

Shelf-life study of salt and garlic treated smoke-dried *Mastacembelus pancalus* (baim) during different storage condition

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Abstract

The antibacterial effect of Salt and Garlic-extract (S+G) in smoke-drying method were studied in freshwater *M. pancalus* (baim) fish species by analyzed their moisture, protein, fat and ash content, TVB-N and sensory score value and bacterial load at two different storage condition (room and refrigeration temperature). At different length of storage period, the percentage of moisture, TVB-N value (mgN/100g) and TVC (cfu/g) were gradually increased whereas protein, fat and ash contents were considerably decreased in the smoke-dried samples. Moreover It was observed that the TVB-N value have inverse relationship with the sensory score of both storage condition. The shelf-life of S+G treated smoke-dried *M. pancalus* fish product was 16 month for room temperature and 24 month for refrigeration storage respectively. Therefore, it can be inferred that S+G treated smoke-dried *M. pancalus* fish products have long shelf life when they are stored at refrigeration temperature (4°C).

Keywords: baim-fish, shelf-life study, smoke-drying, storage condition

1. Introduction

In recent years increasing world population is important in human nutrition deficiency causes protein sources of animal origin, this protein the best degree of vulnerability and take advantage of resources at the beginning of a cheap way to resolve fishery is gaining importance day by day [1].

In Bangladesh 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.

The central concern of fish processing is to prevent fish from deteriorating. Various traditional methods are employed to preserve and process fish for consumption and storage. These include smoking, drying, salting, frying and fermenting and various combinations of these. Of all food preservation methods, drying has received the most widespread and enthusiastic publicity in recent years [2]. Fish smoking prolongs shelf life, enhances flavour and increases utilization in soups and sauces. In addition, smoking demonstrated a better efficient method of fish processing in terms of the retention of protein value and reduction in the moisture content [3].

There has been a constant increase in the search of alternative and efficient compounds for food preservation aimed at a partial or total replacement of antimicrobial chemical additives [4, 5]. Extract from many plants used as flavouring and seasoning agents in food and beverages have been used therapeutically for centuries [6, 7]. Fish is normally salted

before smoking to protect food against bacteria, mold, and spoiling. Different salting methods are being practiced by the smoked fish industry in different parts of the world [8, 9]. Garlic (*Allium sativum* L.) exhibit a broad antibiotic activity against both gram negative and gram positive bacteria [10, 11]. The active compound present in garlic is the allicin, allyl alcohol which is a thiosulfinate compound reported for its antimicrobial activity [12]. In this research work salt with garlic extract used before smoke-drying which are easily available and cheaper cost wise.

Among freshwater fish species, *M. pancalus* (baim) is a delicious, nutritious and popular to consumers. These fishes have unique taste and high demand from all corners of the country as these are economical in price and full of nutrients especially animal protein and fat. Usually fatty fishes are used for smoke-drying but in the present research work, the above mentioned fish are selected because these fish considered widely accepted and preferred by the peoples and they need this fish when it is not available. To make this fish available in off seasons, attempts have been made to cure them using commercial salt and garlic extract. Besides, this highly accepted fish are not yet tried to preserve using salt and garlic extract or in any other forms.

Several works have been done in biochemical and microbial qualities of different kinds of freshwater fishes, but no works on the shelf life study of salt and garlic-extract treated smoke-dried fish has yet been done. Considering these facts, the present investigation was carried out to determine the overall shelf life qualities of freshwater *M. pancalus* fish treated with salt and garlic-extract in smoke-drying process.

2. Materials and methods

2.1 Sample collection

Freshwater fish species, *Mastacembelus pancalus* (baim) was collected from the Meghna River early in the morning. Fresh mature fish samples were transported to laboratory in sterile polythene bag to avoid any type of microbial contamination.

2.2 Place of experiment

The whole experiment was carried out at the laboratory of Fish Technology and Food Microbiology Section of the Institute of Food Science and Technology (IFST) of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka.

2.3 Preparation of Samples

At first, the collected *M. pancalus* fish was beheaded and gutted and washed properly with clean water. The dressed fish samples were then weighed and prepared for further processing.

