International Journal of Advanced Research and Development
ISSN: 2455-4030, Impact Factor: RJIF 5.24
www.advancedjournal.com
Volume 1; Issue 9; September 2016; Page No. 06-09

Changes in the quality and yield of fish chub due to temperature fluctuations persevered with local spices

[1] Jummai AT, [2] Negbenebor CA, [3] Okoli BJ

[1] National Biotechnology Development Agency, Federal Ministry of Science and Technology, Abuja, Nigeria
[2] Department of Food Science and Technology, University of Maiduguri, Nigeria
[3] Department of Chemical Sciences, Bingham University, Karu, Nigeria

Abstract
Temperature is a factor which affects microbiological quality of feeds during their storage. Three storage temperatures of the feeds for fish were taken into account in this study: -11°C, 5°C and 20°C. Analyses comprised the survival of proteolytic, ammonifying, psychrophilic and mesophilic bacteria as well as fungi. It was found that after 72-day storage of fish feed at the three temperatures, fungi showed the highest survival (56% - 80%). As regards the four physiological groups of bacteria, the highest survival was observed for mesophilic bacteria (6.25% - 9.58%), followed by psychrophilic ones (2.5 - 3.25%) and ammonifiers (0.07 -0.11%), while proteolytic bacteria showed the lowest survival (0%). No live cells of the latter bacteria were observed after 64-day storage of the feed at -11 and 5°C, and after 72-day storage at 20°C.

Keywords: micro-organisms, survival, fish feed

1. Introduction
Complete feeds produced commercially are both important and indispensable in fish farming. They are usually in form of dry complex feeds composed of plant (cereal seeds, bran, rapeseed or soybean meal or cake, legume seeds) and animal components (meat-bone and fish meal, poultry off-fall, meat, powdered milk, animal fats) supplemented with vitamins and minerals [1].

Protein represents the major component of fish feeds. It is a source of energy for the fish, but also a medium for micro-organisms, especially proteolytic bacteria and ammonifiers. Good quality of the products used and proper hygiene of the technological processes decrease the risk of microbiological contamination of fish feeds.

Storage conditions, especially temperature and humidity, represent another important factor affecting microbiological quality of feeds. Improper storage temperature may prolong survival of the micro-organisms present in the feed [11], or even enhance their multiplication and production of toxic substances. Toxin-producing fungi are especially dangerous, most of all those producing aflatoxins, patulins, and trichotecens which are strongly cancinogenic and mutagenic [6].

Microbiological analysis should be used in classifying a feed as suitable for use; its results should not exceed the respective standards. At present, fish feeds used in Poland must conform to the Polish Standard (PN-76/R-64791) [9] referring to dry feeds. This standard defines necessary microbiological examination of the presence of active micro-organisms, such as: proteolytic and ammonifying bacteria, saprophytic and toxin-producing fungi. No works have been found in the available literature on the survival of micro-organisms present in fish feeds during storage, although there are papers devoted to the survival of bacteria in other environments, e.g. lake water [3, 7, 10] fish, meat, milk, cheese, broth and soil [3, 4, 5]. These studies dealt with the survival of single strains of bacteria.

The objective of the present study was to examine survival of the physiological groups of proteolytic and ammonifying bacteria, fungi (mentioned in the standard PN-76/64791), as well as psychrophilic and mesophilic bacteria present in fish feeds during their storage at -11, 5 and 20°C.

2. Materials and Methods
Sources of Materials
The fish samples *Clarias gariepinus* used for this study were obtained from Gamboru Maiduguri fresh fish market and transported to Food Science and Technology Department Laboratory and were then processed immediately. The fish samples were stored under frozen condition (-18°C) in freezer before analysis. The spice *Monodora myristica*, common salt (Dicon salt), casing (small intestine of cow), sorbitol (Archer Daniels, Midland UK) were obtained from Monday Market Limited, Maiduguri.

Preparation of the Spice
The *Monodora myristica* (Plate I) fruits were dehulled to remove the outer coat and processed as presented below and samples were wrapped in aluminium foil then autoclaved at 15 Psi 121°C) for 15 minutes to destroy any microorganism present on the sample.

