

Comparative quality assessment of *X. cancila* (kaika) fish smoked with salt and garlic in different storage condition

¹ Mosarrat Nabila Nahid, ² Dr. Gulshan Ara Latifa, ³ Dr. Shubhash Chandra Chakraborty, ⁴ Farzana Binte Farid and ⁵ Mohajira Begum

^{1,2,4} Department of Zoology, University of Dhaka, Dhaka, Bangladesh

³ Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh, Bangladesh

⁵ Institute of Food Science and Technology, Bangladesh Council of Scientific and industrial Research (BCSIR), Dhaka, Bangladesh

Abstract

A study was conducted on the shelf-life quality of salt and garlic treated smoke-dried *X. cancila* under different storage conditions. Sensory evaluation, Biochemical-composition and microbiological-quality were analyzed using standard methods of analysis. Biochemical-composition and some mineral contents were also analyzed in freshly-processed condition. During entire storage period protein, fat and ash content decreased whereas moisture, TVB-N, and TVC increased significantly both room-temperature and refrigeration-temperature, but the increase in room temperature was little prominent than the product stored in refrigeration temperature. According to quality assessment it is clear from present findings that salt and garlic treated smoke-dried *X. cancila* fish-products stored at refrigeration temperature have been found to have longer shelf-life (21 months) than room-temperature (12 months) stored fish-products. The results of the present study indicated that it is necessary to store high quality smoke-dried fish-products in refrigeration-temperature to ensure good quality up to a certain period of time.

Keywords: Quality-assessment, smoke-dried-fish, salt, garlic, room-temperature, refrigeration-temperature

1. Introduction

Fish demand is increasing as a result of the increasing world population, higher living standards and the good overall image of fish among consumers [1]. Fish is the primary source of animal protein in the diet of the most people of Bangladesh. Fish contributes about 9% of the total protein consumption and 63% of the per capita animal intake in the daily diet of the people. Ojutiku *et al.* highlighted that fish is rich in protein with amino acid composition very well suited to human dietary requirements comparing favorably with egg, milk and meat in the nutritional value of its protein [2]. Fish is also contains absorbable dietary minerals [3]. The body usually contains small amounts of these minerals, some of which are essential nutrients, components of many enzyme systems and metabolic mechanisms, and as such contribute to the growth of the fishes [4]. In addition the freshwater small indigenous fish species are a valuable source of macro and micro-nutrients and play an important role to provide essential nutrients for the people of Bangladesh. Analysis of small fish showed that they contain a large amount of calcium and most likely also iron and zinc.

In low income countries like Bangladesh, small fish are only consumed protein sources, as they are accessible, less expensive, affordable well liked, culturally acceptable and can be purchased in small quantities [5]. Popular small indigenous freshwater fish such as, *Xenotodon cancila* (Kaika) which has unique taste is selected for the present study. The people of Bangladesh are habituated and preferred to take fish in fresh condition but small indigenous fish like *X. cancila* (Kaika) is not always available in a fresh condition because major fishing grounds are far away from the cities and the consuming

centers which are not easily accessible. Moreover the peak of fish catch in Bangladesh is seasonal. Seasonal abundance in certain places and a dearth of fish in others stimulates fisherman to preserve their catch.

However, fish is highly perishable because it provides favorable medium for the growth of microorganisms after death [6, 7]. Microbial action has been known to play a large part in the spoilage of fish [8]. Bacterial spoilage is characterized by softening of the muscle tissue and the production of slime and offensive odors [9]. Thus, it is imperative to process and preserve some of the fish caught in the period of abundance, so as to ensure an all year round supply. This will invariably reduce post-harvest losses, increase the shelf life of fish, and guarantee a sustainable supply of fish during off season with concomitant increase in the profit of the fishermen [10]. Proper preservation starts the moment fish is harvested until reaches the consumer's table [11]. Of all the preventive measures to arrest spoilage in fish, at domestic and local levels, smoking remains the cheapest. It is also the most preferred of all the treatment methods. As documented by Tull, smoking impact flavors and preservative effect on fish [12]. Moreover, it has worldwide acceptability as processed fish. Smoked fish are often contaminated with microorganisms including moulds. Due to the consumer awareness of chemical preservatives, extensive studies are been made on natural preservatives for preservation of fish products.

