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Comparative Study of Antimicrobial Activity of the Use of Sodium Benzoate and Brine as Preservative on Catfish (*Clarias gariepinus*)

Amusat Afusat Ibiwumi*, Adedokun Adewole, Ibraheem Abdul-Rasheed Ajamu, Oladokun Pauline Olutoyin

Department of Science Laboratory Technology, Oyo State College of Agriculture and Technology, Igboora, Nigeria

Email address:

wumiamusat@gmail.com (A. A. Ibiwumi)

*Corresponding author

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Abstract: There have been worldwide problems as to which of the preservatives is active in preservation of food among others and that which of them will be able to control the microbes responsible for the food spoilage. This study was carried out to assess antimicrobial activity of sodium benzoate and brine on catfish (*Clarias gariepinus)* as preservatives at room temperature for 6-weeks storage. Raw catfish were subjected to the following treatments: 1g, 3g and 5g of Sodium benzoate and 1g, 3g, 5g of Brine were added to the sample fishes. The fish samples that were not treated with any preservatives served as control. All samples were subjected to microbial culture using laboratory standards. The control and the treated fresh fish samples showed diverse microbial load. All treated catfish sample were negative to *Klebsiella spp* and *Aspergillus flavus*. The treatment effectively reduced the TVC, TFC and TCC Coliform*, Klebsiella spp, Bacillus cereus* and *Aspergillus flavus* after preservation and the low microbial counts was maintained until the end of the 6 weeks' storage. Treatments with 5g sodium benzoate proved best in terms of microbial reduction but organoleptically, 1 or 2% treatments are acceptable to consumers. Treatment with 1g of brine (Wet salt) is acceptable when consumed. Therefore, this study revealed that the fish preserved with sodium benzoate had low microbial count compared to the fresh fish preserved with brine, hence it is advisable that the catfish should be preserved with sodium benzoate.

Keywords: Fish Storage, Organoleptic, Wet Salt, *Aspergillus flavus*, TVC, TCC

1. Introduction

Fish is very rich in protein constituents and of high quality, which is an important vitamins and many unsaturated fatty acids [1]. The high demanding rate of fresh sea food of high quality had prone some researchers to look for the best way to preserve the fishes so as maintain elongated shelf life for human consumption. Modified atmosphere packaging (MAP) and vacuum packaging (VP), along with refrigeration have become increasingly popular preservation techniques, which have brought major changes in storage, distribution, and marketing of raw and processed products to meet consumer demands [2, 3]. Immediately the fishes are killed, they become subject to post mortem changes and damage by microorganisms and insects that quicken the rate of its decomposition. The amino constituent of fish is similar to that contained in milk, egg and meat. Vitamins, proteins, minerals and less carbohydrates with little or no saturated fat were contained in the cat fish. Not less than 1,250 species of *Clarias gariepinus cultured by individuals were* rared in fresh *water and* usually accompanied by loss in quality and quick decomposition particularly shortly after catch [4]. In Nigeria, consumption rate of catfish is high compared to other fish species, this may be due to variety of ways by which it could be made such as pepper soup, barbecue, smoked type etc. Food spoilage means changes of the original nutritional value, texture and flavor, that make it harmful and unsuitable for consumption [5]. Spoilage of food

products can be due to chemical characteristics and enzymatic activities Among the factors which contribute to the spoilage are: degradation of protein, development of oxidative rancidity, vitamin degradation, enzymatic reaction, the action of microorganisms and more importantly water activity [6].

