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Comparative Shelf-life quality analysis of smoked tilapia using two different temperatures

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ABSTRACT

This study evaluated shelf-life quality of two different temperature (60ºC and 80ºC) treated smoked Tilapia (*Oreochromis niloticus*), by analyzed their biochemical (proximate and chemical) composition and sensory evaluation during storage at refrigeration temperature (4°C). For this purpose a locally made improved smoking kiln was used. Treatment "A" was processed at 60ºC temperature for 2 hrs and "B" was processed at 80ºC temperature for 1.5 hrs under laboratory method. There was a general decline in sensory characteristics i.e. color, texture, odor, general appearance and mean of acceptability of fish- product during storage. The differences in the biochemical composition of the fresh and smoke-dried samples were statistically significant ($p<0.05$). Moisture (%) and TVB-N value (mgN/100gm) increased significantly whereas protein (%), lipid (%) and ash (%) content significantly decreased. The initial value of moisture, protein, lipid, ash, salt and TVB-N of freshly smoked Tilapia was 67.55%, 18.77%, 4.26%, 3.52%, 9.37%, 4.29% in treatment A and 72.05%, 17.64%, 4.24%, 4.36%, 5.31%, 5.24% in treatment B respectively. Between these two different temperature treated Smoked Tilapia, product prepared at 60°C treatment A became spoiled at 65 days whereas product prepared at 80°C treatment B smoke-dried product remain in good condition up to 45 days. This is because of the variation of temperature affects the moisture content of product. Due to high temperature (80 ºC) moisture content remain high and product's shelf-life decrease and product comparatively soft. Whereas at 60ºC temperature product comparatively hard and had longer shelf-life. Therefore, considering all the quality parameters it showed that the hot smoked products prepared at 60ºC could be stored for two months after smoking retaining its unique taste and flavor.

Introduction

Smoke-curing as applied to fish, is one of the short-term methods of fish preservation effected by a combination of drying and the deposition of naturally produced chemicals resulting from the thermal breakdown of wood. e.g. saw-dust, wood chips etc. As a traditional preservation technique, smoking is used to prepare fish products with longer shelf life (Burgess and Bannerman.1963).The processing of a fish species inevitably entails a storage period for the finished product prior to marketing and consumption. Since fish are composed of perishable nutrients, storage period should be kept to a minimum with adequate storage conditions provided so as to prevent deteriorative changes occurring through oxidative damage and/or microbial, insect or rodent infestation. Smoking enhances flavour and increase utilization of the fish. Nonetheless, deterioration and spoilage still occur in smoked fish during storage. This study is therefore aimed at assessing the ambient temperature effect on shelf-life of smoke-cured Tilapia fish. The most important environmental factors governing the storage or shelf life of fish are ambient temperature and humidity. These factors dictate the rate at which chemical changes take place. Smoking is one of the traditional fish processing methods aimed at preventing or reducing post-harvest losses. In the early methods of smoking, heavily salted fish was generally used to be smoked for long durations, even few weeks, and the resultant products were called 'hard cures'. These products has long shelf-life at ambient temperatures owing to the high salt concentration; long smoking and drying periods resulting lower water activity (a_w) whereas lightly smoked fish is considered as an alternate to fresh fish having slight pleasant smoke flavor (Balachandran 2001). Smoking of fish in Bangladesh although a less expensive method of fish preservation with variable shelf life, is not yet practiced significantly especially, during rainy season fish catching rate is sometimes higher when sun light becomes unfavorable for drying. Under that situation fish can be preserved for short period of time by smoking. Proper preservation starts the moment it is harvested until reaches the consumer's table [4]. In tropical countries, fish are generally heavily smoked at high temperature so that they are also cooked. The presence of antioxidants in smoke render smoked products resistant to rancidity. Hot-smoking reduces microbiological load at high temperature and antiseptic components (close to 80°C) in fish tissue. Fish is normally salted before smoking. Different salting methods are being practiced by the smoked fish industry in different parts of the world (Espe et al., 2001 and Jittinandana et al., 2002). During hot-smoking, brining is carried out to ensure penetration of about 2% or more of salt into the fish tissue; the salt gives the desired taste to the product. Smoking of fish in Bangladesh although a less expensive method of fish preservation with variable shelf - life, is not yet practiced significantly especially, during