Fish is normally salted before smoking to protect food against bacteria, mold, and spoiling. Different salting methods are being practiced by the smoked fish industry in different parts of the world [13, 14].

Moreover spices are often added while cooking meat and fish in order to add taste, aroma, color and flavor. Many reports are there on the antioxidant and antimicrobial potential of herbs and spices like basil, rosemary, clove, pepper, mustard [15]. Likewise, garlic (*Allium sativum*) exhibit antioxidative activities and inhibit the microbial growth in a variety of foods [16, 17]. In this research work salt with garlic extract used before smoke-drying which are easily available and cheaper cost wise.

Limited work is known with quantifiable information on shelf life of salt and garlic extract treated smoke-dried fish-products under various storage conditions. For this purpose, the present study was therefore initiated to produce high quality smoke-dried *Xenotodon cancila* (kaika) fish-products treated with the combination of salt and garlic extract. Effectiveness of these preservatives (salt, garlic extract) to prolong the shelf-life in different storage condition (room and refrigeration temperature) was also evaluated through assess their sensory evaluation (score), Biochemical compositions and microbiological quality in entire storage-period.

2. Materials and methods

2.1. Sample collection: Freshwater fish species, *Xenotodon cancila* (kaika) was collected from the Meghna River early in the morning. Fresh mature fish samples were transported to laboratory in sterile polythene bag to avoid any type of microbial contamination.

2.2. Place of experiment: The whole experiment was carried out at the laboratory of Fish Technology and Food Microbiology Section of the Institute of Food Science and Technology (IFST) of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka and only mineral work was done in Center for Advanced Research in Science (CARS).

2.3. Preparation of Samples: At first, the collected kaika fish was beheaded and gutted and washed properly with clean water. The dressed fish samples were then weighed and prepared for further processing.

2.4. Preparation of samples for processing: Dressed fishes were then dip in freshly prepared brine, containing of 30% salt with 30% garlic extract in plastic buckets and kept immersed into this solution for 20 minutes followed by draining.

2.5. Fish smoke-drying: The fishes were smoked in improved traditional type of smoking kiln [18]. The fish smoking kiln was operated by first loading tamarind wood chips and rice-husk into the heat chamber, preheating for some minutes and then loading the fish-samples onto removable wire mesh trays in its central chamber for the smoking process. The desired temperature (70-75°C) was maintained manually. Smoking was done approximately for 4 hours. During the smoking fish samples were turned upside down in the middle period, to make the sample smooth and steady in texture and appearance. The smoked fishes showed characteristic attractive golden brown color and acceptable texture with smoky flavor, which was followed by cooling for 20-30 minutes at ambient temperature to make fish muscle compressed and facilitate to prevent breaking of smoked products.

2.6. Storage for shelf life study: The marked, cooled salt treated smoked-dried fish samples were then packed in transparent polythene bags. Bags were then sealed by using an electrical sealing machine (PFS-300). After that, half of the smoke-dried fish products were kept for storage at room (26-31°C) temperature and another half were kept for storage at refrigeration temperature (4°C), for further analysis.

2.7. Parameters of quality assessment

For quality analysis-Sensory evaluation, biochemical and Microbial analysis were done. Sampling was done on every 2nd month for fish kept at room temperature (26-31°C) and at every 3rd month for fish stored at refrigeration temperature (4°C).

The analytical methods are given below:

- Sensory evaluation (score) was assessed by the sensory method described by Larmond [19].
- Moisture, fat, ash and salt contents of the fish were determined by AOAC method [20].
- The crude protein of the fish was determined by Micro-Kjeldahl method [21].
- TVB-N using Conway modified micro-diffusion technique [22].
- Microbiological analysis was done according to the standard methods of AOAC [23].
- Samples for mineral analysis were prepared according to recommendations of Perkin Elmer's procedures of Atomic Absorption Spectrometer [24].

