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The determination of shelf life quality of three smoke-dried freshwater fishes in refrigeration storage

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Abstract
Smoke-drying is one of the most important low cost methods of fish preservation and the products provide nutrients to all categories of people through the world including Bangladesh. This study evaluated shelf-life quality of smoke-dried three different freshwater fishes; chapila, kaika and baim by analyzed their biochemical composition, sensory evaluation and microbial quality during storage at refrigeration temperature (4°C). There was a general decline in sensory characters of these three smoke-dried fish products during storage period. At different length of storage period, the percentage of moisture, TVB-N value (mgN/100g) and TVC (cfu/g) were gradually increased whereas protein and fat contents were considerably decreased in these smoke-dried samples. No yeast or mould was detected in these three smoke-dried fish products. According to quality assessment it is clear from present findings that smoke-dried chapila fish-products stored at refrigeration temperature have been found to have longer shelf-life than kaika and baim fish products.

Keywords: smoke-drying, fresh water fishes, shelf-life quality

1. Introduction
Fish is an important source of food and income to many people in the developing world. Fish play an important role in the Bangladeshi diet, constituting the main and often irreplaceable animal-source food in poor rural households and it contributes about 9% of the total protein consumption and 63% of the per capita animal intake in the daily diet of the people.

'Quality' has defined degree of excellence and includes such things as taste, appearance and nutritional content. It might also be said that quality is the composite characteristics that have significance and used for acceptability [1]. 'Shelf life' is a judgement that must be made by the food manufacturer or retailer. The actual length of the Shelf life of any given product will depend on a number of factors such as processing method, packaging and storage conditions [1]. Fish-quality is all those attributes which fish eater or buyer consciously or unconsciously consider or expect to be present in fish in terms of nutritional benefit, dietary satisfaction and that it does not contain any harmful bacteria or pathogen and that it is caught from unpolluted water [2].

Fish is a highly perishable food which needs proper handling and preservation if it is to have a long shelf life and retain a desirable quality and nutritional value. The quality of fish in generally decreases after death due to chemical reactions (changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine) and microbiological spoilage. As Bangladesh is climatically a paradise for breeding and spreading of flora and fauna so spoilage agents like microorganism, insects and other pests multiply tremendously and causes considerable damage to our fish resources during post harvest period. In our country during post harvest period large amount of fish are spoiled and wasted due to lack of proper measure for processing and preservation because of the fact that neither we can consume all the fishes caught nor can we transport to other places wherever necessary due to our insufficient handling and transportation system. In other words, poor post-harvest technology (handling, preservation and processing) can cause unhealthy situation through massive spoilage.

So, the central concern of fish processing is to prevent fish from deteriorating and solve the fish deficit problem as well as uniform supply of fish in the market and even to the remote areas of the country throughout the season. Moreover development and utilization of proper scientific preservation techniques are very much essential for processors to produce a good quality of fish and fishery products.

Preservation of fish achieved by various traditional methods which are employed to preserve and process fish for consumption and storage. In Bangladesh, different processing methods like salting, drying, fermentation, icing, freezing etc. have been practicing from long time to preserve the fish from spoilage or to increase the shelf-life. Fish in any of these forms give rise to products of great economic importance and the demand for such products has been increasing. The quality of fish processed by the various methods cannot be the same and hence it subsequent effect on the fish's shelf life also varies. It has been observed that different processing methods have different effects on the nutritional compositions of fish. Of all food preservation methods, drying has received the most widespread and enthusiastic publicity in recent years [3]. Smoking is a method of preserving fish which involves drying, cooking and depositing natural wood–smoke chemicals like tars, phenols and aldehydes all of which have powerful bactericidal action and prevent the growth of other microorganisms on the flesh of the fish [4]. Smoking demonstrated a better efficient method of fish processing in

terms of the retention of protein value and reduction in the moisture content [5]. Ward reported that smoke-drying had been used for centuries in preserving fish, and is still widely used for this purpose among several communities in the third world where up to 70% of the catch is smoked [6]. Moreover fish smoking prolongs shelf life, enhances flavor and increases utilization in soups and sauces [7].

Fish smoking especially smoke-drying of freshwater small indigenous species of fish is very limited or new kind of research activities in this country in Bangladesh. Freshwater fishes are commonly used and a popular fish item among a large community of the country as principal animal protein source. Due to high palatable, taste and rich in nutrients three commercially important varieties of Bangladeshi freshwater fish such as chapila (*Gudusia chapra*), kaika (*Xenentodon cancila*) and baim (*Mastacembelus pancalus*) have been selected for the present research work.

