Fisheries
ORIGINAL ARTICLE

Changes in biochemical and microbiological parameters of Mola (*Amblypharyngodon mola*) fish pickle during storage at room temperature

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ARTICLE INFORMATION	ABSTRACT
	Among small indigenous species (SIS) of fishes Mola (*Amblypharyngodon mola*) has high nutritive value. Beside traditional curry preparation it is necessary to make available this highly nutritive fish in other 'ready to eat' forms and also adding value to this fish. Therefore, this study was carried out to prepare fish pickle with Mola (*Amblypharyngodon mola*) and to observe the changes in biochemical parameters and bacterial load of this pickle stored at room temperature (28°C to 32°C). The study was conducted in the Department of Fisheries Technology Laboratory, Bangladesh Agricultural University. The results of the study showed that the percent moisture and protein content decreased while lipid and ash content increased after preparation of pickle than those of fresh fishes. The fish pickle stored at room temperature (28°C to 32°C) in sealed and vacuum sealed packing conditions, the percent moisture, protein and lipid content decreased with the progress in storage period gradually but ash content increased. At this temperature (at both sealed and vacuum sealed pack), pH value of the pickle decreased very slowly but the TVB-N value and bacterial load increased gradually throughout the storage period. So, it was concluded that, at room temperature (28°C to 32°C) shelf life of Mola (*Amblypharyngodon mola*) fish pickle was short, pickle may remain in acceptable condition until 12 days in sealed pack and 30 days in vacuum sealed pack. **Keywords:** Fish pickle, *Amblypharyngodon mola*, biochemical parameters, bacterial load, room temperature storage

1 Introduction

In recent year's value addition have received a wider attention because of increased urbanization. There is a growing demand for value-added products due to social and cultural changes (Pagarkar A U, 2011). Therefore, to increase profitability, development of value added products from low cost fish could be a better option to produce and supply for consumption. Pickles have been of commercial importance in some developing countries like Korea, where pickles are made from freshwater fish (common carp, silver carp and cat fishes), anchovies, shrimps, squid, oyster, sea urchin etc. Several workers have studied on the pickles prepared from different fish in India such as fresh water fish, low cost marine fish and from invertebrates. A study on fish pickle carried out stated that pickles were prepared from two miscellaneous fresh

water fishes *Amblypharyngodon mola* and *Puntius ticto* (Sharma and Sarma, 2012).

Two hundred and sixty species of freshwater fishes available in Bangladesh, over one hundred and forty species are classified as small indigenous fish species (SIS). *Amblypharyngodon mola* (Hamilton-Buchanan, 1822), belonging to the family Cyprinidae, is a small indigenous fish species (SIS) of high commercial importance. It has wide distributional range in India, Pakistan, Bangladesh, Myanmar, Nepal and SriLanka (Nath and Dey, 1990; Talwar and Jhingran, 1991; Jayaram, 1999). Commonly known as 'Mo-lacarplet' or 'Pale carplet', *A. mola*, is widely distributed in fresh water habitats like rivers, streams, ponds, beels, canals, paddy fields etc. *A. mola* has been playing vital role providing the main source of animal protein for all rural and urban households as well. SIS's are also an important source of vitamin A, calcium and iron (Ahmed, 1981; Wahab, 2003). Among the SIS of Bangladesh, Mola (*A. mola*) bears prime importance in terms of availability and popularity. Unlike many other fish species mola is not seasonal fish and is available in ample quantity throughout the year (Kohinoor et al., 2001). So far in Bangladesh, there are a few literatures on the development of pickle from SIS, *Amblypharyngodon mola* and on the quality changes took place in these products. Considering the facts, the present study was conducted to develop pickle from *Amblypharyngodon mola* and to know quality changes at room (28°C to 32°C) under various packing conditions.

2 Materials and Methods

2.1 Duration of the study

The present study was conducted from May 2016 to July 2017 (total three months), in the laboratories of Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh.

