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Exposure Time on Bacteria Flora/Count and Shelf-Life of Canned Mackerel (*Scomberomerus* Sp). Under Ambient and Cold Storage Conditions

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Abstract: Canned mackerel (*Scomberomerus* sp.*)* a widely accepted food product in the tropic, with shelflife of 3 years after production (Dec 2004-Dec. 2007) is being assessed after a 24 h exposure period under both cold (-4°C) and ambient temperatures (27°C). A total of 13 cans (Geisha brand of mackerel in tomato sauce) were analysed. The base line data was obtained at (0 day) with the first can. The remaining 12 cans were grouped into 2 samples in relation to the storage media adopted. After an opening the cans were kept at their respective storage media for 24 h exposure period before analysis. The readings were taken biweekly for 12 weeks. The assessment made were: organoleptic, biochemical Trimethyamine (TMA) Thiobarbituric acid (TBA) and period value (PV), microbial assay (count and identification) and proximate analysis (initial and final).The organoleptic parameters assessed were (texture, colour, rigidity of fillets and reaction of can with the product) which showed no significant variation ($p>0.05$) while odour, taste and appearance showed significant difference ($p<0.05$). The biochemical tests revealed that all parameters increased in their values in both media with increasing exposure/storage time. The ambient (PV = 4.380 meq kg^{-1}), TBA = 1.849 mg kg^{-1} and TMA = 2.531 mgN/100 fish) were greater than the cold medium (PV = 4.300 meq kg^{-1}, TBA = 1.638 md kg^{-1} and TMA = 2.20 gm N 100 g^{-1}). significant ($p<0.05$) increase were recorded for TMA and TBA with strong positive correlations ($r = 0.61$ and 0.63 respectively) with exposure storage time, while PV had a slower rate of increase with $r = 0.40$. The ambient medium showed fourteen *bacteria* sp. and four *fungi* sp. with a higher total viable count range of (2.8-40.7 x 10^4 $cfug^{-1}$) as against the cold medium which had eight *bacteria* sp. and no *fungi* sp. represented with a range of (2.8-7.8 x 10^4 $cfug^{-1}$). The bacteria growth in both media were significant ($p<0.05$) and present progressive increase in growth pattern with storage time. However cold storage at -4°C suppress the rate of multiplication of the identified bacteria. However, the most prominent bacteria spp at ambient temperature were (a) *Staphylococcus aureus* (27.4 x 10^4 $cfug^{-1}$) (b) *Pseudomonas aureginosa* (26.5 x 10^4 $cfug^{-1}$) and (c) *Bacillus cereus* (21.5 x 10^4 $cfug^{-1}$) whole for the cold (a) *Klebisella aerogenes* (6.6 x 10^4 $cfug^{-1}$) (b) *Micrococcus acidiophilus* (5.7 x 10^4 $cfug^{-1}$) and (c) *Bacillus cereus* (4.0 x 10^4 $cfug^{-1}$). All viable counts recorded were still below maximum microbial count limit of (1.0 x 10^6 $cfug^{-1}$). Canned mackerel in tomato sauce is advisably consumed before the 3 years expiry date and also immediately it is opened to prevent food poisoning. Since increase in bacteria count and biochemical values in both media with exposure/storage time were evident in this study.

Key words: Exposure time, ambient/cold storage, bacteria flora/count, shelflife, canned mackerel *Scomberomerus* sp.

INTRODUCTION

Canned fish have gained a successful market in the tropics in the past years. Its consumption is largely accepted like any other ready-to-eat food. Canning of fish involves the use of thermal processes sufficient enough to destroy all heat sensitive bacteria spores, inactivate the enzyme and cook the fish so that the product remains acceptable to the consumer after prolonged storage. During canning, good management practice guidelines are and must be strictly adhered to, in accordance with the Hazard Analysis and Critical Control Point (HACCP) standards in all the stages of the canning process, so that the end product is free from contamination and safe for consumption[1].

Mackerel (*Scomberomerus* sp.) a small pelagic fatty fish (of about 7-20%) is majorly processed and packaged in cans with tomato sauce. The major brands of canned mackerel found in the Nigerian markets are Geisha, Chef,. Diete, Pegion and Marinae brands. They are majorly

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imported products from Thailand and usually have a validity period of 3 years after production (Dec. 2004-Dec. 2007) as it is the case for the batch used in this study.

