**ASSESSMENT OF THE USE OF SODIUM BENZOATE ON THE SAFETY AND SHELF LIFE OF SMOKED TILAPIA**

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**ABSTRACT**

This study was carried out to assess microbial quality and safety of smoked Tilapia (*Oreochromis niloticus*) treated with Sodium benzoate) during 8-week storage at room temperature. Raw catfish steaks were subjected to the following treatments for 5 minutes prior to smoking: 1-5% Sodium benzoate. The non-treated tilapia served as control. The control and the fresh fish treated samples showed diverse microbial load. All treated smoked sample were negative for *E. coli and Streptococcus sp.* The treatment effectively reduced the TVC, Coliform, Staphylococcus and fungi after smoking and these low microbial counts was maintained until the end of the 8 weeks storage. Treatments with 5% sodium benzoate proved best in terms of microbial reduction but organoleptically 1 or 2% treatments are acceptable to consumers. [New York Science Journal 2010; 3(5): (ISSN 1554 – 0200).

**Key words:** Sodium benzoate, storage, microbial load and smoked catfish

**INTRODUCTION**

Smoking of fish and/or meat products is one of the most ancient processing technologies. It has been for centuries used for preservation, and is still widely used for this purpose among several communities in the third world where up to 70% of the catch is smoked for preservation (Ward, 1995). Consumers are rediscovering the good taste of smoked seafood, including smoked catfish. To satisfy the consumer demand, it is necessary to produce good quality and safe smoked seafood products. Fish and fisheries products are among the most perishable commodities worldwide mainly due to microbial spoilage. About one-third of the world’s food production is lost annually as a result of microbial spoilage. In fact, microbial activity is responsible for spoilage of most fresh and of several lightly preserved seafoods (Lund *et al.*, 2000). Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella spp.*, and *Clostridium botulinum* (Heinitz and Johnson, 1998). Omojowo and Ihuahi (2006) reported that smoked fish samples from 4 local Markets in Kainji Lake area of Nigeria were dominated by gram-positive bacteria, Potential pathogens, coagulase-positive Staphylococcus, and *Escherichia coli.* Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods that include the use of smoking and chemical preservatives. This includes sodium benzoate. Though it sounds as if it was created in a laboratory, it is actually natural preservatives.  It is found naturally in cranberries, prunes, greengage plums, cinnamon, cloves and apples.  The compound has antimicrobial properties and prevents the growth of bacteria and mold. For the bacteriostatic and fungicidal properties of Sodium Benzoate, they are used in preservation of fish. It prolongs the shelf-life of fish for a very long period without affecting its texture, taste and appearance.  It is often found in foods such as jams, salad dressings, carbonated beverages, relishes, olives and pickles. The antimicrobial activity of Sodium benzoate against bacteria, moulds and yeast has been published (USFDA, 1978). Looking at this wide range of antimicrobial activity, the impact of Sodium benzoate on smoked Tilapia during storage times at room temperature on microbial, physical, organoleptic and nutritional quality is therefore studied during 8-week storage at room temperature.

**MATERIAL AND METHODS**

Fresh Tilapia (*Oreochromis niloticus*) were obtained from a private Fish pond in National Institute for Freshwater Fisheries Research (NIFFR) Housing Estate, New Bussa, Niger State. The fish samples measuring 12-18cm and weighing 75-90g. The fish were transferred within 30 minutes to the laboratory in a sterile polythene bags and then killed by severing the spinal cord with a sterile scalpel and aseptically eviscerated, washed and rinsed in sterile water. The fish samples were randomly chosen and divided into 6 groups of 5 fish each. The samples were subjected to treatments. The treatments were as follows; (1) control (untreated samples); (2, 3, 4, 5 and 6) treated with 1, 2, 3, 4 and 5% Sodium benzoate for 5 minutes. A sample from each group were separated from each treatment and smoked. Smoking was done according to the methods described by Omojowo and Ibitoye (2005). After smoking and the fish were allowed to cool down and stored in different boxes. This was done to mimic commercial practices. The samples were drawn after two, four, six and eight weeks of storage; then subjected to analysis.

