbenka, 2012).In Nigeria, the social-economic status of rural fish farmers and consumers make smoking the most preferred choice of processing. According to Ighodaro and Abolagba (2010), smoking reduces the moisture content of fish to a point that it impairs the activities of spoilage microbes. In Nigeria, it has also been noticed that fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts

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across socio-economic, age, religious and educational barriers (Adebayo-Tayo et al., 2008). As earlier reported, the microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish are caught and not of the fish species; hence, the indigenous microbial populations of fish can vary significantly (Eyo, 2001). A similar report on fish confirmed that, fish because of their soft tissues and aquatic environment are extremely susceptible to microbial contamination. Millions of bacteria, many of them potential spoilers, are present in the surface slime, on the gills and in the intestines of live fish, although the flesh itself is normally sterile. Bacterial growth and invasion of the fish are prevented by the body's natural defense system during life but after death the defense system breaks down and the bacteria multiply and invade the flesh (Abolagba and Uwagbai, 2011).

Poor postharvest technology (handling, preservation and processing) have been reported earlier to have the ability to cause unhealthy situation resulting in massive spoilage. An estimate of 40% postharvest losses of total fish landings have been reported in Nigeria (Akande,1996). Saliu (2008) similarly reported that 15% of the total fish catch in Kainji Lake is lost because of spoilage and breakage between the sources of supply and the consumers. (Saliu, 2008) also reported that fish spoilage in Nigeria is influenced to a large extent by high ambient temperatures, considerable distances of landing ports to points of utilization and poor as well as inadequate infrastructure for post-harvest processing and landing. This research work was carried out to determine the shelf life of smoked fish using different packaging materials.

II. MATERIALS AND METHODS

Sample Collection

Twenty five (25) samples of frozen fish sold in the market was purchased. 6 replicates of 4 different smoke dried fish including Mackerel (Scomber scombrus), Sardine (Sardinela eba), Panla (Gadus morhua) and Cat fish (Clarias gariepinus) were randomly purchased from 5 different markets; in Awka and environs Anambra state. The fish samples were collected labeled appropriately and kept in sterile polythene bags for microbial analysis.

3.1 Preparation of Materials:

The working tables were swabbed with 70% ethanol to disinfect them. All the wares were washed and air-dried after which they were sterilized in hot air oven at 60^0C for 1hour. The 60 smoked fish samples were taken to Microbiology Laboratory of Nnamdi Azikiwe University, Awka and Anambra State Polytechnic, Mgbakwu for microbial analysis and fishery department of Anambra State Polytechnic, Mgbakwu was used for smoking of fish.

3.2 Sample Preparation:

a) **Smoking of fish: Two methods were used; smoking with traditional kiln and smoking with oven.**
b) **Smoking of fish with traditional kiln:** The collected fish samples were washed cleaned smoked for 4h under monitored ambient conditions. The fish smoking kiln was operated by first loading charcoal into the heat chamber, preheating for some minutes, and then loading the fish onto the trays in its central chamber, which was then closed for some time to allow the smoking to take place. The smoking time, temperature and ambient conditions was monitored during the smoking operations. The smoking was terminated when the fish were properly brown (Olayemi et al., 2011).
c) **Oven drying:** some foil was placed on the oven rack. The bent and pinned fish was placed on the foil lined oven rack and covered with another sheet of aluminum foil. The oven was set to 250°C / 480F and top and down heating (bake). The bake setting cooks the fish before the drying starts. The fish was baked for 15 minutes. All the foil sheets were removed and oven setting changed to Grill/Broil and grilled for 10 more minutes or till the fish browns.

3.2.1 Serial dilution:

Ten gram (10g) of each fish sample were selected at randomly which represented whole body of the fish both bone and skin were weighed aseptically and homogenized in 90ml sterile peptone water using electric blender. Then, serial dilutions were made by mixing I.0ml of the suspension in 9.0mlsterile peptone water to obtain 10^1 dilutions. Analysis was done at 3 months interval until the fish became inedible.

3.3 Media preparation:

Two media were used for isolation of fungi, Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). Nutrient agar was used for isolation of bacteria, Mannitol salt agar (for Staphylococcus spp); MacConkey agar (for E. coli and other enteric bacteria); Robertson cooked meat medium (for Clostridium botulinum) and Eosin Methylene blue agar (for enteric bacteria). Incubation was in an aerobic incubator for 24 hours

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at 37°C. After 24 hours, the bacteria colonies that appeared on plates were counted using a digital colony counter. The average colony counts from duplicate plates was obtained and expressed as colony forming units (c.f.u.) per gram of sample. Colonies on the plates was sub-cultured on nutrient agar plates to ensure purity of cultures. The different pure cultures were cultured in nutrient agar slant for identification and storage.

