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BACTERIOLOGICAL ANALYSIS OF DRIED CRAYFISH AND STOCK FISH IN OZORO MARKET

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Abstract:

Bacteriological analysis of dried Crayfish and stock fish sold in Ozoro market was carried out to evaluate the bacteriological quality of stockfish and dried crayfish and also provide some relevant information on the microflora of the species commonly found in dried crayfish and stockfish. Samples were collected from three different markets and were labeled as sample A and B for crayfish and stockfish respectively. A total of six (6) bacterial isolates were obtained, Bacillus subtilis, Vibrio species, Escherichia coli, Mycobacterium species, Staphylococcus aureus and Pseudomonas aeruginosa. The total heterotrophic count ranged from 2.4 x 10^4 cfu/ml to 7.2 x 10^4 cfu/ml. The occurrence of the bacterial isolate ranged from 11.11% to 22.22% of which E. coli, Staphylococcous aureus, and Pseudomonas aeruginosa appears as the lowest while Bacillus subtilis, Vibrio species and Mycobacterium species was the most predominating isolate. The presence of these isolated bacteria could be due to contamination of the sample due to unhygienic environment in which the stock fish and crayfish were stored, displayed, and sold.

Key Words: ***Bacteriological, Analysis, Dried, Crayfish, Stock Fish, Market***

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INTRODUCTION:

Fish is a low fat, high protein food and contains beneficial fatty acids such as Omega - 3 fatty acids. Omega - 3 fatty acids are important for optimal brain and nervous system development in fat uses and infants. Fish constitute more than 60% of the protein intake in adults especially in rural areas (Adeleye, 1992). Fish flesh is tender and is better digested than beef or other types of animal protein. (Adebayo. *et al*., 2008).

Fish is an extremely perishable food. The quality of caught fish and its usefulness for further utilization in processing is affected by the fish capture method. Unsuitable fishing method does not cause mechanical damage to fish, but also creates stress and conditions which accelerate a symptom that reduced the quality or strength of the fish without any preservatives or processing measures (Okonta and Ekelemu 2005). And for that reason fish is preserved and processed in order to slow down or prevent the enzymatic, bacterial and chemical deterioration of the fish and the different kinds of preservative include drying, smoking, freezing, chilling and banning; and also using crayfish and stock fish as a case study.

Cray fish are decapods crustaceans, rich in sodium, potassium, and phosphorus with good amount of iron, zinc, copper, and manganese. It has a good amount of vitamin A, C, B6, thiamine, riboflavin and is considered in some parts of the world as a delicacy (Willard, 2006). Crabs like crayfish products are available to customers in the tropics as salted, smoke dried or sundried. By far, drying is the commonest processing method and the primary aim is to prolong the shelf life of the products by reducing the water content as much as possible, thus protecting the products.

Stock fish is gutted, beheaded fish (round, split, or fillet) produced by natural or industrial drying, without the addition of salt or other additive (Norwegian Industry standard for fish, 1997). Stock fish can be made out of cod (*Gadius morhua*) haddock (*Melanogrammu aeglefinus*), Saithe (*Pollachius virens*), ling (*Molva molva*) or Task (*Brosmebrosn'ze*). In Nigeria, cod stock fish and saithe stock fish especially their heads are common in the markets and these particular stockfish are popular because of their rich taste and aroma and are sometimes eaten raw, but mostly used in cooking most native soups that complement the grain staples fufu and garri (Junaid, *et al.*, 2010). And this stockfish are mainly called "okporoko" by the Igbos (Eastern Nigerian) refers to the sound the hard fish makes in the pot.

Stockfish is preserved by drying. During drying, 80% of the water is removed while other nutrients are concentrated. Stock fish is known as one of the richest source of protein, vitamin B, Iron, and calcium.

This study evaluates the bacteriological qualities of stockfish and dried crayfish and also provides some relevant information on the microflora of the species commonly found in dried crayfish and stockfish sold in Ozoro market. Thus, the study is also aimed at an attempt to assess whether dry crayfish and stock fish are safe for human consumption as well as to prefer some solutions to the problems and danger involved in the kind of crayfish and stockfish sold in Ozoro market.