rainy season fish catching rate is sometimes higher when sun light becomes unfavorable for drying. Under that situation fish can be preserved for short period of time by smoking. Tilapia (*Oreochromis niloticus)* an exotic fish is one of the most favorite fish throughout the subcontinent is principally a herbivore. This species has come into culture practice in farmer's level to a large extent than any other single fish species in Bangladesh in the last few years. The causes behind this are many such as easily available, relatively faster growth, high survival rate, very good food conversion ratio, comparatively low feed cost, high acceptability among consumers and good market value as well as good taste. Tilapia fish generally grows in ponds, reservoirs and even in small tanks so this fish sometimes called "Aquatic Chicken. This would probably be a good quality smoked-cured fish product to be introduced publicly in the country. With the advanced of new technologies, people are interested in convenience food with good quality. This is the time to introduce this less known and ready to eat fishery product to popularize in the country which would show a considerable length of shelf-life with good taste, and meet the growing demand among all ages including young generation.

Materials and Method

Smoking process

For the study, fresh mature (average wt. 350±5gm).Tilapia (*Oreochromis niloticus)* was collected from the market of "Kamal Ranajit Market", BAU Mymensing. A total number of sixteen fish was selected randomly for the purpose and carried to the laboratory of Fisheries Technology, Bangladesh Agricultural University, Mymensingh, Bangladesh. The fish were gutted and washed thoroughly with clean water. The fishes were then beheaded with a sharp knife and filleted from neck towards the tail.Then fillets prepared for two experiments soaked into 26% brine solution for 30 minutes. The samples were then kept on racks and put in the smoking kiln. Total smoking time used in this experiment was about 2.0 hours for 60^{o}C and 1.5 hrs for 80 ^{o}C. The maximum temperature in the smoking chamber was 100°C.The smoke temperature was recorded every 10 minutes so that the temperature inside the kiln becomes homogenous. The smoke produced by the burning of sawdust was made of black berry (*Syzygium cumini*) tree and collected from a local saw-mill. After smoking, the products were packed in transparent polyethylene bags, sealed with a electrical sealing machine (PFS-300) to reduce microbial infestation and stored at room temperature for further studies.

Smoking kiln

The fishes were smoked in improved traditional type of smoking kiln (Sarkar, 2005).The smoking kiln or smoking chamber was made with steel as a rectangular box of 105×75×45 cm^3 size. Horizontally, the box or chamber was divided into two equal parts by using a horizontal perforated iron net-frame and the bottom portion was used as base for burning saw dust as smoke source. The upper chamber had facilities of hanging 4-6 mm iron rods supported from two sides as rack. Both the chamber had door which could be opened when needed. On the top, there was an outlet for smoke control. By controlling the lid of the outlet the smoke temperature inside the fish chamber i.e. the upper chamber could be controlled. Another small hole on the top was used to provide a thermometer to measure the temperature inside the chamber. Smoking was achieved by burning of wood saw dust. The moderately hot smoke (temperature 75-80^{o}C) arose through the big hole into the upper chamber where fish fillets were hung separately.

Estimation of sensory score value

The quality assessment as well as sensory evaluation (score) was carried out every three months intervals for samples stored at refrigeration temperature (4°C) using trained panel of four judges until it was an acceptable condition (Debnath, 2009). The questionnaires were prepared using 9- point hedonic score described by Larmond to evaluate changes in color, flavor, texture and mean of general acceptability until it was an acceptable condition. Parameters on the questionnaires were as follows: Like extremely = 9; Like very much = 8; Like moderately = 7; Like slightly = 6; Neither like nor dislike = 5; Dislike slightly = 4; Dislike moderately = 3; Dislike very much = 2; and Dislike extremely = 1 (Larmond, 1977).

Estimation of biochemical composition

Using conventional method of AOAC (Association of Official Analytical Chemicals), the proximate composition of fish was determined (AOAC, 1990).