3.3.1 Sub-culture

After incubation period, discrete colonies from bacteria plates were picked with a flamed wire loop and sub-cultured onto a newly prepared nutrient agar plates. Also, a flamed knife was used to sub-culture different colour of mycelia growth from Sabrouraud agar. All plates were incubated appropriately. All nutrient plates were transferred into an incubator at 37^{o}C for 24hrs while all the sabrouraud agar plates incubated at room temperature for 48 hrs. Pure colonies and mycelia were transferred into agar slants and stored properly for further characterization.

3.3.2 Identification of isolates

Isolates were identified with the aid of keys and diagrams presented by Frazier and Westhoff (2004), Kogan (2001) Barnett and Hunter (2000); the following test were carried out: Gram staining, catalase test, citrate test, methyl red test, indole test urea test, coagulase test, sugar fermentation test, oxidase test, lactose test, glucose test. Mannitol test and motility test.

3.3.3 Isolation of Fungal Flora;

Ten gram (10g) of each fish sample was taken and crushed in a sterile mortar with pestle under laboratory condition. Nine milliliters sterile distilled water was added and serially diluted up to 10^6 fold as described by Syllabi and Façade (Ayolabi and Fagade, 2010). 0.1ml aliquots aseptically removed separately with a sterile pipette and transferred into labeled sterile Petri dishes and 20ml melted Potato Dextrose Agar (PDA) was added by pour plate method. The PDA (Biotech, USA) was prepared according to manufactures instruction. After rotating gently, the plates will be incubated at 27°C for 72 hours. Pure colonies was isolated from mixed culture and inoculated onto the surface of freshly prepared PDA which was supplemented with 30mg/ml of Chloramphenicol (Micro Lab Limited) to inhibit bacterial growth. The plates were incubated at 27°C for 72 hours.

3.3.4 Identification of fungi:

Fungal isolates was transferred to sterilized plates for purification and identification. The grown fungi was placed on a slide, stained with gram stain for yeast identification and lacto phenol cotton blue to detect fungal structures covered with a cover slip, examined under microscope and identified on the basis of their colony morphology and spore characteristics (Cheesbrough, 2000).

Macroscopic and microscopic observations was carried out on the cultures. The physical characteristics of the mycelia such as the colour and structure was noted as well as the microscopic characteristics (Barnett and Hunter, 2000).

Identification of isolates

Isolates were identified with the aid of keys and diagrams presented by Frazier and Westhoff (2004), Kogan (2001) Bernette and Hunter (1987); the following test were carried out: Gram staining, spore staining, catalase test, citrate test, methyl red test, indole test, urea test, coagulase test, sugar fermentation test, oxidase test, lactose test, glucose test,mannitol test and motility test.

Bacterial Counting

The Petri dishes containing the overnight culture that was obtained from serially dilution was placed on colony counter and the readingsweretaken. The number of colonies counted on the plates was recorded taking into consideration the dilution factor and used to calculate colony forming units (cfu) per ml.

3.4 Determination of Engineering Properties of Packaging Materials

Methods of Olayemi (2012), was used;

The two properties that were determined are the water absorption rate and oil absorption rate being important factors affecting the quality of smoked fish during storage. Other properties that were measured are thickness of all the packaging materials (with the aid of a micrometer screw gauge) and the opacity using visual inspection

3.5 Determination of Water Absorption Rate

The water absorption rates of the packaging materials were determined in accordance with BS 6504. Samples were immersed in water and the weight gain after 1 hour was measured and recorded.

Water absorption was calculated from the measurement using

Equation 1. $W_{AR} = \underline{W_f - W_i}$

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ATW_i

(1) Where, W_{AR} is the water absorption rate (g/cm²/min)
$W_f$is the final weight (g),
W_i is the initial weight before immersion (g),
A is the surface area of the immersed material (cm²), and
T is the time of immersion (min.)