Smoked fish and shellfish products can be source of microbial hazards such as *Listeria monocytogenes*, *Salmonella spp.,* and *Clostridium botulinum* [7] Omojowo and Ihuahi [8] reported smoked fish samples of 4 local markets in Kanji Lake area to be dominated by gram-positive bacteria, potential pathogens, coagulase-positive *Staphylococus*, and *Escherichia coli*. Pathogenic bacteria identified from whole catfish and fillets, include: *Aeromonas sp., C. freundii, E. coli, H. alvei, K. pneumonia, Listeria sp., P. shigelloides*, *Proteus sp., S. aureus*, and *Vibrio sp*. among others. Among the most perishable commodities worldwide today are fish and fisheries products because of microbial attack. About one out of three world's food production is lost yearly due to microbial spoilage. In fact, microbial activities are responsible for destruction of most fresh and of several other preserved sea-foods [9]. Many times, sodium chloride is added mainly as a flavour and useful ingredient which in most cases the effect could not be feeled directly. Another reason that the anti-microbial effect of sodium chloride may be called indirect is that it reduces the water activities in many foods and thereby indirectly prevents microbial growth [10]. Sodium benzoate ($NaC_6H_5CO_2$) is a preservative found naturally in cranberries, prunes, greengage plums, cinnamon, cloves and apples. The food and drug administration (FDA) includes sodium benzoate in its list of direct food substances affirmed as generally recognized as safe (GRAS). Sodium benzoate has been generally reported to be used at concentration below 0.1% [11]. In some countries, 0.2% and 0.3% concentrations are permitted and are commonly used [12]. Sodium benzoate has anti-microbial properties preventing the growth of bacteria and mold. It prolongs the shelf life of fish for a very long period without affecting its texture, taste and appearance [12]. Therefore, the present research was designed to compare the effectiveness of different preservatives and their antimicrobial activities on the cat fish.

2. Materials and Methods

2.1. Collection of Fish Samples

About Five kilograms (5kg) of fresh cat fish were purchased at OYSCATECH's fish farm. The cat fish (*Clarias gariepinus*) samples were cut into sizes in the Oyscatech fish farm and taken to the Department of Science Laboratory Technology of the Oyo State College of Agriculture and Technology, Igboora where the research wok was carried out and kept inside an air-tight keg until ready to use.

2.2. Grouping of the Catfish (Clarias gariepinus)

The samples (Catfish) were chosen randomly and divided into 3 portions. The samples were subjected to treatments. The treatments were as follows; Portion 1 was not preserved and labeled as group 1 or control. Portion 2 were mixed with 1g, 3g and 5g of sodium benzoate and were labeled as BZ respectively. Portion 3 were mixed 1g, 3g and 5g of brine, labeled and preserved as BR. The samples were then stored in a clean plastic and separated into group according to the methods described by Omojowo and Ibitoye [13]. The catfish (*Clarias gariepinus*) samples were monitored from day one to know organoleptic apearance of the samples.

2.3. Microbiological Examination

Determinations of microbiological load (TVC, TFC and TCC) were recorded according to the procedure described by Olusegun and Jacob [14]. Nutrient agar and Potato Dextrose agar were prepared and used according to manufacturer's instruction. Serial dilution was done using 7 cleaned test tubes.

2.4. Determination of Proximate Analysis

2.4.1. Determination of Crude Protein

The total nitrogen (Crude protein was determined using the Kjeldahl method). About 0.5g of the fish sample was weighed on a nitrogen-free paper. The paper was wrapped round the sample and dropped at the bottom of the kjeldahl digestion flask together with 6-8 glass beads, 4-5 spatulaful of granular mixture of $CuSO_4$ and K_2SO_4 as catalyst and 20ml of concentrated H_2SO_4 was carefully added. The flask was gently heated on a Gerhardt heating mantle in an inclined positionin a fume cupboard until full digestion was achieved (when liquid changed from brown colour to colourless). The content of the flask were then transferred to a clean 100ml volumetric flask, and 25ml aliquot was used for the distillation and total nitrogen was determined calorimetrically.

2.4.2. Determination of Ash Content

Ash content of the fish sample was determined by incineration in a carbonated Sheffield LmFs murffle furnace at 5000°C [15]. The difference in weight of the fish samples before and after heating was taken as the ash content. The formular is as follows:

$$\%\text{Ash content} = \frac{W2 - Wo}{W1 - Wo} x100$$

Where Wo = Empty crucible
W1 = Dry sample
W2 = Ash sample

2.4.3. Determination of Dry Matter

Moisture content of each fish samples was determined using oven dry method. 5g of the samples were placed in weighed crucibles maintained at 80°C in an oven until constant weights indicate the dry matter and was calculated as follows:

$$\% \text{ moisture content} = \frac{W1 - W2}{W1} X100$$

Where W1 = Wet sample W2 = Dry sample

Other proximate analysis such as fat contents and crude fibres were determined using the procedure set by AOAC [16].

