

Shelf-Life Quality Of Smoke-Dried Freshwater SIS Fish; Chapila (*Gudusia chapra*, Hamilton-Buchanan; 1822) Kaika (*Xenentodon cancila*, Hamilton-Buchanan; 1822) And Baim (*Mastacembelus pancalus*, Hamilton-Buchanan; 1822) Stored At Laboratory Condition (26⁰-31⁰C)

Mosarrat Nabila Nahid¹, Dr. Gulshan Ara Latifa¹, Dr. Subhash Chandra Chakraborty², Farzana Binte Farid¹ and Mohajira Begum³

¹Department of Zoology, University of Dhaka, Dhaka 1000, Bangladesh

²Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh, Bangladesh

³Institute of food Science and Technology, BCSIR, Dhaka-1205, Bangladesh.

Abstract : This study assessed the comparative changes in sensory characteristics, microbiological quality and biochemical components of fresh and smoke-dried freshwater small indigenous species of fish, chapila, kaika and baim during storage at room temperature (26-31⁰C) using standard methods of analyses. Important mineral composition was also determined in fresh smoke-dried fish products. The initial value of moisture, protein, fat, ash, TVB-N and TVC of freshly smoke-dried Chapila, Kaika and Baim fish was 12.36%, 45.25%, 32.05%, 10.83%, 6.39 mgN/100gm and 3.3×10⁴ CFU/g ; 11.69%, 74.85%, 5.25%, 8.31% , 11.08 mgN/100gm and 3.6×10⁴ CFU/g; 8.22%, 70.82%, 10.78%, 10.74% , 9.69 mgN/100gm and 3.7×10³ CFU/g respectively. During storage period, the percentage of moisture, TVB-N value (mgN/100g) and TVC (CFU/g) were increased whereas protein, fat and ash contents were considerably decreased. However, there was also a general decline in mean of general acceptability score of smoke-dried fish products during storage. From the overall performance, smoke-dried baim fish (6 month) have better shelf-life than smoke-dried chapila (4 month) and kaika (4 month) fish. The information obtained in this study that smoking demonstrated a better efficient method of fish processing which could be useful to fish consumers, processors and nutritionists in the efficient management of fish resources.

Keywords : Smoke-drying, SIS-fish, Nutritional-composition, Microbiology analysis.

I. Introduction

Compared to other sources of protein, fish are well known to be excellent sources of protein which can be seen from amino acid composition and protein digestibility [1]. Fish provides 20% animal protein intake to about 2.6 billion people globally and at least 50% of animal protein intake for over 400 million in Asia and Africa [2, 3]. In recent times, fish has been reported as the cheapest source of protein used to correct protein deficiency in human diets in the tropic region [4]. In Bangladesh, about 63% animal protein of meal comes from fish resources [5]. Although, fish production is steadily increasing, preservation of the commodity still remains a challenging problem. Susceptibility of fish to rapid spoilage has been attributed to its intrinsic characteristics and to possibilities of microbial contamination from a variety of sources [6]. The processing and preservation of fresh fish were of utmost importance since fish is highly susceptible to deterioration immediately after harvest and also to prevent economic losses [7]. Lack of adequate fish handling, processing techniques and storage facilities contribute significantly to the low supply of fish to poor rural dwellers that form three quarters of the population in developing countries [8]. If fish is not sold fresh, preservation methods should be applied to extend the shelf-life. These include freezing, smoking, drying, canning (wet salting), frying, glazing etc. Fish in any of these forms give rise to products of great economic importance and the demand for such products has been increasing. Processing is carried out with the aim of either to supply distant markets or to produce a range of products with different flavor and texture. The quality of fish processed by the various methods cannot be the same and hence its subsequent effect on the fish's shelf life also varies. Of all food preservation methods, drying has received the most widespread and enthusiastic publicity in recent years [9]. 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 [10]. Smoking demonstrated a better efficient method of fish processing in terms of the retention of protein value and reduction in the moisture content [11].

