Effect of Traditional Fish Processing Methods on the Proximate and Microbiological Characteristics of Laubuka dadiburjori During Storage at Room Temperature

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ABSTRACT
Fish is a major source of protein and post-harvest loss of fish is a key factor of economic and protein wastages in developing countries like Bangladesh. This study was carried out to know the effects of sun drying, smoking, freezing and canning on proximate, biochemical and microbiological characteristics of chela (Laubuka dadiburjori) fish stored at room temperature for 60 days. The proximate compositions of the fish samples were determined. Moisture, protein, lipid, ash and carbohydrate contents of the fresh fish were 76.56, 13.74, 4.25, 2.37 and 1.41%, respectively. Total volatile base nitrogen content was 7.10 mg/100 g. Total plate count was $1.13 \times 10^4$ CFU g$^{-1}$. The proximate compositions found in the different processing methods were statistically different to the fresh fish samples. Water activity and pH value increased significantly in sundried and smoked chela at the end of 60 days of storage. Salt content decreased significantly (p<0.05) in sundried fish than other three processing methods. Total protein content was high in smoke dried fish (61.31%) and low in frozen fish (11.85%) at the end of storage period. The TVB-N was found high in sundried and smoked fish samples after storage period. Smoking demonstrated efficient method of fish processing in terms of the retention of protein value and reduction in the moisture content but canning was the best method for benefit and retention of the product. The information obtained in this study could be useful to fish consumers, processors and nutritionists in the efficient management of fish resources.

Key words: Laubuka dadiburjori, traditional processing methods, proximate composition, biochemical characteristics, microbiological quality

INTRODUCTION
Fish is a good source of animal protein and minerals (Tidwell and Allan, 2001). Fish is widely consumed in many parts of the world because it has high protein content. The quality of fish protein is very high because of its low saturated fat, its riches with essential amino acids and also it’s containing ω-3 and ω-6 fatty acids that known to support good health. According to FAO. (2008) and Gandotra et al. (2012), fish provides 20% of 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. In developing countries, it provides only 13% of animal protein intake. Fish constitute the major source of animal protein intake in Bangladesh due to its availability and low cost. 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 (Venugopal et al., 1997). Preservation of fish can be achieved by various methods, i.e., refrigeration, freezing, salting, canning (wet salting), icing, smoking, glazing, drying, frying, etc. Therefore, 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. It has been observed that different processing methods have different effects on the nutritional compositions of fish. In this context, the aim of the present study is to assess the effects of sun drying, smoke drying, freezing and canning on the quality of chela fish (Laubuka dadiburjori) and to determine the effect of storage on the quality of chela fish in terms of proximate, biochemical and microbiological characteristics.

MATERIALS AND METHODS

Fish collection: Fresh chela fishes (Laubuka dadiburjori) were collected from the river Jamuna in the early hours of the day and fish was stored in ice with a fish/ice ratio of 1:2 (w/w) then transported within 1 h to the laboratory. Upon arrival, fishes were carefully washed with cooled tap water and degutted, then washed again with tap water to remove blood, slime and unnecessary flesh.

Fresh sample: The fresh flesh sample of chela fish was taken for quality analysis of fresh experimental fish. About 6 or 7 slices was taken randomly which represented the parts from whole body of the fish for biochemical and microbiological analyses.

Analyses: The fish samples were subjected to the following analyses.

Proximate composition: For fresh fish, the proximate composition was determined from the body muscle. In case of sun dried, smoke dried, frozen and canned fish, it was carried out from flesh. The analysis was carried in triplicate; the average values were calculated and expressed as Mean±SD of triplicate observation. All chemicals used were of analytical grade and supplied by Sigma Co. (St. Louis, USA).

Moisture: Moisture content was determined by following the AOAC (2005). The 5-10 g of sample was taken in a petriplate and dried in an electric oven at 100±2°C for 16-18 h. The samples were kept in a dessicator until weighing in a digital balance (Toledo, Switzerland). The weight loss in the process was expressed as % moisture content in the sample.