This study was conducted to investigate the effect of exposure time on the suitability of mackerel for consumption under ambient and cold storage conditions over a period of time and determining the bacteria flora and count as well as the shelf life of mackerel for consumption prior to the stated expiry date.

Fishes are the most abundant class of vertebrates, they inhabit various kinds of aquactic environment and are highly proteineous having in its tissue the essential amino acids methionine and lysine[2].

Fish goes bad easily, the cause of which as been attributed to series of complicated changes brought about in the dead fish by bacteria, indigenous enzymes and chemical reactions[3]. Bacteria occur naturally on the skin and in the slime of living fish, where they do no harm, but immediately after the fish's death, the bacteria begins to attack its tissues. In terms of microbial spoilage, *Clostridium botulinum* has been identified especially in canned fish, food poisoning has also been attributed to *Escherichia coli, Bacillus cereus, Staphylococcus aureus* e.t.c.[4]. Chemical and physiological changes that occur in harvested fish include formation of volatile bases particularly Trimethylamine (TMA), dimethylamine (DMA) and ammonia[1].

The two important raw materials in canning are the fish and the can[1]. This two factors must be properly taken care-of in order to ensure a good product.

Can composition and coating: Tin plates, aluminium containers and glass are the commonest packing materials used for canning. Tin plate is produced by the electrolyte deposition of tin on mild steel. When fish is to be packed in tomato sauce or any acid medium, the inside of the tin must be lined with an enamel or acid lacquer. This will prevent corrosion or reaction between the content and tin to form black stain, which is offensive to the consumer. Shell fish produce hydrogen sulphide, which will react with iron sulphide, producing a black precipitate. This black precipitate is offensive to the consumer. The solution is to apply a special lacquer of zinc oxide to the inside of the containers. This will react with sulphur to give a white instead of black stain which the consumer prefers[1].

There is no problem of sulphur staining in aluminium cans or glass since the metals do not dissolve in the usual medium used to can fish. Lacquer may also be prepared from different compounds such as polymerized fish oil, vinyls, Oleoresins and expoxide lacquers[1].

MATERIALS AND METHODS

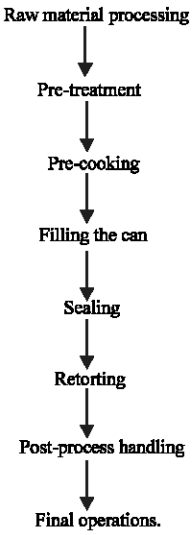
There is a direct and unavoidable, relationship linking raw material quality and end product quality and this hold as much for the production of canned fish as it does for fish which is bought fresh and prepared at home. Handling conditions immediately after catch are responsible for the rapid loss of the "as-fresh quality". The quality suffers whenever the raw material is temperature abused and/or physically damaged between catching and thermal processing[6]. Fish for canning can be trimmed to remove bruises and other localized flesh defects, this does not justify processing badly injured fish[7]. As the quality of fish deteriorates from the moment of death, it is therefore necessary to retard the rate at which undesirable, quality degrading, changes occur. Techniques which are recommended for the rapid inhibition of temperature related spoilage in freshly caught fish as reported by Graham[8] for canning include:

- the use of ice which is applied directly to the fish.
- Immersion in Chilled Sea Water (CSW) tanks,
- Immersion in Refrigerated Sea Water (RSW) tanks; or
- Freezing of fish harvested along distances from the cannery or for fish which is received fresh or chilled to be held in frozen storage until processing.

Other factors important in this stage include observation of hygienic practices, avoid excessive contamination with and proliferation of spoilage microorganisms and elimination of rodents, insect, birds or other vermin FAO/WHO,[9].

Grading of fish: It is often necessary to grade fish prior to canning. Grading systems may be for size and/or any of the sensory attributes which reflect fish freshness and ultimately end-product quality[4].

Canning process involves:



Raw material processing: As mentioned earlier, this involves all activities carried out to ensure that the fish is kept in proper condition.

Pre-treatment: This covers a range of operations during which the product is prepared for canning. This include operations such as; gutting, washing, nobbing, filleting, shelling (peeling), cutting, brining and dipping[10].