2.4 Preparation of samples for processing

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

2.5 Fish smoke-drying

The fishes were smoked in improved traditional type of smoking kiln [13]. The fish smoking kiln was operated by first loading tamarind wood chips and rice-husk into the heat chamber, preheating for some minutes and then loading the fish-samples onto removable wire mesh trays in its central chamber for the smoking process. The desired temperature (70-75°C) was maintained manually. Smoking was done approximately for 4 hours. During the smoking fish samples were turned upside down in the middle period, to make the sample smooth and steady in texture and appearance. The smoked fishes showed characteristic attractive golden brown color and acceptable texture with smoky flavor, which was followed by cooling for 20-30 minutes at ambient temperature to make fish muscle compressed and facilitate to prevent breaking of smoked products.

2.6 Storage for shelf life study

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

2.7 Sampling procedure

3 or 4 slices of salt and garlic treated smoked-dried *M. pancalus* fish samples were taken randomly which represented the parts from 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 every 2nd months interval for fish kept at room temperature (26-31°C) and at every 3rd months interval for fish stored at refrigeration temperature (4°C) until the fish became inedible.

2.8 Parameters of shelf-life study

There are some parameters which determine the shelf-life quality of salt-garlic (S+G) fish products during storage condition, such as-

- Sensory evaluation was assessed by the sensory method as described by Larmond [14].
- Moisture, fat and ash contents of the fish were determined by AOAC method [15].
- The crude protein of the fish was determined by Micro-Kjeldahl method [16].
- TVB-N using Conway modified micro-diffusion technique [17].
- Microbiological analysis was done according to the standard methods of AOAC [18].

3. Results & Discussion

3.1 Changes in moisture content

In present study, the moisture levels in smoke-dried fishes examined were below 20% (Figure-1) which is good or acceptable for smoke-dried fishes suggested by Lilabati and similar result was also found by Adebowale *et al.* in four types of smoked *C. gariepinus* (7.16-10.71%) [19, 20]. Comparatively lower percentage moisture in freshly processed salt and garlic treated smoke-dried fish-products could be as a result of loss of water during smoking which was observed by Salan *et al.* [21]. In case of room and refrigeration-temperature stored S+G treated smoke-dried *M. pancalus* fish-products, moisture (%) content were varied in the range of 7.53-12.43% and 7.53-8.70% respectively (Figure 1 and Figure 2). During the storage period, moisture content increased both room and refrigerated temperature, which was in agreement with the work by Ezembu and Onwuka, Al-Reza *et al.*, Kumar *et al.* and Yanar [22, 23, 24, 25]. In another study 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] which is also more or less similar with present work [26]. This increase can be attributed to absorption of moisture from the surrounding since there was no re-drying during storage [27]. The gutted smoke dried fish samples of African cat fish (*Clarias nigrodigitus*) had moisture content as 6.27 to 10.92% which is similar with present study [28]. Moisture content of 12% is the level beyond which fish products begin to grow moulds after few days [29]. In this study, the final moisture content of refrigerated stored salt-garlic treated smoke-dried fish samples was less than 12%.

3.2 Changes in protein content

During room and refrigeration storage, protein (%) content was varied in the range of 59.07-57.63% and 59.07-58.59% in S+G treated smoke-dried *M. pancalus* fish-products respectively (Figure 1 and Figure 2). In storage condition, the protein content decreased significantly with the time due to water soluble protein diffused out to the surrounding for exosmosis [30]. This could be due to gradual degradation of initial crude protein to more volatile products such as total volatile bases, hydrogen sulphide and ammonia [31]. Similar drop in protein concentration was reported for *Heterobranchus longifilis* [32]. Al-Reza *et al.* was observed a significant decreasing trend in protein content of smoke-dried

chela fish during storage period [23]. 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 [33].

Changes in moisture, protein, fat and ash content of salt and garlic (S+G) treated smoke-dried *M. pancalus* fish samples storage at room (26-31°C) and refrigeration temperature (4°C) are shown in Figure 1 and Figure 2.

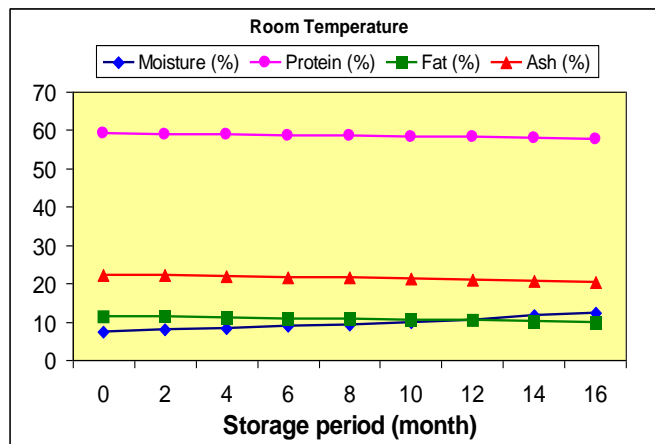


Fig 1: Changes in moisture, protein, fat and ash content of salt and garlic (S+G) treated smoke-dried *M. pancalus* fish samples storage at room (26-31°C) temperature