Hot smoking is the traditional methods of fish smoking in the tropics. 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 [12]. In industrialized countries, however, fish smoking is done for enhancement of flavor and texture, often producing value added products whose preservation is achieved by other means.

In Bangladesh smoked fish is recent addition to the fishery products and preservation of SIS fish is a new trend or new kind of research activities in this country. SIS 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 variety of Bangladeshi freshwater small indigenous species (SIS) of fish such as chapila (*Gudusia chapra*), kaika (*Xenentodon cancila*) and baim (*Mastacembelus pancalus*) have been selected for the present research work. As these SIS fishes are mostly eaten whole with bones, they are also a very high content of bioavailability of protein, calcium, vitamin A, Iron and Zinc which can play an important role in elimination of malnutrition problem in Bangladesh.

Now-a-days consumer wants to know and ensured the nutritional value of the products what they are eating. Also the measurement of quality is often necessary to ensure the fresh and processed fish meet the requirements of food regulations and commercial specifications [13]. However, empirical research database on the nutritional value of processed fish in Bangladesh is just growing. Although a good number of works on the biochemical composition of fishes in Bangladesh have been done by many researchers [14, 15, 16, 17, 18, 19, 20]. But no extensive research work has so far been done on the SIS fishes to preserve them through smoke-drying process as well as using them in different seasons.

Therefore, considering the possible health risk and the nutritional benefits associated with fish consumption; this study was carried out to complement efforts aimed at ascertaining the effect of smoke-drying on the nutritive value and shelf life of three common (SIS) fishes (chapila, kaika and baim) which are available and consumed in Bangladesh. In this context, the aim of the present study is to determine the effect of storage on the quality of smoke-dried fish in terms of sensory evaluation, biochemical composition, mineral contents and microbiological characteristics. The knowledge obtained from this research work will aid fish consumers, nutritionists and processors in determining the nutritional values of stored smoke-dried product and to guarantee its optimal use as a good source of healthy food.

II. Materials and methods

2.1. Sample Collection:

Three freshwater fish species; chapila (*Gudusia chapra*), kaika (*Xenentodon cancila*) and Baim (*Mastacembelus pancalus*) were collected from the Meghna River early in the morning. These experimental fishes were collected from the Meghna River during very early in the morning. They were brought to the laboratory (IFST) in iced condition. Fresh mature fish samples were transported to the research 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 the smoked-dried product:

The experimental fish was quickly carried to the laboratory as early as possible to avoid any spoilage during that period. At first, the collected chapila fish was discaled while kaika and baim 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 [21]. 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 smoke-drying process. The desired temperature (75-80°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. After completing the smoke-drying process, the smoke-dried 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 room (26⁰C-31⁰C) temperature for further analysis.

2.6. Sample preparation:

At first the collected fresh fishes as well as smoke-dried fish samples were fairly minced and homogenized in a blender for the analysis.

2.7. Analysis:

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^oC-31^oC).

Important mineral composition i.e. Ca, Mg, Cu Zn, Fe and Mn were determined in fresh smoke-dried fish products.

The analytical methods are given below:

- Physical changes were assessed by the sensory method as described by Larmond,[22].
- The moisture, fat, and ash contents of the fish were determined by AOAC method [23].
- The crude protein of the fish was determined by Micro-Kjeldhal method [24].
- TVB-N was determined by Conway modified micro-diffusion technique [25].
- Microbial analysis (TVC) was done according to the Standard methods of [26].
- Samples for mineral analysis were prepared according to recommendations of Perkin Elmer's procedures of Atomic Absorption Spectrometer [27].