There is little or no information available on the shelf-life of smoked fish products under storage conditions. So, it is important to evaluate the storage life of smoke-dried fish products under various storage conditions. In this context, the aim of the present study to assess the effect of smoke-drying method on the shelf life quality of three freshwater (chapila, kaika and baim) fishes and to determine the effect on the quality of these three experimental fishes in terms of sensory characters, biochemical composition and microbiological quality during refrigeration storage.

2. Materials and methods

2.1 Sample collection

Three freshwater fish species; *Gudusia chapra* (chapila), *Xenentodn. cancila* (kaika) and *Mastacembelus. pancalus* (baim) were 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.

2.3 Preparation of Samples

At first, the collected chapila (*G. chapra*) fish was discaled while kaika (*X. cancila*) and baim (*M. pancalus*) fish was beheaded. Then three fish samples were gutted and washed properly with clean water. The dressed fish samples were then weighed and prepared for further processing.

2.4 Fish smoke-drying

The fishes were smoked in improved traditional type of smoking kiln [8]. 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.

2.5 Storage for shelf life study

The marked, cooled 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, three groups of smoke-dried fish product were then kept for storage at refrigeration (4°C) temperature for further analysis.

2.6 Sampling procedure

3 or 4 slices of experimental fishes were taken randomly which represented whole body of the fish. Then the slices were chopped with skin and bone and finally ground with an electric blender to make a homogenous sample before being sampled for analysis. Analysis was done at 3 months interval until the fish became inedible.

2.7 Parameters of quality assessment

The analytical methods are given below:

- Sensory evaluation was assessed by the sensory method as described by Larmond [9].
- Moisture, fat, ash and salt contents of the fish were determined by AOAC method [10].
- The crude protein of the fish was determined by Micro-Kjeldahl method [11].
- TVB-N using Conway modified micro-diffusion technique [12].
- Microbiological analysis was done according to the standard methods of AOAC [13].

3. Results & Discussion

3.1 Biochemical composition of smoke-dried chapila (*G. chapra*), kaika(*X. cancila*) and baim (*M. pancalus*) fish in fresh process condition

Biochemical composition of freshly processed smoke-dried chapila, kaika and baim fish products are given in Figure 1.

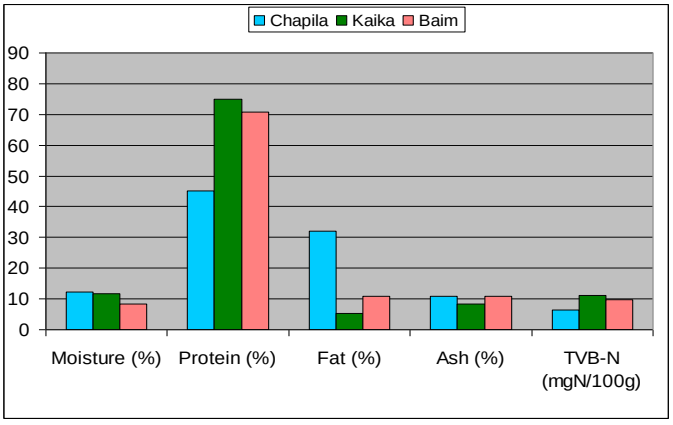

Fig 1: Biochemical composition of freshly processed smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish products

In present study, moisture protein, fat, ash and TVB-N value of freshly processed smoke-dried chapila, kaika and baim fish samples were varied in the range of 8.22% to 12.36%, 45.25%