**Microbiological and other Analysis**

Total viable count (TVC), Coliform, Staphylococci and Fungi count were evaluated according to the methods described by Harrigan and McCance 1976; Speck 1984 and Sneath et. al. 1986). Moisture contents, fat and Crude protein were estimated as per AOAC (1980). All samples were done in duplicates. Sensory evaluation was carried out according to the method of Afolabi et. al. (1984). Statistical analysis was according to SAS, Institute, Inc, (1992) at P < 0.05.

**RESULTS AND DISCUSSION**

**Microbial Analysis**

Total Viable count (TVC), Coliform, Staphylococci and Fungi count in log CFU/g of fresh and smoked Clarias and tilapia samples plated on selective and non-selective media are shown in Tables 1. TVC of the fresh non-treated (control) Tilapia was 5.97 log CFU/g but after the sample were subjected to treatments with 1-5% Sodium benzoate, the reduction was highest in 5% (4.90 log CFU/g and least in 1& 2% which were both 5.56 log CFU/g. Also, the treatments with Sodium benzoate reduced the Coliform to 3.60 log CFU/g in 5% and least in 1& 2% which were both 4.26 log CFU/g (Table 1). In the same vein, Staphylococci count was reduced from 4.51 log CFU/g in the control to 2.86 log CFU/g in 5% and least was with 1% which was 4.15 log CFU/g log Fungi count reduced from 4.68 log CFU/g (control) to 4.61log CFU/g in 1% and highest in 5% (4.08 log CFU/g).

Smoking sharply reduced the total viable count in all samples, but the sample treated with 4% and 5% conc. showed the greatest reduction and maintained a low level throughout 8 weeks of storage. The samples treated with 5% Sodium benzoate was 4.05 log CFU/g initially and at the end of eighth week of storage with TVC of 6.82 while The TVC of smoked control (untreated) samples was the highest throughout the period of storage reaching 8.91log CFU/g on the 8th week. The results obtained were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the lowest microbial load and maximum shelf stability followed by 0.4% Sodium benzoate. Similar to TVC, the coliform count of the smoked samples treated with 5% conc. had the least reduction and remain the lowest of the treatments throughout the period of storage. Significant increases in coliform population of all samples occurred after 4 weeks of storage. Coliform count of all treated samples was less than 3.0 log CFU/g throughout the 8-week storage. In the control samples, the Coliform population of Tilapia showed 5.0 and 5.86 log CFU/g on the 6th and 8th week respectively.

This result was similar to that reported by Virginia, (2002) where the Coliform in the control sample showed 2.6 log CFU/g on the 4th week. The high coliform count recorded in this report may be due to contamination from the animal manure used in fertilizing the ponds at one time or the other. In the Staphylococcus population, the smoked sample treated with 4-5% Sodium benzoate reduced the Staphylococcus count to 0 and remained 0 until the end of 8th week storage. This report is similar to earlier reports where 3-5% Potasium sorbate, Citric acid and Sodium metabisulphite also reduced Staphylococcus population to zero (Omojowo *et.al.,* 2009a, Omojowo *et.al*., 2009b, Omojowo *et.al.,* 2010). The isolation of *Staphylococcus* in smoked samples on day 0 may be attributed to post processing contamination. However, *Staphylococcus* was killed by the treatments 4-5% Sodium benzoate. The population of the Fungi reduced in all the treatments and at the end of the 8-week storage time. However, the control samples were high throughout the period of torage.

The results obtained were similar to those reported by Efiuvwevwere and Ajiboye (1996), where the samples treated with 0.4% potassium sorbate showed the minimum fungal load throughout storage followed by 0.4% Sodium benzoate and presence of profuse mould growth after day 8 in Control and Sodium benzoate-treated sample. It is of interest to observe that in spite of the slightly reduced moisture contents (from 2nd to 6th week) in almost all the samples, microbial load still increases dramatically. This suggests that one single factor may not account for these microbial changes. Cross contamination, pH, purity of preservatives is among other factors that can influence microbial changes. The TVC of the most of the treated samples were all below 5x105 CFU/g to the 6th week which is below m in a three-class attribute plan and signifies good quality. Low levels of coliform bacteria were detected and the pathogens *Staphylococcus aureus* counts were below 103 in all the treated samples till the 8th week except samples treated with 1-2% in which the count exceed 103 in the 8th week (Tables 1). The control however, has TVC higher than 5x105 CFU/g in the second week and higher than the recommended limit 7.0 log CFU/g (ICMSF, 1986) after the 4th week. In addition the Coliform count already exceeded 103 just after smoking. This finding is of concern as a result of the associated public health implications. For example, generally, hot smoked fish are consumed in the tropics with little or no further processing/cooking; thus, they fall into the high-risk category of foods (ICMSF, 1986). Hence there is a need for the use of appropriate percentage of choice antimicrobial agent.

