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Comparative bio-chemical analysis of traditional and experimental dried fish products of Chalan Beel, Bangladesh

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Abstract
The present study attempted to evaluate the quality of eight dried fishes (*Puntius* sp. *Chanda* sp. *Amblypharyngodon mola, Oxygaster gora, Cirrhinus reba, Glossogobius giuris, Channa punctatus, Mastacembelus pancalus*) which were produced employing traditional method in Chalan Beel areas by local dried fish processors and the experimental dried products were prepared through proper processing method in Department of Fisheries, University of Rajshahi, Bangladesh, from February, 2015 to July, 2015. Traditional and experimental samples were subjected to biochemical analysis on 1st, 4th and 6th month. In the storage period of traditional dried fishes, the average highest bacterial load was 7.8×10^5 cfu/g in *Mastacembelus pancalus* on 6th month and lowest APC was 3.6×10^3 cfu/g) in *Channa punctatus* on 4th month whereas in experimental dried fishes the highest Aerobic Plate Count (APC) was 1.42×10^5 cfu/g in *Mastacembelus pancalus* on 1th month and the lowest APC was 5.2×10^2 in *Puntius* sp. on 4th month. From the 1st, 4th and 6th month the highest average value of Total Volatile Base Nitrogen (TVB-N) was 38.08 mg/100g in *Oxygaster gora* while lowest TVB-N value was 23.13 mg/100g in *Amblypharyngodon mola* in traditional dried fishes whereas the highest TVB-N value was 31.77 mg/100g in *Channa punctatus* and lowest TVB-N value was 17.02 mg/100g in *Chanda* sp in experimental dried fishes. The lowest and highest average values of TMA in traditional dried fishes were 6.91mg/100g and 8.70 mg/100g in *Oxygaster gora* and *Mastacembelus pancalus* respectively whereas in experimental dried fishes the highest TMA value was 10.58 mg/100g in *Channa punctatus* and lowest TMA value was 4.98mg/100g in *Puntius* sp. In experimental dried fishes, no pathogenic bacteria were present but *Escherichia coli* and *Klebsiella* sp. were found in *Cirrhinus reba* and *Oxygaster gora* respectively in traditional dried fish.

Keywords: Fish products, nutrition, TVB-N, APC, TMA, Chalan Beel

1. Introduction
Drying as a very simple and common fish preservation method had been practiced perhaps longer than any other food preservation technique. In Bangladesh dried fish is an important source of protein and is relished by many people of coastal, central and north-eastern districts, however the physical and organoleptic qualities of many traditional sun dried products are un-satisfactory Nowsad [20]. Nevertheless, according to DoF [8] Bangladesh exported 2634 mt. dried fisheries product which plays an important role in the national economy by earning TK. 29.67 in the 2014-2015 fiscal year.

The quality of dried fish as well as its shelf-life is adversely affected by the occurrence of microorganisms with the accompanying chemical changes including hydrolysis of carbohydrates, proteins and fats to simpler forms, which are ultimately utilized by bacteria and other microorganisms Fraizer and Westhoff [10]. Metabolic Activity of microorganisms also cause the spoilage of food through undesirable enzymatic changes affecting the quality of the food, which include the formation of products resulting in off-flavors and affecting the organoleptic and textural quality and ultimately decrease Shelf-life Sivasankar [26]. Therefore, determination of microbiological quality of processed fishes is very important for guarding consumer's health and hygiene Lilabati *et al*. [17]. The presence of the pathogenic bacterial loads in dried fishes is acquiring importance in view of safety and quality of the sea food Ponda [22].

Microbial spoilage can be reduced by good handling and processing practices through keeping fresh fish separate from spoiled fish, clean surfaces of landing and processing sites, clean utensils and ice, which ensure hygiene and contribute to lowering microbial load Bremner [5].

In monsoon when humidity is high complete drying is not achieved in traditional methods which facilitate to absorb moisture enhancing microbial growth, and contribute to reducing shelf-life of dried fish Azam [2]. In increasing ambient temperature favorable for microorganisms, the quality of dried fishes is considerably affected and spoilage soon sets in Clucas and Ward [6]. Consumer's demand of safe food has received considerable attention and needs to be satisfied ensuring safety and quality of dried fish following hygienic method Horner [12].