to 74.85%, 5.25% to 32.05%, 8.31 to 10.83%, 6.39 to 11.08 mgN/100g respectively. During smoke-drying, the percentage of moisture content decreased protein, fat and ash content increased due to water loss. This observation is in agreement with the findings of Atlantic mackerel and European eel, pike perch and rainbow trout [14, 15]. The low moisture content is to reduce to minimum conditions in the fish that allow for spoilage organisms and chemical activities. This decrease was caused by loss of water during smoking which was observed by Salan *et al* [16]. The moisture levels in all the smoke-dried fishes examined were below 20% which is good or acceptable for smoke-dried fishes suggested by Lilabati and similar result was also found by Hei and Sarojnalini in some smoke-dried hill stream fishes (9.36±0.01-15.77±0.02%); Oladipo and Bankole in smoke-dried *Clarias gariepinus* (18.32%) and *Oreochromis niloticus* (8.22%) [17, 18, 19]. In general, fish is said to be an excellent source of protein content. The wide arrays of results detected in this study have attested the same. Fapohunda and Ogunkoya reported that smoke drying methods increased the protein and fat contents of *C. gariepinus* which is in line with the present research work [20]. The increase in crude protein level can be explained by Kumolu-Johnson *et al*. who stated that smoking resulted in concentrating crude protein components of fish [21]. This concentration was resulted from the loss of moisture by the smoking process as opined by Koral *et al* [22]. However, the contents varied in their concentrations when compared with earlier reports in the identical line (Hei and Sarojnalini; Ezembu and Onwuka; Effiong and Fakunle) [18, 7, 23]. This variation can be due to differences in environmental conditions, age and size of fish, season of sample collection, food availability, etc. [24, 25, 26]. After smoke-drying, there was an increase in fat content (Figure 1.) and this variation could be the result of evaporation of moisture contents which is in agreement with the previous works of Chukwu and Shaba [27].

3.2 Shelf life study

Shelf-life is the length of time that corresponds to a tolerable loss in quality of a processed food and other perishable items. In present research work, shelf-life study of smoke-dried chapila, kaika and baim fish-products were done by assessing their sensory evaluation, biochemical composition (moisture, protein, fat and TVB-N value) and microbiological quality.

3.2.1 Sensory evaluation

The sensory characters of smoke-dried chapila, kaika and baim fishes were evaluated on the basis of the color, odor, texture and remarks which are presented in Table 1.

Table 1: Sensory evaluation of smoke-dried chapila (*G. chapra*), kaika (*X. cancila*), and baim (*M. pancalus*) stored at refrigeration temperature for different lengths of storage period

Storage period (month)	Product	Color	Flavor/Odor	Texture	Remarks
0	Chapila	Bright golden brown	Fresh smoky flavor	Firm & semi-elastic	Excellent
	Kaika	Golden brown	Fresh smoky flavor	Firm & semi-elastic	Excellent
	Baim	Golden brown	Fresh smoky flavor	Firm & semi-elastic	Excellent
3	Chapila	Bright golden brown	Fresh smoky flavor	Moderately firm & semi-elastic	Very much acceptable
	Kaika	Moderately golden brown	Smoky flavor	Slightly firm & semi-elastic	Moderately acceptable
	Baim	Golden brown	Fresh smoky flavor	Moderately firm & semi-elastic	Very much acceptable
6	Chapila	Moderately golden brown	Smoky flavor	Slightly firm & semi- elastic	Moderately acceptable
	Kaika	Slightly golden brown	Slightly smoky flavor	Slightly elastic	Just Satisfactory
	Baim	Light golden brown	Moderately smoky flavor	Semi-elastic	Nice and satisfactory
9	Chapila	Light golden brown	Moderately smoky flavor	Moderately elastic	Acceptable
	Kaika	Deep brown	Unpleasant odor	Slightly soft	Rejected
	Baim	Slightly golden brown	Slightly smoky flavor	Slightly elastic	Just satisfactory
12	Chapila	Slightly golden brown	Slightly smoky flavor	Slightly elastic	Just satisfactory
	Kaika	*	*	*	*
	Baim	Deep brown	Unpleasant odor	Slightly soft	Rejected
15	Chapila	Brownish	Less smoky flavor	Loss of firm texture	Slightly acceptable
	Kaika	*	*	*	*
	Baim	*	*	*	*
18	Chapila	Deep brown	Unpleasant fishy odor	Slightly soft	Rejected
	Kaika	*	*	*	*
	Baim	*	*	*	*

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 [28, 29, 30, 31, 32]. Sowumi stated that smoked fish is highly desirable because of its enhanced flavour and texture in fish in addition to the protection offered by smoking against microbiological, enzymatic and chemical deteriorative alterations [33]. From the sensory evaluation, results shows the color of smoke-dried chapila fish was 'bright golden brown' color, but 'golden brown' color was found in smoke-dried kaika and baim fish and 'firm and semi elastic' texture was found in all smoke-dried products at fresh process condition. At the beginning of the storage 'fresh smoky flavor' was found in smoke-dried chapila, kaika and baim. In '0' day the remarks of all the smoke-dried products were considered excellent which had

turned to 'slightly acceptable' or 'Just Satisfactory' to the consumer at 15, 6 and 9 months and considered to be rejected at 18, 9 and 12 months for smoke-dried chapila, kaika and baim fish samples kept at refrigeration temperature respectively. The shelf-life of these three types of smoke-dried fish products were found to be related to the temperature and the length of storage. At the beginning of storage all the sensory parameters of these three smoke-dried chapila, kaika and baim fish samples were rated as excellent based on the grading scale (Table 1.). Moreover, there was found no broken parts of the experimental fishes after smoke-drying process.