 **TABLE 1: MICROBIAL LOAD OF TILAPIA TREATED WITH SODIUM BENZOATE (Log10)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Microbial group** | **Control** | **1%** | **2%** | **3%** | **4%** | **5%** |
| **B/4 Smoking** | **TVC** | 5.97 **a** | 5.56 **b** | 5.56 **b** | 5.34 **bc** | 5.06 **cd** | 4.90 **d** |
| **After ,,** | **TVC** | 4.51 **b** | 4.04 **c** | 4.00 **c** | 3.90**cd** | 3.65 **cd** | 3.28 **d** |
| **2nd week** | **TVC** | 6.16 **c** | 4.23 **d** | 4.23 **d** | 4.27 **d** | 4.18 **d** | 4.05 **d** |
| **4th ,,** | **TVC** | 6.75 **c** | 5.0 **d** | 4.75 **de** | 4.80 **de** | 4.75 **de** | 4.30 **e** |
| **6th ,,** | **TVC** | 7.37 **d** | 5.60 **e** | 5.28 **e** | 5.20 **e** | 5.06 **ef** | 5.00 **f** |
| **8th ,,** | **TVC** | 8.91 **e** | 7.75 **f** | 7.70 **f** | 7.68 **f** | 7.31 **f** | 6.82 **g** |
|  |  |  |  |  |  |  |  |
| **B/4 smoking** | **Coliform** | 4.56 **a** | 4.26 **b** | 4.26 **b** | 4.02 **b** | 3.68 **b** | 3.60 **b** |
| **After ,,**  | **Coliform** | 3.32 **b** | 1.68 **c** | 1.38 **c** | 1.26 **c** | 1.28 **c** | 1.04 **c** |
| **2nd week** | **Coliform** | 3.41 **b** | 1.80 **c** | 1.80 **c** | 1.74 **c** | 1.62 **c** | 0.98 **d** |
| **4th ,,** | **Coliform** | 4.06 **c** | 2.35 **d** | 2.24 **d** | 2.20 **d** | 2.07 **de** | 1.82 **e** |
| **6th ,,** | **Coliform** | 5.00 **d** | 2.70 **e** | 2.60 **e** | 2.44 **e** | 2.41 **e** | 2.36 **e** |
| **8th ,,** | **Coliform** | 5.86 **e** | 2.80 **f** | 2.81 **f** | 2.84 **f** | 2.69 **f** | 2.68 **f** |
|  |  |  |  |  |  |  |  |
| **B/4 smoking** | **Staph.** | 4.51 **a** | 4.15 **b** | 4.06 **b** | 4.00 **b** | 3.80 **b** | 2.86 **c** |
| **After ,,**  | **Staph.** | 3.20 **b** | 1.60 **c** | 1.51 **c** | 1.30 **d** | 0.30 **e** | 0.0 **f** |
| **2nd week** | **Staph.** | 4.15 **c** | 1.66 **d** | 1.57 **d** | 1.38 **e** | 0.0 **f** | 0.0 **f** |
| **4th ,,** | **Staph.** | 4.74 **d** | 2.40 **e** | 2.17 **f** | 1.90 **g** | 0.0 **h** | 0.0 **h** |
| **6th ,,** | **Staph.** | 5.10 **d** | 3.02 **e** | 2.86 **e** | 2.43 **f** | 0.0 **g** | 0.0 **g** |
| **8th ,,** | **Staph.** | 6.32 **e** | 3.72 **f** | 3.20 **g** | 2.84 **h** | 0.0 **i** | 0.0 **i** |
|  |  |  |  |  |  |  |  |
| **B/4 smoking** | **Fungi**  | 4.68 **a** | 4.61 **b** | 4.60 **b** | 4.44 **b** | 4.36 **b** | 4.28 **b** |
| **After ,,**  | **Fungi**  | 3.34 **b** | 2.00 **c** | 1.98 **c** | 2.00 **c** | 1.96 **c** | 1.87 **c** |
| **2nd week** | **Fungi**  | 4.64 **c** | 2.65 **d** | 2.51 **d** | 2.36 **d** | 2.29 **d** | 2.24 **d** |
| **4th ,,** | **Fungi**  | 5.20 **d** | 3.61 **e** | 3.62 **e** | 3.58 **e** | 3.52 **e** | 3.45 **e** |
| **6th ,,** | **Fungi**  | 5.58 **d** | 4.26 **e** | 4.19 **e** | 4.11 **e** | 4.06 **e** | 4.04 **e** |
| **8th ,,** | **Fungi**  | 7.52 **e** | 6.37 **f** | 6.33 **f** | 6.15 **f** | 6.06 **fg** | 5.60 **g** |