Estimation of moisture

About 5 gram of previously prepared fairly minced samples were taken into each known weight basin and weighed in a digital balance (Toledo, Switzerland). The samples were allowed to dry into the oven (Memmet 854 Schwabach) at 105°C for 24 hours in order to remove the moisture until constant weight. After that, the basins are taken out of the oven, cooled in a desiccators and were weighed in a digital balance.

$$\% \text{ of moisture content} = \frac{E}{C} \times 100$$

Where, E= Weight of moisture
C=Weight of sample

Estimation of Protein

The protein content was estimated using conventional micro-kjeldahl method (Pearson, 1999). Calculation For most routine purposes the percent of protein in the sample is then calculated

by multiplying the% of N2 with an empirical factor of 6.25 for fish. Total Nitrogen%=

$$\frac{\text{ml. acid titrated} \times \text{strength of standard acid titrated} \times \text{milliequivalent of N}(0.014)}{\text{Wt. of sample}} \times 100$$

Estimation of Fat

About 5 g of the homogenous sample was taken into conical flasks and 10 ml of folch reagent (Chloroform: Methanol = 2:1) was added into the sample and homogenized properly and kept in air-tight condition for 24 hours. Fat contents of the fish muscle react with that solvent and remains in the solution. After 24 hours the solution of the flask was filtered in another weighed conical flask through a filter paper. Then these flasks were given in a hot water bath to dry up and removed the solvent. After that the flasks were kept into an oven for an hour to get the actual fat content. Then the flasks were weighed in an electronic balance to get the amount of fat content.

$$\text{Lipid content \%} = \frac{\text{weight of lipid}}{\text{weight of sample}} \times 100$$

Estimation of Ash

About 4-5 g fish sample was weighed into a pre-weighed crucible. The crucible with the contents was heated first over a long flame till all the material was completely churned. Then it was transferred in the Muffle Furnace held at dark red at a rate of 600°C for 5 hours until the residue become white. The crucible were cooled in desiccators and weighed. Finally the% of ash content was calculated.

$$\text{\% of ash content} = \frac{E}{C} \times 100$$

Where, E= Weight of ash
C=Weight of sample

Estimation of TVB-N (Total Volatile Base Nitrogen)

TVB-N value was determined by using Conway modified micro-diffusion technique (Conway and Byrne, 1933). Samples that were in the different levels of acceptability from highly acceptable condition to unacceptable condition had been selected for TVB-N analysis. 25 ml of 10% Trichloro Acetic Acid (TCA) was added to 2 gm of ground fish sample and kept overnight and then filtrated with known volume. 2% boric acid, TCA, K2CO3 and the solutions made from the fish samples were taken into the Conway dishes. After the addition of Potassium Carbonate (K2CO3), each dish was covered by a piece of glass that was stacked with glue (Paraffin soft white) initially. Then it was kept for 24 hours. The samples and Potassium Carbonate (K2CO3) reacts to form NH3 which was absorbed by the boric acid and then the solution of each Conway dish had been titrated by N/70 H2SO4 with the help of a micro-burette. Finally TVB-N was calculated.

$$\text{Amount of TVB-N (mg/100g sample)} = \frac{\text{ml titrant} \times 0.014 \times 1}{\text{Sample weight}} \times 100$$

Estimation of Salt Content

The salt content determined by filtering 1.0g fish sample mixed with 10 ml distilled water with filter paper and an aliquot of 0.2 ml from the solution (filtered) was taken in another conical flask to which 10 ml of distilled water was added followed by an addition of 2 drops of 5% Potassium Chromate and mixed properly. Titration was done with 0.05 N $AgNO_3$ solution up to the end point which was indicated by the brick-red colour.

Salt content was determined by the following formula:

$S_1 \times V_1 = S_2 \times V_2$
% of NaCl = $S_1 \times 58.5$ (molecular wt. of NaCl)
Where, V_1= Volume of sample
V_2= Volume of titrant

Microbiological Analysis

Standard plate count (SPC) expressed as Colony Forming Units per gram (CFU/g) of fresh fish muscle and smoked fish fillets were determined by using consecutive decimal dilution technique using spread plates. Five (05) g sample was taken from fresh fish muscle and smoked fish products separately. The sample was homogenized in 95 ml sterile physiological saline solution (0.85% NaCl solution) in a sterile blender jar for a few minutes until homogenous slurry was obtained. Total SPC expressed as colony forming units per gram of muscle (CFU g^{-1}) of the representative samples was determined by standard plate count methods using plate count agar according to (Collins and Lyne, 1933). No. of bacteria per gram of the fish sample (CFU/g) was calculated by using the following formula:

$$\text{CFU/g} = \frac{\text{No. of colonies on Petridish} \times 10 \times \text{dilution factor} \times \text{wt. of total sample solution}}{\text{Wt. of fish sample (g)}}$$