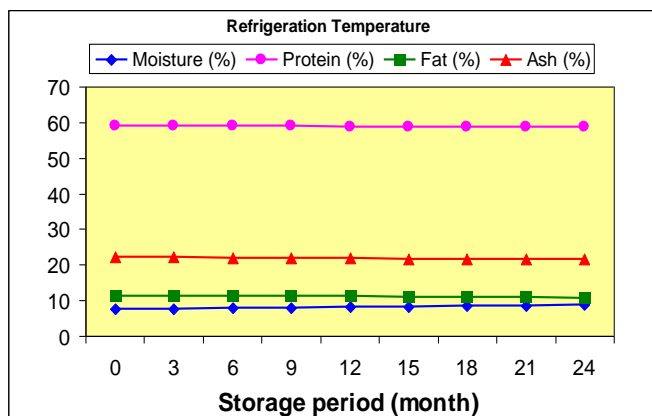


Fig 2: Changes in moisture, protein, fat and ash content of salt and garlic (S+G) treated smoke-dried *M. pancalus* fish samples storage at refrigeration temperature (4°C)

3.3 Changes in fat content

In case of room and refrigeration temperature stored S+G treated smoke-dried *M. pancalus* fish-products; fat (%) content was varied in the range of 11.32-9.81% and 11.32-10.77% respectively (Figure 1 and Figure 2). Horner found that reduction in fat content over the storage period may be attributed to oxidation of poly-unsaturated fatty acids contained in the fish tissue to products such as peroxides, aldehydes, ketones and free fatty acids [34]. Egbal *et al.* stated that decrease in fat during storage may also be due to the effect of packaging and storage condition [35]. Al-Reza *et al.* was also observed the reduction in fat content of smoked chela (*Laubuka dadiburjori*) fish during storage at room temperature [23]. In this study it has been observed that during storage condition the fat content decreases very slowly which

may be due to hydrolytic nature of smoked products which is in line with the findings of Islam [36].

3.4 Changes in ash content

Clucas and Ward reported that the inorganic content remain as ash after the organic matter is removed by incineration [37]. Ash (%) content was found to vary from 22.15% to 20.42% and 22.15% to 21.48% for S+G treated smoke-dried *M. pancalus* fish respectively during storage at room and refrigeration temperature (Figure 1 and Figure 2) respectively. Salan *et al.* observed 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 result for ash content of smoked fish products have been reported in previous study [21, 38, 39]. Also Doe and Olley reported that smoking resulted in the concentration of nutrients, such as, protein and ash [40]. There was decrease of ash content of S+G treated smoke-dried *M. pancalus* fish over the period of room and refrigeration storage which is similar with the findings of Ezembu and Onwuka who reported the decrease of ash content in both improved and traditional smoked three fish species over the period of storage [22]. The ash content changes with the time of storage due to absorbance of moisture and loss of protein [30].

3.5 Sensory score value

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 [41, 42, 43, 44, 45]. At room and refrigeration storage the sensory scores of salt and garlic (S+G) treated smoke-dried *M. pancalus* fish samples are presented in Figure 3 and Figure 4. The quality assessment as well as sensory evaluation (score) was carried out every two months intervals for samples stored at room temperature (26°C-31°C) and every three months intervals for samples stored at refrigeration temperature (4°C) using trained panel of four judges following 9-point ascending scale to evaluate changes in color, odor, texture, general appearance and mean of general acceptability score until it was an acceptable condition [46]. At the beginning of storage all the sensory parameters of S+G treated smoke-dried fish samples were rated as good based on the grading scale. Moreover, there was found no broken parts of the experimental fish after smoke-drying process. The highest mean of general acceptability score was found 8.85 in freshly process S+G treated smoke-dried fish samples. The reduction in the sensory qualities with increase in storage period of processed fish could be attributed to higher activities of the spoilage agents which is in agreement with the findings of Olatunde *et al.* and Daramola *et al.* [47, 27]. Similarly, Llobreda *et al.* and Reilly and Dela-cruz was also observed the results of research into storage of crustaceans (Oyster and Shrimps) revealed quality loss during storage both at ambient temperature and chilling [48, 49]. The mean of general acceptability score of the end product of salt and garlic (S+G) treated smoke-dried *M. pancalus* fish samples were 4.37(16

month) and 3.54 (24 month) during storage at room (26-31⁰C) and refrigeration temperature (4⁰C) respectively. Sensory score value decrease with the increase of TVB-N value and storage period which is presented in Figure 3 and Figure 4.