3.6 Determination of Oil Absorption Rate
Methods of Olayemi (2012), was used;
The oil absorption rates for the packaging materials were determined using method BS 6504. Packaging materials were immersed in oil and increase in weights was determined after
one hour. The oil absorption rate will be calculated using

Equation 2. $O_{AR} = \frac{M_f - M_i}{ATM_i}$

(2)Where;
O_{AR} is the water absorption rate (g/cm²/min),
M_f is the mass of the immersed material (g),
M_i is the mass of the material before immersion (g),
A is the surface area of the material in cm²,
T is the time

3.7 Determination of Shelf life of Fish
3.7.1 The shelflife of smoked fish was determined using the following:
The cooled smoked fish samples were packaged in different packaging materials produced they are;

a. Polyethylene (PP) serve as control
b. Polyethylene carton (PCP)
c. Polyethylene cardboard paper (PCDP)
d. Polyethylene foil (PFP)
e. and in an air tight container,(ATCP)

The samples were stored at room temperature. Polythene bags were sealed using an electrical sealing machine (PFS-300). The samples were packaged in triplicate After that, five groups of smoke-dried fish product were kept for storage at refrigeration (4°C) temperature for further analysis. During determination of shelf life microbial loads, sensory test of the samples were determined at intervals of 0day,3rd day, 7th day, 14th day 21day, and 30th day of storage.

3.8 Sensory Evaluation
Method of Obi et al., (2010), was used;
The sensory characters of smoke-dried Mackerel (Scomber scombrus), Sardine (Sardinela eba), Panla (Gadus morhua) and Cat fish (Clarias gariepinus) fishes was evaluated on the basis of the color, odor, texture, taste and remarks.

All the samples was evaluated for organoleptic characteristics and overall acceptability by 10 panelists that comprised undergraduate students, teaching and non-teaching staff members of Anambra State Polytechnic, Mgbakwu, Anambra State, Nigeria; using nine (9) point hedonic scale ranging from like extremely (score = 9) to dislike (score = 1) as extremes (Obi et al., 2010). Prior to each assessment, the panelists were informed about the task of the test. In addition to the information, a detailed set of written instruction on testing method were available in each table. A portion of different samples of fish was served to each panelist and asked to freely evaluate, comment and score the samples and asked to taste, color, flavor, texture. Scale used was as follows, 9-Like extremely, 8-Like very much, 7-Like moderately, 6- Like slightly, 5-Neither like nor dislike, 4-Dislike slightly,3-Dislike moderately, 2-Dislike very much, 1-Dislike extremely.

To eliminate bias, un-labeled samples was presented to the panelist individually with sufficient privacy to guarantee independent judgment. The acceptability of the samples was based on the scores and remarks made by the panelists. The result of the test was assessed using the Hedonic preference test. The scores for the samples were analyzed statistically using the method of analysis- Anova (Snedecor and Cochran, 1976). Mineral and water was made available as neutralizers. The test was performed under conditions of standard light and temperature 20°C. The same subjects were used in all the steps of the sensory evaluation, so near accurate data collection could be obtained.
The sensory evaluation of the samples was done on 0day, 3rd day and 7th day till the samples gets bad. The analysis was done in triplicate.

III. RESULTS

Table 1: Total viable count of aseptically smoked and oven dried fish smoked in the lab packaged in polyethylene packaging and stored at room temperature

Days	Samples	Bacteria isolates Smoked fish	Fungi	Bacteria Oven dried	Fungi
0day	Mackerel	-	-	-	-

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	Sardine	-	-	-	-
	Panla	$0.8x10^2$		$0.2x10^2$	
	Catfish	$0.5x10^3$	-	-	-
	Mackerel	-	-	-	-
3rd day	Sardine	-	-	-	-
	Panla	$1.0x10^2$	$2.2x10^3$	$0.9 x10^2$	-
	Catfish	$1.6x10^2$	$1.4x10^2$	-	-
7th day	Mackerel	$1.3x10^2$	$2.0x10^3$	$0.6x10^2$	-
	Sardine	$1.7x10^2$	$0.9x10^2$	-	$1.7x10^2$
	Panla	$3.1x10^2$	$4.2x10^2$	$0.3x10^2$	$3.5x10^2$
	Catfish	$3.8x10^2$	$5.1x10^2$	$1.1x10^2$	$3.0x10^2$
14th day	Mackerel	$3.7x10^2$	$7.2x10^2$	$2.6x 10^2$	$4.2x10^2$
	Sardine	$3.4x10^2$	$6.0x10^2$	$3.3x10^2$	$3.6x10^2$
	Panla	$3.9x10^2$	$4.9x10^2$	$2.5x10^2$	$4.1x10^2$
	Catfish	$5.2x10^2$	$8.5x10^2$	$3.3x10^2$	$3.2x10^2$
21st day	Mackerel	$2.2.x10^6$	$7.8x10^6$	$1.2.x10^6$	$6.0x10^6$
	Sardine	$3.7x10^6$	$5.7x10^6$	$7.4x10^2$	$4.5x10^2$
	Panla	$7.1x10^6$	$8.2x10^6$	$5.3x10^6$	$6.6x10^6$
	Catfish	$3.5x10^6$	$6.9x10^6$	$4.1x10^6$	$5.0x10^6$
30th day	Mackerel	$9.4x10^6$	$8.9x10^6$	$7.3x10^6$	$7.3x10^6$
	Sardine	$7.5x10^6$	$4.8x10^6$	$6.2x10^6$	$5.1x10^6$
	Panla	$9.0x10^6$	$7.4x10^6$	$6.6x10^6$	$6.6x10^6$
	Catfish	$5.1x10^6$	$7.5x10^6$	$9.3x10^6$	$5.6x10^6$

