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Exposure Time on Bacteria Flora/Count and Shelflife of Canned Sardine (*Sardinella pilchardus)* Under Ambient and Cold Storage Conditions

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Abstract: This study is aimed at investigating the exposure time on bacteria flora/count and shelflife of conned sardine (*Sardinella pilchardus)* under ambient and cold storage conditions. Twenty five cans with an average weight of 165.05 g of the Titus (with an expiry date of 4 years(30/9/2004-30/9/2008 pf batch no 1432) were purchased and stored under the ambient at an average temperature of 27°C and cold (-4°C) storage conditions as samples for 12 weeks. Proximate analysis of the samples were taken at the beginning of the experiment and at the end for both the ambient stored and cold stored (after an exposure time of 24 h). Initial base line and biweekly studies were carried out for 12 weeks for (a) Ogranoleptic (odour, taste, texture, appearance, rigidity of fillet, colour and reaction of fish with can (b) Chemical (Trimethylamine (TMA), Peroxide Values (PV) and Thiobarbituric Acid (TBA) and lastly © Microbiological analysis for bacteria count and identification of the bacteria on the samples from each storage environment after an exposure time of 24 h in each cases. All the chemical parameters (TMA, TBA and PV) were significantly ($p<0.05$) correlated with exposure/storage time. Correlation coefficients r = 0.60, r = 0.66 and r = 0.54 were recorded respectively with all indicating spoilage rate increases progressively with exposure time/storage period. Highest PV ranges of (0.023-0.715), TBA (0.057-1.056) and TMA (1.01×10^3-$3.63 \times 10_3$) were recorded for canned sardines stored at ambient temperatures of 27°C. However these are still within acceptable tolerance limit. Organoleptic assessment with average scores of 5.5 and 6.0 recorded for cold and ambient stored samples. No viable bacteria count was recorded for cold stored samples throughout the experiment. However the range initial 0.1×10^4 and final 5.0×10^4 cfug $^{-1}$ total viable count recorded for ambient storage were still below the minimum bacteria count for spoilage, that could cause significant or deleterious effect that could result in food poisoning. Traces of the following bacteria sp. were recorded at ambient temperatures (a) *Bacillus subtilis* (1.2×10^4cfng^{-1}) (b) *Streptococcusfaecium* (0.9×10^4 cfng^{-1}), © *Proteus vulgaricus* (0.7×10^4 cfng^{-1}) (d) *Pediococcus halophilus* (0.6×10^4 cfng^{-1}) (e) *Micrococcus acidiphilus* (0.4×10^4 cfug^{-1}) (f) *Streptococcus lactis* (0.4×10^4 cfng^{-1}) and (g) *Aerobacter aerogenes* (0.4×10^4 cfng^{-1}) w hile fungi sp. *Aspergillus terrens* (0.1×10^4 cfng^{-1}) *Aspergillus niger* (0.3×10^4 cfng^{-1}) were recorded also for samples stored at ambient temperature of 27°C. Hence in view of this the four years recommended expiry data may be upheld for canned sardine *(Sardinella pilchardus* fish products in oil sources provided the HACCP (Hazard Analysis and Critical control points) and closely monitored. It is therefore recommended that exposure of c anned sardine in oil should not exceed 12-24 h under whatever food storage temperatures to avoid food poisoning.

Key words: Exposure time, ambient/cold storage, bacteria flora/count, shelflife, canned sardine *(Sardinella pilchardus)*

INTRODUCTION

Fish spoilage is one of the greatest problems affecting the finishing industry the world over and attempts are being made to reduce fish spoilage to the barest minimum. Wastage of fish through spoilage has been estimated at 20-50% of domestic fish production in tropical countries Eyo[1]. In an attempt to bridge the gap between demand and supply and avert the problems association to unbalanced nutritional status of the citizenry particularly in places where fish contributes significantly to the protein intake of the people. Huge