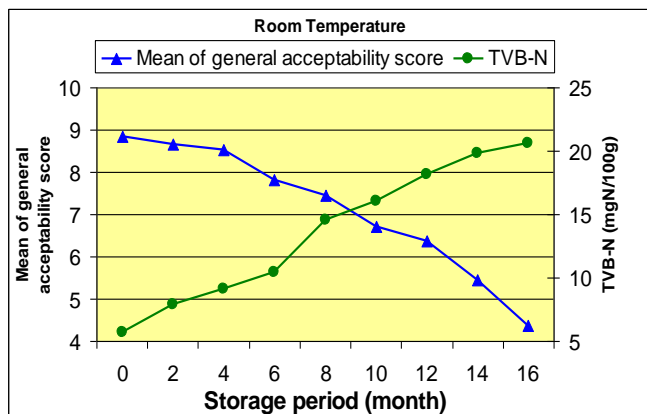


Fig 3: Changes in TVB-N value and mean of general acceptability score of salt and garlic (S+G) treated smoke-dried *M. pancalus* fish samples storage at room (26-31⁰C) temperature

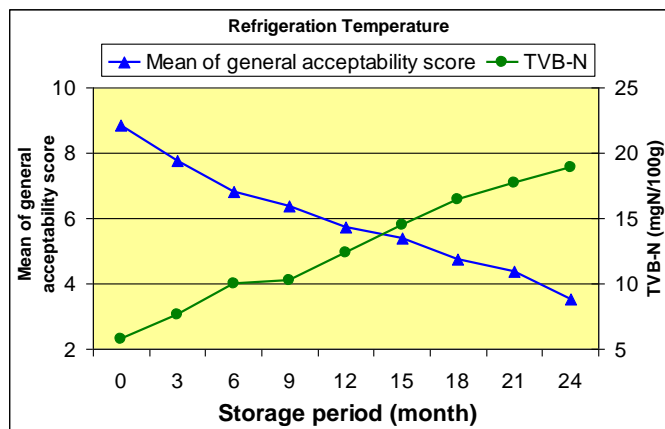


Fig 4: Changes in TVB-N value and mean of general acceptability score of salt and garlic (S+G) treated smoke-dried *M. pancalus* fish samples storage at refrigeration temperature (4⁰C)

3.6 Changes in TVB-N value

Total Volatile Base Nitrogen is mainly contributed by ammonia in the muscle produced by deamination of muscle proteins [50]. It helps to measure the level of fish spoilage and to explore the shelf life of fish. The ratio of Total Volatile Base Nitrogen (TVB-N) has been recommended as a useful index of quality in fish which was increased during storage. There was continuous increase in the TVB-N value of the S+G treated smoke-dried fish samples all throughout the period of room and refrigeration storage. The increase in TVB-N throughout the storage period may be due to microbial activity, storage temperature, and absorption of moisture which is in line with Kumar *et al.* [24]. 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 [33, 22]. Similarly Hassan *et al.* also reported that the TVB-N values of smoked shrimp were increased with the

time of storage [30]. Likewise, Duyar *et al.* found the increasing trend of TVB-N value in refrigeration storage which is similar with the present findings [51]. In salt-garlic treated smoke-dried *M. pancalus* fish product, the TVB-N values ranges from 5.74 (0 day) to 20.58 mgN/100g (16 month) during storage at room temperature whereas ranges of TVB-N value was 5.74 (0 day) to 18.92 (24 month) mgN/100g in refrigeration storage respectively (Figure 3 and Figure 4). 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]. Such differences reflect different products, specific treatments and processing conditions.

3.7 Changes in Total Viable Count (TVC)

Total Viable Counts (TVC) of bacteria in S+G treated smoke-dried *M. pancalus* during storage at room temperature (26-31⁰C) and refrigeration temperature (4⁰C) is given in Figure 5 and Figure 6.