MATERIALS AND METHODOLOGY:

STUDY AREA

This experiment was conducted in Ozoro, Delta State of Nigeria. Ozoro is in Isoko North Local Government of Area Delta State. The people are Isoko speaking and hospitable. Their main activities are food crop, farming accompanied by some hunting. They are also engaged in trade of food crop for cash to meet the other basic house hold needs. The region experience higher rainfall and humidity most of the year.

Sterilization of Glassware

The glassware that was used for this study was washed with detergent rinsed thoroughly and sterilized using autoclave at 121°C for 15 minutes.

Methods

Sample Collection

Stock fish and crayfish samples were bought from three different markets; Ozoro main market, Ozoro small market and Owhelogbo junction market in Isoko North L.G.A of Delta State.

The samples were collected into sterile universal containers after properly covered at the place of purchase and the samples were labeled A for all stockfish and B for all crayfish. Both were transported to the laboratory in sterile universal container, where analyses were carried out.

Analysis

Isolation of test organisms

The samples were serially diluted according to the method of (Cheesbrough, 2002). Media prepared was according to the manufacturer's instruction and then used for enumeration of isolated bacteria.

The plates were incubated at 37°C for 24 hours. Purified isolates where identified according to their

morphological characteristics and reactions to biochemical test.

Morphological Characteristics

Gram Staining

Smear of each bacterial isolate was made on a grease free clean glass slide with a drop of normal saline, air dried, and heat fixed by quickly passing the slides over flame. The smears was flooded with crystal violet for one minute (1 min) then wash the crystal and add lugol's iodine solution for 1 minute and then washed with water which it was decolourized with 95% alcohol for l5secs and rinsed off with water again. The slide was then flooded with safranine red for one minute to counter stains, washed off with water, dried, and examined under the microscope using oil immersion and x 100 objective.

Biochemical Test

The biochemical analyses carried out were in accordance with procedures reported by (Cheesbrough, 2002).

Citrate Test

The bacterial isolate were tested for their ability to utilize citrate as the sole carbon source. Simmons citrate medium was used.

Bacterial isolates were inoculated into Simmons citrate medium in test tubes and incubated at 37°C for 24-48hours. The culture media was observed for a colour change from green to blue. Positive showed no growth with intense blue color, while negative test showed no growth and the colour of the medium remained green (Bello, 2002).

Triple Sugar Iron Agar Test (TSI)

Bacterial isolates were stabbed into **TSI** slant media and also streaked on the surface of the slant after which the media was incubated at optimal temperature of 37°C for 24 hours. The TSI slant medium was used to check for the present of the following:

Gas: If bubble is present in the media (gas positive).
H2S: If black is present in the media (H2S positive)
Lactose: If the top of the media turn from pink to yellow (lactose positive)
Glucose: If the bottom of the media turns from pink to yellow (glucose positive).

Oxidase Test

This test determines the presence of oxidase enzymes in the tested organism. The reagent contains tetramethyl-p-phenylenediamine which serves as an alternative substrate for the cytochrome oxidase reaction. A piece of filter paper was placed on a clean sterile petri-dish and 3 drops of oxidized reagent was added.

The bacterial isolates were smeared on the filter paper by means of sterile rod. Organism indicates positive when it retains the purple colouration within five to ten seconds of the analysis.

Catalase Test

This test detects the presence of catalase enzyme when present in a bacterium, it catalyse the breaking down of hydrogen peroxide with the release of oxygen as bubble.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

With a wire loop, a colony was picked from the pure culture and was transferred to the centre of a glass slide. 1- 2 drops of 3% hydrogen peroxide was added to the bacterial isolates. Immediate production of bubbles indicated positive result and if no bubble indicated negative.

Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which then accumulates in the medium for indole production.

Bacterial isolates were inoculated not peptone water medium contained in a sterile test .tubes then incubated at 37°C for 48 hours. After the incubation period about 3 drops of kovac's indole reagent was added to the peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for colour development. A red colour ring at the interface of the medium denotes a positive result. And if the isolate is negative, the reagent layer will remain yellow or slightly cloud (Bello, 2002).

RESULTS AND DISCUSSION:

Results

The bacteria isolated from the sample were *Bacillus subtilis*, *Mycobacterium species, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus* and *Vibrio species*. Bacterial isolated have shown the ability to utilize sugar as their substrate which was shown in table 1. Table 2 shows the occurrence of bacteria isolates identified and their total heterotrophic count.