Fat, ash and carbohydrate: Proximate composition analyses for fat, ash and carbohydrate were carried out according to the methods given in AOAC (2005). Fat content of the fish samples was determined as per using the Soxhlet method. Ash content was determined by heating the 1 g sample at 550°C for 24 h.

Protein: The total nitrogen content was determined using a micro system of Kjeldahl (Kjeltec System 1002, Sweden) method (AOAC., 2005). Crude protein was estimated by multiplying the total nitrogen content (% N) by the factor 6.25.
**Total Volatile Base Nitrogen (TVB-N):** The total volatile base nitrogen (TVB-N) was determined by Conway’s micro-diffusion analysis (Osman et al., 2001). In this procedure, the Trichloro Acetic Acid (TCA) extract prepared sample was treated with potassium carbonate, ammonia liberated and absorbed by boric acid. The quantity of ammonia absorbed was volumetrically determined by titrating the ammonium borate against standard sulphuric acid. The results were expressed as mg TVB-N kg\(^{-1}\) muscle.

**Sodium chloride content:** Salt content of the sample was determined by the method prescribed for salted fishery products (FAO, 2008). The 5 g of the muscle sample was treated with distilled water against standard silver nitrate solution using potassium chromate as indicator.

**Water activity (aw):** In order to enumerate the influence of water on the flourishing of microbial fishery, the water activity of the sample was determined at room temperature using water activity value analyzer (model no. 5803, Glufft Gmbh and Co, Stuttgart-1). The instrument consists of a thermocole case, holding two sample containers each with a detachable sensor head, which indicates water activity directly on the dials at the required level of temperature. Calibration of the \(a_w\) meter was carried out by keeping a filter paper soaked with the standard solution of barium chloride in the sample container (\(a_w = 0.9\) at 20°C). The holding period was 3 h to get the \(a_w\) at room temperature. The instrument was manually calibrated to get the correct water activity.

**pH:** The 1 g sample of the fish flesh was homogenized in 10 mL of distilled water and the mixture was filtered. The pH of the filtrate was measured using a pH meter (Mettler Toledo 320-s, Shanghi, China).

**Microbiological analysis**

**Total Plate Count (TPC):** The 10 g of muscle tissue samples from each fish was aseptically cut out and homogenized aseptically with 90 mL of Buffered Peptone Water (BPW) solution (Oxoid, Hampshire, UK). The tissues were stomached for 30 sec in a stomacher. Appropriate dilutions were made according to decimal dilution method and planted onto nutrient (plate count agar) agar plates by spread plate technique (BAM., 1995). The plates were incubated at 30±1°C for 24 h. Total Plate Count (TPC) was enumerated and expressed as number of colony forming U g\(^{-1}\). Strict aseptic procedures were followed in every step of analysis.

**Halophillic bacterial count:** Halophilic bacterial count was determined using 3.5% sodium chloride solution as diluent. Planting was done onto nutrient (plate count) agar with 10% salt plates by spread plate technique. The colonies developed in the planter were counted and expressed as number of colony forming units/g of the sample.

**Total fungal count:** Total Fungal Count (TFC) was enumerated using potato dextrose agar by BAM. (1995). The 10 g of fish sample was weighed aseptically and homogenized with 90 mL of physiological saline solution. Appropriate dilutions were made from the 9.0 mL physiological saline and plated onto Potato dextrose agar plate containing antibiotics or tartaric acid solution. The plates were incubated at room temperature for four days and all colonies were counted and the data was reported as Colony Forming Units CFU g\(^{-1}\).
The pathogenic bacteria like *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Vibrio* etc and fungi were enumerated by following the method of BAM. (1995). All culture media were purchased from Oxoid (Hampshire, UK).