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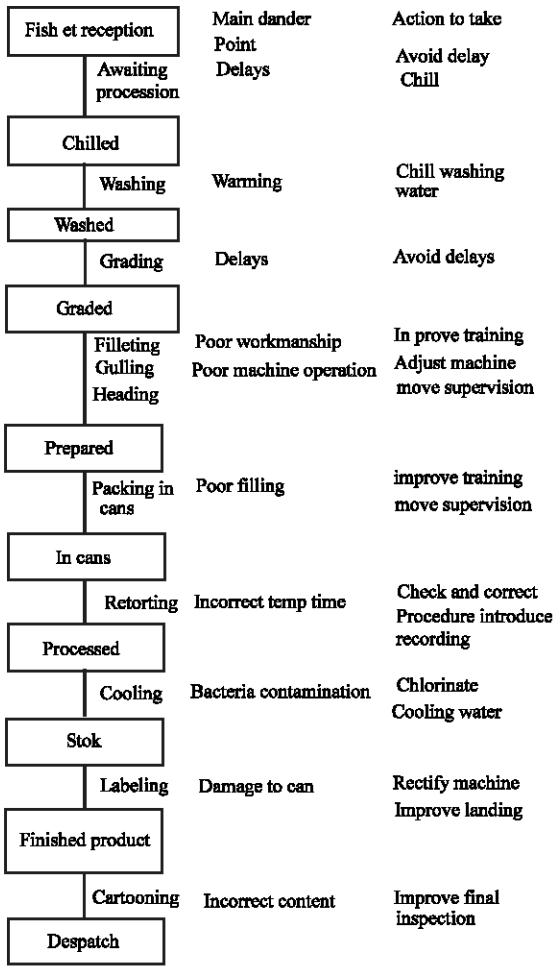
amount and efforts have been invested into the various preservations means, so as to reduced the wastage associated. This brought about the much cherished canned fish products. It is all about subjecting a good quality fish into a process involving heat treatment of the fish in a sealed container made of tin plate, aluminium can or glass, until the product has been fully sterilized. The degree of freshness of the raw material (fish) and it's preservation go a long way to determine the quality and the shelflife of the processed canned product. Fish intended for canning must be in first class condition and must be handled in an hygienic manner to reduce microbial load on the fish.

During post harvest handling in transit to Cannery, the fish inevitably become contaminated with other bacteria, these will further accelerate spoilage FAO[2]. Eyo[1] also reported that studies with marine and fresh water species have shown that the longer the delay before fish are chilled after death, the faster will bacteria and enzymic spoilage occurs, hence the shorter the storage life on ice. Spinelli *et al.*[3] also said the conformational structure of fish proteins is easily changed by changing the physical environment. During storage, peroxide formation is slow at first, that is at an induction period which may vary from few weeks to several months according to the particular oil or fat, the temperature etc Oyelese *et al.*[4]. Peroxide, tolerance limit value is set at 10-20 mg e.g per 100 g extracted fat and value of 20-40 mg e.g per 100 g extracted fat are said to correspond with noticeable rancid tastes Dyer[5], Likewise acceptable set limit thiobarbituric acid value (TBA) for fresh fully food is 2 x 7.8 ma/on a/hyde Pearson[6]. Connell[7] also proposed an acceptable trimethylamine value (TMA) of 10-15 mg TMAN per 100 g fish. Eyo[1] revealed that the dominating micro flora of tropical fish are the Gram positive forms. Food spoilage micro-gunisms are capable of inducing deleterious changes in various food products through their characteristic features such as heat resistance, growth requirements and adaptation to extrinsic and process parameters Efiuvwevwere *et al.*[8]. Thus their survival in processed foods makes then a potential source of food spoilage and health hazards Peck[9]. similarly lipolytic microorganisms are commonly associated with the spoilage of fatty and oily food Mossel *et al.*[10] some of these microorganisms include *Clostriduim botulinum, Bacillus cereus* and *Clostridium perfringes* etc. It is therefore of interest to ensure that quality assurance and HACCP (Hazard Analysis and Critical Control Point) are put is place.

Conning-simplified flow chart:



Source:-Connell[7]

Since sardine were packaged under vaccum atmosphere during processing, The little time of exposure will render the passive micro-organisms active and also lead to their rapid multiplication due to the high ambient temperature in the tropics which hastens fish spoilage by accelerating the activities of bacteria, enzymes and chemical oxidation of fat in fish flesh. It is therefore the aim of this study to investigate.

- The effect of exposure time on the suitability of sardine *(Sardinella pilchardus)* for consumption under ambient and cold storage conditions.
- Assess organoleptically chemically and determine the bacterial flora count over a period at ambient and cold storage conditions.
- Determine the shelflife of the canned sardine and the suitability for consumption prior to expiration of labeled expiry date.