Results and Discussion

Sensory evaluation of smoked fish products

The standard method was adopted as such, extraneous flavour were not added to it**.** A total of 16 fishes were used for the organoleptic characteristic property of smoked Tilapia (*O. niloticus)* samples. It was found that almost all panel members judged the smoked fish produced at 60ºC. The smoked fish in the treatment 'A' showed better in respect of all sensory attributes and had an overall acceptability score of 8.64 in a nine point hedonic scales (Larmond, 1977). According to scores given by the panelists 60ºC treated smoked fish products were considered as the better quality

and all treatments were selected for subsequent shelf-life study, biochemical and bacteriological analysis. The products were packed in sealed polythene bags and stored at refrigeration temperature (4^{o}C) and an ambient temperature for further evaluation. At 7 days of intervals smoked samples were taken out from the refrigerator and their sensory quality was evaluated by panel members. The overall acceptability of smoked fish in treatment B showed no significant changes up to 30 days and samples A up to 45 days of observation (Fig 1-3).

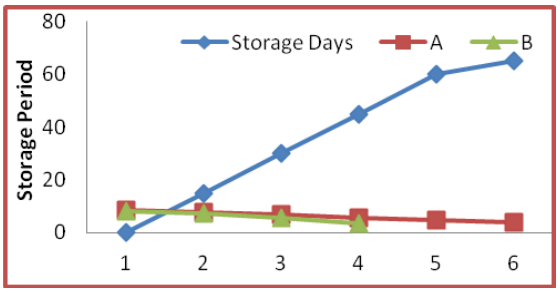

Figure 1. Color assesment of smoked Tilapia.

However, after 45 days of storage samples B and after 65 days samples A were considered rejected because all the sensory attributes were more or less changed and signs of quality deterioration were observed.

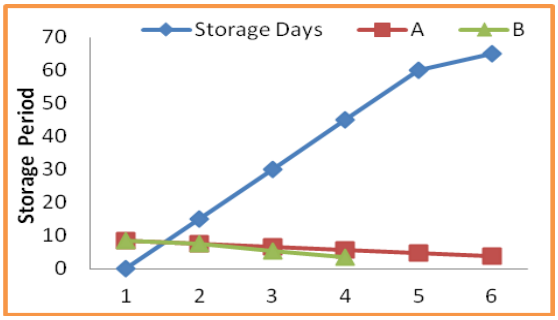

Figure 2. Odor assesment of smoked Tilapia.

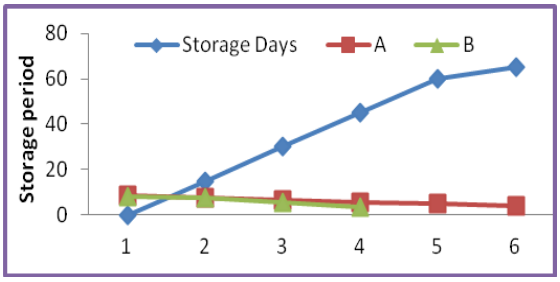

Figure 3. Texture assesment of smoked Tilapia.

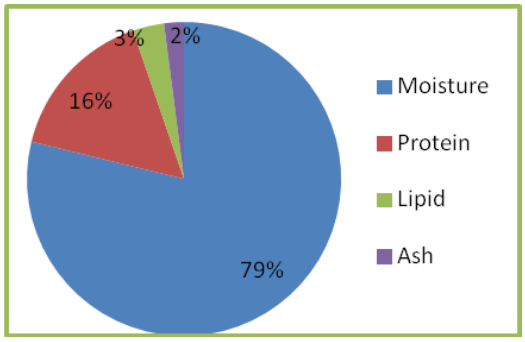

Figure 4. Initial composition of fresh Tilapia.

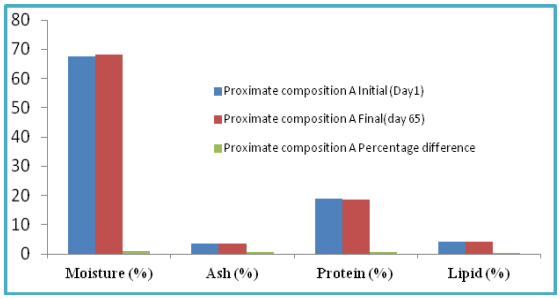

Figure 5. Mean proximate composition variation of smoked fish product in treatment A and B during the storage period at refrigerated temperature.

