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. 01-05

Effect of temperature on pH and free fatty acid content of *Clarias gariepinus* chubs persevered with local spices

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Abstract
This study was conducted to determine the effect of storage temperature on changes in pH and free fatty acid content of *clarias gariepinus* chubs persevered with local spices. Cooled smoked samples were packaged and stored at refrigerated and frozen storage conditions and examined for 1-12 weeks under frozen and days 1-6 under refrigerated condition at intervals. Frozen fish chubs stored at (-18 °C) had a relatively low free fatty acid content and fairly constant pH(5.9 $\pm$ 0.01 to 6.09 $\pm$ 0.0) irrespective of treatment while the refrigerated fish chub (7-10 °C) sample showed a progressive increase in the free fatty acid content as the period of preservation increases. The combined use of local nutmeg and sorbitol increased the shelf life under both storage conditions. The production of fish chub could be a viable economic venture in places where catfish is readily available and it is likely to improve protein intake of the consumers and reduce post-harvest losses.

Keywords: *Clarias gariepinus*, *Mondora myristica*, Fish chub, Sorbitol, Free Fatty Acid Content and pH

1. Introduction
The recent awareness on the effect of white meat like fish and the high nutritional contents to man has caused a drift in the choice of meat to favour its consumption (Oriakpono *et al.*, 2011) [17]. Aquaculture practices are considered today as one of the most promising sources of animal protein. During the recent past, the potential and prolific nature of fish culture has been directed towards its large-scale adoption and promotion in developing countries. A reason for the steady increase in aquaculture production and maintenance is the lack of research and technical input and the expansion of areas under culture (Mahboob, 2014) [13].

Fish and shellfish contain about 19% protein similar in amino acid composition to that found in muscle meats. The protein content varies up to 20%, depending upon the species and the season of the year. Fish contains considerably lower fat content than beef (Ndome *et al.*, 2010a) [14].

Nutritional quality and organoleptic acceptability in terms of colour, texture, smell, flavour and appearance may be affected by the environmental degradation and quality of nutrition and feed provided during culture, especially in semi-intensive and intensive culture systems compared to wild fish (Thomas, 1973; Grigorakis *et al.*, 2003) [18, 11].

The prevailing climatic conditions in the tropics experience an increase in temperature and relative humidity of over 25^0C and 70%, respectively (Adaga, 2014) [1]. Such conditions accelerate mould growth and lipid oxidation (Berger, 1989; Coppen, 1989; Van den Bergh, 1990) [5, 23]. According to Bautista *et al.* (1992) [4] and Ramezandeh *et al.* (1999), feed storage at high temperature results in an increase in both oxidative and hydrolytic rancidity with loss in feed quality.

Studies by Van den Bergh *et al.* (1990) [23] and Ruiz *et al.* (2000) [20] indicate that fats are intrinsically unstable when subjected to high temperature above 30^0C. Under such conditions, fats are hydrolysed to release ketonic acids, which further undergo auto-oxidation with degeneration of free radical products (Hamilton, 1989) [5]. The spoilage of fresh fish can be attributed to series of metabolic processes that deteriorate fish quality and render it undesirable and unacceptable for human consumption due to changes in sensory and biochemical characteristics (Ndome *et al.*, 2010b) [15]. The noxious smells of spoiled fish are suspected to be produced by microbes to repulse large animals, thus reserving the food resource for them while increasing spoilage and reducing organoleptic properties (Sherrat *et al.*, 2006; Braun and Sutherland, 2005) [21, 6]. Due to the increase in fish consumption, there seems to be a habit of storage for a long period with an assumption, that the fish maintains the nutritional quality and safe for human consumption. The objective of this study was to assess overall changes in proximate composition and organoleptic quality of flesh of fresh *Clarias gariepinus* and those stored at two temperatures at 7-10°C for 1 week, and -18°C for 12 weeks.

2. Materials and Methods
Preparation of the Spice
The *Monodora myristica* 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 (Fig 1).