MATERIALS AND METHODS

Titus sardine *(Sordinella pilchardus)* brand was selected for the study. Twenty five cans were purchased at Agbeni market, ensuring that they were all of the same batch number 1432, of known manufacture date (30 September 2004) and expiry (30 September 2008). The average weight of the can was 65.05 g twenty-four can were divided into two groups A and B samples A were labeled A_1, A_2, A_3 ……. And were placed on the shelf at room temperature of 27°C (the ambient samples) samples b were labeled B_1 B_2 B_3 ……. and were also placed in the freezer at temperature of-4°C (the cold stored samples). While the remaining one was used to generate the base line sata the day (o day) the experiment started.

Samples of sardine were picked on forthright basis and opened for 24 h (i.e., exposure time of 24 h before analysis were carried out) in their various storage environment before the tests were carried out. The study lasted for 12 weeks and readings were taken biweekly.

The proximate analysis according to AOAC[11] was carried out for the sardine initial and final proximate analysis for both the shelf and freezer stored samples after exposure time of 24 h in each case. The organoleptic test was also done. Likewise. The chemical assessment (Peroxide Value (PV) determination through the method described by Lea[12]. The Thiobarbituric acid determination through the method described by Pearson[6], while the trimethylamine through the method described by Dyer[13]. The isolation and identification of bacteria in both ambient and freezer stored samples were done using methods described by Harrigan and McCance[14] and Collins and Lyne[15]. The procedures for phenotypic characterization of bacteria which includes Cultural profiles on different culture media, growth in air gram staining Coagulase, catalase and oxidase test and carbohydrate fermentation for microbiological analysis.

ICMSF International Commission on Microbiological Specification for Foods.

RESULTS

The final proximate analysis revealed a fall in the value of the various constituents of the sardine stored at both ambient and cold conditions form the initial while the effect is higher with the sample stored at ambient temperature as shown Table 1. grude potein initial percentage 22.38% felt to 19.38% in ambient tropical temperature (27°C) and 2.25 in cold storage temperature (-4°C). Ether extract initially 32.50% felt to 29.52% at ambient storage and 30.40% at cold storage etc as shown in Table 1. the organolpetic assessment revealed that there is no significant changes in the average score of the various parameters tested. The average score for taste, odour and appearance was 5.5 while parameters like colour, reaction with can, texture and rigidity of fillet 6.0 for ambient stored product, as shown in Table 2.

The chemical assessment revealed that all the chemical indices (TMA, TBA and PV) were significant ($p<0.050$) increase with the period of storage in both media e.i ambient tropical temperature and cold temperature. Only that the rate of increament is slower in cold stored sardine, as shown in Table 3.

The PV rose from (1.01×10^3m e.g kg^{-1}) at 0 week, to (3.63×10^3m e.g kg^{-1}) and from (1.01×10^3m e.g kg^{-1}) to 1.01×10^3m e.g kg^{-1} in both ambient and cold storage conditions respectively, at the 12th week. The thiobarbituric acid at an initial of (0.057 mg kg^{-1}) at 0 week rose to (1.056 mg kg^{-1}) and (1.014 mg kg^{-1}) in both ambient and cold storage temperature respectively, at the 12 week. The trimethylamine of an initial of (0.023 mg 100 g^{-1}) at 0 week, rose to (0.715 mg 100 g^{-1} and (0.687 mg 100 g^{-1}) in both ambient and cold storage temperature, respectively at the 12th week. As shown in Table 4.

Seven different types of bacteria were found and two types of fungi from 0-12th week. Majority of the isolated bacteria were gran positive. The bacteria included *Bacillus subtilis, Streptococus lactis, Micrococcus acidiopholus, Proteus vulgaricus, streptococcus foecium Aerobacter aerogenes*. While the fungi included *Aspergillus niger* and *Asperhillus terreus*. The isolated bacteria and fungi were from the ambient stored samples whole there was no bacteria or fungi isolates from the cold stored samples. The total viable count witnessed was 5.0×10^4 $cfug^{-1}$ throughout the study, with *Bacillus subtilis* dominating (1.2×10^4 $cfng^{-1}$) followed by *Streptococcus faecium* (0.9×10^4 $cfng^{-1}$) and *Proteus vulgaricus* (0.7×10^4 $cfng^{-1}$), as shown in Table 5.

The statistical analysis reveals that there is a negatively correlated relationship between the total viable count and the length of storage while the relationship between oil-form and length of storage is a positive one.