Table 2: Engineering properties of packaging materials used

Packaging material	Thickness	Water absorption rate	Oil absorption rate
PCDP	0.4	5.628	8.7999
PFP	0.45	0.003	0.001
PCP	0.5	7.56	8.9
PP	0.5	0.045	0.0001
ATCP	3.7	0.052	0.0003

Keys:
PP: Polyethylene packaging
PCP: Polyethylene carton polyethylene packaging
PCDP: Polyethylene cardboard paper packaging
PFP: Polyethylene foil packaging
ATCP: Airtight container packaging

Table 3: Taste and Smell of smoked and oven dried fish samples from day 0 to 30

Fish Samples	Method	0 Day	3rd Day	7th Day	14th Day	21st Day	30th Day	Grand Mean
Fish Taste Ratings								
Mackerel	Smoked	8.67±0.577 b	7.67±0.577 b	7.67±0.577 a	5.67±0.577 b	0±0	0±0	4.94
	Oven dried	7.67±0.577 b	6.67±0.577 a	6.67±0.577 a	6.67±0.577 b	0±0	0±0	4.61
Sardine	Smoked	8.67±0.577 b	7.67±0.577 b	6.67±0.577 a	4.67±0.577 a	0±0	0±0	4.61
	Oven dried	8.67±0.577 b	8.67±0.577 b	6.67±0.577 a	6.67±0.577 b	0±0	0±0	5.11
Catfish	Smoked	8.67±0.577 b	8.67±0.577 b	7.67±0.577 a	6.67±0.577 b	0±0	0±0	5.28
	Oven	8.67±0.577	8.67±0.577	6.67±0.577	6.67±0.577	0±0	0±0	5.11

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	dried	b	b	a	b			
Panla	Smoked	6.67±0.577 a	7.67±0.577 b	6.67±0.577 a	6.67±0.577 b	0±0	0±0	4.61
	Oven dried	7.67±0.577 b	7.67±0.577 b	7.67±0.577 a	6.67±0.577 b	0±0	0±0	4.94
P-value (Fish Samples)		0.001	0.002	0.305	0.024	NA	NA	NA
P-value (Method)		1.000	1.000	0.368	0.006	NA	NA	NA
Fish Smell Mean Ratings								
Mackerel	Smoked	7.67±0.577 b	6.67±0.577 a	6.67±0.577 c	4.67±0.577 a	4.67±0.577 b	3.67±0.577b	5.67
	Oven dried	7.67±0.577 b	7.67±0.577 b	7.67±0.577 c	5.67±0.577 b	4.67±0.577 b	4.67±0.577b	6.33
Sardine	Smoked	7.67±0.577 b	6.67±0.577 a	3.67±0.577 a	3.67±0.577 a	2.67±0.577 a	2.67±0.577a	4.50
	Oven dried	8.67±0.577 b	8.67±0.577 b	5.67±0.577 b	5.67±0.577 b	3.67±0.577 a	3.67±0.577b	6.00
Catfish	Smoked	8.67±0.577 b	8.67±0.577 b	6.67±0.577 c	4.67±0.577 a	4.67±0.577 b	2.67±0.577a	6.00
	Oven dried	8.67±0.577 b	8.67±0.577 b	7.67±0.577 c	5.67±0.577 b	4.67±0.577 b	3.67±0.577b	6.50
Panla	Smoked	6.67±0.577 a	7.67±0.577 b	6.67±0.577 c	4.67±0.577 a	2.67±0.577 a	3.67±0.577b	5.33
	Oven dried	8.67±0.577 b	8.67±0.577 b	7.67±0.577 c	4.67±0.577 a	4.67±0.577 b	3.67±0.577b	6.33
P-value (Fish Samples)		0.024	0.002	0.000	0.235	0.001	0.024	
P-value (Method)		0.006	0.001	0.000	0.001	0.006	0.006	

Results are in Mean± Standard deviation. Means with the same letters of alphabet in a column are not significantly difference (P>0.05, DMRT)

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Table 4: Texture and Colour of smoked and oven dried fish samples from day 0 to 30