2.2 Sources of samples

Fresh Mola (*Amblypharyngodon mola*) fishes were collected from Kamal- Ranjit (KR) Market of Bangladesh Agricultural University (BAU), Mymensingh. Total 3 kg of fishes were collected and were immediately transported to the Fish Processing and Quality Control Laboratory, BAU and kept at room temperature (28°C to 32°C) in trays. Then the proximate composition of fresh fish was analyzed.

2.3 Preparation of fish pickle

2.3.1 Fish pickle preparation procedure

The fishes were thoroughly washed with tap water to remove contaminants on the skin. After gutting and

beheading, the samples were washed with tap water and salt water. The excess water was removed from fish with a fresh dry cotton cloth. Then the fish were marinated with required amount of turmeric, red chili, coriander powder and salt for at least an hour in a refrigerator (at 5°C to 8°C), fried in mustard oil, added other ingredients (Table 1) and finally heated till vinegar absorbed. During packing (in sealed and vacuum sealed packs) care was taken to see that there was enough oil in the pickle packs (Fig. 1). Standard recipe for the preparation of pickle is given in the Table 1.

2.3.2 Sample storage

A total of 7 pack pickle samples were stored at room temperature (28°C to 32°C) for quality analysis. Among them 4 packs were stored in sealed condition and 3 packs were stored in vacuum sealed condition for about 30 d.

2.4 Quality analysis

2.4.1 Proximate composition

To observe the changes in proximate composition (moisture, protein, lipid and ash) of pickle samples chemical analysis was done according to the methods described in Association of Official Analytical Chemists (AOAC, 2005) with certain modifications. Triplicate samples were taken for each samples to carry out the analysis.

Table 1. Ingredients for fish pickle preparation

Ingredients	Quantity
Mola fish	1 kg
Chili powder	20 g
Cumin powder	10 g
Turmeric powder	5 g
Garlic powder	10 g
Clove powder	2 g
Mustard oil	100 mL
Salt	35 g
Sugar	40 g
Tamarind	50 g
Pachforon	5 g
Cinnamon	2 g
Coriander powder	2 g
Vinegar	20 mL
Sodium benzoate	1 g

2.4.2 Determination of pH value

pH was measured at room temperature following the method described in (AOAC, 2005). At first accurately 5 g sample was taken and homogeneously mixed in 50 mL distilled water. pH was measured
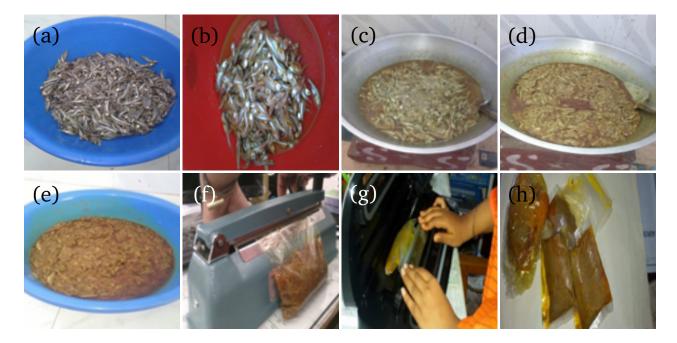
Figure 1. Procedure of Mola fish pickle preparation. (a) Fresh washed fish, (b) Marinated fish, (c) Frying with mustard oil, (d) Cooking with water, (e) Cooked fish, (f) Sealing, (g) Vacuum sealing, and (h) Pickled fish ready for storage

using an electronic pH meter (HANNA pH 211 Microprocessor pH Meter) with a glass electrode using expandable scale.

2.4.3 Determination of TVB-N value

Total Volatile Base Nitrogen (TVB-N) was determined according to the methods given in AOAC (1984) with certain modification.

2.4.4 Determination of bacterial load

The colonies units (CFU) were counted under a Quebec dark field colony counter (Leica, Buffalo, NY, USA) equipped with a guide plate ruled in square centimeters. Agar plates containing 30-300 colonies were used to calculate bacterial load using following formula:

$$BL = \frac{C \times D \times V}{W} \times 10 \qquad (1)$$

where BL = bacterial load (CFU g^{-1}), C = number of colonies on petridish, D = dilution factor, V = volume of stock solution (mL), and W = weight of pickle or condiment sample (g).