The prediction equation Table 6 gives the shelflife of the product at a known trimethylamine (TMA) or Peroxide Value (PV) levels.

The proximate analysis revealed a decrease in the values of most of the constituents of the canned sardine, for both stored at ambient tropical temperature (27°C) and cold (-4°C) storage conditions.

Crude protein initially was 22.38% and decreased to 19.38% at ambient temperature storage and 20.25% in cold storage temperature. Moisture content initially 50.515 decreased to 45.62% at ambient temperature and 47.755 at

Table 1: Proximate composition of sardine stored at ambien temp. (27°C) and cold storage (-4°C)

Parameters	Initial reading (%)	Ambient temperature .Storage final reading (%)	Cold storage temperature final reading (%)
Crude protein	20.38	19.38	20.25
Esther extract	30.80	29.52	30.40
Crude fibre	0.24	0.18	0.22
Ash	2.05	2.98	3.70
Moisture content	45.81	45.62	44.12
Nitrogen free extract	0.72	2.32	1.31

Table 2: Avrage scores of organoleptic assessment of canned sardine fish

	Ambient temp. Storage (27°C)							Cold temp. Storage (-4°C)						
Week parameters Determined	0	2	4	6	8	10	12	0	2	4	6	8	10	12
Taste	6	5.5	5.5	5.5	5.5	5.5	5.5	6	5.5	5.5	5.5	5.5	5.5	5.5
Texture	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Odour	6	5.5	5.5	5.5	5.5	5.5	5.5	6	5	5	5	5	5	5
Appearance	6	5.5	5.5	5.5	5.5	5.5	5.5	6	6	6	6	6	6	6
Rigidity of fillet	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Colour	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Reaction with can	6	6	6	6	6	6	6	6	6	6	6	6	6	6

Table 3: The relationship between the chemical assessment and period of assessment

Independent variable	Dependent variable correlation coefficient (R)	Decision
Peroxide Values (PV)	0.54* +ve	Significant
Thiobarbituric Acid value (TBA)	0.66* +ve	Significant
Trimethylamine acid value (TBA)	0.60* +ve	Significant

Source:- Field Survey 2005 * Correlation in significant at 5%

Table 4: Chemical assessment of samples under ambient and cold storage conditions

	Ambient Temp (27°C)							Cold Storage Temp (-4°C)						
Week	0	2	4	6	8	10	12	0	2	4	6	8	10	12
Peroxide value (PV) m eq kg^{-1}	$1.01x10^3$	$1.70x10^3$	$1.82x10^3$	$2.17x10^3$	$2.60x10^3$	$2.91x10^3$	$3.63x10^3$	$1.01x10^3$	$1.6x10^3$	$1.79x10^3$	$2.15x10^3$	$2.15x10^3$	$2.87x10^3$	$3.58x10^3$
Thiobarbituric Acid value Mgkg^{-1} (TBA)	0.057	0.081	0.236	0.415	0.858	0.840	1.056	0.057	0.078	0.234	0.390	0.702	0.858	1.014
Trimethylamine Mg/100g (TMA)	0.023	0.114	0.248	0.245	0.315	0.553	0.715	0.023	0.098	0.196	0.196	0.294	0.419	0.687

The trimethylamine at an initial of (0.023 mgN/100g) at 0 week, rose to (0.715mgN/100g0 and (0.687 mgN/100g) in both ambient and cold storage temperature respectively at the 12th week. As shown in Table 4

Table 5: Microbial load of canned sardine (titus) under ambient temperature (28°c) storage

Isolated micro Week Organisms	O $cfug^{-1}$	2 $cfug^{-1}$	4 $cfug^{-1}$	6 $cfug^{-1}$	8 $cfug^{-1}$	1O $cfug^{-1}$	12 $cfug^{-1}$	
Bacteria								
Bacillus subtilis	-	$0.5x10^4$	-	$0.1x10^4$	$0.2x10^4$	$0.2x10^4$	$0.2x10^4$	$1.2x10^4$
Streptococcus lactis	-	-	-	-	$0.1x10^4$	$0.2x10^4$	$0.1x10^4$	$0.4x10^4$
Micrococcus acidiphilus	-	-	-	-	-	$0.2x10^4$	$0.2x10^4$	$0.4x10^4$
Proteus vulgarius	-	$0.3x10^4$	-	-	$0.2x10^4$	$0.2x10^4$	-	$0.7x10^4$
Pediococcus halophilus	-	$0.4x10^4$	-	-	$0.1x10^4$	-	$0.1x10^4$	$0.6x10^4$
Aerobacter aerogenes	-	-	-	-	$0.1x10^4$	$0.3x10^4$	-	$0.4x10^4$
Streptococcur faecium	-	$0.8x10^4$	-	-	-	$0.1x10^4$	-	$0.9x10^4$
Aspergithus terreus	$0.1x10^4$	-	-	-	-		-	$0.1x10^4$
Aspergillus niger	-	$0.1x10^4$	-	-	-	$0.2x10^4$	-	$0.2x10^4$
TVC	$0.1x10^4$	$2.1x10^4$	-	$0.1x10^4$	$0.7x10^4$	$4.1x10^4$	$0.6x10^4$	$5.0x10^4$