Pre-cooking: Usually carried out in steam, water, oil, hot air or smoke or a combination of these. For fish like tuna, mackerel and other tuna-like fish, pre cooking is carried out in steam at temperatures between 100°C and 105°C for as little as one hour for small species or over eight h for large specimens. The common aim is to raise backbone temperatures to between 60 and 85°C FAO/WHO,[11].

Filling: Usually done either automatically using machines or carried out manually. Filling is done such that allowance is given for the headspace to avoid excessive build up of pressure and damage to the hermatic seal FAO/WHO,[11].

Sealing: Is usually done using a seaming machine which hooks the lid and can body together by means of fairly tight but unwrinkled double seam[4].

Retorting: Is carried out under low temperature in order to reduce the risk of operational errors in the commonest sterilization equipment in the tropics[1].

Post-process handling: This involves chlorination and cooling water, used to cool the cans so as to prevent post-leaker spoilage[1].

Final operations: These are carried out to ensure that the product are in good condition before it is sent-out, it includes operations such as storage, labelling and cartoning[1,2].

Microbiology of fish: Micro-organisms are present on the surfaces of the fish and do not affect the fish during life, but after death, saprophytic and commensal residents invade the flesh and bring about its decomposition . The numbers and types of bacteria occurring on freshly caught fish will vary with geographical area, season and whether they are pelagic or demersal. In practice viable counts on marine fish using sea water media are only slightly higher than counts on fresh water[3]. Total viable numbers of bacteria on fish in the environment vary considerably. The commonest group of bacteria found are the Gram-negative Pseudomonas, *Alteromonas Moraxella, Acinetobacter, Flavobacterium, Cytophaga, Vibrio, Aeromonas* and the Gram-positive *Micrococcus* and *Coryneform* group[3].

Some bacteria that cause foodborne infections: Although many different organisms can grow in foods, only a few elaborate toxins can make foods dangerous to eat. Among the bacteria, the best known are *Staphylococcus aureus, Clostridium perfringens, C. botulinum* and *Bacillus cereus* as shown in Table 1. Various fungi-including several species of mushrooms and *Claviceps purpurea,* which produces ergot are poisonous when ingested. Certain members of the genera *Fusarium* and *Aspergillus* produce elaborate toxins that if they are in foods and feeds can be carcinogenic for humans or domestic animals. Other species of *Penicillium, Sclerotina, Pithomyces* and *Rhizoctina* that are not plant pathogens produce metabolites that have powerful estrogenic, toxic phototoxic or cholinergic effects.

Staphylococcal *enterotoxin*: Staphylococcal food poisoning is one of the most commonly reported food borne illness in the united states *S. aureau* resides as a saprophyte in the mucus secretions of the nasopharyhgeal region, it occurs more often in the posterior areas than the pharynx. The bacteria is forcefully ejected in forms of droplets during coughing and sneezing. It also cause localized infections such as pimples, boils, carbuncles and more generalized infections such as meningitis, osteomylitis and mastitis of human and animal[4].

Clostridium *botulinum*: Is a rare neuroparalytic disease caused by consuming foods containing toxin of *Clostridium botulinum.* Being a saprophyte, the organism seldom grows or produces toxin in live animal. It can only do so by growing in food. Orally ingested toxins, absorbed chiefly through the upper intestinal tract are transported to the lymphatic system. This inhibits endings to effector elements. Synapse blockage is accomplished by interfering with some unknown step in the release of acetylcholine from the nerve endings[4].

Bacillus *cereus*: Is readily isolated from nearly all plant foods, cooked food, dehydrated food and spices. Small numbers of spores and vegetative cells are swallowed daily with impunity. When larger numbers are present in foods, they produce an intoxication characterized by acute abdominal pain, flatulence and diarrhea which may result in death. The illness appears within 2-8 h after consumption of the food[4].