3. Results

From the table 1 above, the numbers of microbial colonies formed by each sample was obtained. The microbial load varied significantly with the preservatives (A and B). In the fresh catfish before storage (i.e. week zero), the highest TVC of 5.33×10^3 was recorded in control sample followed by sample B of 4.97×10^3 while sample A recorded 3.47×10^3. It was shown that in all week intervals, there were high TVC (cfu/g), TFC (cfu/g) and TCC (cfu/g) counts across the control samples than the preserved fish counts, except For week 2 where TVC was 3.75×10^5 which was a bit lower. Generally, week zero showed high microbial and fungi load from preserved fish with brine (B) compared to preserved fish with Sodium benzoate (A) which ae TVC A (3.47×10^3), B (4.97×10^3), TFC A (6.03×10^3) B (6.47×10^3) and TCC A (3.20×10^3) and B (5.30×10^3) cfu/g respectively. Week 2 revealed highest microbial load of fish preserved with Sodium benzoate A for TVC (6.77×10^3) cfu/g and least was recorded for TCC (2.43×10^3) cfu/g. Sample B recorded least TVC of (1.50×10^3) and highest TFC of (6.70×10^3) cfu/g respectively. As week increasing i.e. week 4-6, it was shown that fish preserved with brine B had elevated TFC across the weeks and also TCC showed no growth of microbes across samples.

Table 1. *Microbial Analysis of Fresh Fish Samples with Preservatives.*

SAMPLE	TVC (cfu/g)	TFC (cfu/g)	TCC (cfu/g)
WEEK 0			
A	3.47×10^3	6.03×10^3	3.2×10^3
B	4.97×10^3	6.47×10^3	5.30×10^3
C	5.33×10^5	7.30×10^5	7.07×10^3
WEEK 2			
A	6.17×10^3	6.77×10^3	2.43×10^3
B	1.50×10^3	6.70×10^3	2.03×10^3
C	3.75×10^5	7.93×10^5	7.43×10^5
WEEK 4			
A	3.33×10^3	2.00×10^3	NG
B	5.27×10^3	7.47×10^3	NG
C	5.90×10^3	7.00×10^3	NG
WEEK 6			
A	3.70×10^3	4.30×10^3	NG
B	2.33×10^3	7.52×10^3	NG
C	6.21×10^3	7.23×10^3	NG

Keys: A = Sample with sodium benzoate, B = Sample with brine and C = Control (untreated).

Sample preserved with sodium benzoate indicate some suspected bacteria which are *Klebssiella spp, Bacillus cereus Bacillus subtilis, Proteus mirabilis,* while suspected fungi includes *Aspergillus flavus, penicillium spp, Neurospora spp and Aspergillus spp. In sample B that is preserved with Brine, suspected bac*teria were detected which are *Bacillus subtilis, Proteus mirabilis Bacillus subtilis, Proteus mirabilis, Klebssiella spp, Bacillus cereus, Klebssiella spp, Bacillus cereus and suspected fungi are Neurospora spp, Aspergillus fumigatus, Mucor and penicillium spp*. However, control sample which is unpreserved revealed some of the suspected bacteria such as and with the associated fungi which include *Neurospora spp, Aspergillus fumigatus, Aspergillus flavus, Mucor and penicillium spp*. (Table 2).

Table 2. *Suspected Microbes Associated with Fresh and Preserved Cat fishes.*

Samples	Suspected Bacteria	Suspected Fungi
A	*Klebsiella spp., Bacillus cereus, Bacillus subtilis, Proteus mirabilis.*	*Neurospora spp., Aspergillus flavus, Aspergillus spp., Penicillium spp.*
B	*Klebsiella spp., Bacillus cereus, Bacillus subtilis, Proteus mirabilis.*	*Aspergillus fumigatus, Mucor, Penicillium spp.*
C	*Klebsiella spp., Bacillus cereus, Bacillus subtilis, Proteus mirabilis.*	*Neurospora spp., Aspergillus flavus, Aspergillus spp., Penicillium spp., Mucor, Aspergillus fumigatus.*

Keys: A = sample with sodium benzoate, B = sample with brine C= Control (Untreated).