The total viable count recorded was 5.0×10^4 $cfug^{-1}$ throughout the study, with *Bacillus subtilis* dominating (1.2×10^4 $cfng^{-1}$) followed by *Streptococcus faecium* (0.9×10^4 $cfng^{-1}$) and *Proteus vulgaricus* (0.7×10^4 $cfng^{-1}$), as shown in Table 5

Table 6: Shelflife of canned sardine (*Sardinella pilchardus)* prediction

Parameter	R	R^2	Prediction equation
Trimethykamine (TMA)	0.60049	0.361	$TMA = A + Bn^m \pm SE$ = 0.099 + 0.47 x 0.849±0.192
	R	R^2	
Peroxide Value (PV)	0.43142	0.186	$In\ (PV) = A + Bn^m \pm SE$ = 7.404 + 0.109 n 0.576±0.344

cold storage. Crude fibre initially 0.245 decreased to 0.18% at ambient temperature and 0.22% at cold storage etc. This is in line with FAO[2] that the values of the constituents are affected due to the various chemical reactions and changes induced by bacteria. Also Spinelli *et al.*[3] revealed that the conformational structure of fish proteins is easily changed by changing the physical environment.

During the organioleptic assessment of canned sardine the taste panel results showed that they are kept in a good condition for the period of the study. Due to the oil content of the fish product the taste, odour and colour were all maintained at ambient temperature while the cold stored samples recorded no alternations, therefore no off odour/flavour was also recorded in both. This in line with the first phase of characteristics pattern of deterioration of fish stored in ice according to FAO[2]. The chemical indices are means of detecting extent or degree of deterioration both in fish and lipid. Peroxide Value (PV) produced was 2.62×10^3 m e.g kg^{-1} 2.57×10^3 m e.g kg^{-1} for both ambient and cold storage conditions respectively throughout the period of the study. Since the data obtained from the canned product revealed that before the experiment, a period of 34 weeks were spent on the shelf in the open market, which yielded 1.01×10^3 m e.g kg^{-1} of PV. This indicates that during storage, peroxide formation is slow at first, during an induction period which may vary from few weeks to several months according to the particular oil or fat, the temperature. This conforms with Oyelese *et al.*[4] and Dyers[5] work in which peroxide value was (0.2-3.5) from 10th-30th week, which has not exceeded the PV for fresh oils (10-20 m eg kg^{-1} extracted fat) PV of 20-40 m eg kg^{-1} extracted fat are said is correspond with noticeable rancid taste.

Thiobarbituric acid (TBA) for the ambient temperature steadily increases from (0.057 to 1.056) mg kg^{-1}. This values are not above the acceptable set limit by Pearson[6] of 2 x 7.8 malonaldehyde. Since TBA is an index for measuring secondary stage (last stage) of lipid oxidation. Has not taken place to any great extent that could course damage.

Trimethylamine values produced by both ambient and cold storage were 0.692 mg 100 g^{-1} and 0.6644 mg per 100 g, respectively throughout the period of the study. The values are not above the acceptable value limit proposed by Connell[7]. 10-15mg TMA N/100 g tissues should be considered maximum allowable.

All the chemical parameters (TMA, TBA and PV) were significantly ($p<0.05$) correlated with time of storage. Correlation coefficients (r) between PV, TMA, TBA and length of storage/exposure time were 0.54, 0.60 and 0.66 respectively, indicating that spoilage rate increases progressively throughout the duration of the experiment.