III. Results and Discussion

Moisture, protein, fat, ash, TVB-N and TVC of fresh Chapila fish was 76.41%, 10.53%, 11.62%, 1.50%, 2.40 mgN/100g and 3.1×10⁵CFU/g ; fresh Kaika fish was 77.67%, 14.27%, 4.66%, 2.94% , 4.92mgN/100gm and 4.1×10⁵ CFU/g ; baim fish was 77.21%, 15.17%, 6.13%, 1.72% , 3.16 mgN/100g and 5.8×10⁴CFU/g respectively (Fig.1). Fresh sample presented low protein content [28].

3.1. Biochemical analysis:

In the present study the values obtained from the analysis of biochemical composition includes proximate composition (moisture, protein, fat, ash) and TVB-N value.

During smoke-drying, the percentage of moisture content decreased and protein, lipid 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 [29, 30]. Similarly, smoke drying methods increased the protein, ash and fat contents of *C. gariepinus* [31].

3.1.1. Moisture (%) Content:

The moisture content can be used as a pointer to the rate at which deterioration occurred in fish samples resulting in the early decomposition. The initial(0 day) percent of moisture were found 12.36%,11.69% and 8.22% for smoke-dried chapila, kaika and baim fish respectively (Fig. 2 , Fig. 3 and Fig. 4). There was a gradual increase in the moisture content of these three types of smoke-dried fish samples with increasing storage period. In the present study, the moisture content of smoke-dried chapila, kaika and baim fish rose to 14.05 % (4 month), 13.93% (4month) and 15.37% (6 month) respectively during storage at room temperature (Fig. 2 , Fig. 3 and Fig. 4). The increase can be attributed to absorption of moisture from the surrounding since there was no re-drying during storage [32]. The gutted smoke dried fish samples of African cat fish (*Clarias nigrodigitus*) had moisture content as 6.27 to 10.92% which is more or less similar with present study [33]. The shelf-life of three types of smoke-dried 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 [34].

3.1.2. Protein (%) Content:

Fish protein is of high quality and contains sufficient amounts of all the essential amino acids required by the body for growth, maintenance of lean muscle tissue and active metabolism [35]. In this study, the protein content increased in smoked chapila, kaika and baim fish , when compared with the fresh fish, suggested that protein nitrogen was not lost during smoke-drying [36,37]. Also this is in harmony with the findings of Ogbonnaya and Shaba [38]. 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 [39]. This concentration was resulted from the loss of moisture by the smoking process as opined by Koral *et al.*[40]. Highest protein value was found in smoke dried kaika fish (74.85%) while least was found in smoke-dried chapila fish (45.25%) in fresh process condition. Protein decomposes with passing time [41]. Protein (%) were found to vary from 45.25% (o day) to 44.81% (4 month), 74.85%(0 day) to 74.12% (4 month) and 70.82% (o day) to 68.89% (6 month) for smoke-dried Chapila, kaika and baim fish respectively during storage at room temperature (Fig. 2 , Fig. 3 and Fig. 4). In storage condition, the protein content decreased significantly with the time due to water soluble protein diffused out to the surrounding for exosmosis [42]. This could be due to gradual degradation of initial crude protein to more volatile products such as total volatile bases, hydrogen sulphide and ammonia [43]. Similar drop in protein concentration was reported for *Heterobranchus longifilis* [44] . 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 [45].

3.1.3. Fat (%) Content:

After smoke-drying, there was an increase in fat content and this variation could be the result of evaporation of moisture contents which is in agreement with the previous works of Ogbonnaya and Shaba; Daramola *et al.*; Bouriga *et al.* and Bilgin *et al.* [32,38,46,47]. Moisture content of fresh fish was higher than that of smoke-dried fish as a result of dehydration of water molecule present in dry fish. During storage at room temperature the fat content varied in a range of 32.05-31.28%, 5.25-4.27% and 10.78-7.66% respectively (Fig. 2, Fig. 3 and Fig. 4). Usually moisture and fat contents in fish flesh are inversely related and their sum is approximately 80% [48]. This inverse relationship was also well defined in this experiment.