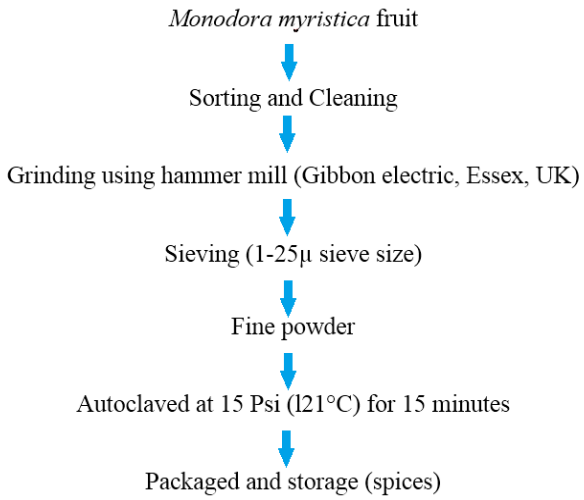


Fig 1: Flow diagram for the processing of the spice *Manodom myristica*

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 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) [16].

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. 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 changes in pH and free fatty acid 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).

Estimation of Fats

Fat extraction was carried out by the soxhlet method as described by AOAC (1984) [2]. Three grams of macerated fish chub sample were weighed into a thimble, which was inserted into the extractors. The extraction flasks were inserted into the flasks containing 150ml of petroleum ether. The heaters were turned on after assembling the apparatus and extraction was carried out for about 3hrs.

After extraction, the petroleum ether was recovered from the chamber and the flask containing the extracted fat was placed in the oven for about 10mins after which they were put in the desiccators to cool for about 15mins and then reweighed.

$$\% \text{ Fat} = \frac{\text{wt of flask(empty)} + fat - \text{wt of flask}}{wt\ of\ sample} \times 100$$

These were done in triplicate.

Chemical Analysis

Determination of pH

Three grams of each sample were mixed thoroughly in 15ml of distilled water. The pH meter was standardized with buffer solutions of pH 4 and 9. The pH of the samples was taken by dipping the pH electrode into the slurry. The pH meter (Kent 7046/46) was used. This was carried out in triplicates.

Determination of Free Fatty Acid

Five grams of each sample was added to 50ml of neutral 95% ethanol in a 250ml Erlenmeyer flask the flask was warmed and shaken thoroughly to dissolve the free fatty acids. Two drops of 1% phenolphthalein indicator solution was added and titrated against 0.l N KOH, while shaking thoroughly during titration, until the pink coloration persisted for about 15 seconds.

Free fatty acid value= Acid value × 0.503

Triplicate determination were made.

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 changes in pH and free fatty acid content for three months period.

Statistical Analysis

All data for statistical analysis were subjected to analysis of variance (ANOVA) as described by Amerine *et al*, (1965) [3]. The differences between means were determined using Duncans multiple range test (DMRT) as described by Gomez and Gomez (l984) [11].

3. Results and Discussion

Free Fatty Acid at Frozen Temperature (-18°C)

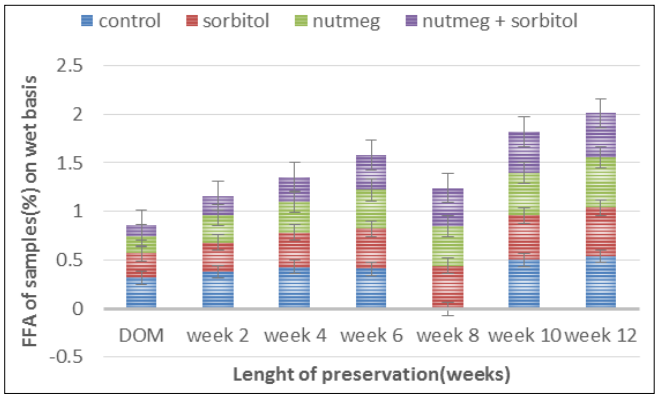
The initial free fatty acid content on week 0 was 0.32% for the control and 0.11- 0.25% for the treated samples under frozen storage conditions (Fig 2). All treated samples significantly (P< 0.05) had lower free fatty acid values when compared to the control samples, on week 0.