Fish Samples	Method	0 Day	3rd Day	7th Day	14th Day	21st Day	30th Day	Grand Mean
Mean Texture Rating								
Mackerel	Smoked	6.67±0.577a	5.67±0.577a	5.67±0.577a	5.67±0.577a	5.67±0.577c	3.67±0.577b	5.50
	Oven dried	8.67±0.577b	7.67±0.577c	7.67±0.577b	6.67±0.577a	5.67±0.577c	4.67±0.577c	6.83
Sardine	Smoked	7.67±0.577b	6.67±0.577b	6.67±0.577b	6.67±0.577a	3.67±0.577a	1.67±0.577a	5.50
	Oven dried	8.67±0.577b	7.67±0.577c	7.67±0.577b	6.67±0.577a	5.67±0.577c	3.67±0.577b	6.67
Catfish	Smoked	8.67±0.577b	8.67±0.577c	7.67±0.577b	6.67±0.577a	5.67±0.577c	1.67±0.577a	6.50
	Oven dried	8.67±0.577b	8.67±0.577c	7.67±0.577b	6.67±0.577a	6.67±0.577c	2.67±0.577b	6.83
Panla	Smoked	6.67±0.577a	7.67±0.577c	7.67±0.577b	6.67±0.577a	5.67±0.577c	5.67±0.577d	6.67
	Oven dried	8.67±0.577b	7.67±0.577c	7.67±0.577b	6.67±0.577a	4.67±0.577b	4.67±0.577c	6.67
P-value (Fish Samples)		0.024	0.000	0.024	0.368	0.002	0.000	
P-value (Method)		0.000	0.006	0.006	0.305	0.050	0.006	
Mean Colour Rating								
Mackerel	Smoked	6.67±0.577a	6.67±0.577a	7.33±0.577c	3.67±0.577a	3.67±0.577a	0.67±0.577a	4.78
	Oven dried	7.67±0.577b	6.67±0.577a	6.67±0.577c	5.67±0.577c	3.67±0.577a	2.67±0.577c	5.50
Sardine	Smoked	7.67±0.577b	7.67±0.577b	3.67±0.577a	4.67±0.577b	2.67±0.577a	1.67±0.577b	4.67
	Oven dried	8.67±0.577b	7.67±0.577b	5.67±0.577b	5.67±0.577c	2.67±0.577a	1.67±0.577b	5.33
Catfish	Smoked	8.67±0.577b	8.67±0.577b	6.67±0.577c	5.67±0.577c	3.67±0.577a	2.67±0.577c	6.00
	Oven dried	8.67±0.577b	8.67±0.577b	6.67±0.577c	4.67±0.577b	5.67±0.577b	2.67±0.577c	6.17
Panla	Smoked	7.67±0.577b	6.67±0.577b	7.67±0.577c	4.67±0.577b	3.67±0.577a	3.67±0.577c	5.67
	Oven dried	8.67±0.577b	8.67±0.577b	8.67±0.577d	6.67±0.577c	3.67±0.577a	4.67±0.577d	6.83
P-value (Fish Samples)		0.003	0.000	0.000	0.062	0.000	0.000	
P-value (Method)		0.006	0.050	0.025	0.001	0.018	0.006	

Results are in Mean Standard deviation. Means with the same letters of alphabet in a column are not significantly difference (P>0.05, DMR

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Table 5: General Acceptability Ratings of smoked and oven dried fish samples from day 0 to 30

Fish Samples	Method	0 Day	3rd Day	7th Day	14th Day	21st Day	30th Day	Grand Mean
General Acceptability Ratings								
Mackerel	Smoked	7.67±0.577a	6.67±0.577a	7.33±0.577c	3.67±0.577b	1.67±0.577a	0.67±0.577a	4.61
	Oven dried	7.67±0.577a	7.67±0.577b	7.67±0.577c	4.67±0.577c	1.67±0.577a	0.67±0.577a	5.00
Sardine	Smoked	8.67±0.577a	6.67±0.577a	4.67±0.577a	2.67±0.577a	1.67±0.577a	0.67±0.577a	4.17
	Oven dried	8.67±0.577a	7.67±0.577b	5.67±0.577b	4.67±0.577	2.67±0.577b	1.67±0.577b	5.17
Catfish	Smoked	8.67±0.577a	8.67±0.577b	5.67±0.577b	5.67±0.577d	4.67±0.577c	1.67±0.577b	5.83
	Oven dried	8.67±0.577a	8.67±0.577b	6.67±0.577c	5.67±0.577d	3.67±0.577c	2.67±0.577c	6.00
Panla	Smoked	8.67±0.577a	8.67±0.577b	6.67±0.577c	4.67±0.577c	3.67±0.577c	3.33±1.528c	5.94
	Oven dried	7.67±0.577a	8.67±0.577b	7.67±0.577c	6.67±0.577d	2.67±0.577b	2.67±0.577c	6.00
P-value (Fish Samples)		0.024	0.000	0.000	0.000	0.000	0.000	
P-value (Method)		0.305	0.050	0.003	0.000	0.305	0.301	