3.1.4. Ash (%) Content:

Ash content of smoke-dried fish samples was higher than that of fresh fish. Clucas and Ward reported that the inorganic content remains as ash after the organic matter is removed by incineration [49]. Salan, Juliana and Marilia observed an increase of ash content in smoked *C. gariepinus* and the authors further noted that the increase in the ash content in the smoked fish was due to the loss of humidity and that the significant reduction in the moisture content when the fish was smoked as a result of the loss in moisture during hot smoking which was in agreement with the present study and also similar results for ash content of smoked fish have been reported in previous studies [50, 47, 51]. Also Doe and Olley reported that smoking resulted in the concentration of nutrients, such as, protein and ash [52]. Ash (%) content was found to vary from 10.83% (0 day) to 10.57% (4 months), 8.31% (0 day) to 7.82% (4 months) and 10.74% (0 day) to 8.08% (6 months) for smoke-dried chapila, kaika and baim fish respectively during storage at room temperature (Fig. 2, Fig. 3 and Fig. 4). The ash content changes with the time of storage due to absorption of moisture and loss of protein [42]. Smaller sized fish species has higher ash content due to the higher bone to flesh ratio [32].

3.1.5. TVB-N value:

Total Volatile Base Nitrogen (TVB-N) levels were monitored as the main parameter of fish muscle freshness. Total Volatile base Nitrogen (TVB-N) is widely used as an indicator of the degree of lipid oxidation [45]. It helps to measure the level of fish spoilage and to explore the shelf life of fish. TVB-N are produced by decomposition of proteins into simpler substances (ammonia, trimethylamine, creatine, purine bases and free amino acids) [53]. In this study the higher values of TVB-N were reported in fresh smoke-dried chapila, kaika and baim fish compared with fresh fish. An increase of TVB-N after smoking was most likely caused by an autolytic process which produces volatile amine compounds [54]. This agrees with the report of Adeyemi *et al.* which stated that the TVB-N of *T. trachurus* before smoking was 28.12 ± 0.38 mg N/100g and rose to 31.90 ± 0.3 mgN/100g after smoking [55]. Vasiliadou *et al.* also observed an increase in the TVB-N value after smoking [56]. The initial (0 day) TVB-N values obtained from smoke-dried chapila, kaika and baim fish were 6.39 mgN/100g, 11.08 mgN/100g and 9.69 mgN/100g (Fig. 5). There was a continuous increase in the TVB-N value of all the smoke-dried samples throughout the period of storage (Fig. 5) which could be due to gradual degradation of the initial protein to more volatile products such as total volatile base nitrogen [32]. The increase in TVB-N throughout the storage period may be due to microbial activity, storage temperature, absorption of moisture. Pearson recommended that the limit of acceptability of fish is 20-30 mg N per 100g [57]. While Kirk and Sawyer suggested a value of 30-40 mg N/100g as the upper limits [58]. Increase in final values of TVB-N in this study is similar with other researchers [44, 59]. During hot smoking fish are exposed to heat and atmospheric oxygen. These factors can accelerate the oxidation of the fish lipids resulting in an increase in TBA [47].

3.2. Mineral composition:

The mineral compositions of fresh processed smoke-dried chapila, kaika and baim fish are given in (Table-1). Smoke-dried chapila fish had the highest Ca (575 mg/100g) while baim fish had the least Ca (325 mg/100g) content. Magnesium was highest (107.75 mg/100g) in kaika fish whereas lowest (22.25 mg/100g) was found in smoke-dried chapila fish. The highest amount of Fe was observed in kaika fish (9.50 mg/100g) while smoke-dried chapila fish contains the least amount of Fe (2.10 mg/100g). Zn also highest (7.70 mg/100g) in kaika fish whereas lowest in (1.10 mg/100g) smoke-dried chapila fish. Cu (0.35 mg/100g) and Mn (0.87 mg/100g) was highest in smoke-dried baim fish whereas lowest Cu (0.30 mg/100g) and Mn (0.77 mg/100g) was found in smoke-dried kaika fish.