**Sensory evaluation:** The sensory evaluation was carried out by a group of panelist according to the method described by Potter (1968). The boiled samples of sundried, smoked, frozen and canned fish were evaluated for quality attributes include texture, flavor, color, appearance, general taste and overall acceptability by using 9-point hedonic scales (9 = excellent, 8-9 very good, 6.5-7.9 good, 5-6.4 fair, <5 bad) and score 5.0 was considered the borderline of fish acceptability. A sensory panel formed consist of ten experienced judges (5 males and 5 females; 25-55 years old) who had been involved in sensory analysis of different kinds of fish and fish foods. Previously to the present experiment, a special training was carried out concerning different quality conditions and to check the panelists’ understanding of the descriptors. The sensory evaluation was conducted between 10:30 and 11:30 am and Panelist received four samples per session. Fishes were cooked in a glass bowl covered with a cap in a microwave oven (Samsung) during 1.8 min at 600 W. Samples were coded with 3-digit random numbers and a randomized complete block design was used in which the samples were randomly assigned to each panelist. Panelists were asked to consume the dorsal part of the fillet. Sessions were performed in individual partitioned booths. These conditions were conducive to concentration and avoided communication between assessors and disturbance by external factors (ISO., 1988). Scores among panelists were averaged.

**Processing techniques**

**Sun drying:** The fishes were aseptically gutted, eviscerated and washed in chilled water. Before drying, fishes were salted by dry commercial salt (NaCl) (fish weight: salt weight, 3:1) in clean plastic basins. The salting was done by placing crude salt in the gut cavity and outside the fish. The salting process was allowed to continue for several hours. Then fishes were dried by exposing to ambient sunlight at temperatures of 35-42°C on drying racks made of plastic coated metallic wire mesh racks. The racks with fishes were covered with fishnets during day time to prevent insects and other pests. At night, the racks were covered with plastic sheets to prevent water condensing on the drying fishes. After drying, they were allowed to cool naturally to ambient temperatures of 23-25°C. Sun-dried product was packaging with plastic bag maintaining aseptic condition as far as possible and was stored at room temperature.

**Smoke drying:** The fish samples were gutted, washed thoroughly with clean water and prepared for smoke curing. Fishes were dip in freshly prepared salt solution (mix four parts of clean water and one part of salt) for 15 min followed by draining and then. The fishes were smoked in a mechanical kiln (AFOS-Torry Mini Kiln). Heat was generated by the burning of charcoal from tamarind wood chips. Pre-heating for 15 min and then loading the fish samples onto removable wire mesh trays in its central chamber for the smoking process. The desired temperature (75-80°C) was maintained manually by using a thermometer. Smoking was done approximately for 4 h. During smoking, fish samples were turned upside down in middle period, to make the sample smooth and steady in texture and appearance. Then the samples were cooled for 20-30 min at ambient temperature. The cooled smoked fish samples were then packed and sealed in vacuum condition in polythene bags. Smoke-dried fish product were then kept for storage at room temperature for further analysis of sensory and biochemical compositions.
Freezing: The fishes were eviscerated and washed with chilled water to remove traces of blood. It was wrapped tightly in plastic wrap then kept in air tight plastic container and immediately stored in the freezer (model: Haier Thermocool BD-428A) for 60 days at below -16±2°C. Before analysis, frozen fish were thawed using running water (25-26°C).

Canning: The fishes were eviscerated and washed with chilled water, then precooked in steam pressure of 10 psi for 60 min to attain a final backbone temperature of 90-92°C. Precooking removes the fish oils and coagulates the protein in the fish to loosen the meat. The fish were then cooled at room temperature (15-18°C) for about 2 h. The 100 g fish were placed in small flat rectangular cans (105×60×25 mm; 150 mL). Can was then filled with brine (mixing four parts of clean water and one part of salt) as coating medium. The cans were vacuum-sealed and sterilized in a horizontal steam heated retort (115°C, 45 min, F_0 = 7 min). When the heating time was completed, steam was cut off and air was used to flush away the remaining steam. Cans cooling was carried out at reduced pressure.

