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Aspects of the Microbiology of Fermented African Catfish, *Clarias gariepinus*

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Abstract: Microbial analysis was carried out to determine the quality of fish sauce prepared using the African catfish *Clarias gariepinus*. The samples were prepared in three jars namely: Treatment A, B and C. Treatment A was prepared with a salt concentration of 10% and was incubated at a temperature of 40°C, (accelerated fermentation process), Treatment B was prepared without the addition of salt and was also incubated at a temperature of 40% (Putrefaction indicator) while Treatment C was prepared with a salt concentration of 25% without incubation (Traditional method of fermentation). The three jars were tightly sealed and left to ferment for 28days in a cool and dry environment. The microbial and proximate values of each treatment was analyzed weekly. The strains isolated in the three jars belonged to twelve genera of microorganisms which are *Bacillus*, *Staphylococcus*, *streptococcus*, *Clostridium*, *Lactobacillus*, *Micrococcus*, *Aeromonas*, *Pleismonas*, *Moraxella*, *Aspergillus*, *Rhizopus* and *Penicillium* with *Bacillus* having a predominant occurence of 47.05%. Treatment B had the highest number of microbial counts of public health significance, while the microbial counts in Treatments A and C had microbial counts that were safe for human consumption. There was a concomitant decrease in pH values of Treatment A and C while treatment B increased steadily. It was concluded that the microbial counts were within the level of consumption.

Keywords: Fish sauce; Microbiology; *Clarias gariepinu;* Fermentation.

1. Introduction

Fish sauce is a condiment derived from fish that has been highly salted and allowed to ferment until the fish flesh is transformed into simpler compounds. It is an essential ingredient in South-east Asian cuisine, with a characteristic flavour and taste. It is developed microbiologically with halophilic bacteria which are majorly responsible for its aroma and flavor. Lopetcharat, *et al.* [1]. The fermentation of the fish sauce involves the breakdown of protein in the fish tissues to release about 12% free amino acids and a high amount of methionine and lysine. Thongthai [2].

Fish sauce produced traditionally has a fragnant aroma and taste, but due to its cost and time consumption, accelerated methods of production are now being put into practice such as incubation, addition of plant proteolytic enzymes, addition of histidine e.t.c. Sanceda, *et al.* [3]. Various types of salt are used for the salting and fermentation of fish. They include solar salt, rock salt and vacuum salt with each salt containing its own unique micro flora. Solar salt which is the most widely salt in fish curing and has been found to contain the largest amount of microorganisms majorly the bacillus types. The microorganisms present in the salt also contribute to the degradative changes in the fish.

The salt present in the sauce initiates the production of organic acids and a concomitant decrease in pH which is the major difference between putrefaction and fermentation. Chemical changes that occurs during putrefaction includes, the oxidative rancidity of the fat present, breakdown of protein to produce hydrogen sulphide, ammonia and indole and the production of volatile trimethylamine which has an ammonical smell. Pearson [4].

Pathogens rarely multiply at high salt concentration, however, *Pediococcus halophilus* is able to produce histamine during long storage, toxins produced by *clostridium botilinum* in poor quality fish before salting may be stable in the fish sauce. Huss and Petersen [5]. This study aims to determine the proximate composition of the fish sauce to determine its nutrition value in curbing malnutrition in developing countries and also to identify the fungi and bacteria present to determine if it presents a public health concern.

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2. Materials and Methods

2.1. Collection of Samples

Live specimens of catfish, *Clarias gariepinus* were purchased from a local fish in Lagos were used for this study. The specimens were transported live to the Department of Marine sciences Laboratory.

2.2. Preparation of the Fish Sauce

Headed Catfish was eviscerated and washed with distilled water.
Treatment A – 500g of fish + 25% salt
Treatment B – 500g of fish + 0% salt
Treatment C – 500g of fish + 25% salt

2.3. Equipments and Glassware

The following glass wares were used in the course of this study; McCartney bottles, conical flasks, pipettes, measuring cylinder, test tubes, disposable petri dishes, inoculating loop, hockey sticks and beakers. The equipments used were autoclave, oven, weighing balance, water bath, and spirit lamp

2.4. Sterilization

2.4.1. Dry heat Method

All glass wares were washed, rinsed in distilled water and then air dried. They were all then wrapped in aluminium foil and placed in the oven at 170C for 2hours to be sterilized by dry heat.

2.4.2. Moist Heat Method

Distilled water used for serial dilution as well as the growth media for microbial isolation contained in Mc Cartney bottles and conical flasks respectively were sterilized by autoclaving at a pressure of 1.1kg/cm3 at 121C for 15minutes.

2.4.3. Aseptic Techniques

The work bench was wiped with 70% w/v ethanol after which the spirit lamp with open flame was allowed to burn throughout the whole process. The hockey stick was sterilized by dipping in ethanol and flaming off afterwards while the inoculating loop was heated in flame until red hot before and after use.