Figure 6. Product of treatment -A at 60^{o}C.

Figure 7. Product of treatment -B at 80^{o}C.

The initial moisture content of fresh Tilapia fish was 78.82%. However the moisture content in the smoked fish at 0 day was found 67.55 % in treatment A at 60 ^{o}C and 72.05% in treatment B at 80 ^{o}C. The variation in the moisture content in the smoked product A and B clearly shows that treatment A had much higher moisture loss during smoking. During the 45th days of observation the moisture contents were 67.90% and 72.65% in A and B respectively (Fig 5). Experiment conducted by (Gopal, 2005) with pangas (*Pangasius hypophthalmus*) showed that medium hot smoked

(about 55°C) pangas had moisture content of 64.26% on 1st day to 63.21% on 21st day of observation with the product prepared by dipping in saturated brine. This study with Tilapia also agrees with the findings of the above study.

Experiment conducted by (Rayhan et al. 2017) with pangas (*Pangasius hypophthalmus*) showed that fresh pangas had initial moisture content was 72.82% and the 45th days of observation the moisture content was 68.10%, and 67.05% in 60°C and 75 °C. Nketsia and Sefa-Dedeh (2000) determined the moisture content of the smoked fish products ranged from 11.70% to 69.20%.The present study with smoked Tilapia using two treatments had moisture content of higher ranges similar to the above report. The initial ash content of fresh Tilapia fish was 3.47%. However the Ash content in the smoked fish at 0 day was found 3.5 2% in treatment A at 60 °C and 4.36% in treatment B at 80 °C. During the 45th days of observation the ash content was 3.45% & 4.18 % in A and B respectively. And at 65 days ash content was 3.45 at 60 °C.

The initial ash content of fresh Tilapia fish was 3.47%. However the Ash content in the smoked fish at 0 day was found 3.5 2% in treatment A at 60 °C and 4.36% in treatment B at 80 °C. During the 45th days of observation the ash content was 3.45% & 4.18 % in A and B respectively.

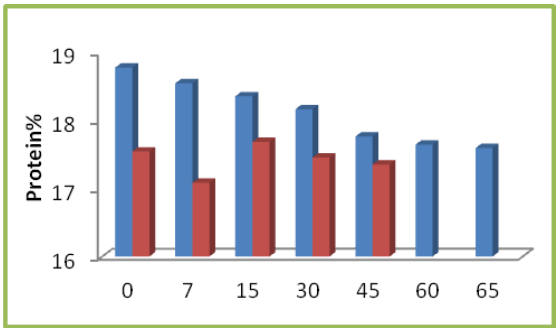

Figure 8. Protein and Lipid content of smoked fish product in different treatments A and B during the storage period at Refrigeration temperature.

The initial protein content of fresh Tilapia fish was 15.91%. However the protein content in the smoked fish at 0 day was found 18.77 % in treatment A at 60 °C and 17.64% in treatment B at 80 °C. During the 45th days of observation the protein contents were 17.76% and 17.35% in A and B respectively (Fig 8). And at 65 days protein content was 17.59% at 60 °C. However, there was found no significance change of protein content during the storage at refrigeration condition on different days of observation. Chakraborty (2007) reported that in sun dried salted hilsa products, protein content increased from 17.06% to 35.00%. This was due to a significant loss of moisture and high uptake of salt by fish muscle.

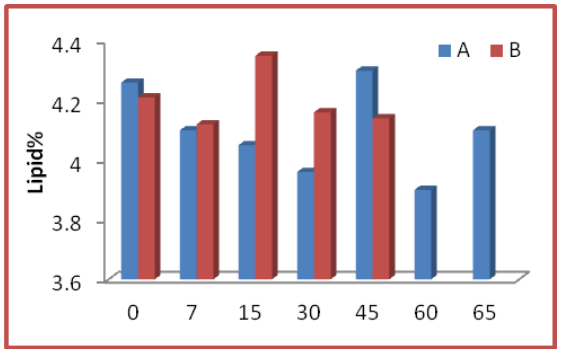

Figure 9. Lipid content of smoked fish product in different treatments A and B during the storage period at Refrigeration temperature.