3. Results & Discussion

3.1. Biochemical and mineral composition of Salt-Garlic (S+G) treated smoke-dried *X. cancila* (kaika) fish in fresh process condition

Biochemical composition of freshly processed salt and garlic (S+G) treated smoke-dried *X. cancila* (kaika) fish products are given in Figure 1. In present study, moisture protein, fat, ash, salt and TVB-N value of freshly processed S+G treated smoke-dried *X. cancila* (kaika) fish samples were 9.57%, 62.76%, 5.37%, 22.30%, 2.98% and 5.97 mgN/100g respectively. During smoke-drying, the percentage of moisture content decreased and protein, fat and ash content increased significantly ($p < 0.05$) due to water loss. This observation is in agreement with the findings of Atlantic mackerel and European eel, pike perch and rainbow trout [25, 26].

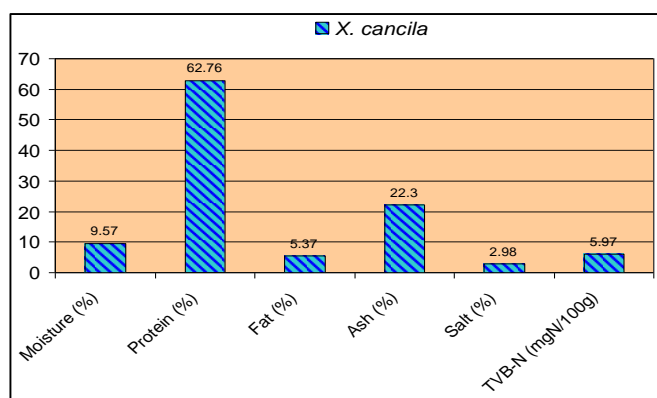


Fig 1: Bio-chemical composition of freshly processed salt and garlic (S+G) treated smoke-dried *X. cancila* (kaika) fish-products

The mineral compositions of freshly processed smoke-dried *X. cancila* (kaika) fish are given in Table 1.

Table 1: Mineral composition of Salt-Garlic (S+G) treated smoke-dried *X. cancila* (kaika) fish in fresh process condition

Minerals	Mg/100g
Ca	560
Mg	127.50
Fe	12.75
Zn	9
Cu	0.87
Mn	1.10

In this research work freshly processed S+G treated smoke-dried *X. cancila* (kaika) fish contain 560 mg/100g Ca, 127.50 mg/100g Mg, 12.75 mg/100g Fe, 9 mg/100g Zn, 0.87 mg/100g Cu, 1.10 mg/100g Mn. The mineral composition showed that Ca, Mg recording the most abundant while Fe, Zn, Cu and Mn recording the trace amounts in fresh processed S+G treated smoke-dried *X. cancila* (kaika) fish samples. In this study S+G treated smoke-dried *X. cancila* fish contained appreciable concentrations of major elements (mineral) like Calcium(Ca), Magnesium(Mg) and minor elements like Iron (Fe), Zinc(Zn), Copper (Cu) and Manganese (Mn) suggesting that this fish could be used as good sources of minerals in fresh process condition. Ca and Mg were observed to dominate other minerals in all fresh smoke-dried samples. Eyo reported that the mineral content of fish makes fish unavoidable in the diet as it is a source of different minerals that contribute greatly to good health [8]. However mineral composition recorded variations in their concentrations in the experimental fish species used for the study. Variations in the concentration of minerals in fish muscles could be due to their concentration in the water bodies where they live, the fish physiological state and or the ability of the fish to absorb the elements from their diets and the water bodies [27, 28, 29].

3.2 Quality assessment of Salt-Garlic (S+G) treated smoke-dried *X. cancila* (kaika) fish during Shelf-life study (room and refrigeration temperature):

Quality of fishery products compromises many aspects like freshness, sensory properties, nutritional relevant components and hygienic status. Assessment of the actual freshness of fish and fish products can be done by a number of methods belong to the areas of sensory, physical, chemical and microbiological analysis [30] (Connell *et al.*). The present work deals with the changes in sensory evaluation (score), biochemical composition and microbiological analysis in salt and garlic (S+G) treated smoke-dried *X. cancila* (kaika) fish products stored in room and refrigeration temperature.