The table below showed that proximate composition was increased in Moisture content (A 62.19, B 62.05 and C 60.11%), Crude protein (A 55.21, B 59.77 and C 50.23%), Dry matter (A 35.21, B 36.49 and C 35.83%) and Ash content (A 21.19, B 21.13 and C 19.05%) respectively. There was a drastic reduction in the value of percentage crude fibre (A 5.04, B 4.25 and C 4.37%) across the samples while Fat content has the least values (A 09.11, B 11.02 and C 07.11%) across samples A, B and C respectively (Table 3).

Table 3. *Average values of Proximate Composition of the fish samples.*

Samples	%Crude Protein	%Ash Content	%Fat Content	%Crude Fibre	%Dry Matter	%Moisture Content
A	55.21	21.19	09.11	5.04	35.21	62.19
B	59.77	21.13	11.02	4.25	36.49	62.05
C	50.23	19.05	07.11	2.37	35.83	60.11

4. Discussion

The microbial load count of the fish studied that were higher i.e. TVC, TFC and TCC (cfu/g) at zero week before storage could be associated with the handling and processing of the fish and also carriage from one place to another. Similar observation was made by [17, 18, 12], they all reported highest

no of microbes in untreated samples. The present results have also shown the highest bacteria count (TVC) recorded in A, B, and control sample at the end of 6 weeks. The bacterial load (TVC) count results for samples (A and B) used for this study are below the maximum bacteria count of 5.5×10^5cfu/g while that of control was higher than the recommended value for good fish product according to the International Commission on Microbiology Safety for Food. The present finding disagree with those observed by Olusegun and Jacob [14] while similar observation was observed by Efiuvwevwere and Ajiboye [19, 20] who also reported highest TVC in control samples after 8th weeks. During storage of fish sample there was significant increase in the fungi count with length of storage as seen in this study. This is in line with Oyebamiji and Oyebimpe [21] who worked on stored fish products marketed in the open market. The presence of fungi may be due to the difference in the chemical composition of the fish species and to which different moulds react differently [18]. The decreased in (TCC) of some fresh fish samples (A and B) and no growth shown as weeks increases may be due to presence of water or impact of preservatives.

However, the average values of proximate composition of Cat Fish samples from this study fall within the range reported by the earlier study of Idah and Nwanko [22]. All samples from this study that revealed high moisture content could be related to high microbial load because moisture is one of the growth factors of the microbes. Similar observation of high moisture content and low fat content was also reported by Kedar *et al.* [20] while deviated report was observed by Omojowo *et al.* [12] who also reported reduced moisture contents in all samples examined. The treated fish samples in the present study that showed high level of crude protein than the control samples was also reported by Vlieg [23] and Omojowo and Raji [24]. The bacteria encountered across the samples examined were *Klebssiella spp*, *Bacillus cereus Bacillus subtilis, Proteus mirabilis,* while suspected fungi isolates includes *Aspergillus flavus, penicillium spp*, *Neurospora spp, Aspergillus spp*. and Mucor. Similar isolates of bacteria was reported by Omojowo and Raji [24] however, Kedar *et al*. [20] reported E-coli, *Staphylococcus aureus* as organisms isolated from fish examined. The bacteria and fungi isolated from fish preserved with Sodium Benzoate and brine were similar with the report of Omojowo *et al.* [12, 25] who also reported similar isolates like *Bacillus cereus, Klebsiella spp*. *E-coli, S. aureus*, e.t.c., however, *Neurospora spp., Penicillium spp., B. subtilis, Proteus mirabilis* isolated from the present study differs from their observation.

5. Conclusion and Recommendation

This study revealed that the percentage of sodium benzoate accepted organoleptically was 1-2% addition to fish and 5% is highly useful for fish preservation because it prevents the growth of microbes. However, brine is also recommended, preferably at low concentration. Higher concentration can be used in preservation for effective result while Sodium benzoate is more preferable for preservation at higher and lower concentration. The present study also indicated that some of the microbial species encountered were pathogenic that can cause serious diseases in patient that consume the contaminated fishes. In order to reduce the microbial attack of *C. gariepinus* and other fishes, suitable preservative like sodium benzoate could be adopted. This study was only carried out for 6 weeks and therefore more research is recommended for prolonged storage of *Clarias gariepinus* (Catfish) to see more effect of the preservatives on the growth of pathogens with longer period of storage.

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