 Means in the same rows with different superscript are significantly different (p < 0.05).

**BACTERIAL ISOLATES**

All treated smoked sample were negative for *E. coli and Streptococcus sp*

The control and the fresh fish treated samples showed the following bacteria flora *Bacillus coagulans, B. cereus, Klebsiella ozanae, Proteus vulgaris, Escherichia coli, Staphylococcus aureus, and Streptococcus sp,* while the fungi isolated include *Penicillium verrucosum, Aspergillus niger, A. candidus, A. flavus and A. nidulan* while the smoked untreated catfish sample (control) were dominated by the following organisms *B. coagulans,* (about 70% of the isolates) while the remaining being *S. aureus*, and *Streptococcus sp*. Smoked untreated tilapia sample also showed bacteria load above except that *Streptococcus sp* was not isolated in the sample. The treated sample showed the microbial load in the following pattern; 1% 2, and 3% Sodium benzoate treated samples contains the following isolates *B. coagulans, S. aureus, K. ozanae, A. niger, A. candidus, A. nidulan, and P. verrucosum while* 4 and 5% Sodium benzoate treated samples have the following isolates *B. coagulans, K. ozanae,* A*. nidulan and A. flavus* in 4% only.

**Visual Observation**

The actual external colour of smoked Tilapia varied from light brown to grayish brown. There were generally, no major difference between the control and most of the treated samples. Generally, the external colour of the treated samples did not change during the eighth week of storage.

**Proximate Analysis**

The proximate analysis of raw and Smoked Tilapia are presented in Figure 1 to 3. There were no significant (p<0.05) differences in Protein (18.3 – 20.2%), Fat (2.6 – 3.0%), and Moisture contents (73.4 - 77.0%) of fresh tilapia when subjected to different treatments. The moisture content of fresh samples decreased sharply after the smoking. This decrease was due to loss of water during smoking (Asiedu et al., 1991). Most of the treated sample decrease on the second week, increase at the fourth week and decrease from the 6th week again and the result of the 8th week is close to the one obtained in day 1 of smoking. The moisture content of all treatments remained similar throughout 8 weeks of storage. This reduction in the fat content can be due to a sampling problem, since on the 8th week of storage most the treated samples increased and showed a result close to that of the day 0. The variation in fat contents follows the pattern of moisture variation and this may be a strong factor responsible for the variation also. Storage time may not have significant effect on the fat content of smoked fish. In addition, the average protein content of the fish samples increases after smoking, and increases till the 4th week and later decreases till the end of the 8th week of storage. There was an inverse relationship between the moisture and protein content in the smoked samples. The initial increase in protein content in smoked fish and till the 4th week may be due an increase in the dry matter content per unit of weight following sample dehydration during smoking and reduction in the moisture contents during the early part of the storage before autolysis becomes pronounced.