3.2.1. Sensory evaluation (Score): The sensory attributes of quality and the shelf life of smoke-dried fish products are mainly affected by the initial microbial contamination, processing conditions, handling of the product after processing and storage temperature [31, 32, 33, 34, 35]. At room and refrigeration storage the sensory scores of salt and garlic (S+G) treated smoke-dried *X. cancila* fish samples are presented in Figure 2 and Figure 3.

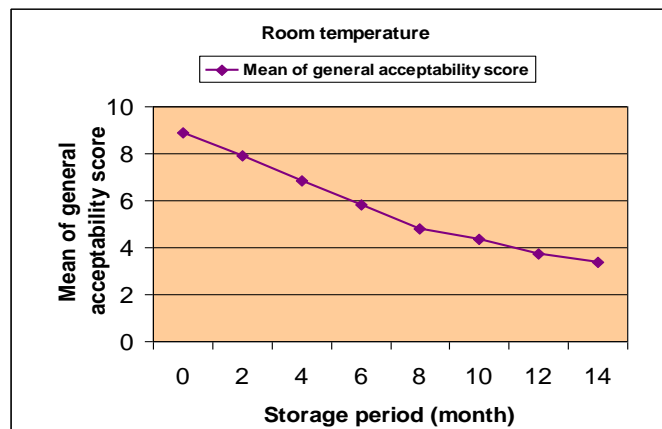


Fig 2: Changes in sensory evaluation (score) of salt and garlic treated smoke-dried *X. cancila* during storage at room temperature (26-31°C)

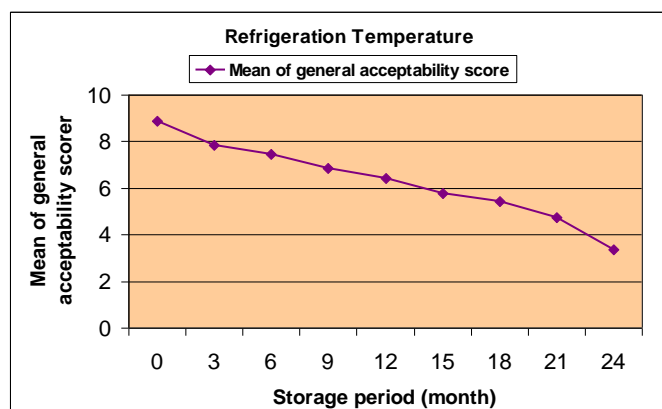


Fig 3: Changes in sensory evaluation (score) of salt and garlic treated smoke-dried *X. cancila* during storage at refrigeration temperature (4°C)

The quality assessment as well as sensory evaluation (score) was carried out every two months intervals for samples stored at room temperature (26°C-31°C) and every three months intervals for samples stored at refrigeration temperature (4°C) using trained panel of four judges following 9-point ascending scale to evaluate changes in color, odor, texture, general appearance and mean of general acceptability score until it was an acceptable condition [36]. At the beginning of storage all the sensory parameters of S+G treated smoke-dried fish samples were rated as good based on the grading scale. Moreover, there was found no broken parts of the experimental fishes after smoke-drying process. The highest mean of general acceptability score was found 8.90 in freshly process S+G treated smoke-dried fish samples. The mean of general acceptability score decreased as storage-duration increased and the acceptability score <3.5 is considered as rejected or spoiled for S+G treated smoke-dried products during room and refrigeration storage. The mean of general acceptability score of the end product of S+G treated smoke-Dried *X. cancila* fish was 3.37 (14 months) and 3.36 (24 months) for room and refrigeration storage respectively (Figure 2 and Figure 3). The reduction in the sensory qualities with increase in storage period of processed fish could be attributed to higher activities of the spoilage agents which is in agreement with the findings of Olatunde *et al.* and Daramola *et al.* [37, 38]. Similarly, Llobreda *et al.* and Reilly and Dela-cruz

was also observed the results of research into storage of crustaceans (Oyster and Shrimps) revealed quality loss during storage both at ambient temperature and chilling [39, 40].