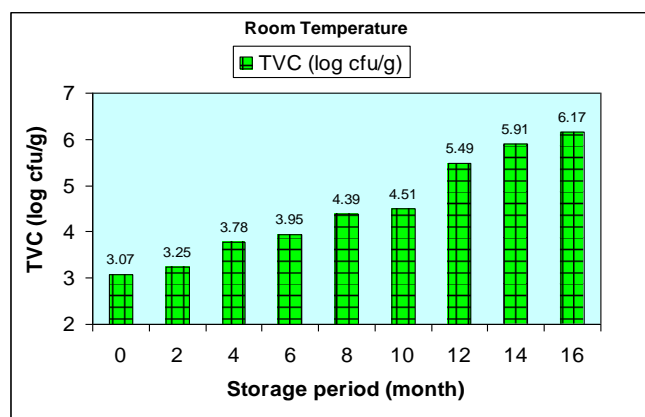


Fig 5: Changes in Total Viable Count (TVC) log cfu/g of salt and garlic treated smoke-dried *M. pancalus* fish during storage at room temperature (26-31⁰C)

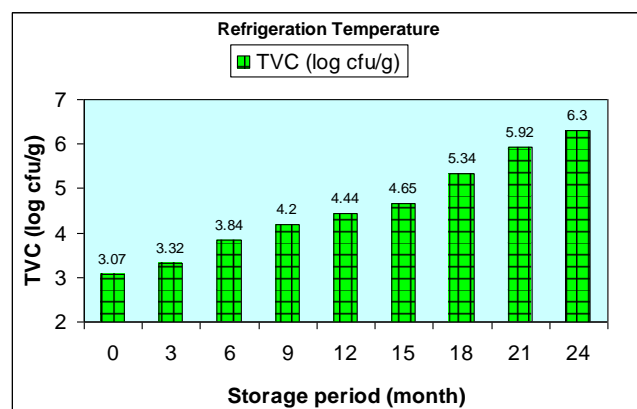


Fig 6: Changes in Total Viable Count (TVC) log cfu/g of salt and garlic treated smoke-dried *M. pancalus* fish during storage at refrigeration temperature (4⁰C)

The amount of bacteria in foods serves as a general indicator of hygiene. Determination of Total Viable Count (TVC) is widely used to assess the bacterial quality of fish. Kumolu-Johnson and Ndimele stated that spoilage of fish resulting from action of enzymes and bacteria can be slowed down

during smoking ^[56]. Goulas and Kontominas also reported that the effect of temperature and smoke, addition of salt and low water activity are important for inhibiting the growth of microbes in food ^[57]. In fresh process condition, TVC of salt and garlic treated smoke-dried *M. pancalus* fish sample was 3.07 log cfu/g which was found to be increased with storage period or time and these counts rose to 6.17 log cfu/g (16 month) and 6.30 log cfu/g (24 month) at room and refrigeration temperature respectively. TVC of smoke-dried fish samples were increased with increase in the duration of storage due to growth and multiplication of the microbes ^[38]. As the duration of storage increased processed fish samples may absorb small amounts of moisture from surrounding atmosphere providing enabling environment for microbial growth ^[58]. Similarly Hassan *et al.* also observed that a close relationship exists between the moisture content and the bacterial load in smoked shrimp products which is harmony with the present research work ^[30]. In Bangladesh, DOF and BSTI recommended the TVC of processed fish to be not more than 10⁶ cfu/g. If any sample contains more than 10⁸ cfu/g bacterial counts then these microbes can cause spoilage of that product ^[59]. Kleickman and Schellece evaluated the microbial numbers of smoked fish and discovered that the average plate count amounted to 10⁶/g of fish ^[60]. According to Cheesbrough, it is generally accepted that fish with microbial load >10⁶cfu/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 ^[61]. In this study, TVC of S+G treated smoke-dried fish-products increases during room and refrigeration storage period but were within the range of 10⁶cfu/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 10⁵ (5.7 log¹⁰ cfu/g) ^[62]. In another study Kumolu-Jhonson *et al.* suggested that fresh garlic possesses antioxidant and antimicrobial properties, which can extend the shelf life of *Clarias gariepinus* which is in line with the S+G treated smoke-dried fish *M. pancalus* samples ^[63].

4. Conclusions

The present study provides information about the suitability of salt and garlic treated smoke-drying method of the commercially important fish like baim to produce a very stable and safe product with long storage life. By assessing the shelf-life quality as well as the feasibility of the method, it can be recommended to explore the smoke-drying method treated with natural preservatives like salt and garlic extract in commercial scale which further will contribute in national economy of Bangladesh.

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