The mineral composition showed variable values in all fresh processed smoke-dried fishes analyzed; with Ca, Mg recording the most abundant while Fe, Zn, Cu and Mn recording the trace amounts. All the fish samples examined in this study 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 these fishes 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[43] 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. The wide array of minerals detected from these species attest to this. However mineral composition recorded variations in their concentrations among the selected 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 [60, 61, 62].

In this research work the order of mineral concentrations in smoke-dried chapila, kaika and baim fish were $Ca > Mg > Fe > Zn > Mn > Cu$ which is more or less similar with the findings of the Kirchessner and Schwall [63].

3.3. Sensory score value:

Sensory methods are considered to be the most useful and dependable criteria for assessing the degree of freshness for quality determination. Human being is capable to detect defects from visual signs of deterioration such as loss of freshness and changes during storage period. Sensory quality assessment is an easy, quick and efficient method of getting idea about the quality of the product. This method is based on the response or tendency of sense organ for accepting the food products.

The quality assessment as well as sensory evaluation (score) was carried out every two months intervals for samples stored at room temperature ($26^{\circ}C$ - $31^{\circ}C$) 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.[64]. Smoked fish is highly desirable because of its enhanced flavor and texture in fish in addition to the protection offered by smoking against microbiological, enzymatic and chemical deteriorative alterations [65]. The aroma from all these smoke-dried fish samples was characteristically desirable. The smoke determines the color which is one of the qualities that attracts consumers. The color ranges from black, dark brown, golden brown or light brown to dirty white [66].

The shelf-life of these three types of smoke-dried fish product was found to be related to the temperature and the length of storage. At the beginning of storage all the sensory parameters of these three samples were rated as good based on the grading scale. The highest mean of general acceptability score was found 8.66, 8.68 and 8.90 in fresh process smoke-dried chapila , kaika and baim fish products respectively (Fig. 6). The mean of general acceptability score decreased as storage-duration increased.

The acceptable shelf life was found to be 4 month, 4 month, 6 month during storage at room temperature for smoke-dried chapila, kaika and baim fish respectively. The mean of general acceptability score of the end product of smoke-dried chapila, kaika and baim was 5.36 (4 month), 4.59 (4 month) and 4.42 (6 month) respectively (Fig. 6). The results of the sensory analysis indicated that the storage lives of these smoke-dried fishes were different. At the end of 4 month smoke-dried chapila and kaika fish products became spoiled whereas smoke-dried baim fish products were still remain in good condition. This agrees with the results of research into storage of smoke dried fish and crustaceans (Oyster and shrimps) which revealed quality loss during storage both at ambient temperature and chilling[32,67,68].

3.4. Microbiological analysis:

The total viable counts (TVC) of smoke-dried chapila, Kaika and baim fish samples are presented in (Table-2). Results showed that fresh smoke-dried fish samples had relatively lower total viable counts of bacteria when compared with the fresh fishes. This can be explained by the bactericidal effect of smoke constituents such as acids, aldehydes and phenols [69]. In view of present study, Total Viable Counts (TVC) of smoke- dried chapila, kaika and baim fish samples ranged from 3.3×10^4 (0 day) to 4.2×10^6 CFU/g (4 month), 3.6×10^4 (0 day) to 4.0×10^6 CFU/g (4 month) and 3.7×10^3 (0 day) to 3.3×10^6 CFU/g (6 month) respectively (Table-2). 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 [47]. As the duration of storage increased processed fish samples may absorb small amounts of moisture from surrounding atmosphere providing enabling environment for microbial growth [69]. Smoking inhibits microbial growth in stored fish products [50]. 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 [70].