Results are in Mean Standard deviation.Means with the same letters of alphabet in a column are not significantly difference (P>0.05, DMRT)

Table 6: Sensory attributes of smoked and oven dried Fish Sample in different packaging

Samples	Packing	Taste	Smell	Texture	Colour	General Accept.
Ratings at 14 days						
Catfish	PP	6.33±0.577	6.67±0.577	7.0±1.000	5.67±0.577	5.67±0.577
	PCP	5.67±0.577	6.67±0.577	6.67±0.577	4.67±0.577	4.67±0.577
	PCDP	5.67±0.577	5.67±0.577	6.67±0.577	4.67±0.577	5.67±0.577
	PFP	4.67±0.577	3.67±0.577	5.67±0.577	5.67±0.577	5.67±0.577
	ATCP	5.67±0.577	6.67±0.577	7.33±0.577	5.67±0.577	5.67±0.577
	P-value	0.062	0.000	0.109	0.092	0.205
Sardine	PP	6.67±0.577	7.33±0.577	7.67±0.577	6.33±0.577	6.33±0.577
	PCP	5.67±0.577	6.67±0.577	7.67±0.577	4.67±0.577	4.67±0.577
	PCDP	5.67±0.577	5.67±0.577	4.67±0.577	4.67±0.577	4.67±0.577
	PFP	6.67±0.577	6.67±0.577	6.67±0.577	6.67±0.577	5.67±0.577
	ATCP	7.67±0.577	7.67±0.577	7.67±0.577	7.67±0.577	7.67±0.577
	P-value	0.008	0.015	0.000	0.000	0.000
Mackerel	PP	6.67±0.577	5.33±0.577	7.33±0.577	4.67±0.577	5.00±0.000
	PCP	6.33±0.577	4.67±0.577	6.67±0.577	4.33±0.577	3.67±0.577
	PCDP	5.67±0.577	4.33±0.577	6.33±0.577	4.00±1.000	4.00±0.000
	PFP	5.67±0.577	4.33±0.577	6.67±0.577	4.67±0.577	4.00±0.000
	ATCP	7.00±1.000	5.67±0.577	7.67±0.577	5.33±0.577	5.67±0.577
	P-value	0.132	0.057	0.092	0.256	0.000
Panla	PP	6.67±0.577	7.00±0.000	7.67±0.577	5.67±0.577	6.33±0.577

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	PCP	5.67±0.577	5.00±1.000	6.33±0.577	4.67±0.577	4.67±0.577
	PCDP	5.33±0.577	5.00±1.000	5.67±0.577	4.00±1.000	5.33±1.155
	PFP	5.00±1.000	4.33±0.577	5.67±0.577	4.33±0.577	4.33±0.577
	ATCP	7.00±1.000	6.67±0.577	8.00±0.000	6.00±0.000	6.67±0.577
	P-value	0.043	0.004	0.000	0.013	0.011
Ratings at 30 days						
Catfish	PP	-	2.67±0.577	3.00±0.000	2.33±0.577	2.33±0.577
	PCP	-	1.67±0.577	2.67±0.577	2.33±0.577	1.67±0.577
	PCDP	-	2.67±0.577	2.67±0.577	0.67±0.577	1.67±0.577
	PFP	-	2.67±0.577	3.67±0.577	1.67±0.577	1.67±0.577
	ATCP	-	3.67±0.577	2.67±0.577	1.67±0.577	2.33±0.577
	P-value	NA	0.024	0.152	0.030	0.171
Sardine	PP	-	4.33±0.577	5.33±0.577	2.33±0.577	3.0±0.000
	PCP	-	2.67±0.577	4.67±0.577	2.67±0.577	2.33±0.577
	PCDP	-	3.67±0.577	5.67±0.577	1.67±0.577	2.33±0.577
	PFP	-	1.67±0.577	4.67±0.577	1.67±0.577	1.67±0.577
	ATCP	-	3.67±0.577	5.67±0.577	2.67±0.577	2.67±0.577
	P-value	NA	0.002	0.130	0.130	0.089
Mackerel	PP	-	2.67±0.577	6.33±0.577	2.33±0.577	3.0±0.000
	PCP	-	1.67±0.577	5.67±0.577	2.67±0.577	1.33±0.577
	PCDP	-	2.67±0.577	5.33±0.577	1.67±0.577	1.33±0.577
	PFP	-	2.00±1.000	6.67±0.577	1.33±0.577	1.33±0.577
	ATCP	-	3.67±0.577	6.67±0.577	2.33±0.577	2.67±0.577
	P-value	NA	0.0040	0.057	0.092	0.042
Panla	PP	-	3.00±1.000	6.67±0.577	2.33±0.577	2.33±0.577
	PCP	-	2.67±0.577	5.67±0.577	1.33±0.577	1.33±0.577
	PCDP	-	2.33±0.577	5.00±1.000	2.00±0.000	1.00±0.000
	PFP	-	2.67±0.577	5.33±0.577	2.33±0.577	1.67±0.577
	ATCP	-	3.33±0.577	7.00±0.000	2.67±0.577	3.00±0.000
	P-value	NA	0.485	0.013	0.080	0.002