Table 1: Cultural, Morphological and Biochemical Characteristics .of Bacteria isolates.

Isolates	Gram stain	Morphological characteristics	Citrate	Oxide	Catalase	Indole	Glucosse	Latose	H_2S	Gas
Mycobacterium species	GPB	Rods	+	-	+	-	+	+	-	-
Vibrio species	GNB	Rods	+	+	+	-	+	-	-	+
Staphylococcus aureus	GPC	Cocci		-	+	+	+	+	-	+
Bacillus subtilis	GPB	Rods	-	-	+	-	+	+	-	-
Escherichia coli	GNB	Rods	-	-	+	+	+		-	+
Pseudomonàs	GNB	Rods	-	+	+	-	+	+	-	+
aeruginosa										

Key = + = positive, - = negative, GPB = Gram Positive Bacillus, GNB Gram Negative Bacillus, GPC = Gram positive cocci.

Table 2: heterotrophic count isolates identified and their total heterotrophic count.

Sample	Cfu/ml		
A1	4.0	x	10^4
A2	7.2	x	10^4
A3	6.0	x	10^4
A4	5.2	x	10^4
A5	4.8 x 10^4		
B1	4.4.	x	10^4
B2	4.4.	x	10^4
B3	2.4	x	1O4
B4	6.8 x 10^4		

Table 3: Percentage of occurrence of isolated bacteria

Bacterial isolate	Occurrence (%)
Bacillus subtilis	22.22%
Vibrio species	22.22%
E.coli	11.11%
Mycobacterium species	22.22%
Staphylococcus aureus	11.11%
Pseudomonas aeruginosa	11.11%

Discussion

From table 1, the result reveals the biochemical test and bacterial isolated from the study. The total number of (6) six bacterial species were isolated from the samples; *Mycobacterium species, Vibrio species, Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa*.

The presence of food borne pathogens in a fish product is a function of the harvest environment, sanitary conditions, and practices associated with

equipment and personnel in the processing environment (FDA 2001). The handling of fish products during processing involves a risk of contamination by pathogenic bacteria. *Vibrio parahaemolyticus* and *Staphylococcus aureus* causing food borne human intoxication (Huss, *et al*., 1998; Shena and Sanjeev 2007).

The Isolation of *Pseudomonas spp.* from the dried Cray fish and stock fish samples analyzed is of high importance because the bacterium plays a considerable role as potential pathogenic bacteria for human and as an indicator of food spoilage (UNBS, 2012)

Table 2 shows the total heterotrophic count From sample A (crayfish), the heterotrophic count ranged from $4.0x10^4$ cfu/ml to $7.2 x10^4$ cfu/ml of which, *Vibrio species* has the highest count while *Bacillus subtilis* has the lowest count. From Sample B, (stockfish), the heterotrophic count ranged from $2.4 x10^4$cfu/ml to $6.8x10^4$ cfu/ml. *Vibrio species* has the highest count while *Pseudomonas aeruginosa* has the lowest count. Table 3 shows the occurrence of bacteria isolated which ranged from 11.11% to 22.22% of which *E.coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* appears as the lowest while *Bacillus subtilis*, *Vibrio species* and *Mycobacterium species* was the most predominating isolates. The contamination of the sample may be due to improper handling and exposure to the environment.
This research work is in consonance with Kasozi *et al*., (2016) who isolated *E. coli, Staphylococcus aureus, Pseudomonas spp, Bacillus cereus* from traditionally dry salted Pebbly fish (*Alestes baremoze*) sold in different markets of West Nile Region in Uganda.

CONCLUSION AND RECOMMENDATIONS:

Conclusion

From the result obtained from this study, contamination of dried crayfish and stockfish may be due to improper handling, how they are been prepared and the environment they are been sold and improper storage.

Recommendations

It is hereby recommended that:

1. Crayfish and stockfish should be properly and effectively preserved and handled to avoid been contaminated
2. Crayfish and stockfish should be displayed in hygienic manner.
3. Crayfish and stockfish should be properly cooked before consumption.
4. Consumers should be enlightened about sanitary measures.

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