Microbial Toxemias. (sp.) of bacteria associated with food poisoning

Disease	Etiotigal agent	Principal types	Incubation period	Symptoms
Staph. Food poisoing or staph enterotoxin	*Staphylococcus aureas*	$A_1 B_1 C_1 C_2 D$ and E	1 to 6 h average 2 to 3 h	Abdominal Cramps, diarrhea, nausea, vomilting, acute prostration
Botulism	*Clostridium botulinum*	A, B, E and F (others not implicated in foods)	1/2 day to more than a week usually 1-2 days	Nausea, vomiting, dirrlea, early, double vision and later, difficulty in swallowing and speech, respiration. Often respiration paralysis and death. 8-24 h usually 10-12h
Perfringens food poisoning	*Clostridium perfringens*	A and C (other not implication in foods	8-16 h usually 11-12 h	Abdominal cramps, diarrhea, nausea malaise, vomiting rare.
B. cereus food poisoning	*Bacillus cereus*	-	10-24 h usually 12-14"	Abdominal cramps, diarrhea, nausea vomiting rare.
V. parahaemdyticus food poisoning	*Vibrio parahaemolyticus*		Usually 12-36 h	Abdominal cramps diarrhea fever, nausea, vomiting
Salmonelosis	Salmonella	-		Diarrhea, paino, fever and vomiting
	Source; colins and Lyre (1984)	-		

Mould infestation: The moulds commonly associated with fish product especially dried cured fish in storage are *Aspergillus hallophillus: A. restrictus; A. glaucus Wallemia sebi* and *Penicillum* sp.[12].

Aspergillus sp. and *Penicillus* sp. grow on fermented fish, smoked fish, salted fish and dried fish products and produce toxic substances known as mycotoxins. Ingestion of mycotoxins have been found to cause kidney dysfunction, liver damage, cancer and hepatoxin in mammals including man[13-15]. Certain types of fungi of the *Aspergillus flavus* group produce aflatoxin, which is a mycotin. Four commonly occurring aflatoxin are classified as B_1, B_2 G_1 and G_2. The letter refers to the colour of the fluorescence exhibited on thin layer chromato-garms (B = blue and G = green) and the suffixes to their respective positions on such chromatogram[15].

When moulds are discovered in dried fish, the fish should be properly cooked to destroy the toxin before it is consumed[1].

Mould infestation can be controlled by practicing general hygiene incorporating humectants, by reducing water activity and by the use of 1% solution of sorbic acid[16].

Quality Tests on Fish

Trimethylamine (TMA) test: The non-odorous trimethylamine Oxide (TMAO), common component of marine fish but not common component of fresh water fish is reduced to the odourous TMA characteristic of stale fish. The measurement of Volatile Reducing Substances (VRS) was developed to determine spoilage in protein food stuffs. This test (VRS) is applicable to fish that have been at sea during periods of sluggish catch and in which the changes in decomposition have not proceeded to the point of organoleptic detection. Such fish do not yield a canned product of good quality[4]. The level of TMA found in fresh fish rejected by sensory panels varies between fish species but is typically around 10-15 mgN 100 g^{-1} fish in aerobically stored fish and at a level of 30 mgN 100 g^{-1} fish in packed cod. At level of 40mgN/100g the fish is regarded unfit for consumption[17].

Thiobarbitaric acid test (TBA): The TBA test measures deterioration in both extractible and non-extractible lipids and is therefore applicable to flesh foods rather than pure oils. Advances in oxidative rancidity is indicated by the increase in the amount of red pigment in the reaction between 2-thiobarbituric acid and oxidized lipids. TBA is mostly applicable to fatty fish, the rancidity of the fat is considered to be a more useful indicator of fitness for consumption. It's accepted value limit is 2.7-8 malonaldhyde for fresh fatty food[5].

Peroxide Value (PV): The peroxide value is a measure of the peroxide contained in the oil. It is used to measure the degree of deterioration in pure oils. PV is usually determined volumetrically by methods developed by Lea[18]. Accepted value for grading noticeable rancid taste is 10-20 meq kg^{-1} which correspond to incipient spoilage[5].