The total microbial count witnessed in the ambient stored sample 5.0×10^4 $cfng^{-1}$. This is low to the limit bacterial count of sardine (1.0×10^6 $cfng^{-1}$) Brasil[15]. Though low microbial count should not be regarded as indicator of good quality unless the types of micro-organisms colonizing the media are knopwn Eyo[1]. Meanwhile most of the isolated bacteria were gram positive bacteria, they include *Bacillus subtilis* (1.2×10^4 $cfug^{-1}$), *Streptococcus faecium* (0.9×10^4 $cfug^{-1}$), *Proteus vulgaricus* (0.7×10^4 $cfng^{-1}$), *Pediococcus halophilus* (0.6×10^4 $cfng^{-1}$), *Aerobacter aeropenes* (0.4×10^4 $cfng^{-1}$), *Micrococcus acidiphilus* (0.4×10^4 $cfng^{-1}$) and *Streptococcus lactis* (0.4×10^4 $cfng^{-1}$) and fungi spp isolated are *Aspergillus niger* and *Aspergillus terreus* of (0.3×10^4 $cfng^{-1}$) and (1.0×10^4 $cfug^{-1}$), respectively. This is in line with Shewan[16] that Gram positive bacteria such as *Bacillus* dominate tropical waters. All were isolated for samples stored at ambient tropical temperature. Based on the revelation of the statistical analysis of a negative relationship of the total viable count with length of storage which could be due to the oil used in the product, which tend to prevent multiplication of the micro-organisms by suppressing them till they are destroyed. The positive relationship of the coliform with length of storage implies that the longer the period of storage, the higher the coliform formation in the fish product, that is the higher the risk of contamination if formed coliforms are poisonous spare producers. It can easily initiate spoilage. Some of the these isolated bacteria are coliform (i.e., pathopenic). Most coliform bacteria have been implicated in food poisoning outbreak of some products Frazier *et al.*[17]. *Bacillusi* species are spore formers whose spore could survive high temperature of processing, as it is the situation in this study with the incidence of *Bacillus subtilis* (1.2×10^4 $cfng^{-1}$) tripling the counts for *Streptococcus lactisi.* (0.4×10^4 $cfng^{-1}$), *Micrococcus acidiphilus* (0.4×10^4 $cfng^{-1}$) and *Aerobacter aerogenes* (0.4×10^4 $cfng^{-1}$) also found in the study.

For spoilage to be quickly detected in stored canned *Sardinella pilchardus* the first three bacteria *Bacillus subtilis, Streptococcus faecium* and *Proteus vulgarius* should be looked out for especially *Bacillus subtilis* (1.2×10^4 $cfng^{-1}$) under ambient tropical temperature. However the fact that this study did not implicate these bacteria under cold storage, the possibility of contamination by these bacteria cannot be possibly ruled out. The fungi isolated could be as result of contamination, this can be reflected in their numbers and times of occurrences.

The shelflife of the canned fish product is determined with the prediction equation for known values of Trimethylamine or Peroxide value.

DISCUSSION

The proximate analysis revealed a decrease in the values of most of the constituents of the canned sardine, for both stored at ambient temperature (27°C) and cold (-4°C) storage conditions.

Crude protein initially was 20.38% and decreased to 19.38% at ambient temperature storage and 20.25% in cold storage temperature. Moisture content initially 45.81 decreased to 45.62% at ambient temperature and 44.12 at cold storage. Crude fibre initially 0.24 decreased to 0.18% at ambient temperature and 0.22% at cold storage etc. This is in line with FAO[2] that the values of the constituents are affected due to the various chemical reactions and changes induced by bacteria. Also Spinelli *et al.*[3] revealed that the conformational structure of fish proteins is easily changed by changing the physical environment.

During the organioleptic assessment of canned sardine the taste panel results showed that they are kept in a good condition for the period of the study. Due to the oil content of the fish product the taste, odour and colour were all maintained at ambient temperature while the cold stored samples recorded no alternations, therefore no off odour/ flavour was also recorded in both. The chemical indices are means of detecting extent or degree of deterioration both in fish and lipid. Since the data obtained from the canned product revealed that before the experiment, a period of 34 weeks were spent on the shelf in the open market, which yielded 1.01 x 10^3 meq kg^{-1} of PV from the baseline result at 0 day. This indicates that with storage, peroxide formation is slow at first, during an induction period which may vary from few weeks to several months according to the particular oil or fat. This conforms with Oyelese O.A. and Adejumo C.O.[4] and Dyer[13] work in which peroxide value was 0.2-3.5 meq kg^{-1} from 10th-30th week, which has not exceeded the PV recommended for fresh oils of 10-20 meq kg^{-1} and extracted fat of PV of 20-40 m eq kg^{-1} extracted fat which is corresponds with noticeable rancid taste.