IV. CONCLUSION

This research provides basic nutritional information on three selected freshwater SIS fish, both fresh and smoke-dried which is necessary to formulate guideline for common people to help them to plan better nutritional diet for good health. The present study also provides a possible application of smoke-drying as an efficient drying process for fish especially in developing countries where all the required sophisticated storage equipment is not available. Smoke-drying methods are efficient in the post harvest management of fishery

products which could be improved the preservative strategies of dried fish and thus prolong the shelf life of one of the commercially important food commodities in the tropics.

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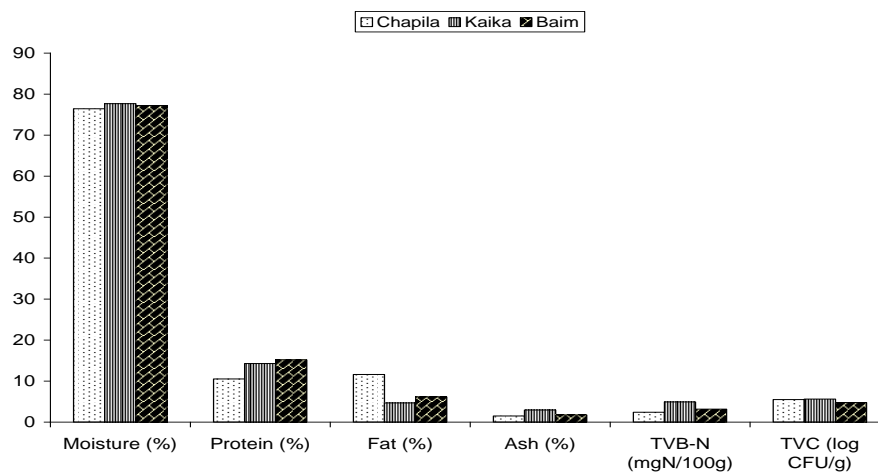


Figure 1. Changes in biochemical composition and other features of fresh chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish.

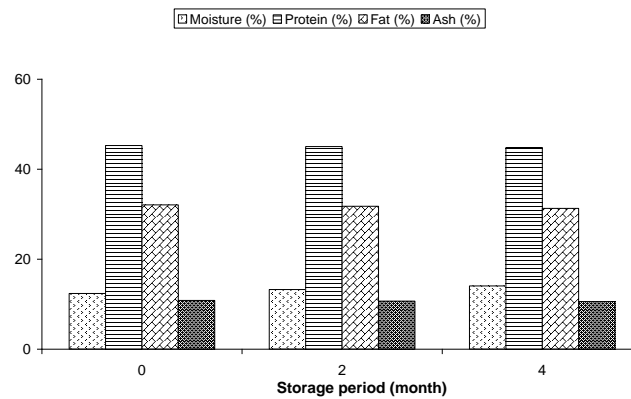


Figure 2. Changes in Proximate Composition of smoke-dried chapila (*G. chapra*) fish during storage at room temperature (26⁰C-31⁰C).

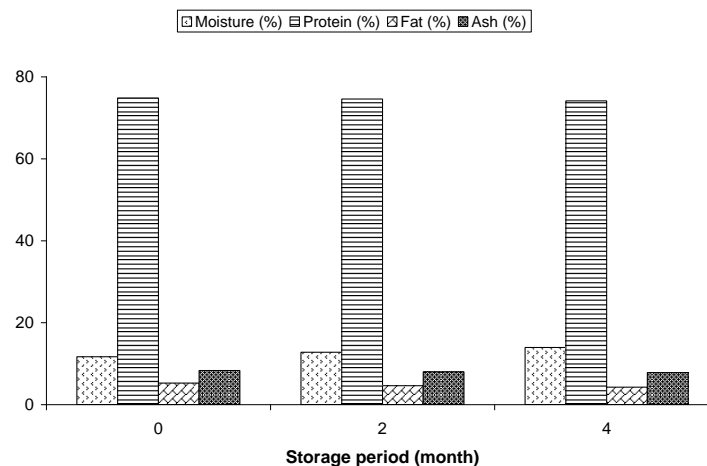


Figure 3. Changes in Proximate Composition of smoke-dried Kaika (*X. cancila*) fish during storage at room temperature (26⁰C-31⁰C).