Keys:
PP: polyethylene packaging, PCP: polyethylene carton polyethylene packaging
PCDP: Polyethylene cardboard paper packaging, PFP: Polyethylene foil packaging
ATCP: Airtight container packaging

IV. RESULT AND DISCUSSION

The total viable counts (TVC) of aseptically prepared smoked fishes and oven dried fishes shows no growth of bacteria and fungi in both mackerel and sardine samples of smoked and oven dried fishes, $0.8x10^2$ and $0.2x10^2$ was recorded on panla smoked and oven dried respectively, $0.5x10^3$ bacterial load was recorded in smoked catfish, no fungi was isolated. The presence of microorganism in freshly smoked fish at 0day might be as the result of post processing contamination during cooling and packaging (FDA, 2001).

On 3rd day neither bacterial nor fungi was isolated from both mackerel and sardine smoked fish and oven dried fish. There was a slight increase on bacterial load on both panla and catfish smoked fish. $1.0x10^2$ bacterial loads were recorded and $2.2x10^3$ fungi load was recorded on panla smoked fish. Then $0.9x10^2$ bacterial load was recorded on panla oven dried fish, no fungi was recorded on oven dried fish while $1.4x10^2$ fungal load was recorded on panla smoked fish. $1.6x10^2$ bacteria $1.4x10^2$ fungal count was recorded on smoked catfish neither bacteria nor fungi was isolated on oven dried catfish. In almost all of the samples were records of bacteria growth and fungi growth in 7th day, the bacterial load ranges from $0.3x10^2$ -$3.8x10^2$ in which oven dried panla has lowest bacterial load and smoked catfish recorded the highest bacterial load respectively, mackerel oven dried recorded no fungal load. At 14th day all the samples recorded growth of both bacteria and fungi with an increase in bacterial and fungal load.

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The bacterial load of isolates ranges from $2.6x10^2$-$5.2x10^2$ in which smoked catfish recorded highest bacterial load and mackerel oven dried fish recorded lowest bacterial load. Fungal load ranges from $3.2x10^2$-$8.5x10^2$ in which oven dried catfish recorded the lowest TVC and smoked catfish recorded the highest TVC respectively. At 21st day both bacterial and fungal loads continued increasing, it was recorded in all the samples an increase in all the species of fishes used in which both bacteria and fungi counts continued increasing ranging from $1.2x10^6$-$7.1x10^6$ in which smoked panla fish recorded the highest count bacterial counts and oven dried mackerel recorded lowest bacterial counts. Fungi counts ranges from $4.5x10^2$-$8.2x10^6$, sardine oven dried recorded lowest while panla smoked fish recorded highest fungal count respectively. At day 30th it was deduced that both the fungi and bacteria count keep increasing. The bacterial counts ranges $5.1x10^6$-$9.0x10^6$ in which it was recorded that smoked catfish recorded the highest bacterial count respectively. The fungi count ranges from $4.8x10^6$-$8.9x10^6$ in which smoked sardine recorded the lowest and mackerel recorded the highest fungal counts respectively.

The increase in TVC during storage period might be attributed to multiplication of microorganisms as a result of changes in environment and temperature during storage. Smoked fish samples may have a relatively low water activity level which is a prerequisite for fungal growth. The increase in TVC of the sample is in agreement with (Dutta, et al., 2018) who worked on bacterial and fungal population assessment in smoked fish during storage period.