2.5 Statistical analysis

Data from different biochemical measurements were subjected to statistical analyses. The statistical analysis package SPSS 11.5 (SPSS Inc, Chicago, IL, USA) was used to calculate mean and standard deviation of the values.

3 Results

3.1 Biochemical composition

Biochemical composition of fresh Mola (*Amblypharyngodon mola*) was determined under laboratory condition immediately after collection of the sample. The percent moisture, protein, lipid and ash content were 79.11±7.46, 11.51±1.51, 6.51±0.58 and 2.50±1.28, respectively (Table 2). While pickle was prepared the amount of these major constituents changed. The values for moisture, protein, lipid and ash content on '0' day after preparation of pickle were 37±0.02, 11.89±1.66, 21.47± 0.01 and 5.71±0.88, respectively (Table 2).

Table 2. Proximate composition of fresh Mola (*Amblypharyngodon mola*) fish and fish pickle

Component	Fresh fish	Fish pickle
Moisture (%)	79.11±7.46	37.00±0.02
Protein (%)	11.51±1.51	11.89±1.66
Lipid (%)	6.51±0.58	21.47±0.01
Ash (%)	2.50±1.28	5.71±0.88

3.2 Changes proximate composition

In order to study changes in the proximate composition and quality of fish pickle at room temperature (28°C to 32°C), total 7 packs of fish pickle were stored. There were 4 sealed packs and 3 vacuum sealed packs. Sealed packs were stored for 16 days and vacuum

Table 3. Changes in moisture, ash, protein and lipid contents, and pH and TVB-N content of fish pickle at room temperature (28°C to 32°C) in sealed and vacuum sealed pack

Day	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	pH	TVB-N (mg $100g^{-1}$)
	Sealed pack					
0	37.00±0.02	5.71±0.88	11.89±1.66	21.47±0.01	5.53±0.00	1.64±0.04
4	35.98±0.13	5.39±0.67	10.04±1.06	18.58±0.49	5.31±0.01	1.79±0.01
8	34.53 ±0.07	7.04±0.41	9.33±0.89	13.70±0.07	5.07±0.03	2.58±0.02
12	33.97±0.67	7.47±0.54	9.01±0.62	8.18±0.67	4.87±0.02	2.42±0.01
16	33.00±0.43	9.59±1.03	7.02±0.49	7.48±0.32	4.53±0.01	3.18±0.01
	Vacuum sealed pack					
0	37.00±0.02	5.71±0.88	11.89±1.66	21.47±0.01	5.53±0.00	1.64±0.04
10	37.56±0.78	6.02±0.94	9.79±0.92	17.49±0.13	5.08±0.01	3.91±0.01
20	35.34±0.89	7.64±0.16	8.78±0.17	13.01±0.72	4.43±0.05	4.14±0.04
30	32.80±0.67	9.87±0.07	5.06±0.33	9.92±0.21	4.12±0.07	7.15±0.08

sealed packs were stored for 30 d at room temperature. Biochemical analyses were done for the samples in sealed packs at every 4 days of interval and the samples in vacuum sealed packs at every 10 d interval.

In this experiment initial moisture content (%) of pickle was found 37±0.02% which was much lower than that of fresh fish. After 4 d of storage moisture content of pickle in sealed pack decreased to 35.98±0.13% and then after 16 d of storage value reduced to 33.00±0.43% (Table 3). On the other hand moisture content in pickle was obtained 37.56±0.78% after 10 d of storage at vacuum sealed packed which further decreased to 32.80±0.67% on 30th day of storage (Table 3). In the case of ash content, the initial value was 5.71±0.88%. With lapse of storage period the percent ash content of pickle both in sealed and vacuum sealed packs increased. In sealed pack the percent ash content increased to 9.59±1.03% after 16 d of storage and 9.87±0.07% in vacuum sealed pack after 30 days of storage of pickle samples.