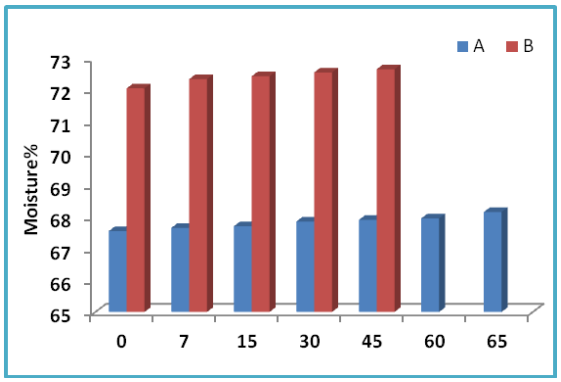

Figure 10. Moisture content of smoked fish product in different treatments A and B during the storage period at refrigeration temperature**.**

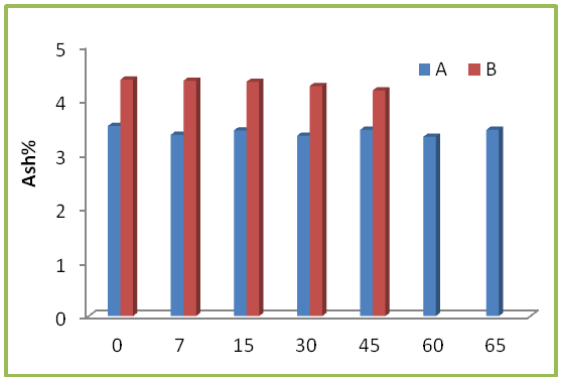

Figure 11. Ash content of smoked fish product in different treatments A and B during the storage period at refrigeration temperature.

The initial lipid content of fresh Tilapia fish was 3.23%. However the moisture content in the smoked fish at 0 day was found 4.26 % in treatment A at 60 °C and 4.24% in treatment B at 80 °C. During the 45th days of observation the lipid content was 4.40% and 4.14 % in A and B respectively (Fig 9). And at 65 days lipid content was 4.10 % at 60 °C. However, only a small but no significant change of lipid content during the storage in refrigeration condition on different days of observation. This small variation of lipid content might be due to the complex biochemical process of salting in and out during this small ripening period. Borgstrom (1965)

also reported that lipid content of hot smoked herring was 15-20%. Nketsia and Sefa-Dedeh (2000) determined the fat content of the smoked fish and obtained the values between 7.20-19.00% which shows the similar values of this study. Kosygin et al. (2001) found that lipid content ranged from 9.00-14.50% in six smoked hill stream fishes. The present study of smoked pangas also agrees with the lipid content with the above reports.

At 65 days ash content was 3.45 at 60 ^{o}C (Fig 11). However, there was no significant change of ash content during the storage at refrigeration temperature on different days of observation. Study done by Gopal (2005) with pangas (*Pangasius hypophthalmus*) showed that medium hot smoked pangas fish found the ash content values ranging 1.39-1.60% in different days of observation of the product in refrigeration storage. This study with pangas also agrees with the findings of the above study. Kosygin et al. (2001) in his experiment found that ash content on dry basis ranged from 5.02-7.00% in six smoked hill stream fishes.

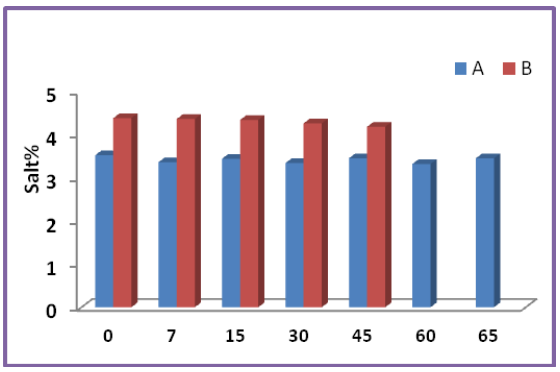

Figure 12. Salt content of smoked fish product in different treatments A and B during the storage period at refrigeration temperature.