MATERIALS AND METHODS

Thirteen cans of Geisha were bought at Agbeni market Ibadan and were divided into 2 groups/samples labeled A and B. The samples A were allotted to the cold

Table 1: Proximate composition of samples

	Initial	Final proximate	
		Cold	Ambient
Parameters %	Proximate	Storage (-4°C)	Storage (27°C)
Crude protein	20.14	18.24	16.05
Ether extract	10.75	8.75	6.24
Crude fibre	6.53	6.22	6.18
Ash	4.75	3.70	2.31
Moisture content	54.34	56.26	59.88
Nitrogen free extract	2.42	6.84	9.34

Table 2: Organoleptic assessment score sweet for samples under Cold (-4°C) and ambient (27°C) conditions
A samples under cold storage medium (-4°C)

Length of storage period (wks)								(x)		
Samples	0	2	4	6	8	10	12	Mean	0	2
Parameters determined	AB	A_1	A_2	A_3	A_4	A_5	A_6	Score	AB	B_1
Taste	6.0	5.5	5.5	5.5	5.5	5.5	5.5	5.5	6.0	5.0
Texture	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Odour	6.0	5.5	5.5	5.5	5.5	5.5	5.5	5.5	6.0	5.0
Appearance	6.0	5.5	5.5	5.5	5.5	5.5	5.5	5.5	6.0	6.0
Rigidity of fillets	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Colour	6.0	5.5	5.5	5.5	5.5	5.5	5.5	5.5	6.0	5.5
Reaction of can with product	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.00	6.0	6

Key: 1-Extremely Unacceptable, AB-Base line sample, 2-Poor, A-Cold Stored sample, 3-Unsatisfactory, B-Ambient Stored sample, 4-Fair, 5 -Satisfactory 6-Good, 7-Extremely acceptable

storage medium of -4°C temperature and samples B to the ambient storage medium of average temperature 27°C. The remaing can was used to generate base line data and was labeled AB. All the cans had the same production date (Dec 2004) and expiry date (Dec 2007). The average weight of the cans was 198.35 g.

The experiment was designed to last for 12 weeks analysis of samples were taken on a b_1-weekly basis. One sample from each of the storage medium was opened and returned back to there respective medium and are analysed after 24 h exposure. The base line data was first analysed marking the commencement of the experiment at Oday. Analysis carried out on the base line sample and other samples (A and B) are: Proximate analysis (initial and final), Biochemical analysis (TMA; TBA and PV), microbial identification and count and organoleptic assessment.

The proximate analysis according to AOAC[19] was carried out for initial and final fish samples for both the shelf and freezer stored samples after exposure time of 24h in each case. The organoleptic test was also done using a 5 man trained panel. The chemical assessment (Peroxide value (PV) determination through the method described by Lea[18]. The Thiobarbituric acid (TMA) determination through the method described by Pearson[20] while the trimethylamine levels were analysed through the method described by Dyer[21]. The isolation and identification of bacteria in both ambient and freezer stored samples were done using methods described by Harrigan and Mccance[22]. The procedures for phenotypic characterization of bacteria which includes Cultural profiles on different culture media Growth in air Grandstaining Coagulase ctalase and oxidase test and Carbohydrate fermentation for microbiological analysis.

Result obtained were subjected to; Analysis of variance using the complete randomized block design (CRBD).

- Least Significant Difference (LSD)
- Correlation analysis of the parameters
- Regression Analysis

The above tools were used to ascertain the viability of Geisha (*Scomberomerus* sp.) under ambient (27°C) and cold storage (-4°C) with exposure, time in view of the 3years expire labeled date.

RESULTS

The proximate composition of the samples revealed that lower values were recorded for the two media (ambient and cold) when compared to the initial. Although the cold stored samples had values close to the initial proximate as shown in Table 1.

The remaining indices scored 6.0. Parameters like odour, taste and appearance showed significant difference ($p<0.05$) with storage period/exposure time, while other parameter were not significant ($p>0.05$) as shows in Table 2.

Table 3: Biochemical assessment of samples under cold (-4°C) and Ambient (27°C) Condition A. Samples under cold storage medium (-4°C) B. Samples under Ambient storage (27°C)

Length of storage Period (wks)								(x)			
Samples	0	2	4	6	8	10	12	Value	0	2	4
Parameters determined	AB	A_1	A_2	A_3	A_4	A_5	A_6		AB	B_1	B_2
Peroxide Value (PV)											
Meq/kg × 10^3	0.200	1.610	2.330	2.870	3.220	3.760	4.300	18.290	0.200	1.820	2.570
Thiobarbituric Acid value0											
(tba) MG/KG	0.029	0.078	0.468	0.780	1.092	1.404	1.638	4.709	0.029	0.090	0.575
Trimethylamine acid value											
(TMA) mgN/100 gm fish	0.58	0.205	0.631	0.947	1.368	1.683	2.209	7.001	0.058	0.653	1.107