2.5. Culture Media

Three different culture media were used in the course of this study. This includes,

- Nutrient agar

This is an all-purpose, non-selective medium for obtaining the total viable count of bacteria

- De Man, Rogosa sharpe agar

This is a selective medium used for the isolation of lactic acid bacteria (including Lactobacilli)

- MacConkey agar

This is a differential medium for isolating enteric gram-negative pathogens

2.6. Isolation of Micro-organisms

Using spread plate technique, 1ml of fish sauce sample was taken aseptically into the first bottle using a sterile pipette and diluted serially. Aliquots of 0.1ml of dilutions 10-1, 10-2, 10-3, 10-5 and 10-8 were aseptically transferred in triplicates onto the center of the sterile agar plates. The Nutrient agar, Mc conkey and MRS plates were incubated at 37C for 72hours. The total number of the isolated discrete colonies makes up the total plate count. For the isolation of fungi, the same procedure is carried out by using PDA and incubating at room temperatures for 72hours.

2.7. Characterization and Identification of Isolates

2.7.1. Bacterial Isolates

a) Cutural Chrateristics

The characteristics of the colonies on the pure culture plates such as shape, margin, elevation and colour were observed.

b) Cultural Characteistics

Gram positive bacteria were observed as purple colouration while gram negative cells were pinkish in colour.

c) Biochemical Test

Catalase, coagulase, oxidase, urease, indole, Citrate utilization test were carried out on the bacterial isolates.

2.7.2. Fungal Isolates

a) Cultural Characteristics

The fungal colonial characteristics on the pure culture plates include shape, margin, consistency, elevation, sporulation, mycelia formulation and colour were all observed and recorded.

b) Cellular Characteristics

Fungi isolates were stained with methylene blue (positive stain) this results in a positive blue staining of the fungal cell. The stained isolates are viewed under the microscope.

c) Statistical Method

One-way analysis of variance (ANOVA) and comparism by means of DMRT (Duncan multiple Range Test) was also used to test for statistical differences in the result obtained from the microbial analysis

3. Results

Bacterial colonies represented the greatest number of all the isolates, isolates were mainly creamish white, red-pink, white and some light yellow. The bacterial viable counts of Nutrient agar, DeMan Rogosa Sharpe agar and MacConkey agar are presented in Table1.

Table-1. Viable counts of bacteria (Log10CFU/g) isolated in treatments A, B, C.

Day	NA(10^{-8})	MC CONKEY(10^{-8})	MRS(10^{-2})
7	9.4	9.46	3.66
14	9.56	9.26	3.97
21	9.62	9.3	3.99
28	9-49	9.53	3.96
7	9.95	9.9	0
14	10.14	9.88	0
21	9.18	10.01	0
28	10.4	10.23	0
7	9.59	9.72	3.48
14	9.8	9.74	3.7
21	10.01	9.86	3.84
28	10.07	9.69	3.96

The results from the table above indicate that the highest bacteria counts were recorded from Treatment B with a mean count of 10.40× 108CFU/g. Counts on Treatment A varied from (Log10 CFU/g) 9.30 – 9.62, that of Treatment B varied from (Log10CFU/g) 9.18-10.40 while that of Treatment C varied from (Log10 CFU/g) 9.59 – 10.07. Counts of Lactic acid bacteria (LAB) in MRS agar varied from (Log10 CFU/g) 3.66 – 3.96 in Treatment A, there was no observable growth in Treatment B and from (Log10 CFU/g) 3.48-3.98 in Treatment C. Bacterial isolates from the various fish sauce samples is summarized in Table 2.

Table-2. Bacterial isolates from the various fish sauce samples

SAMPLES	ISOLATES	NO.	FREQ. OF OCCURENCE
JAR A	*Bacillus cereus*	5	14.71%
	Bacillus	2	5.88%
	Staphylococcus	3	8.82%
	Lactobacillus	2	5.88%
	Micrococcus spp.	1	2.94%
JAR B	*Moraxella*	4	11.77%
	Aeromonas	2	5.88%
	Pleismonas	1	2.94%
JAR C	*Clostridium*	1	2.94%
	Lactobacillus	3	8.82%
	Bacillus subtilis	4	11.76%
	Bacillus pulminus	3	8.82%
	Bacillus	2	5.88%
	Streptococccus spp.	1	2.94%

Bacillus cereus had the highest occurrence with a total of 14.71%, while the least occurring bacteria isolates were *clostridium botulinum*, *micrococcus*, *streptococcus* and *pleismonas* all with an occurrence of 2.94% each. All the fish sauce samples studied was free from both *Escherichia coli* and *salmonella shigella*. Cultural, morphological and biochemical characteristic of the bacteria isolates is described in Table 3.