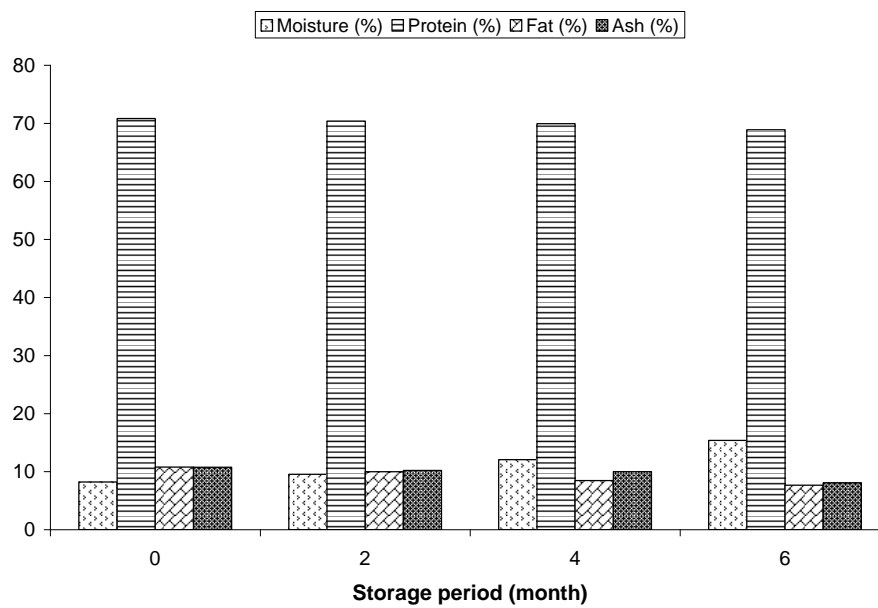


Figure 4. Changes in Proximate Composition of smoke-dried baim (*M. pancalus*) fish during storage at room temperature (26°C-31°C).

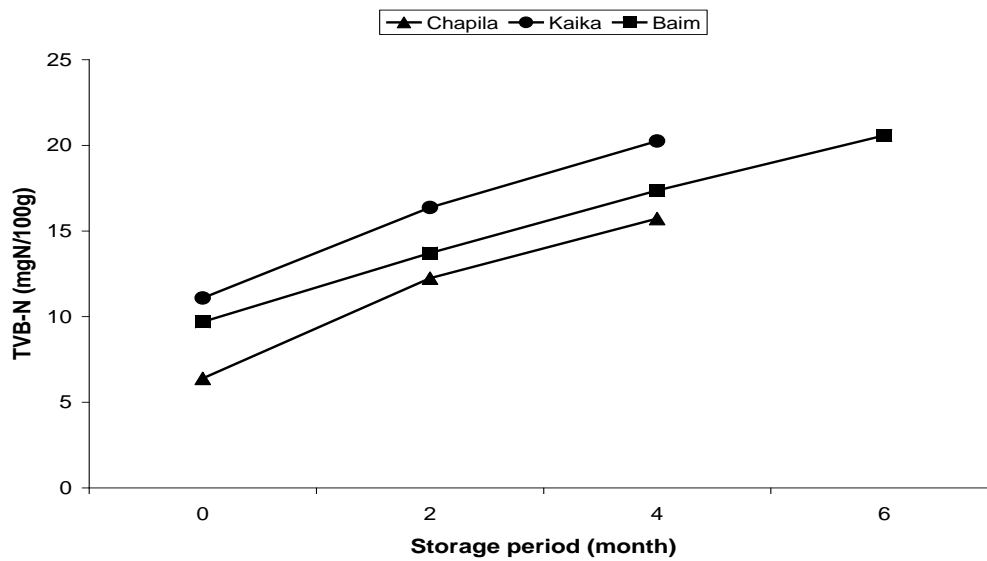


Figure 5. Changes in TVB-N value of smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish during storage at room temperature (26°C-31°C).