The biochemical assessment Table 3 revealed that all the indices considered (TMA, TBA and PV) increased with the period of storage in both media

Table 4: The correlation (r) between biochemical assessment/microbial assessment with exposure time/ storage period

Independent variable	Dependent variable correlation coefficient (R)	Decision
Peroxide Value (PV)	0.40 +ve	Significant
Thiobarbituric acid (TBA)	0.63* +ve	Significant
Trimethylamine acide (TMA)	0.61* +ve	Significant
Total viable count (TVC)	0.56* +ve	Significant

These indices showed a significantly ($p<0.05$) positive correlation ($r = 0.61$ and 0.63 for TMA and TBA) with storage length exposure time except for PV which showed a lower positive correlation ($r = 0.40$) shown in Table 4

Table 5: Isolated organism and their founts for samples under cold storage medium (-4°C) and ambient storage medium (27°C)

Length of storage Period (wks)								No.						
Samples	0	2	4	6	8	10	12	of	0	2	4	6	8	10
Isolated Organism cfug^{-1} (x10^4)	AB	A_1	A_2	A_3	A_4	A_5	A_6	Prevalence	AB	B_1	B_2	B_3	B_4	B_5
Bacteria Spp														
Bacillus subtilis	0.5	0.2	0.2	0.4	0.2	0.3	0.5	2.3	0.5	1.0	0.8	1.2	2.0	2.2
Bacillus cereus	0.4				1.0	1.2	1.4	4.0	0.4	2.5	3.0	3.2	3.8	4.0
Staphylococcus aureus	0.6				0.5	0.6	1.0	2.7	0.6	3.5	4.0	4.5	5.0	5.8
Pseudomanas aureginosa	0.5				-	-	-	0.5	0.5	4.0	5.0	4.0	4.5	4.0
Streptococcus faecium	0.3				-	-	-	0.3	0.3	0.3	2.5	3.0	3.8	3.5
Bacillus frimus					-	-	-	-	-	2.0	1.5	2.0	2.5	3.0
Pseudomanas florescences					-	-	-	-	-	0.1	0.4	0.5	0.8	1.0
Micrococcus acidiophilus		-	0.2	0.6	1.5	1.7	1.7	5.7	-	0.5	1.2	1.5	1.8	2.0
Streptococcus lactis					0.8	0.6	0.6	2.0	-	-	0.8	0.6	0.8	1.0
Proteus vulgariscus	0.3				0.4	-	-	0.7	0.3	-	2.0	1.2	1.6	2.5
Proteus morganii					0.3	0.3	0.4	1.0	-	-	1.0	1.0	1.5	2.8
Escherichia coli					-	-	-		-	-			0.5	1.0
Websiella aerogenes				0.2	2.0	2.2	2.2	6.6	-	-			0.3	0.6
Pediococcus halophilus									-	-			0.2	0.5
Fungi sp.														
Actinomycetes sp.	-	-	-	-	-	-	-	-		0.1	-	-	-	-
Fusarium oxysporium	-									-	-	-	0.1	0.4
Aspergillus niger										-	-	-	0.2	0.2
Penicillium oxalium										-	-	-	0.1	-
Total viable count (TVC)	2.8	0.2	0.4	1.2	6.7	6.9	7.8		2.8	14.0	22.2	22.9	29.5	34.5

In Table 5., The ambient medium was found to contain fourteen bacteria species and four fungi species. The most prominent bacteria in this medium are *Staphylococcus aureus* (27.4×10^4 cfug^{-1}), *Pseudomonas aureginosa* (26.5×10^4 cfug^{-1}) and *Bacillus cereus* (21.5×10^4 cfug^{-1})

The proximate composition of the samples revealed that lower values were recorded for the two media (ambient and cold) when compared to the initial. Although the cold stored samples had values close to the initial proximate as shown in Table 1. The percentage crude protein content in the initial sample was 20.14, which reduced to 18.24 and 16.05 in the cold and ambient media, initial ether extract was 10.75% which dropped to 8.75% and 6.24% in the cold and ambient media. Ash content and crude fibre also dropped from the initial values 4.75% and 6.53 to 3.70 and 6.22% in the cold medium for ash and crude fibre. The ambient medium recorded 2.31% and 6.18% for the ash and crude fibre content.