This result shows that storage time causes a decrease in the protein content of smoked fish which agreed with earlier work of Ufodike and Obureke (1989) where they attributed the decrease to hydrolysis of protein during the process of autolysis in the fish muscle. However, the treated samples show some corresponding higher value of protein more than the control especially as the concentration of the preservatives increases from 1-5. This increase may be due to the effects of the preservatives which slow down autolysis in the fish muscles and consequently slow down the protein break down.





**Note, in x-axis 1= Day 1, 2= 2n d Wk, 3 = 4th Wk, 4= 6th Wk and 5= 8th Wk**



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**ORGANOLEPTIC ASSESSMENT**

The quality of the smoked fish (both treated and untreated) was evaluated immediately after smoking and after storage for 8th week on taste, flavour, texture, appearance and overall acceptability. The fish flesh overall score was given to both untreated (control) and the one of various treatment using a hedonic scale of 1- 5 fish scoring less than 2 being regarded as unacceptable. This assessment was done for both tilapia and the catfish. Table 2 summarizes the taste panel results. From the result, the trend of scores for the overall acceptability of freshly smoked tilapia is as follows: 1% = 2 % > C = 3 % = 4% > 5% while on the 8th week the trend is 2% > 1% >C >3% > 4 % > 5 %. N.B. The panelist were made of people with no formal training in fish assessment representing the ordinary consumers outside that needs no training before deciding the acceptability of fish in the markets.

**TABLE 2. ORGANOLEPTIC ATTRIBUTES OF FRESHLY SMOKED AND 8TH**

 **WEEK STORED TILAPIA TREATED WITH SODIUM BENZOATE**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Tilapia Treatment** | **Taste** | **Flavour** | **Texture** | **Appearance** | **Overall-acceptability** |
|  **CONTROL** | **4.2** | **4.1** | **4.2** | **4.1** | **4.1** |
| **FRESHLY SMOKED - 1 %** | 4.0 | 4.1 | 3.9 | 4.0 | 4.4 |
|  **2 %** | 4.0 | 4.0 | 4.1 | 4.2 | 4.4 |
|  **3 %** | 2.6 | 2.5 | 2.3 | 2.7 | 2.1 |
|  **4 %** | 2.1 | 2.0 | 2.0 | 1.6 | 2.1 |
|  **5 %** | 1.6 | 1.7 | 1.6 | 2.3 | 1.5 |
|  |  |  |  |  |  |
| **CONTROL (8TH WK)** | **3.3** | **3.5** | **3.6** | **3.6** | **3.0** |
| **8TH WEEK OLD 1%** | 3.6 | 3.8 | 3.8 | 3.9 | 3.2 |
|  **2%** | 3.6 | 3.6 | 3.7 | 3.9 | 3.7 |
|  **3%** | 3.0 | 2.8 | 3.0 | 4.0 | 2.8 |
|  **4%** | 2.0 | 2.1 | 2.5 | 3.9 | 2.0 |
|  **5%** | 1.4 | 1.6 | 1.6 | 3.7 | 1.2 |

Values are arithmetic means of duplicate determinations

**CONCLUSION AND RECOMMENDATION**

Though, 5% concentration of Sodium benzoate showed the greatest reduction of TVC, Fungi, and even Staphylococcus population to 0 in most of the treatments however, organoleptic study has reveals that the samples treated with 1 and 2% Sodium benzoate are preferred by the consumers. In most of the various microbial count except Staphylococcus population, the output of 1% treatment is not significantly different from 2% treatment. And since the lower the concentration the better, 1% concentration will be highly recommended. This 1% concentration was able keep the fish to ICMSF (1986) standard of good quality till the 6th week by reducing the TVC from 7.37 in the control to 5.60 log CFU/g. It also reduced the coliform in the control from 5.86 log CFU/g to 2.70 log CFU/g in tilapia. Also in the staphylococcus count the reduction is from 5.10 log CFU/g in the control to 3.02 CFU/g in tilapia. Also the Fungi count was reduced from 5.58 log CFU/g in the control to 4.26 log CFU/g. Hence, 1% Sodium benzoate may be used as a preservative in smoked fish without adversely affecting quality in terms of lipid oxidation, color, microbial and nutritional quality for a period of 6 week.

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