Flavor/odor is an important quality parameter, as poor odor will discourage people from accepting food products. The aroma of smoke-dried chapila, kaika and baim fish samples were characteristically desirable. The smoke determines the color which is one of the qualities that attracts consumers. Clucas stated that the color ranges from black, dark brown, golden brown or light brown to dirty white [34]. From Table 1. It is clearly shown that the end of shelf-life loss of firm texture was observed in smoke-dried chapila, kaika and baim fish during storage at refrigeration temperature. However, slightly soft texture was observed in all the rejected smoke-dried fish samples.

3.2.2 Bio-chemical Composition

Changes in bio-chemical composition of smoke-dried chapila, kaika and baim fish are shown in Figure 2, 3, 4 and 5.

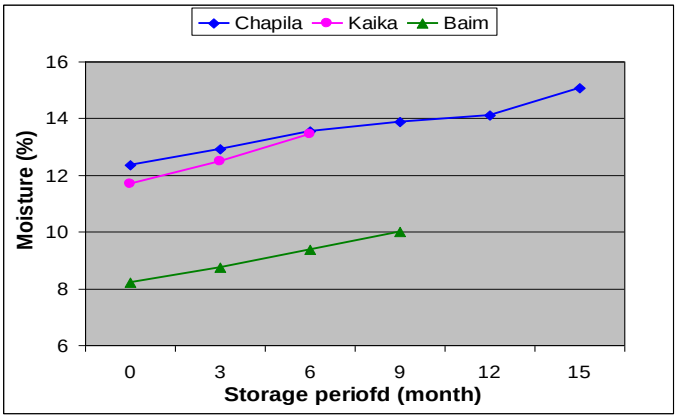

Fig 2: Changes in moisture (%) content of smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish during storage at refrigeration temperature (4°C)

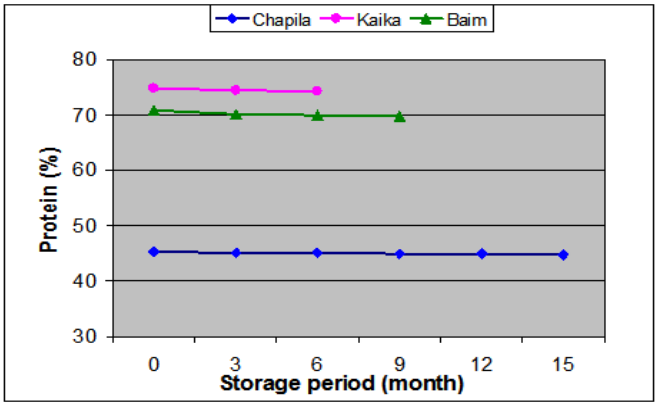

Fig 3: Changes in protein (%) content of smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish during storage at refrigeration temperature (4°C)

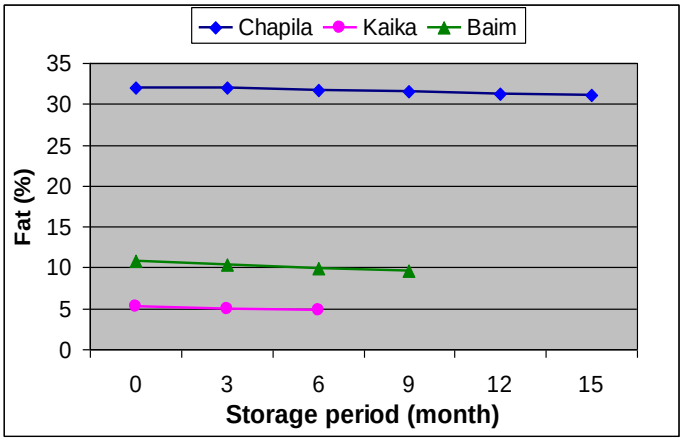

Fig 4: Changes in fat (%) content of smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish during storage at refrigeration temperature (4°C)