The initial protein content in fish pickle was 11.89±1.66%. As the time passed protein content in fish pickle started to decrease, on 12th day of storage protein content (%) decreased to 9.01±0.62% the after 16 d of storage it reduced to 7.02±0.49% (Table 3). For the pickle sample stored at vacuum sealed pack a similar trend was observed. Here, the initial percent protein content in pickle 11.89±1.66% reduced to 5.06±0.33% after 30 d of storage. The same Table 3 shows the changes in percent lipid content in fish pickle. Irrespective of packing condition percent lipid content in pickle samples decreased with the progress of storage period. The initial lipid content in fish pickle found 21.47±0.01% in sealed pack which decreased to 13.70±0.07 on 8th day and then to 7.48±0.32 after 16 d of storage. While the fish pickle stored in vacuum sealed pack the initial percent lipid value 21.47±0.01 reduced to 9.92±0.21 30 after 30 d of storage.

3.3 Changes in the pH and TVB-N values

The changes in the pH value of fish pickle during storage at room temperature (28°C to 32°C) are shown in Table 3. The initial pH value of fish pickle was found 5.53±0.0 which decreased to 4.53±0.01 after 16 d in sealed pack and 4.12±0.07 in vacuum sealed pack after 30 d of storage. The changes in the TVB-N value (mg 100 g^{-1}) of fish pickle during storage in both packing condition at room temperature (28°C to 32°C) also presented in the same table. Here the initial value for TVB-N was found 1.64±0.04. With progress in storage period TVB-N value gradually increased. After 16 d of storage TVB-N value for pickle stored in sealed pack increased to 3.18±0.01 whereas the value was obtained 7.15±0.08 after 30 d of storage for pickle stored in vacuum sealed pack.

3.4 Changes in the bacterial load

In this experiment bacterial load of pickle stored in sealed pack at room temperature (28°C to 32°C) was observed only. Result showed that bacterial load of pickle increased with the lapse of storage period from its initial value 2.10×10^3 to 2.30×10^7 within 16 d of storage (Table 4).

Table 4. Changes in bacterial load (BL) of fish pickle at room temperature (28°C to 32°C) in sealed pack

Day	BL (CFU g^{-1})
Day 0	2.10×10^3
Day 4	2.90×10^4
Day 8	3.30×10^4
Day 12	1.60×10^6
Day 16	2.30×10^7

4   Discussion

The chemical composition of fish varies greatly from one individuals to another depending on age, sex, environment and season with protein levels ranging from 16-21%, lipid 01-25%, ash 0.4-1.5%, moisture 60-81% with extremes of 96% having been reported (Huss, 1995). Devi and Sarojnalini (2012) reported that fresh Mola contains protein level of 3.56%, lipid 5.83%, ash 1.40% and moisture 74.72%. According to Ahmed et al. (2012), chemical composition of fresh Mola contains protein 17.95%, lipid 2.87%, ash 2.50% and moisture 76.86%. Mazumder et al. (2008) obtained protein level 18.46%, lipid 4.10%, ash 1.64% and moisture 76.38% in fresh Small Indigenous Species Mola. All these findings coincide with the values obtained in the present study. The effects of cooking methods on moisture and other contents of fish muscle had been studied (Stephen et al., 2010; Weber et al., 2008; Rosa et al., 2007; Ersoy et al., 2006; Kucukgulmez et al., 2006). The proximate composition of fish pickle is affected by moisture loss which concentrates the nutrient. Moisture content influences the quality of the products (Sen, 2005). However, moisture content of the final product has economic implications as the retention of moisture by the product resulting in economic gains by increasing weight of the product (Rao et al., 2013). In the present study, moisture content of fresh fish was found higher than fish pickle. (Holma and Maalekuu, 2013) reported reduced moisture content (%) in fresh fish immediately after frying. Here, at room temperature (28°C to 32°C) moisture content (%) started to decrease slowly which is similar to the previous finds. The present study showed high level of ash content (%) in fish pickle just after preparation. Devi and Sarojnalini (2012) reported, after deep frying of Mola fish ash content reached to 6.01±0.21%. The higher ash content in the cooked fish might be due to its higher bony consistency and high scaly nature.