3.2.2. Biochemical analysis: In the present study the values obtained from the analysis of biochemical composition

includes proximate composition (moisture, protein, fat, ash) and chemical composition (TVB-N value). Changes in proximate composition (moisture, protein, fat and ash) of salt and garlic (S+G) treated smoke dried *X. cancila* (kaika) storage at room (26-31°C) and refrigeration temperature (4°C) is shown in Figure 4 and Figure 5.

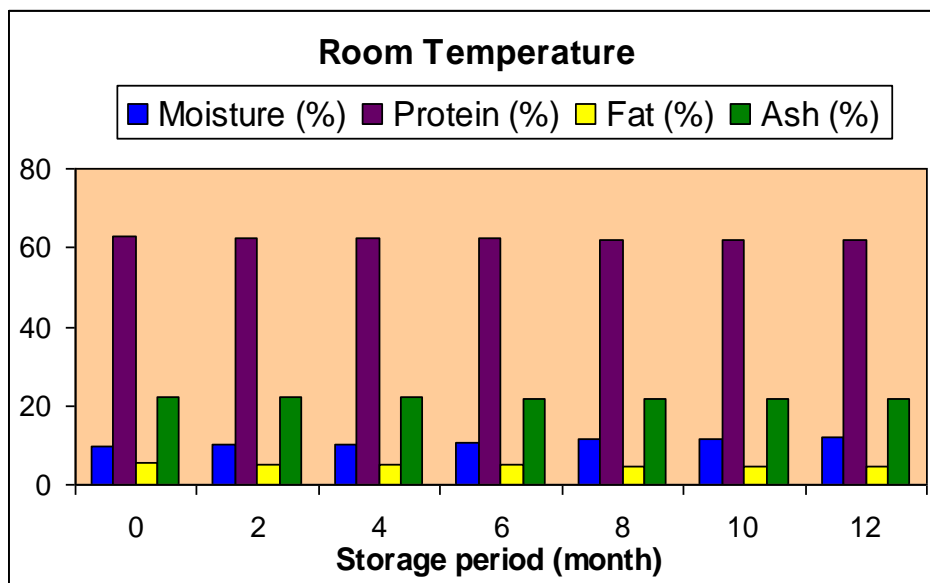


Fig 4 Changes in proximate composition of salt and garlic treated smoke-dried *X. cancila* fish during storage at room temperature (26-31°C)

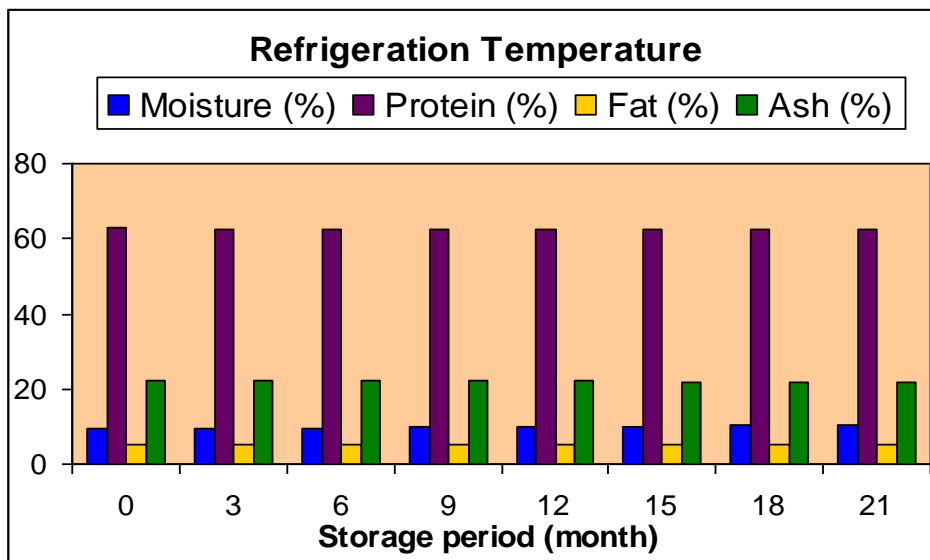


Fig 5: Changes in proximate composition of salt and garlic treated smoke-dried *X. cancila* fish during storage at refrigeration temperature (4°C)