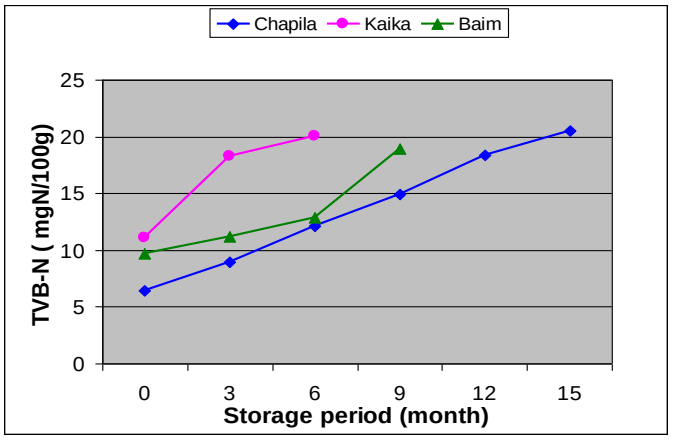

Fig 5: Changes in TVB-N value of smoke-dried chapila (*G. chapra)*, kaika (*X. cancila*) and baim (*M. pancalus*) fish during storage at refrigeration temperature (4°C)

The figures indicated that moisture and TVB-N value were gradually increased whereas protein and fat content were gradually decreased at refrigeration storage. Moisture, protein and fat contents were the major constituents, which had been considered in evaluating the nutritional value of the fishes studied. In this research work the nutritional elements showed variable values in all the fishes analyzed; with crude protein recording the highest values and lipid recording the lowest.

Moisture determination is one of the most important and most widely used measurements in the processing and testing of food. During different length of storage period Moisture (%) content were varied in the range of 12.36-15.06%, 11.69-13.45% and 8.22-10.00% in smoke-dried chapila, kaika and baim fish respectively (Figure 2). There was a gradual increase in the moisture content of these three types of smoke-dried fish samples with increasing storage period (Figure 2.) which was in agreement with the work by Ezembu and Onwuka, Al-Reza *et al*., Kumar *et al.* and Yanar [7, 35, 36, 37]. An experiment conducted by Kiin-Kabari *et al.* with Cat fish (*Clarias lezera*) showed that the average moisture content of the smoke-dried fish at the start of storage was 6.6% which rose to 13.5% and 15.2% during storage in okada and ehuru treated smoke-dried fish samples, respectively [38]. Daramola *et al.* reported similar effects that moisture percentage increased over the weeks of storage of Bony tongue (*Heterotis niloticus)*, African carp

(*Labeo coubie)*, Snake fish (*Parachanna obscura)*, Nile Tilapia (*Oreochromis niloticus)* and African mud catfish (*Clarias gariepinus)*[39]. These changes of moisture content during storage period also showed a similar trend of increasing with present experiment at refrigeration temperature. The increase can be attributed to absorption of moisture from the surrounding since there was no re-drying during storage [39]. The shelf-life of smoke-dried chapila, kaika and baim fish samples were more or less similar with the observation of Jallow who stated that fish at 10-15% moisture content, reportedly had a shelf-life of 3-9 months when stored properly [40]. Protein (%) were found to vary from 45.25% (0 day) to 44.78% (15 month), 74.85 (0 day) to 74.26% (6 month) and 70.82% (0 day) to 69.75% (9 month) for smoke-dried chapila, kaika and baim fish respectively during storage at refrigeration temperature (Figure 3). In storage condition, the protein content decreased significantly with the time due to water soluble protein diffused out to the surrounding for exosmosis [41]. This could be due to gradual degradation of initial crude protein to more volatile products such as total volatile bases, hydrogen sulphide and ammonia [42]. Similar drop in protein concentration was reported for *Heterobranchus longifilis*[43]. According to Ghazala, protein decomposes with passing time [44]. The decreasing trend in protein content of smoke-dried chapila, kaika and baim fish samples during storage period agree with the findings of Hoq *et al.*, Ezembu and Onwuka, Al-Reza *et al.* and Kumar *et al.*[45, 7, 35, 36].