Samples treated with a combination of local nutmeg and sorbitol had the lowest rate of free fatty acid production on week 12 during the period of frozen storage and were significantly (P<0.05) different from other treatments and control, suggesting that sorbitol and a combination of local nutmeg + sorbitol are better inhibitors of free fatty acid production after 12 weeks during frozen storage of fish chub.

The control had the highest (P<0.05) free fatty acid content when compared to the treated samples and remained so throughout the period of frozen storage of twelve (12) weeks. All samples irrespective of treatments had their maximum free fatty acid value at week twelve (12) and ranged from 0.45 ± 0.02 to 0.54 ± 0.01%.

After 10 days of frozen storage there was no significant difference in FFA value for he treated samples. However the FFA values for the treated samples were significantly lower than that of the control.

According to Eyo (2004) most oils rancidity is noticeable when the free fatty acid calculated as oleic acid is in the region of 0.5 - 1.5%. Therefore, the Free Fatty acids level obtained in this study was within the safe level. The presence of stearic, oleic acids and myristin in nutmeg may be instrumental in the inhibition of FFA values by the treatment.



Key: DOM-Day of manufacture

Fig 2: Effect of local nutmeg and sorbitol on free fatty acid value mgN/100g of fish chub stored at frozen temperature for 12 weeks

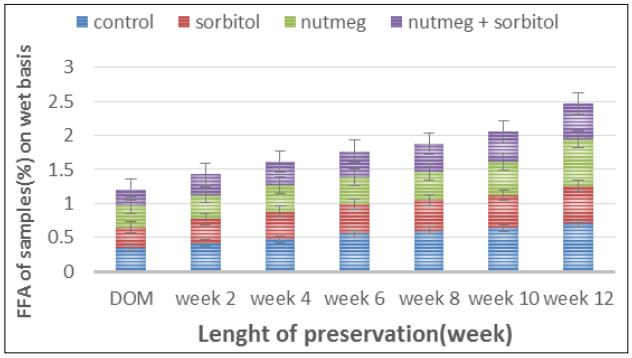
Changes in Free Fatty Acid at Refrigerated Temperature (7-10 º C)

The initial free fatty acid values ranged from 0.24 to 0.35% on day 0, and there was little or no difference between the treated and the control samples, (Fig 3). Free Fatty acid values for refrigerated stored samples were higher than those of frozen stored samples. This is as should be expected because of the higher temperature, which favour bacterial activity. Control samples had higher (P<0.05) free fatty acid content when compared to other treated samples and remained so throughout the period of refrigerated storage temperature for seven days.

Sample treated with local nutmeg + sorbitol had the lowest free fatty acid values at 0, (0.24 ± 0.01%) and remained so after twelve days with value of 0.53 ± 0.05% when compared to other treated samples.

All samples irrespective of treatment had their maximum flee fatty acid value.at day and ranged from 0.53 ± 0.05% to 0.71 ± 0.13%. Result suggests that a combination of local nutmeg + sorbitol is a better inhibitor of free fatty acid production during refrigerated age condition when compared to other treatments. This may be due to the presence of myristia, oleic and stearic acids present in nutmeg, (Stein, *et al*., 2001).

Regardless of treatments, there was an increase in the free fatty acid values for all samples during storage at refrigerated temperature, suggesting that none of the treatment could completely inhibit the free fatty acid production during storage. The free fatty acid value is an indication of the deterioration of fats, which could result in off flavour and odour (Clucas, 1985).