In case of room and refrigeration-temperature stored S+G treated smoke-dried *X. cancila* (kaika) fish-products, moisture (%), protein (%), fat (%), ash (%) content were varied in the range of 9.57-11.90%, 62.76-61.91%, 5.37-4.50%, 22.30-21.71% and 9.57-10.60%, 62.76-62.26%, 5.37-4.98%, 22.30-21.87% respectively. Comparatively lower percentage moisture in freshly processed salt and garlic treated smoke-dried fish-products could be as a result of loss of water during smoking which was observed by Salan *et al.* [41]. During the storage period, moisture content increased both room and refrigerated temperature, but the increase in room temperature was little prominent than the product stored in refrigerated

temperature. The increase can be attributed to absorption of moisture from the surrounding since there was no re-drying during storage [38]. The gutted smoke dried fish samples of African cat fish (*Clarias nigrodigitus*) had moisture content as 6.27 to 10.92% which is similar with present study [42]. Moisture content of 12% is the level beyond which fish products begin to grow moulds after few days [43]. In this study the final moisture of salt-garlic treated smoke-dried fish samples was less than 12%. In storage condition, the protein content decreased significantly with the time due to water soluble protein diffused out to the surrounding for exosmosis [44]. This could be due to gradual degradation of initial crude

protein to more volatile products such as total volatile bases, hydrogen sulphide and ammonia [8]. Similar drop in protein concentration was reported for *Heterobranchus longifilis* [45]. Al-Reza *et al.* was observed a significant increasing trend in moisture content and decreasing trend in protein and fat content of smoke-dried chela fish during storage period [46]. Also Daramola *et al.* was found the decreasing trend of protein content in hot smoked *C. gariepinus* during storage period which is in line with the present findings [47]. Horner found that reduction in fat content over the storage period may be attributed to oxidation of poly-unsaturated fatty acids contained in the fish tissue to products such as peroxides, aldehydes, ketones and free fatty acids [48]. Egbal *et al.* stated that decrease in fat during storage may also be due to the effect of packaging and storage condition [49]. In this study it has been observed that during storage condition the fat content decreases very slowly which may be due to hydrolytic nature of smoked products which is in line with the findings of Islam [50]. There was decrease of ash content of four types of S+G treated smoke-dried *X. cancila* fish over the period of room and refrigeration storage which is similar with the findings of Ezembu and Onwuka who reported the decrease of ash content in both improved and traditional smoked three fish species over the period of storage [51]. The ash content changes with the time of storage due to absorbance of moisture and loss of protein [44].

Total Volatile Base Nitrogen (TVB-N) is one of the most widely used measurements of fish quality and appears as the most common chemical indicator of fish spoilage. It helps to measure the level of fish spoilage and to explore the shelf life of fish. The ratio of Total Volatile Base Nitrogen (TVB-N) has been recommended as a useful index of quality in fish which was increased during storage. There was continuous increase in the TVB-N value of the S+G treated smoke-dried fish samples all throughout the period of room and refrigeration storage. The increase in TVB-N throughout the storage period may be due to microbial activity, storage temperature, and absorption of moisture which is in line with Kumar *et al.* [52]. In another study, Daramola *et al.* reported the increasing trend of TVB-N value with storage time in the hot-smoked *Clarias gariepinus* sample whereas Ezembu and Onwuka also reported the increase of TVB-N value in both improved and traditional smoked three fish species over the period of storage [47, 51]. Similarly Hassan *et al.* also reported that the TVB-N values of smoked shrimp were increased with the time of storage [44]. Likewise, Duyar *et al.* found the increasing trend of TVB-N value in refrigeration storage which is similar with the present findings [53]. In salt-garlic treated smoke-dried *X. cancila* fish product, the TVB-N values ranges from 5.97 (0 day) to 21.39 mgN/100g (12 month) during storage at room temperature whereas ranges of TVB-N value was 5.97 (0 day) to 20.48 (21

month) mgN/100g in refrigeration storage respectively (Figure 6 and Figure 7).