Statistical analysis: The obtained data were statistically analyzed using the Statistical Package SPSS 20.0 version. The differences in fish sample parameters due to time of storage (BS, 30 and 60 days) were analyzed using an analysis of variance (ANOVA) procedure. The significance level was set at the probability level of p<0.05.

RESULTS AND DISCUSSION
Biochemical characteristics of raw chela fish: Fresh chela used in this study contained 76.56% moisture, 13.74% protein, 4.25% lipid, 2.37% ash and 1.41% carbohydrate (Table 1). Sankar and Ramachandra (2001) mentioned that proximate composition of fish varying with species, body size, season, environmental factors and nutritional status. The TVB-N content of fresh chela was found 7.10 mg/100 g of sample, which below the level of 35 mg/100 g, has been suggested as borderline for various fish and fish products (Ghaly et al., 2010). The carbohydrate content in fresh chela was less in amount as compared to protein and lipid.

Microbiological characteristics of raw chela fish: Results in Table 1 show that the Total Plate Count (TPC) and total fungal count in fresh chela were 1.13×10^4 and 1.50×10^2 CFU g^-1, respectively. Some dominant genera of bacteria were identified. Among those Pseudomonas spp., Micrococcus spp., Streptococcus spp. and Vibrio spp. were found in higher percentage. Surendran and Thampurah (2002), previously mentioned that Aeromonas spp. and Pseudo-monas spp. were the main microorganisms associated with fresh water fish. The dominant fungi isolated from the samples of chela were Aspergillus spp. and Mucor spp.

| Biochemical characteristics | Chela fish |
|-----------------------------|------------|
| Moisture (%)                | 76.56±1.62 |
| Protein (%)                 | 13.74±1.22 |
| Lipid (%)                   | 4.25±0.85  |
| Ash (%)                     | 2.37±0.56  |
| Carbohydrate (%)            | 1.41±0.79  |
| TVB-N (mg/100 g)            | 7.10±1.72  |

| Microbiological characteristic | Chela fish |
|-------------------------------|------------|
| TPC (CFU g^-1)                | 1.13×10^4  |
| TFC (CFU g^-1)                | 1.50×10^2  |

*Mean±Standard deviation value for triplicate observations, TFC: Total fungal count, TVB-N: Total volatile base nitrogen.
Table 2: Proximate composition of sundried, smoke dried, frozen and canned chela fish at different storage period

| Sample and time (Days) | Moisture content | Protein content | Lipid content | Ash content | Carbohydrate content |
|------------------------|------------------|-----------------|---------------|-------------|----------------------|
| **Sun dried**          |                  |                 |               |             |                      |
| BS                     | 5.88±0.72a       | 62.80±1.51a     | 14.00±0.43a   | 8.91±0.79a  | 4.01±0.41a           |
| 30                     | 10.83±0.86b      | 60.12±0.89b     | 13.80±0.55a   | 10.96±1.11a  | 3.47±0.26b           |
| 60                     | 14.90±1.77c      | 57.34±1.31c     | 11.23±0.86b   | 13.64±0.90b  | 2.97±0.59c           |
| **Smoke dried**        |                  |                 |               |             |                      |
| BS                     | 1.41±0.33a       | 67.32±1.56a     | 19.27±0.68a   | 6.54±0.46a  | 2.62±0.13a           |
| 30                     | 5.51±0.70b       | 64.01±1.44b     | 18.10±0.70a   | 8.28±0.69b  | 2.12±0.18b           |
| 60                     | 9.11±0.97c       | 61.31±1.11c     | 16.67±0.83b   | 9.18±0.31b  | 1.48±0.05c           |
| **Frozen**             |                  |                 |               |             |                      |
| BS                     | 76.25±1.16       | 13.21±0.78      | 4.08±0.15     | 2.15±0.09   | 1.17±0.08            |
| 30                     | 75.16±2.13       | 12.74±0.67      | 3.44±0.05     | 2.89±0.07   | 1.25±0.20            |
| 60                     | 75.05±1.27       | 11.85±0.98      | 3.00±0.27     | 2.85±0.11   | 1.93±0.16            |
| **Canned**             |                  |                 |               |             |                      |
| BS                     | 67.15±1.69       | 16.68±0.88      | 5.46±0.34     | 8.15±0.83a  | 1.35±0.07            |
| 30                     | 68.13±2.06       | 15.15±0.95      | 5.51±0.56     | 10.43±0.91b | 1.36±0.09            |
| 60                     | 68.88±1.89       | 15.62±0.45      | 5.96±0.48     | 12.60±1.12c | 1.39±0.07            |