Thiobarbituric acid (TBA) for the ambient temperature steadily increases from (0.057 to 1.056) mg kg^{-1}. These values are not above the acceptable set limit by Connell[7], of 2 x 7.8 malonaldehyde. Since TBA is an index for measuring secondary stage (last stage) of lipid oxidation. This implies that lipid oxidation has not taken place to any great extent that could cause damage.

All the chemical parameters (TMA, TBA and PV) were significantly ($p<0.05$) correlated with exposure time/ time of storage. Correlation coefficients (r) between PV, TMA, TBA and length of storage/exposure time were 0.54, 0.60 and 0.66, respectively, indicating that spoilage rate increases progressively throughout the duration of the experiment.

The total microbial count witnessed in the ambient stored sample 5.0 x 10^4 $cfng^{-1}$. This is low to the limit bacterial count of sardine (1.0 x 10^6 $cfug^{-1}$) reported by FAO[2]. Though low microbial count should not be regarded as indicator of good quality unless the types of microorganisms colonizing the media are known Eyo[1]. Meanwhile most of the isolated bacteria under the ambient storage condition were gram positive bacteria, they include *Bacillus subtilis* (1.2 x $10^4$$cfug^{-1}$), *Streptococcus faecium* (0.9 x 10^4 $cfug^{-1}$), *Proteus vulgaricus* (0.7 x 10^4 $cfug^{-1}$), *Pediococcus halophilus* (0.6 x 10^4 $cfug^{-1}$), *Aerobacter aerogenes* (0.4 x 10^4 $cfug^{-1}$), *Micrococcus acidiphilus* (0.4 x 10^4 $cfug^{-})^1$ and *Streptococcus lactis* (0.4 x 10^4 $cfug^{-1}$) and fungi species isolated are *Aspergillus niger* and *Aspergillus terreus* of (0.3 x 10^4 $cfug^{-1}$) and (1.0 x 10^4 $cfug^{-1}$), respectively. However the negative relationship of the total viable count with length of storage/exposure time could be due to the oil used in processing the product which tend to prevent multiplication of the microorganisms. Suppressing them. This is further aided under cold storage since no bacteria was noticed in the cold stored product in this study. The positive relationship of the coliform with exposure time/length of storage implies that the longer the period of storage, the higher the coliform formation in the fish product, that is the higher the risk of contamination if formed coliforms are poisonous spore producers. It can easily initiate spoilage. Some of these isolated bacteria are coliform (i.e., pathopenic). Most coliform bacteria have been implicated in food poisoning outbreak of some products[12]. *Bacillusi* sp. are spore formers whose spore could survive high temperature of processing, as it is the situation in this study with the incidence of *Bacillus subtilis* (1.2 x 10^4 $cfug^{-1}$) tripling the counts for *Streptococcus lactis.* (0.4 x 10^4 $cfug^{-1}$), *Micrococcus acidiphilus* (0.4 x 10^4 $cfug^{-1}$) and *Aerobacter aerogenes* (0.4 x 10^4 $cfug^{-1}$) also found in the study.

For spoilage to be quickly detected in stored canned *Sardinella pilchardus* the first three bacteria

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The shelflife of the canned fish product is determined with the prediction equation for known values of Trimethylamine or Peroxide value.

CONCLUSION

All the chemical parameters implicates progressive spoilage occurring however minimal with length of storage/exposure time in both ambient and cold storage. However the fact that incidence of bacteria was only shown in the ambient samples, possibility of bacteria contamination cannot be ruled out totally for stored samples under cold storage of-4°C (or much lower temperatures) especially if their labeled expiry date I about to or already exceeded. It is finally concluded that the most deadly bacterium, that could immediately result in food poisoning of *Sardinella pilchardus* under ambient tropical temperature (of average 27°C) is *Bacillus subtitis*.

It is therefore recommended that exposure of canned sardine in oil should not exceed 12-24 h exposure time under whatever food storage temperature available. However the longer the exposure time in whatever medium, the higher the risk of bacteria contamination and incidence of food poisoning.

On the other land exposure time at ambient temperature should not be tolerated at all, the canned product should be emptied immediately it is opened since any slight exposure will render the passive bacteria active.

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