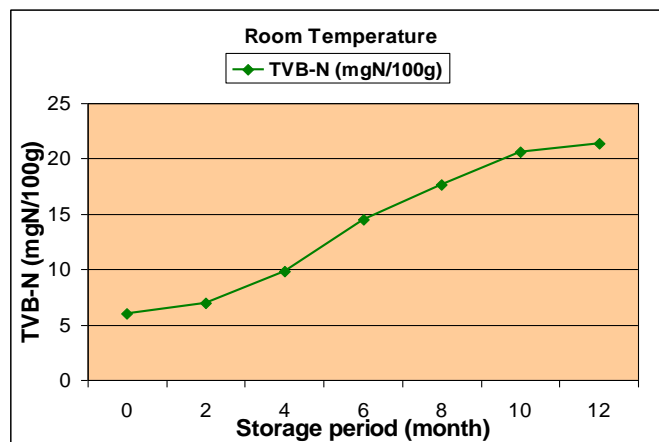


Fig 6: Changes in TVB-N value of salt and garlic treated smoke-dried *X. cancila* fish during storage at room temperature (26-31°C)

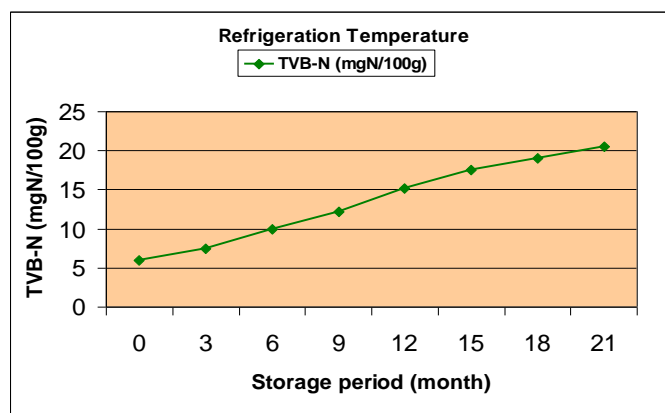


Fig 7: Changes in TVB-N value of salt and garlic treated smoke-dried *X. cancila* fish during storage at refrigeration temperature (4°C)

Various authors have reported different acceptability levels for TVB-N value: 20-30 mg N/ 100g (Pearson and Connell); 25–30 mg /100 g (Lopez- Caballero *et al.*); 20–25 mg/100 g (Kim *et al.*) which is more or less similar with the increase in final values of TVB-N in this study [54, 55, 56, 57]. Such differences reflect different products, specific treatments and processing conditions.

3.2.3. Microbiological quality: Total Viable Counts (TVC) of bacteria in S+G treated smoke-dried *X. cancila* during storage at room temperature (26-31°C) and refrigeration temperature (4°C) is given in Figure 8 and Figure 9.