BS: Before storage, Mean±Standard deviation value for triplicate observation. a,b,cValues in the same column with different superscripts are significantly different (p<0.05) for the different storage period (BS, 30 and 60 days)

**Effect of traditional processing methods on the proximate composition of chela fish before storage:** Proximate composition of the chela fish subjected to different processing methods (sun drying, smoke drying, freezing and canning) is presented in Table 2. Smoke dried fish recorded the lowest (1.41%) moisture content than sundried (5.88%), canned (67.15%) and frozen fish (76.25%). Protein contents were high in the smoke dried fish (67.32%) and then sundried fish (62.80%) compared to canned (16.68%) and frozen (13.21%) fish, this finding was in conformity with the findings of Chukwu (2009) and Kumolu-Johnson *et al.* (2010). According to Tidwell and Allan (2001) fish is a good source of protein. Indeed, the highest crude protein content in smoked and sundried chela fish compared with the raw, canned and frozen chela fish, suggests that protein nitrogen was not lost during drying. The increase in crude protein level can be explained by Kumolu-Johnson *et al.* (2010) who stated that smoking resulted in concentrating crude protein components of fish. This concentration was resulted from the loss of moisture by the smoking process as opined by Koral *et al.* (2009). The highest lipid content observed in smoked fish (19.27%) and then sundried fish (14.00%). The difference in their lipid contents was owing to oxidation of fat during the period of sun drying as mentioned by McGill *et al.* (1974).

On the other hand, canned and frozen fish had the lowest lipid content (5.46 and 4.08%, for them, respectively). This reduction was related to their highest content of moisture. Aberoumad and Pourshafi (2010) stated that the lower the percentage of water, the greater the lipids and protein content and the higher the energy density of the fish.

High value of total ash content (8.91%) in sundried chela and then canned chela (8.15%) was attributed to high salt content. Similar levels of ash content in salted fish were recorded by Kiin-Kabari *et al.* (2011). Sundried fish also recorded the highest carbohydrate content (4.01%), meanwhile the lowest carbohydrate content (1.17%) was observed in frozen storage fish.

**Effect of traditional processing methods on changes in proximate composition of chela fish after stored for 30 days and 60 days:** A significant (p<0.05) increase in moisture content was observed of all post-storage fish products compared with the pre-storage products except frozen and canned storage fish (Table 2). Increase in moisture content was attributed to the difference in the moisture of the processed fish relative to the surroundings mentioned by Daramola *et al.* (2007) and moisture absorption during monsoon due to high relative humidity difference.
This increase in moisture uptake during the storage period was higher in sundried and smoked fish than canned fish. The low moisture uptake by canned products indicates the advantage of processing chela fish. Reversely, a negative trend was observed in frozen fish.

Reduction in crude protein during the storage period may be due to gradual degradation of the initial crude protein to more volatile products, such as Total Volatile Bases (TVB). Low protein content recorded in 60 days stored sample may be due to denaturation of fish protein associated with frozen fish (Reay, 1933) and leaching out of some extractable soluble protein fraction (Daramola et al., 2007). Sundried, Smoked and frozen chela fish showed a reduction in crude fat during storage period. This may be due to breakdown of tissue cells during salting, followed by the heating effect of drying (Pace et al., 1989) and oxidation of Poly-Unsaturated Fatty Acids (PUFA) to products such as peroxides, aldehydes, ketones and the free fatty acids (Daramola et al., 2007). The highly susceptible of fish to oxidative rancidity resulted from the high degree of unsaturation in the form of multiple double bonds in fatty acids Obemeata et al. (2011). By comparing pre-storage and post-storage crude fat content, a significant difference (p<0.05) was recorded in both of sundried and smoked chela fish.