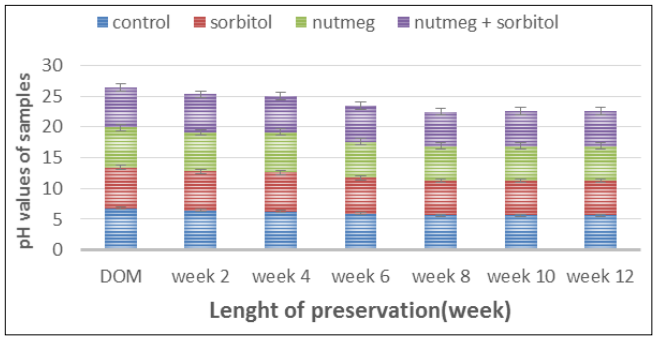


Key: DOM-Day of manufacture

Fig 3: Effect of local nutmeg and sorbitol on free fatty acid value mgN/100g of fish chub stored at refrigerated temperature (7-10 º C) for 12 weeks

Changes in pH During Refrigerated Storage (7-10 °C)

Initial pH values on day 0 ranged from 6.43 ± 0.03 to 6.80 ± 0.01 and were not different (p>0.05) from each other (Fig 4). Results suggest that the addition of the treatment had no effect (P<0.05) on the initial pH values of the samples. Most bacteria do not survive low pH value (Jay, 1987) There was a gradual decrease in pH value day 0 - 4 followed by a further decrease in day 6-l0 for all samples. This suggests that was marked deterioration on day 6. At day 10 there was no significant (P>0.05) in pH values between the treatments and the control The decrease in pH values between the treatment and the control. The decrease in pH may have been due to toxic metabolites produced by the microorganisms during storage.



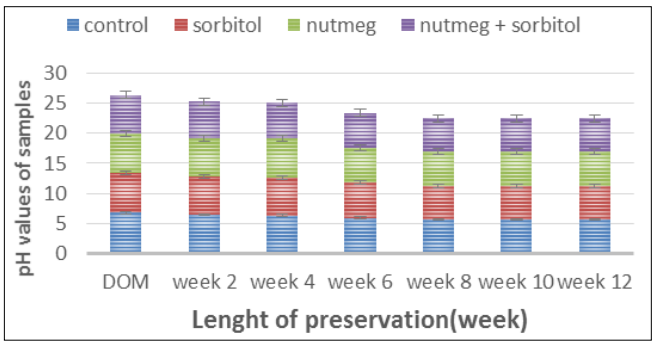
Key: DOM-Day of manufacture

Fig 4: Effect of local nutmeg and sorbitol on pH value of fish chub stored at refrigerated temperature (7-10 º C) for 12 weeks

Changes in pH change during frozen (-18 °C)

At week 0, the pH ranged from 5.9 ± 0.01 to 6.09 ± 0.0 (Fig 5) and were not significantly different irrespective of treatment at week 0 and remain so up to week 12. The constant pH may be related to the retarded microbial growth at -18 °C. The low pH had been known to help improve microbial quality of fish products as microorganisms are inhibited by such low pH values (Jay, 1987).

Frozen storage inhibits the growth of some micro-organism as the chemical and enzymatic reactions proceed slowly (Jay, 1987) due to the death of some of the microorganism as a result of probably freezer shock which could have led to less toxic alkaline metabolites being produced resulting in the drop in pH. The pH values obtained in this study are considered normal and this is in line with the of Eyo (2004).



Key: DOM-Day of manufacture

Fig 5: Effect of local nutmeg and sorbitol on pH value of fish chub stored at frozen temperature (-18 ° C) for 12 weeks

4. Conclusion

In conclusion, the refrigerated sample became a little bit acidic after week 8 while the pH of the frozen sample remain stable throughout the preservation period. Generally, increase in pH up to 0.3 units in stored meat or fish under refrigeration is often due to aerobic microbial activities (Desrosier and Desrosier, 1977) for fish chub an increase in 0.9 unit was observed, which suggest high microbial activities. Therefore it may be desirable if the refrigerated products are stored under the atmosphere of CO; and N_2 gas

Irrespective of treatments, there was an increase in the free fatty acid values for all samples during storage at refrigerated temperature, suggesting that none of the treatment could completely inhibit the free fatty acid production during storage. At frozen storage there was no significant difference in free fatty acid value for he treated samples. However the free fatty acid values for the treated samples were significantly lower than that of the control. The preservation of fish chubs with local spices at frozen temperature is the most cost effective method of preservation in this study.

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