This is also might be due to moisture loss in the processed fish pickle during frying (Kocatepe et al., 2011). In this experiment ash content (%) increased very fast in both packing conditions at room temperature (28°C to 32°C). In this study, it was observed that with the progress in storage period the protein content (%) gradually decreased. Denaturation of fish protein and leaching out of water soluble protein is associated with the decrease in protein in fishery products (Arannilewa et al., 2006; Siddique et al., 2011; Gandotra et al., 2012). Might be these are the reasons of decrease of protein (%) content in pickle prepared from Mola irrespective of packing condition in this experiment. Lipid content (%) of fish pickle in the present study was higher than that of fresh fish might be because of deep frying in high quantity mustard oil. This is similar to finding of Emilin (2005) worked on seafood pickle. Frying and cooking of fish resulted in increased lipid content (%) by condensing lipid of the pickle. The findings of the present study and available literatures Other workers (Kocatepe et al., 2011; Türkkan et al., 2008; Gokoglu et al., 2004; Weber et al., 2008; HassabAlla et al., 2009; Puwastien et al., 1999) indicate that the frying cause higher water loss and lipid gain mainly and this is might be due to the absorption of fat by fish muscle. Marimuthu et al. (2011) also reported that the lipid content in fried fish fillet increased due to absorption of oil during frying. In this experiment lipid content (%) started to decrease in both packing conditions. McGill et al. (1974) reported that lipid content (%) in fishery products started to decrease when oxidation occurs. Immediately after cooking, oxidation didn't take place here, might be due to the use of sodium benzoate as preservative. Besides addition of salt, frying and cooking in mustard oil played roles to stop lipid oxidation because salt and mustard oil acted as preservative. At later phase of storage oxidation took place in fish pickle and lipid content (%) started to decrease.

The detection of pH value is one of the most frequently used physical quality parameter for fish and fishery products, which is affected by the changes in the lipid hydrolysis, microorganisms or enzymes (Varlik et al., 2000). In this experiment pH content in both packing condition decreased gradually with time. The decrease in the pH might be due to the addition of vinegar and tamarind during processing and its gradual uptake by fish pickle. pH in prawn pickle reduced significantly by sodium benzoate (Abraham and Setty, 1994). Fishery products are acceptable up to a pH of 6.8 but are considered to be spoiled above 7.0 (Huss, 1995). Erkan et al. (2010) recommended pH level of 6.8 to 7.0 as the limit of acceptability for fishery products. The preliminary evaluation of market fish pickle has indicated that, with pH range 4.4 to 4.7, the products are objectionably sour; consequently its acceptability decreased (Sahu et al., 2012).

TVB-N is commonly used in chemical method to determine spoilage of fish, and the amount of TVB-N permitted in a product is regulated by the European commission, if sensory evaluation gives any doubt about the freshness of fish. Clucas (1982) reported that in freshwater fish and their products TVB-N mainly comes from ammonia. Different sensory effect of TVB-N varies in species to species. Samples could be considered consumable if the TVB-N level is less than 20 mg $100g^{-1}$ and level more than 30 mg determines the product is not consumable (Conell, 1980; Pearson, 1997). TVB-N is a better index of spoilage (Wallace, 2000). In the present study, the TVB-N value in both packing conditions increased very slowly throughout the storage period which was similar to the changes of Total Volatile Base Nitrogen (TVB-N) content below the range suggested by various researchers.

Mukundan et al. (1981) reported that pickle contains very low bacterial counts due to the inhibitory action of low pH and high salt content of the pickles. Higher amount of mustard oil also reduced the bacterial growth in fish pickle. Erichsen (1967) reported that pickled fish normally carry low level of bacteria in the range of 101 to 103 CFU g^{-1}. Chandrasekar (1979) reported total plate count in fish pickle within the range of 103 to 105 CFU g^{-1}. These are similar to the present study. Garg et al. (1977) have reported a viable count of halophiles in the range of 106 to 107 CFU g^{-1} which is higher than the present study. Bacterial load in fish pickle was within the permissible limit of 107 (ICMSF, 1986).

5   Conclusions

The results obtained for biochemical parameters and bacterial load of fish pickle prepared with Mola showed that at room temperature (28°C to 32°C) pickle may remain in acceptable condition until 12 d in sealed pack and 30 d in vacuum sealed pack.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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