Sun drying recorded the highest ash content (13.64%) after 60 days storage. The significant (p<0.05) increases in ash content recorded in sundried, smoke dried and canned fish due to the crude salt. According to Beauchamp and Engelman (1991), fish muscle absorbed more salt which was put into their gut during processing and same the finding was also observed in smoked fish samples during the storage period (Cardinal et al., 2001). Table 2 indicates that there was slightly increase in the post-storage carbohydrate content of the frozen and canned chela fish and a significant (p<0.05) reduction in sundried and smoked chela fish.

Changes in physiochemical characteristics: Initial salt content of sundried, smoke dried and canned chela was 17.54, 19.57 and 28.02%; these values decreased to 15.67, 18.20 and 27.24% respectively after 60 days of storage (Table 3). Dewi et al. (2011) showed that decrease in salt content attributed to the uptake of moisture during the storage period due to hydrostatic nature of salt.

There was an increase in water activity of chela fish except that in both frozen (0.70) and canned (0.59) fish, which almost remained constant. These value is lower than 0.70 and according
Table 4: Changes in microbial characteristics

| Sample and time (days) | Total plate count (CFU g\(^{-1}\)) | Halophilic count | Total fungal count (CFU g\(^{-1}\)) |
|------------------------|------------------------------------|------------------|-------------------------------------|
| **Sun dried**          |                                    |                  |                                     |
| BS                     | 1.97×10\(^4\)                     | 1×10\(^3\)      | 1.84×10\(^2\)                       |
| 30                     | 5.20×10\(^4\)                     | 1×10\(^3\)      | 4.31×10\(^2\)                       |
| 60                     | 7.34×10\(^4\)                     | 1.21×10\(^2\)   | 6.80×10\(^2\)                       |
| **Smoke dried**        |                                    |                  |                                     |
| BS                     | 1.56×10\(^4\)                     | 1×10\(^3\)      | 1.13×10\(^2\)                       |
| 30                     | 4.60×10\(^4\)                     | 1.04×10\(^3\)   | 3.31×10\(^2\)                       |
| 60                     | 5.50×10\(^4\)                     | 1.31×10\(^3\)   | 4.80×10\(^2\)                       |
| **Frozen**             |                                    |                  |                                     |
| BS                     | 1.30×10\(^4\)                     | 1×10\(^3\)      | 1.13×10\(^2\)                       |
| 30                     | 2.20×10\(^4\)                     | 1×10\(^3\)      | 2.28×10\(^2\)                       |
| 60                     | 2.64×10\(^4\)                     | 1×10\(^3\)      | 3.31×10\(^2\)                       |
| **Canned**             |                                    |                  |                                     |
| BS                     | 1.44×10\(^4\)                     | 1×10\(^3\)      | 0.95×10\(^2\)                       |
| 30                     | 1.88×10\(^4\)                     | 1×10\(^3\)      | 1.13×10\(^2\)                       |
| 60                     | 2.57×10\(^4\)                     | 1×10\(^3\)      | 2.18×10\(^2\)                       |

to Brewer (1999) bacteria can’t grow at a level of 0.70. So, frozen and canned fish remained safe. Significant (p<0.05) upper trends in a\(_w\) observed in sun dried and smoke dried fish. Bacteria should not grow at a a\(_w\) level of 0.70 (Brewer, 1999).

pH is an indicator of the degree of freshness or spoilage. The pH in fresh fish flesh is almost neutral. In our study the pH value of fresh chela fish was 6.8. Drop off pH value after addition of salt was 6.6, 6.2 and 5.9 for sundried, smoked and canned fish respectively and after that pH value increased with time. In the post-mortem period, decomposition of nitrogenous compounds leads to an increase in pH in the fish flesh (Shenderyuk and Bykowski, 1989). The pH value of sundried and smoke dried chela fish were increased significantly (p<0.05) with storage period. According to Simeonidou et al. (1997) increase in pH values associated with the production of basic components induced by the growth of bacteria. Most microorganisms grow best at pH values between 6.6 and 7.5, whereas only a few grow at a pH below 4.