Preparation of Fish Chubs
The fish sample was thoroughly cleaned with 4% salt solution to remove the slime and to minimize contamination. The fish was weighed, headed, gutted, filleted and chopped into smaller sizes. The fish sample was divided into 4 groups, each of the groups was treated separately as indicated below.

i) Control sample + Nitrate (0.33%) + Salt (1.5%).
ii) Sorbitol (0.4%) + Nitrate (0.33%) Salt (1.5%).

iii) Nutmeg (*Monodora myristica*) (0.2%) + nitrate (0.33%) + Salt (1.5%)
iv) A combination of (*Monodora myristica*) (0.2%) + sorbitol (0.4%) + Nitrate (0.33%) + Salt (1.5%). (Negbenebor *et al*., 1999)

The samples were cured for one hour separately and allowed to drain. The processing was carried out at ambient temperature of 28°C to 35°C. The casing (small intestine of cow) was washed thoroughly with salt solution and cut to the desired length (30 cm). The fish was stuffed into the casing with the ends knotted with strings, then placed in a rack and smoked for six hours at 60°C (Plate II). The product was cooled to room temperature. Packaged in polythene bag, separately and stored under refrigerated temperature at 7-10°C for 1 week, and frozen temperature of -18°C for 12 weeks for quality determinations. The samples were analysed for yield, physical quality, proximate composition and trimethylamine content at predetermined intervals. The frozen samples were analysed at week 0, 2, and every two weeks for a period of 12 weeks, while the refrigerated samples were analysed at day 0, 1, and every day for a period of 7 days.

Experimental Design

The experimental design was factorial arrangement with 4 x 2 treatments. The fixed factors were sample treatment consisting of control, Nutmeg, sorbitol and mixed Nutmeg and treatments. The variable factors were refrigeration (1-7 days) and deep freezing 1-12 weeks).

3. Micro-biological analysis

Preparation of Media

i) Nutrient Agar: This is a basic culture medium used to support the growth of micro-(bacteria) and has special nutritional requirements. The medium was prepared by 28g of nutrient agar in l0O0ml of distilled water, stirred to dissolve and autoclaved 121°C for l5 minutes at l5 Psi, cooled to 40°C before plating.

ii) MacConkey Agar: This agar is a differential and low selecting medium used to distinguish lactose and non-lactose fermenting bacteria It was prepared by dissolving 52g of MacConkey agar in l0O0ml of distilled water, stirred to dissolve and autoclaved at l2l"C for p I5 minutes at l5 Psi, cooled to 40°C before plating.

iii) Eosin Methylene Blue agar: This agar medium was used for detection of enterobacteriaceae like klebsiella spp. This medium was prepared by mixing 30g of the agar l000ml of distilled Water, stirred to dissolve and autoclaved at 121"C for 15 minutes at 15 Psi and cooled at 40°C before plating.

iv) Mannitol Salt Agar: This is a differential and selective medium used to isolate *Staphylococcus aureus*. It was prepared by dissolving 111 gm of manitol salt agar in l000ml distilled water, stirred to dissolve and autoclaved at 121°C for 15 minutes and 15 Psi and 40"C before plating.

v) Potato Dextrose Agar (PDA): This is a general purpose medium used to support of the yeast and mould that have special nutritional requirements. The media composition as follows:—Potatoes - 200g, Dextrose (Glucose) - 20g and Agar-agar - 15g.

vi) Procedure: Two hundred grams of peeled and sliced potatoes were boiled in 500ml of water until sofi (20 min). It was filtered and the filtrate made up to l000ml with distilled water. Twenty grams of glucose was dissolved in the extract with 15g of agar and 5ml solution of chloramphenicol in alcohol solution was added. The medium was autoclaved at 121°C for 15 min at 15 Psi and cooled to 40"C before plating.

vii) Inoculation preparation: Ten grams of the homogenized samples were suspended in 90ml of sterile distilled water in a diluent bottle, tightly corked and mixed thoroughly by shaking for 1 min. Serial dilution was carried aseptically as described by Harrigan and McCance (1976).

Sample Plating

One ml of inoculum was pipetted into sterile petri dishes and the media was poured in the inoculum in the petri dishes using the pour plate method (Collins and Lyne, 1976). The media were allowed to set and inverted before incubating at 32"C for 24-48 hours for bacteria count and for 5-7 days at 35°C for mould count. Triplicate determinations were made and counts determined after incubation using a Gallen Kamp Colony Counter.