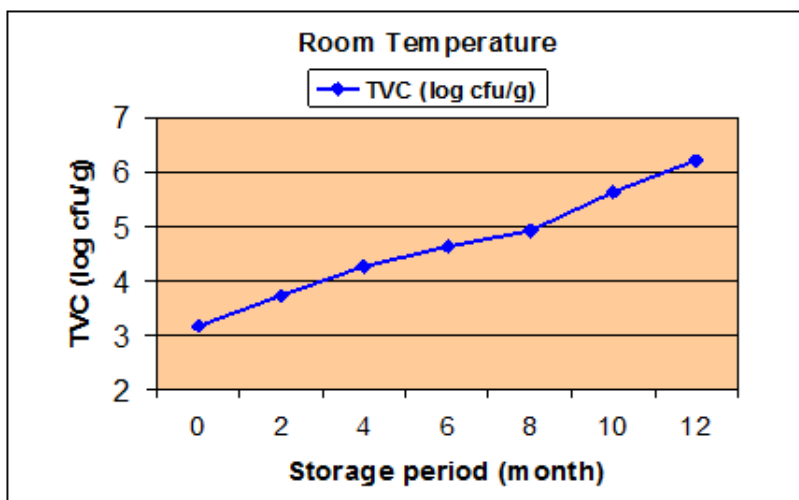


Fig 8 Changes in Total Viable Count (TVC) log cfu/g of salt and garlic treated smoke-dried *X. cancila* fish during storage at room temperature (26-31°C)

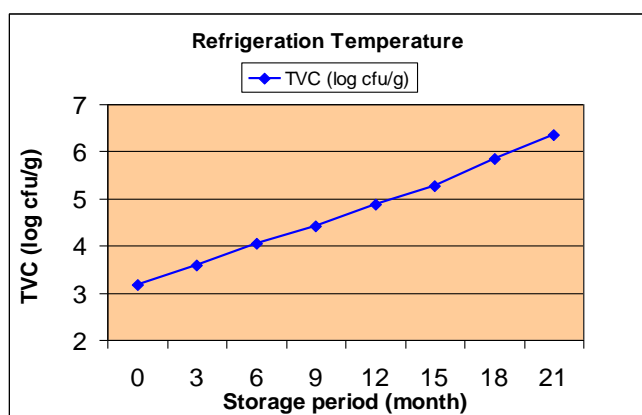


Fig 9 Changes in Total Viable Count (TVC) log cfu/g of salt and garlic treated smoke-dried *X. cancila* fish during storage at refrigeration temperature (4°C)

In case of room and refrigeration-temperature stored salt and garlic treated smoke-dried *X. cancila* fish-products, the range of TVC were 3.17 to 6.23 log cfu/g (12 months) and 3.17 to 6.36 log cfu/g (21 months) respectively. In Bangladesh, DOF and BSTI recommended the TVC of processed fish to be not more than 10^6 cfu/g. If any sample contains more than 10^8 cfu/g bacterial counts then these microbes can cause spoilage of that product [58]. In this study, TVC of these smoke-dried fish-products increases during both room and refrigeration storage period but were within the limits of 10^7 cfu/g specified for quality grading of fish by the International Commission of Microbiological Standards for Foods [59]. Goulas and Kontominas reported that the effect of temperature and smoke, addition of salt and low water activity are important for inhibiting the growth of microbes in food [60]. Total Viable Counts (TVC) of smoke-dried fish samples were increased with increase in the duration of storage due to growth and multiplication of the microbes [61]. As the duration of storage increased processed fish samples may absorb small amounts of moisture from surrounding atmosphere providing enabling environment for microbial growth [62]. Similarly Hassan *et al.* also observed that a close relationship exists between the moisture content and the bacterial load in smoked shrimp products which is harmony with the present research work [44].

In another study Kumolu-Jhonson *et al.* suggested that fresh garlic possesses antioxidant and antimicrobial properties, which can extend the shelf life of *Clarias gariepinus* which is in line with the S+G treated smoke-dried fish samples [63].

4. Conclusions

During storage at room temperature, after 12 months it was found that the salt-garlic treated smoke-dried *X. cancila* fish products were spoiled while the refrigeration stored products were found to be in normal characteristics up to 21 months. From this research it proves that, fish smoke-drying using salt and garlic extract has antibacterial and antioxidant agent that can be used for the preservation and shelf-life extension of fish products which enhance the value in local as well as export market. This work has elucidated more on the importance of smoke-dried freshwater fishes as good sources of protein and minerals. Commercial traders those who produce market smoke-dried fishes in our country may be asked to follow the suggestions made over here on the basis of the findings of the present study.

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