The organoleptic assessment showed that average score for parameters such as texture, rigidity of the fillets and reaction of can with product was 610, while the average score for taste, odour, appearance and colour has 5.5 under the cold stored medium. Considering the ambient condition, colour has an average score of 5.5, taste and odour scored 5.0 while the remaining indices scored 6.0. Parameters like odour, taste and appearance showed significant difference ($p<0.05$) with storage period/exposure time, while other parameter were not significant ($p>0.05$) as shows in Table 2.

The biochemical assessment Table 3 revealed that all the indices considered (TMA, TBA and PV) increased

with the period of storage in both media. These indices showed a significantly ($p<0.05$) positive correlation ($r = 0.61$ and 0.63 for TMA and TBA) with storage length exposure time except for PV which showed a lower positive correlation ($r = 0.40$) shown in Table 4.

Peroxide value at the ambient condition was 0.20-4.38 x 10^3meq kg^{-1} and 0.20-4.30 x 10^3meq kg^{-1} is for the cold medium. TMA values was 0.058-2.513 mg^{-1} N/100 and 0.058-2.209 mgN/100 for ambient and cold conditions. While the TBA values ranged from 0.029-1.849 mg kg^{-1} and 0.029-1.638mg kg^{-1} was recorded for ambient and cold mediums, respectively.

In Table 5, The ambient medium was found to contain fourteen bacteria species and four fungi species. The most prominent bacteria in this medium are *Staphylococcus aureus* (27.4 x 10^4 $cfug^{-1}$), *Pseudomonas aureginosa* (26.5 x 10^4 $cfug^{-1}$) and *Bacillus cereus* (21.5 x 10^4 $cfug^{-1}$). In the cold medium, eight bacteria species was found with no fungi growth. The predominant bacteria shown in this medium are: *Micrococcus acidiophilus* (2.3 x 10^4 $cfug^{-1}$).

The fungi implicated in the ambient medium are: *Fusarium oxysporium* (1.0 x 10^4 $cfug^{-1}$), *Aspergillus niger* (0.6 x 10^4 $cfug^{-1}$), *Pencillum oxalium* and *Actinomycetes* sp. has (1.0 x 10^4 $cfug^{-1}$) as shown in Table 5.

The microbial count for both media showed a gradual increase in its values as the period extends, value increase from 2.8 x 10^4 – 40.7 x 10^4 $cfug^{-1}$ in the ambient medium. The cold medium had 0.2-1.2 x 10^4 $cfug^{-1}$ for the first 6 weeks which was lower than that of the base line bacteria count (2.8 x 10^4 $cfug^{-1}$). Although the values still increased from 2-12 week (0.2-7.8x 10^4 $cfug^{-1}$). Microbial values increased significantly ($p<0.05$) and also showed a positive correlation ($r = 0.56$) with length of storage/ exposure time.

DISCUSSION

The proximate analysis showed that the exposed samples (under cold and ambient media) recorded lower values in their proximate constituents when compared with the baseline or initial proximate. Initial crude protein was 20.14% and was reduced to 18.24 and 16.06% for the cold and ambient conditions. Initial ether extract was 10.75% and while the final was 8.75 and 6.24% for cold and ambient conditions. The general decrease in most of the proximate constitutents of canned mackerel can be related to the effect of microograism which causes degradation of the conformational structure of the fish due to metabolic and chemical reactions they carryout Connell, FAO,[2,3,9].

From the organoleptic assessment, slight variations was noticed in both media (cold medium has an average score of 5-5-6.0 and the ambient medium has 5.0-6.0). This indicates that organoleptic assessment is not sufficient enough to be used for measuring deteriorative changes in canned mackerel exposed within the 24 h exposure time. The biochemical parameters showed a slight but progressive increase in their values as the length of storage period increases. The higher values recorded in the ambient medium supports claims that temperature plays an important role in fish deterioration, higher temperatures favours deterioration and lower temperatures keeps down spoilage[24,25].