Sugar Preparation

The following sugars were prepared (sucrose, sorbitol, lactose etc) by standard methods described by Harrigan and McCance (1976). The sugars were prepared using 1% concentration. One gram of the sugar was dissolved in 10ml of sterile distilled water, 15g of Andrade peptone was also dissolved in 1 litre of sterile distilled water, sterilized at 121°C for 5 min at 15 psi and then allowed to cool to 56°C. Ten ml of the sugar solution was added to 90 ml of Andrade peptone water, mixed very well md 50 ml of the mixture was put into Bijou bottles, corked very well, sterilized using a water bath at 80°C for 30 min. and was then ready or use (amber in color). Durham tubes were inserted into the mixture and then inoculated.

There was incubation for 24 hours and 48 hours. Observations were made after 24 hours and 18 hours for late fermenters. These were carried out for the various sugars.

Mould and Bacteria Identification

Colonies of moulds were isolated, purified and identified using methods described by Eilman (1959). Bacteria were isolated, purified; gram stained and identified using methods described by Collins and Lynes (1976).

Storage Studies Determinations

Samples were packed in polythene bags separately knotted and the storage studies Were carried out for the samples during storage at refrigerated and frozen temperatures. Samples were analysed every two weeks for physical quality, proximate composition and trimethylamine content for three months period to determine the shelf life, and refrigerated samples were analysed day 0,1 and every day for a period of 7 days.

Statistical Analysis

All data for statistical analysis were subjected to analysis of variance (ANOVA) as described by Amerine *et al*, (1965) [2]. The differences between means were determined using Duncans multiple range test (DMRT) as described by Gomez and Gomez (l984) [6].

Microbial Storage

Total Plate Count (Frozen Storage, -18ºC)

The initial total plate count ranged from mean log 3.76 ± 1.2, to 3.84 ± 0.6 on week 0 during frozen storage.

There was no significance difference (P< 0.05) in total aerobic plate count between the treatments and the control on week 0 since there was no significant deterioration in the sample. Therefore the effects of the treatment could not be evaluated (Fig 1).

During frozen storage, the formation of ice tied down the moisture making it unavailable for microbial growth (Jay, 1986). This compared favourably with the rating of les by the taste panel as acceptable (Clucas and Ward, 1996) [3].

The mean log bacteria count of the control sample was 3.84cfi1/g; this could be due to pocessing contamination. Mean log bacteria counts for treated samples decreased at p time from week 2 to week 12 for most of the treatments (Fig 2). This could be mllt of the changes in the environments of the bacteria resulting in shock and death for of them (Adams and Moss, 1995).

After 12 weeks of storage, the total plate count ranged from mean log 3.43i0.5 to k 0.Icfi1/g and was not significantly affected by treatments. In general frozen seafoods lower microbial count compared to fresh product. (Jay 1987).

4. Refrigeration

Total plate count (refrigerated storage, 7 - 10°c)

On day 0, the bacterial count ranged from mean g, (Fig 2) and were not significantly different (P <0.05) from each other and the lat nt for all samples during samples. There was increase in total aerobic storage.

After seven (7) days of storage the total place count rose to 6.10 ± 0.01 to 6.9 ± 0.08 some of the samples had off-flavour and odour. According to Jay (1987) samples with total aerobic plate count of log 6 and above produce of 'flavour.

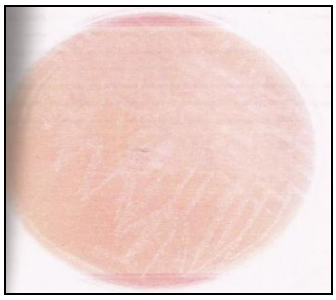

Fig 1

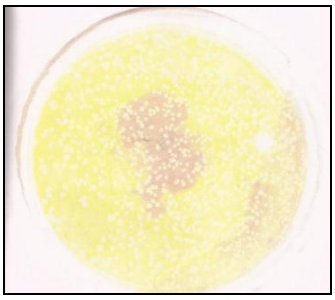

Fig 2

Mould count (frozen storage, -1s"(3)

No mould was detectable from week O to week 8 (Fig 3) irrespective of Result suggests that the processing was effective in inhibiting mould growth. The nee of mould growth in week 10 for the control sample suggests post-processing Ininations. Afier I2 weeks of storage at frozen storage, sample treated with Local |eg + Sorbitol did not show any evidence of mould growth, which suggest that sample ad with Local Nutmeg+ Sorbitol may be mycostatic. Mould growth is a problem in most ed fish products, so the ability of the combination of Local Nutmeg + sorbitol to control nould growth after I2 weeks suggest that it is important or beneficial to fish processing tries. The presence of mould in smoked fish product has been associated with the Iuction of aflatoxin, which is known to be carcinogenic (Clucas and Ward, 1996) [3].