Microbiological changes

**Total Plate Counts (TPC):** The Total Plate Count (TPC) shows an overall increasing trend in sundried and smoke dried chela and increase was greater in sundried stored chela (Table 4).

No substantial TPC was detected in the frozen and canned fish samples of BS, 30 days and 60 days stored chela. This was due to their low water activity comparing to the other two species. The considerable counts were recorded during storage probably due to increase in water activity as well as storage duration. Hood et al. (1983) mentioned that microbial load increased with duration of storage and temperature. However, TPC is positively correlate with a\(_w\) and TVB-N but negatively correlate with overall acceptability.

**Halophilic count:** Halophiles require salt for growth and grow mostly in salted dried fish products (Khan et al., 2005). They reported that halophiles are aerobic and usually not found in canned fish where limited oxygen. As shown in Table 4, the halophilic bacteria could not be detected initially but gradually increased from 60 days of storage due to higher moisture and salinity in the product. The counts of more than 1×10\(^2\) cells g\(^{-1}\) could be due to their higher water activity. AOAC. (2005) observed that a\(_w\) for a saturated salt solution is 0.75 and if the equilibrium relative humidity is greater than 75% of the salted products it will take up moisture from the atmosphere increasing the O\(_2\) and consequently increase the possibility of contamination by microbes.
Total Fungal Count (TFC): Not all fungi which recur in fish are deleterious. Moulds cause of spoilage of fish products and produce mycotoxins. They can grow in salt concentrations between 5 and 26% (Reilly, 1986). Results in Table 4 show an increasing trend in the salt content during the storage period. A considerable higher fungal growth was recorded in sundried and smoke dried chela after 30 and 60 days of storage compared to frozen and canned chela. This may be due to increase in $a_w$ and moisture content. According to Gandotra et al. (2012), products deteriorate by growth of moulds when water content is about 15%. This observation supports our present study. Generally, the rapid reduction in water activity ($a_w <0.75$) is a controlling factor of fungi/mould contamination during storage (Kolakowska, 2002).

Changes in Total Volatile Bases (TVB-N): The TVB-N in fresh chela recorded 7.10 mg N/100 g. TVB-N represented very small quantity in fresh fish and was produced by putrefaction process in spoiling fish. According to Wallace (2000) TVB-N is better index of spoilage. The TVB-N presence in the fish can be explained by means of two different pathways: (1) As a result of bacterial catalysis breakdown of trimethylamine oxide (TMAO) during the storage and (2) From thermal breakdown of proteins, amino acids and other nitrogenous compounds during the heat processing steps (Chia et al., 1983). The TVB-N content quantifies a wide range of basic volatile compounds (NH3, methylamine, dimethylamine, trimethylamine, etc).

The TVB-N values were found to vary from 27.14 mg N/100 g in BS to 32.57 mg N/100 g in 30 days and 36.73 mg N/100 g in 60 days for sundried chela (Fig. 1). For smoke dried chela, it increased from 2.063 mg N/100 g in BS to 29.31 mg N/100 g in 30 days and 33.77 mg N/100 g in 60 days. The TVB-N values were high in sun and smoke dried chela after storage has exerted a higher bacterial and thermal action during storage period. The TVB-N values of frozen and canned chela were 12.63 mg N/100 g and 15.50 mg N/100 g, respectively in BS condition. It increased up to 21.14 mg N/100 g and 17.68 mg N/100 g after 30 days. After that it continued to increase to 25.14 mg N/100 g and 21.95 mg N/100 g after 60 days of storage period. The rate of spoilage increases with the time but few values exceeded the recommended value set for fish regarded as acceptable condition. The increase in TVB-N throughout the storage period may be due to microbial activity, storage temperature, absorption of moisture and relative decrease in salt content. However, the limiting level for rejection of TVB-N is 30-40 mg N/100 g for storage at ambient temperature (Connell, 1995).