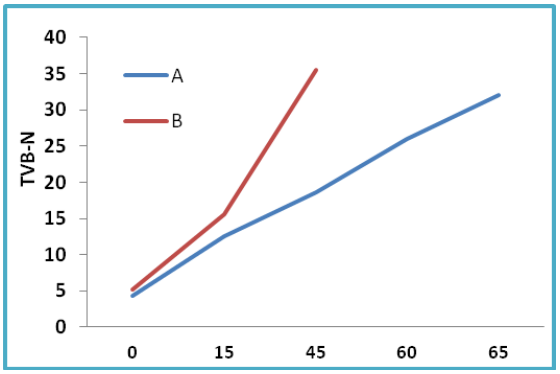

Figure 13. TVB-N content of smoked fish product in different treatments A and B during the storage period at refrigeration temperature.

Samples kept at refrigeration temperature had salt content of 9.34% and 9.90% in treatment A and B respectively on 45 days of storage. The changes in salt content of smoked Tilapia fish tissues of the two different types of products A and B during storage at ambient temperature and Refrigerated temperature are presented in figure 12) where the salt content was found to show no significant change. Debnath (2009) reported that initial salt content of pangas fish fillet was nearly negligible (0.01%) and found the salt content values of smoked product ranging from 4.81-6.91% during different storage condition. Borgstrom (1965) reported that salt content was 2-3% in hot smoked herring. Nketsia and Sefa-Dedeh (2000) determined the salt content of the smoked fish products ranging between 0.4 to 1.2%. This experiment with Tilapia provides more or less similar result with the findings of the above studies. The changes TVB-N (mg/100g) content of smoked Tilapia fish tissues of the two different types of products A and B during storage at refrigerated temperature are presented in figure 13 where the TVB-N content was found to show no significant change. Study conducted by Gopal (2005) with pangas (*Pangasius hypophthalmus*) showed that medium hot smoked pangas had TVB-N (mg/100g) values ranging 6.04-18.12 in 21 days of observation of the product in refrigerator storage. This study with Tilapia also agrees with the findings of the above study.

During storage of smoked fish at refrigeration temperature (4^{o}C) the bacterial load on the 45 days of observation was 3.98×10^{4} and 5.10×10^{6} CFU/g in the treatments A and B respectively (Fig 14). The changing pattern of bacterial population in the treatments A and B during 65days of storage. This shows the increase of bacterial population in all the treatments with the increases of time at refrigeration temperature (4^{o}C). A study by Kolodziejska et al. (2002) on determination of microbial status the aerobic plate count after smoking, chilling and packing in bags was observed at 1st week as 1.66×10^{3} CFU/g in the fresh fish and 1.25×10^{5} CFU/g in flesh which remained as such for 3 weeks at 2^{o}C, while after 14 days at 8^{o}C, the bacterial population of the fresh flesh was 1.86×10^{7} CFU/g. Microbial count of the fish flesh did not increase up to 21 days at 2 and 8^{o}C.

Salim (2005) reported that fresh pangas had initial bacterial load of 1.56×10^{5} CFU/g and after smoking the bacterial load increased to about 2.92×10^{8} CFU/g during 21days of storage at refrigeration temperature (4^{o}C).This study with Tilapia also agrees with the findings of the above study.

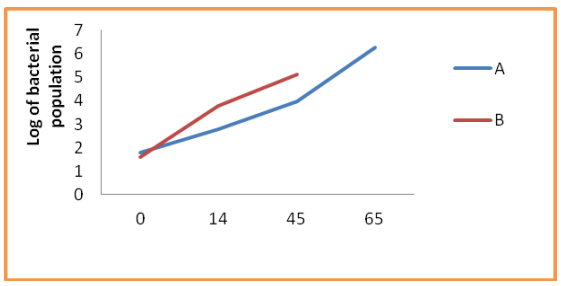

Figure 14. Total Bacterial count (APC) of smoked fish product in different treatments A and B during the storage period at refrigeration temperature.

Conclusion

The results of biochemical analysis and sensory evaluation carried out proves that the overall shelf-life quality of Smoked Tilapia prepared at two different temperature 60ºC and at 80ºC had longer shelf-life of 65 days and 45 days in treatments A and B respectively showing the best 'A' which had 65 days of shelf-life with all its sensory qualities as good condition. The variation of temperature affects the moisture content of product. Due to high temperature (80 ºC) moisture content remain high and product's shelf-life decrease and product comparatively soft. Whereas at 60ºC temperature product comparatively hard and had longer shelf-life. From this research, it can be concluded that smoke-dried Tilapia products can provide satisfactory nutrition to the nation.

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