The result of the sensory evaluation of smoked and oven dried fish samples are shown in Tables 28-30. Findings on the taste of the fish samples revealed that in comparison between methods, the smoked mackerel fish (8.67±0.577 to 5.67±0.577) and smoked catfish fish (8.67±0.577) have better taste than oven dried fish samples while oven dried sardine (8.67±0.577 to 6.67±0.577) and oven dried panla fish (7.67±0.577 to 6.67±0.577) gave better taste than smoked fish samples. In comparison between fish types, the smoked catfish gave the best taste than any other fish type and methods. The taste of the fishes however decreased with days. Analysis of variance showed that the taste of the fishes differ significantly between fish types from Day 0 to 14 ($p<0.05$) and by method of preparation at day 14 ($p<0.05$, Table 28)

Result of the smell of the fish samples revealed that in comparison between methods, the oven drying methods gave better smell for all the fish type than smoke method. In comparison between fish types, the oven dried catfish gave the best fish smell (8.67±0.577 to 3.67±0.577) than any other fish types or method. The smell of the fishes however decreased with days, this might be as the result of multiplication of microorganisms which leads to spoilage of the samples. Analysis of variance showed that the smell of the fishes differ significantly between fish types for all observation days except day 14 ($p<0.05$) and by method of preparation from Day 0 to 30 ($p<0.05$, Table 28).

Result of the texture of the fish samples revealed that in comparison between methods, the oven drying methods gave better texture for all the fish type than smoke method. In comparison between fish types, the oven dried catfish gave better fish texture (8.67±0.577 to 3.67±0.577) than any other fish types or method. The texture of the fishes however decreased with days. Analysis of variance showed that the texture of the fishes differ significantly between fish types for all observation days except day 14 ($p<0.05$) and by method of preparation from Day 0 to 30 ($p<0.05$, Table 29)

Findings on the colour of the fish samples revealed that in comparison between methods, the oven drying methods gave better colour for all the fishes than smoke method. In comparison between fish types, the oven dried panla fish (8.67±0.577 to 4.67±0.577) gave best fish colour than any other fish types or method. The colour of the fishes however decreased with days. Analysis of variance showed that the colour of the fishes differ significantly between fish types for all observation days except day 14 and by methods from Day 0 to 30 ($p<=0.05$, Table 28). The decrease in taste, colour and smell of the fish samples is in agreement with that of Mosarrat, (2017) who also observed changed in smell, texture and color of the fish samples they studied. Result of the ratings of general acceptability of the fishes revealed that in comparison between methods, the oven drying methods had higher acceptability ratings for all the fishes than the smoke method. In comparison between fish types, the oven dried catfish (8.67±0.577 to 2.67±0.577) and oven dried panla fish (7.67±0.577 to 2.67±0.577) got higher ratings on general acceptability than any other fish types or method. The general acceptability of the fishes however decreased with days. Analysis of variance showed that general acceptability of the

fishes differ significantly between fish types for all observation days except day 0 and by methods from Day 3 to 14 ($p<=0.05$, Table 30).

Proper packaging serves to protect products from various contaminants and extends products shelflife (Coles et al., 2003; Marsh and Bugusu, 2007). Products that are properly packaged attract constmers and improve, and increased sales and expand market. Result on the sensory attributes of smoked and oven dried fish samples in different packaging methods are shown in Table 31. Findings revealed that between the 14 and 30 days of observation, the sensory ratings of catfish were higher in PP packaging method while sensory rating of sardine, mackerel and panla fish were relatively higher in ACTP packaging method. ACTP which is air tight container packaging was rated best among the other packaging method followed by polyethylene packaging, this deduction is in agreement with Osibona et al., (2018) who researched on storage fungi and mycotoxins associated with stored smoked catfish (Clarias gariepinus). It is also in agreement with Gopal and Shankar (2011), who also stated that requirements for suitable storage and packaging of smoked fish include inertness, leak proof, impermeability to oxygen and moisture, low transparency and resistance to abrasion and puncture. In catfish, the packaging methods examined showed a significant difference in the smell and texture of catfish at 14^{th} day and then smell, texture and colour at the 30^{th} day of observation ($p<0.05$). In sardine, the packaging method showed a significant difference in all sensory characteristics of sardine examined at the 14^{th} day and a significant difference in only smell and texture in at 30^{th} day of observation ($p<0.05$).

In mackerel, the packaging method showed a significant difference in the general acceptability of the fish at the 14^{th} day and then smell and general acceptability at the 30^{th} day ($p<0.05$). In panal fish, the packaging methods showed a significant difference in all sensory characteristics of the fish examined at the 14^{th} day and a significant difference in only colour, texture and general acceptability of the fish at the 30^{th} day ($p<0.05$, Table 31).

V. CONCLUSION

Proper packaging of smoked fish improves the keeping quality and shelf life of smoked fish, airtight container and polyethylene packaging proves to be a better packaging materials than other packaging materials used in this research work.

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Conflict of interest

The authors confirm no conflict of interest involved with any parties in this research work

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