Dominant bacterial and fungal flora: Drying of fish is reported to impart a degree of microbiological stability to the product, which is a function of reduced water activity and heating.
Table 5: Dominated bacteria and fungi in sundried, smoke dried, frozen and canned chela

| Dominant bacteria            | Dominant fungi              |
|------------------------------|-----------------------------
| Aeromonas spp.               | Aspergillus niger           |
| Pseudomonas spp.             | A. flavipes                 |
| Vibrio spp.                  | Rhizopus niger              |
| Streptococcus spp.           | Pencillium spp.             |
| Staphylococcus spp.          | Mucor                       |
| Flavobacterium spp.          | Archaean halococcus         |
| Micrococcus spp.             |                             |

Table 6: Sensory evaluation of sundried, smoke dried, frozen and canned fish sample

| Preservation method and time (days) | Degree of liking/hedonic scale score (1-9) | Acceptance (%) |
|-------------------------------------|--------------------------------------------|----------------|
| Sun dried                           |                                            |                |
| BS                                  | 9.0                                        | 100.00         |
| 30                                  | 6.6                                        | 73.33          |
| 60                                  | 5.9                                        | 53.10          |
| Smoke dried                         |                                            |                |
| BS                                  | 9.0                                        | 100.00         |
| 30                                  | 6.9                                        | 62.10          |
| 60                                  | 6.1                                        | 54.88          |
| Frozen                              |                                            |                |
| BS                                  | 9.0                                        | 100.00         |
| 30                                  | 7.9                                        | 87.77          |
| 60                                  | 7.4                                        | 82.22          |
| Canned                              |                                            |                |
| BS                                  | 9.0                                        | 100.00         |
| 30                                  | 8.7                                        | 96.66          |
| 60                                  | 8.3                                        | 92.22          |

1: Dislike extremely, 2: Dislike very much, 3: Dislike moderately, 4: Dislike slightly, 5: Neither like nor dislike, 6: Like slightly, 7: Like moderately, 8: Like very much and 9: Like extremely

The dominant bacterial and fungal flora during storage period is shown in Table 5. Fortunately, *E. coli* and *salmonella* bacteria were not detected during the storage period. The previous observation of *Sur Micrococcus spp* and *Pseudomonas spp* are the main micro-organisms associated with fresh water fish.

The dominant fungi found during storage were *Aspergillus* sp. and *Pencellium*. Turkkan *et al.* (2008) reported that the production of aflatoxin when dried and smoked fish was inoculated with *Aspergillus flavus*. Although, there are no reported poisoned by mycotoxins in fishery products; there is a definite risk to human health considering how fish are traditionally processed.

**Sensory evaluation results:** The sensory evaluation result expressed in mean value (Table 6). The result shows that, there were changes in all the sensory parameters after subjecting the fish to different processing methods and storage period. In this study, high quality canned and frozen fish with excellent sensory and physical properties were obtained through storage period. The sensory properties of canned and frozen fish were in more acceptable condition throughout storage period than sundried and smoke dried fish.

**CONCLUSION**

In conclusion, this study shows that chela fish (*Laubuka dadiburjori*) can serve as a good source of dietary protein and lipid. Smoking and sun drying demonstrated efficient method of fish processing in terms of the retention of protein value and reduction in the moisture content. The results from microbiological study demonstrated that after sun drying, smoking, freezing and canning processing of chela, the fish was safe to be consumed after 60 days of storage period. Canning and freezing demonstrated efficient method of chela fish processing in terms of the retention of wholesomeness of stored product.

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