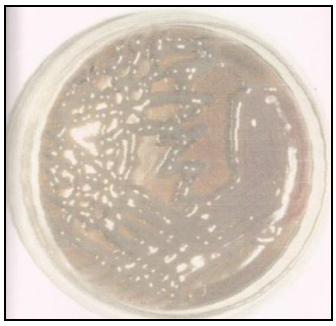

Fig 3

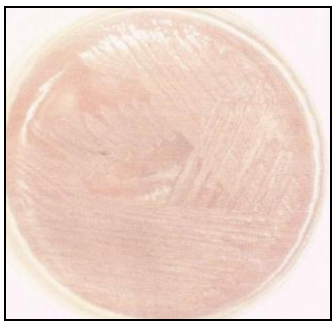

Fig 4

Mould count (Refrigerated Storage, 7 — l0"C)

From day O to day 4, no mould growth was observed in any of the samples of treatment (Fig 4). This suggests that the processing was effective in mould growth during refrigerated storage. Sample treated with Nutmeg + sorbitol show evidence of mould growth up to day 6 which suggest that sample treated with Nutmeg+ sorbitol was more efiective in inhibiting mould growth than the control and treated samples. The inhibition on day 5 may have been due to the presence of acid, stearic acid and oleic acid in nutmeg and sorbitol. On day 7, there was evidence growth on all the samples. This could be as a result of post processing or that the treatments had lost their potency. The control of mould growth is important in most foods, as some of these moulds been implicated as carcinogens.

Coliform count (Frozen Storage, -18°c)

From week 0 to 10 weeks of storage, there was no evidence of coliform growth in any samples (Fig 5), suggesting that the treatments either inhibited the growth of or was as an overall indication of good sanitary quality. The presence of coliform in 12 (Plate 11) in all the samples suggests post-processing eontammations as a result of contamination, (FDA, 2001). However, the values obtained for the coliform were not high enough to cause any food poisoning. The coliform standard for frozen is 3cfiJ/g (Jay, 1986). The incidence of microorganism in sea foods such as catfish will greatly upon the quality of water fiom which they were harvested. Assuming, good water was used than most of the organisms were picked up during the various stages of (Jay, 1987). Coliform is an indicator orgamsm. The absence of coliform during most of the storage is desirable since it indicates the food is of high hygienic quality.

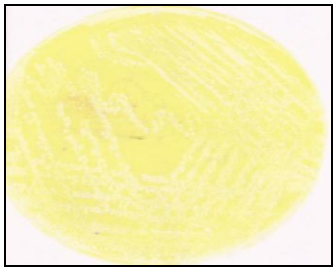

Fig 5

Fig 6

Coliform count (Frozen Storage, 7 – 10º C)

There was no coliform growth from day 0 to day 4 for all the samples irrespective of (Fig 6). Samples treated with Local Nutmeg alone and of a Local Nutmeg + sorbitol showed no evidence of coliform growth up and this suggests that the treatment inhibited the growth of coliform on day 5. There was evidence of coliform growth (Plate 11) in all the samples. However, the for the coliform in this study were not high enough to cause food poisoning The coliform standard for frozen precooked food falls within 0 to over 3cfi1/g or ml by Jay (1987). Coliforms have been implicated in gastrointestinal problems in the elderly.

5. Conclusions

Fungi showed the highest survival during storage of the fish feed. After 72 day-storage at -11 and 20"C survival of these group amounted to 80%, and at 5"C - to 56%. From among the four physiological groups of bacteria, the highest survival after 72 days of feed storage at the three temperatures was observed for mesophilic bacteria (from 6.25 to 9.58%). It was lower for psychrophilic bacteria (2.5 - 3.25%) and ammonifiers (0.07 - 0.11%), and the lowest (0%) for proteolytic bacteria.

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