PV values obtained in both media (Cold; 0.200-4.300 meq kg^{-1}, ambient: 0.200-4.380 mgq kg^{-1}) falls under the accepted value for grading noticeable rancid taste in the fish. Kirk and Sawyer,[5] Connell,[23] states that 10-20 mg kg^{-1} is the accepted value for testing rancidity. However the lower values recorded in the study confirm that peroxide formation is slow and may take weeks or months for any noticeable changes according to Oyelese *et al.*,[26].

Thiobarbituric acid (TMA) value also exhibited a gradual increase in both media. The 1.638 mg kg^{-1} for cold medium and 1.849 mg kg^{-1} in the ambient also falls below the recommend TBA range of 2.7-8 malonaldhyde August 26, 2006August 26, 2006August 26, 2006for fresh fatty fish Kirk and Sawyer,[5]. Like the earlier discussed biochemical parameters, the TMA values also increased from 0.058-2.209 mgN/100 fish for cold medium and 0.058-2.531 mgN/100 fish for the ambient condition. These values also falls below the stated total nitrogen values of 40 mgN/100 fish which is regarded as the value which shows that a fish is not fit for consumption[17,27].

The microbial count and identification exhibited a wide range of variations between the two media. From the ambient medium, 14 bacteria species and 4 *fungi* sp. were identified as against the cold medium which had only 8 *bacteria* sp. with no fungi growth which probably implies that cold storage suppresses bacteria multiplication to extent moreso the base line data sample (AB) value was 2.8 x 10^4 $cfug^{-1}$ which was much higher than values recorded for the first 6weeks (0.2-1.2 x 10^4 $cfug^{-1}$) in the cold medium, this implies that the cold storage initially suppressed bacteria multiplication has oppossed to the ambient medium which had a progressive increase from 2.8-40.7 x 10^4 $cfug^{-1}$.

The prominent bacteria under cold storage are; *Micrococcus acidiophilus* (5.7 x 10^4 $cfug^{-1}$), *Bacillus cereus* (4.0 x 10^4 $cfug^{-1}$) and *Bacillus subtilis* (2.3 x 10^4 $cfug^{-1}$). At ambient storage the predominant bacteria are: *Staphylococcus aureus* (27.4 x 10^4 $cfug^{-1}$), *Pseudomonas aureginosa* (26.5 x 10^4 $cfug^{-1}$) and *Bacillus cereus* (21.5 x10^4 $cfug^{-1}$). However all the values recorded are still

within the limit of acceptability since they are still below the limit of the maximum bacteria load (1.0×10^6 cfug^{-1}) recorded by Brasil[28]. Implicated fungi are *Fusarium oxysporium* (1.0×10^4 cfug^{-1}), *Aspergillus niger* (0.6×10^4 cfug^{-1}), *Penicillium oxalium* and *Actinomycetes* sp. has 0.1×10^4 cfug^{-1}.

Most of the identified bacteria and fungi are of concern to public health, as they are capable of causing food borne intoxications. *Bacillus cereus* is capable of causing abdominal cramp, nausea and diarrhea which may eventually lead to death. The toxin is destroyed when foods are reheated at 60°C[4]. The presence of *Escherichia coli* in the samples may suggest an indication of feacal contamination through man during handling. This bacteria causes diarrhea in man[4,29]. Likewise *Staphylococcus* sp. and *Pseudomanas* sp. were possible contaminant from handlers and utensils used especially after processing as reported by Amusa *et al.*,[19] *Staphylococcus aureus* is capable of causing food poisoning as well as causing localized infections such as pimples, boils, carbuncles and abcesses and more generalized infections such as meningitis, osteomyelitis, pneumonia and mastitis in human and animals[4].

CONCLUSION

Canned Mackerel in tomato sauce is advisably consumed before the 3 years expiry date and also consumed immediately the can is opened in view of results obtained in this study (biochemical and bacteria count) which showed progressive increase in both media (ambient and cold storage) with length of storage period/exposure time. It is therefore recommended that opened canned mackerel especially in tomato sauce should be consumed immediately it is opened to reduce the risk of food poisoning. Since as shown in this study the choice of a particular media of storage (whether cold or ambient) for the exposed canned mackerel in tomato sauce may not reduce the incidence of bacteria multiplication or risk of food poisoning. However *Micrococcus acidiophilus* with the highest bacteria count is a sensitive indicator for cold stored cans and *Staphylococcus aureus* for ambient stored products.

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