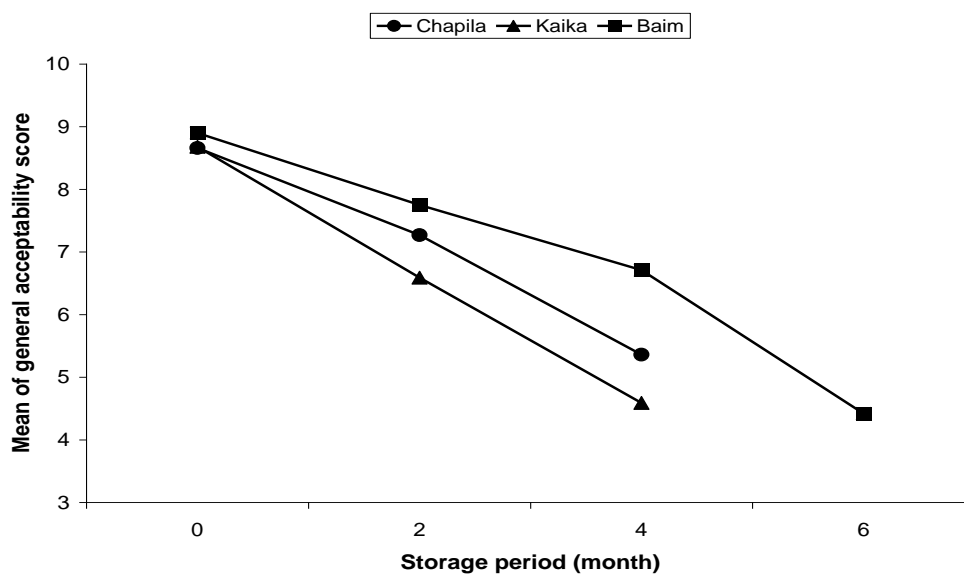


Figure 6. Changes in mean of general acceptability score of smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish during storage at room temperature (26^oC-31^oC).

Table-1. Mineral composition of fresh smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish.

| Important minerals | Ca mg/100g | Cu mg/100g | Fe mg/100g | Zn mg/100g | Mn mg/100g | Mg mg/100g |
|------------------------------|------------|------------|------------|------------|------------|------------|
| Chapila (<i>G. chapra</i>) | 575 | 0.32 | 2.10 | 1.10 | 0.82 | 22.25 |
| Kaika(<i>X. cancila</i>) | 477 | 0.30 | 9.50 | 7.70 | 0.77 | 107.75 |
| Baim (<i>M. pancalus</i>) | 325 | 0.35 | 5.80 | 3.60 | 0.87 | 83 |

Table-2. TVC (CFU/g) of smoke-dried chapila (*G. chapra*), kaika (*X. cancila*) and baim (*M. pancalus*) fish during storage at room temperature (26^oC-31^oC).

| Storage period (month) | Chapila(<i>G. chapra</i>) | Kaika (<i>X. cancila</i>) | Baim (<i>M. pancalus</i>) |
|------------------------|-----------------------------|-----------------------------|-----------------------------|
| 0 day | 3.3×10 ⁴ | 3.6×10 ⁴ | 3.7×10 ³ |
| 2 nd month | 6.7×10 ⁵ | 7.2×10 ⁵ | 8.1×10 ⁴ |
| 4 th month | 4.2×10 ⁶ | 4.0×10 ⁶ | 5.5×10 ⁵ |
| 6 th month | - | - | 3.3×10 ⁶ |