Table-3. Cultural, morphological and biochemical characteristic of the bacteria isolates

Morphology	Gram-stain test	Cat	Coag	Oxid	Ind	Urease	Cit	Glu	Suc	Lac	Malt	Mann	Xylo	motil	Probable organism
Cocci	+	+	+	-	NP	+		+	+	+	+	+	-		*Staphylococcus aurreus*
Cocci	+	+	-	-	NP	+		+	d	d	d	d	d		*Micrococcus spp.*
Cocci	+	-		-	NP				+	d	+	-			*Streptococcus spp*
Rods	+	-							+	+	-				*Lactobacillus fermenti*
Rods	+				-	-		+	-	-				+	*Clostridium botulinun*
Rods	+	+		d	-	d	+	+				-	-	+	*Bacillus cereus*
Rods	+	+		d	-	-	+	+				+	+	+	*Bacillus pulminus*
Rods	+	+		d		d	+	+				+	+	-	*Bacillus subtilus*
Rods	+	+		d		d	+	+				+	+	+	*Bacillus licheniformis*
Coccobacilli	-	+		+		-	-	-		-	-		-	-	*Moraxella spp.*
Rods	-	+		+	+		+	+	+	d				+	*Aeromonas spp.*
Rods	-	+		+	+		-	+	-	+				+	*Pleismonas spp.*

Key

+ : positive
- : negative
***NP**: Not produced*
***d**: Varies in different reaction*
***Cat**: Catalase*
***Coag**: Coagulase*
***Oxid**: Oxidase*
***Ind**: Indole*
***Cit**: Citrate Utilization*
***Glu**: Glucose*
***Suc**: Sucrose*
***Lac**: lactose*
***Malt**: Maltose*
***Mann**: mannitol*
***Xylo**: Xylose*
***Motil**: Motility*

Fungal Isolates from the various fish sauce and their frequency of occurrence is presented in Table4.

Table-4. Fungal Isolates from the various fish sauce samples

SAMPLES	ISOLATES	NUMBER	FREQ. OF OCCURRENCE
JAR A	*Aspergillus flavus*	9	10.98%
	Rhizopus spp.	6	7.32%
	Penicillium spp.	5	6.10%
JAR B	*Aspergillus niger*	7	8.54%
	Aspergillus flavus	15	18.29%
	Rhizopus spp.	15	18.29%
	Penicillium spp.	8	9.76%
JAR C	*Rhizopus spp.*	9	10.98%
	Penicillium spp.	5	6.10%
	Aspergillus niger	3	3.66%

While the mean values for the pH in the three treatments is listed in Table 5.

Table-5. mean values for the pH in the three treatments

Sample\Time	DAY 7		DAY 14		DAY 21		DAY 28	
	Values	Mean	Values	Mean	Values	Mean	Values	Mean
Jar A	6.2		6		5.9		5.6	
	6.1		6		5.9		5.5	
	6.2	6.166667	6	6	5.9	5.9	5.6	5.566667
Jar B	6.7		6.6		6.9		7.2	
	6.7		6.7		6.9		7.1	
	6.7	6.7	6.7	6.666667	6.8	6.866667	7.1	7.133333
Jar C	6		6.1		6		5.9	
	6		6		5.9		5.9	
	6	6	6	6.033333	6	5.966667	6	5.933333

4. Discussion

The microbiological quality of any food is highly influenced by the total number of viable counts of pathogenic organisms present in it. Prescott, *et al.* [6]. In this study, microbial composition and load in three fish sauce samples were studied. Treatment B had the highest colony counts of public health ≥ significance. This indicates that the absence of salt created a favourable environment for bacteria to thrive as salt is a form of preservative inhibiting bacterial growth. Ingram and Kitchell [7].

There was an increase in counts of Lactic acid bacteria (LAB) in Treatment A and C, this is an indication of fermentation. The lack of fermentative growth of Lactic acid in Treatment B shows that LAB are halophilus and require salt for growth and the absence of salt triggers the breakdown of the fish tissues by autolysis and microorganisms origin to the fish to release hypoxanthine which as an offensive odour Pearson [4].

Enteropathogenic isolates which includes salmonella and Escherichia coli of which a total count of ≥106cfu/g is suggested as dangerous and harzadous to the health by Keichumum and Schellhans [8] was not isolated in this study. This indicates that hygienically prepared fish sauce is safe for human consumption. The pH level of Treatments A and C decreased variably over time due to the presence of LAB leading to more acidic values which is a sign of a good quality fish sauce. This study revealed evidence of fungal contamination on all samples of fish sauce. Fungi is ubiquitous and could have been introduced during the preparation of the fish sauce.

Analysis of variance (ANOVA) showed that there was no significant difference ($P< 0.05$) in the counts obtained from the treatments A, B and C using nutrient agar at days 7 and 14 while a significant difference was observed in the three Treatments at days 21and 28. For LAB counts on MRS agar, ANOVA showed a significant difference ($P> 0.05$) in the counts obtained in Treatment A, B and C at days 7, 14, 21, 28. There was no significant difference in the values obtained at days 7, 14 and 28 on the Mac Conkey agar while a significant difference was obtained at day 21. Post-Hoc Tests (Duncan) was done after each ANOVA analysis.

The summary of the Post-Hoc test shows that counts on Treatment A and C are not significantly different but treatment B has values that are significantly different. This test proves the fact that Treatment B which is a set-up for putrefaction shows a lot of variation when compared to Treatments A and C.

5. Conclusion

It was concluded that the inclusion of salt concentration of up to 10% suppressed the bacterial load of treatments A and C. Catfish should be properly processed under optimum conditions to prevent the spread of organisms that are of public health importance.

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