During storage at refrigeration temperature the fat content varied in a range of 32.05-31.11%, 5.25-4.86%% and 10.78-9.55% in smoke-dried chapila, kaika and baim fish respectively (Figure 4). It is evident that smoke-dried chapila, kaika and baim fish showed a reduction in fat content during refrigeration storage (Figure 4.). Horner, found that reduction in lipid 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 [46]. Egbal *et al*. stated that decrease in fat during storage may also be due to the effect of packaging and storage condition [47]. In this study it has been observed that during storage condition the fat content decreases very slowly (Figure 4.) which may be due to hydrolytic nature of smoked products which is in line with the findings of Islam [48]. According to Egbal *et al*. fat content of hot smoked *C. lazera* significantly decreased after 28th day of refrigeration storage which is harmony with the present research work [47].

Total Volatile Base Nitrogen (TVB-N) is mainly contributed by ammonia in the muscle produced by deamination of muscle proteins [49]. It helps to measure the level of fish spoilage and to explore the shelf life of fish. The ratio of 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 three smoke-dried fish samples all throughout the period of 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* [36]. 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 [50, 7]. Likewise, Duyar *et al*. found the increasing trend of TVB-N value in refrigeration storage which is similar with the present findings [51]. In smoke-dried chapila, kaika and baim fish product, the TVB-N values ranges from 6.39 (0 day) to 20.48 mgN/100g (15 month), 11.08 (0 day) to 20.01 mgN/100g (6 month) and 9.69 (0 day) to 18.97 mgN/100g (9 month) during storage at refrigeration temperature respectively (Figure 5). 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 [52, 53, 54, 55].

3.2.3 Microbiological quality

Hilderbrand reported that the lower count in smoked fish may be due to high temperature killing the food poisoning and spoilage bacteria as well as the chemical compounds contained in smoke that can inhibit the growth of bacteria [56]. The changes of Total Viable Counts (TVC) in terms of cfu/g (colony forming unit) of bacteria in smoke-dried chapila, kaika and baim fish samples during storage period are given in Table 2. In fresh process condition, TVC of smoke-dried chapila, kaika and baim fish samples were 3.3×10^4cfu/g, 3.6×10^4cfu/g and 3.7×10^3cfu/g respectively. TVC were found to be increased with storage period or time and these counts rose to 3.8×10^6 cfu/g, 3.4×10^6cfu/g and 4.0×10^6 cfu/g in smoke-dried chapila, kaika and baim fish samples respectively during storage at refrigeration temperature. As the duration of storage increased processed fish samples may absorb small amounts of moisture from surrounding atmosphere providing enabling environment for microbial growth [57]. Similarly Hassan *et al*. also observed that a close relationship exists between the moisture content and the bacterial load in smoked shrimp products [41]. In storage condition, the moisture increases as a result the bacterial load also increases which is harmony with the present research work with smoke-dried chapila, kaika and baim fish. 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].

Kleickman and Schellece evaluated the microbial numbers of smoked fish and discovered that the average plate count amounted to 10^6/g of fish [59]. According to Cheesbrough, it is generally accepted that fish with microbial load $>10^6$cfu/g is likely to be at the stage of being unacceptable from the microbiological point of view and unit for consumption which agrees with the present research work [60] (Table 2.). In this study, TVC of these smoke-dried fish-products increases during refrigeration storage period but were within the range of 10^6cfu/g of specified microbiological limits recommended by ICMSF (1986) for fish and fishery products, the maximum recommended bacterial counts for good quality products is 5 x 10^5 (5.7 $\log^{10}$ cfu/g) [61].

Table 2: TVC (cfu/g) of smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish during storage at refrigeration temperature (4°C)

Storage period (month)	Chapila	Kaika	Baim
0	3.3×10^4	3.6×10^4	3.7×10^3
3	4.0×10^4	5.9×10^5	8.8×10^4
6	0.9×10^5	3.4×10^6	6.2×10^5
9	3.6×10^5	-	4.0×10^6
12	7.8×10^5	-	-
15	3.8×10^6	-	-

4. Conclusions

The present study revealed an efficient and effective model for smoke-drying of freshwater fish species (chapila, kaika, baim) for the production of high quality smoke-dried products and transfer the technology to the rural small-scale fisher folks allover Bangladesh. Also this research has added information that smoke-dried chapila, kaika, baim fishes have greater nutritive value in terms of percentage crude protein and experimentally it has been proved that these products have longer shelf life and has found better way for preservation at refrigeration condition. Moreover, the improvement and development of packaging and good storage condition (refrigeration temperature) will protect the smoke-dried fish-products against spoilage.

5. Acknowledgments

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