In this aspect, bio-chemical examinations of fish products ware done to evaluate the possible presence of pathogenic bacteria of public health significance and to give an impression with respect to hygiene and quality of the dried fish. Very few studies have been conducted on the quality assessment of dried fish in different region in Bangladesh such as: Azam *et al.* [3], Hassan *et al*. [11], Islam *et al.* [14], Farid *et al*.[9], Latifa *et al*. [16] and Mansur [18] have conducted on the bio-chemical quality and shelf -life of dried fishes. But no such work has been done on the bio-chemical quality and shelf-life of the specific freshwater dried fish species of Chalan beel area. Therefore, the present study was carried out to determine the qualities of eight traditional and experimental sundried species of fish which are abundantly available in Chalan beel areas of Bangladesh.

2. Materials and Methods

2.1 Collection of Traditional Sun Dried Fishes

The study was conducted for a period of 6 months (February, 2015 to July, 2015). Eight common and available indigenous fish species such as Punti (*Puntius* sp.), Chanda (*Chanda* sp.), Mola (*Amblypharyngodon mola*), Chela (*Oxygaster gora*), Raikhor (*Cirrhinus reba*), Bele (*Glossogobius giuris*), Taki (*Channa punctatus*) and Guchi (*Mastacembelus pancalus*) were collected from different fish drying points namely Ninguin under Singra Upazila of Natore, Austomonisha, under Bhangura Upazila of Pabna, Ahsanganj under Atrai upazila of Naogaon and Mohisluti under Tarash Upazila of Sirajganj districts, and these places cover the vast areas of Chalan beel. Dried fish samples were then packed tightly in separate polythene bag and brought to the laboratory for chemical and bacteriological analyses.

2.2 Preparation of Experimental Sun Dried Fishes

The same fresh eight species of fishes were collected and washed with tap water to remove the dirt. Then the fishes were gutted and viscera were removed using knives and washed again with tap water to remove blood, slime and other undesirable substances. The fishes were then treated with salt-fish ratio of 1:10 and were exposed under sun covering with nets for 3 to 4 days or until complete drying. The dried fishes were packed in an air tight polythene bag so that moisture cannot be absorbed. Finally, the dried fish products were stored in a clean, cool and dry place for six month and analyzed on 1st, 4th and 6th month.

2.3 Quantitative bacteriological analysis

Bacteriological analysis and identification were done in the laboratories of Institute of Biological Science (IBSc), University of Rajshahi, Rajshahi. Aerobic plate count (APC) was done by consecutive decimal dilution technique and finally calculated by the following formula:

$$\text{Aerobic plate count (APC) in cfu/g} = \frac{C \times D \times 10 \times V}{S}$$

2.4 Identification of Bacteria

LB (Lauryl Broth) agar media were prepared for bacterial culture which indicates the pathogenecity of bacteria. *E.coli* in the dry fish sample was enumerated using standard Most Probable Number (MPN) technique. *Klebsiella sp.* was identified by non automated established biochemical test USFDA [28].

2.5 Determination of Total Volatile-base Nitrogen (TVB-N) and Trimethyl Amine (TMA)

Total volatile Base Nitrogen (TVB-N) was determined according to the methods given in AOAC [1]. The result was calculated by the following formula:

$$\text{TVB-N (mg/100 g sample)} = \text{ml of titrant} \times 0.14 \times \frac{100}{\text{sample weight}}$$

To measure TMA the same method as for TVB-N was used but 20 ml of formaldehyde was added to the distillation flask to block the primary and secondary amines.

2.6 Data processing and analysis

For each measurement, analyses were repeated 3 times and results obtained were analyzed using SPSS version 20. The independent samples t-test was carried out to determine significant differences between traditional and experimental sun dried fish products. Trends were considered significant when the means of compared sets differed at $P < 0.05$ and statistical significance is indicated with appropriate letters on the data tables.

3. Results and Discussion

In this experiment air temperature and relative humidity were recorded. The APC, TVB-N and TMA of traditional and experimental sun dried fish were determined on 1st, 4th and 6th month in the storage period with respect to temperature and humidity respectively.

3.1 Bacteriological Quality

In the study period, the total bacteria count in colony forming unit per gram of the fish sample was counted for traditional and experimental dried fishes. Lowest temperature was recorded 28°C in the month of February and relative humidity was 78% with gradual increase in successive month and reached up to 36 °C and 86% relative humidity.

On the 1st month, when temperature and relative humidity were 28°C and 78%, the highest bacterial load was found in traditional dried fishes 1.42×10^5 cfu/g of *Chanda* sp. and lowest APC was 4.44×10^4 cfu/g in *Channa punctatus* whereas in experimental fishes the highest APC was 1.42×10^5 cfu/g *Mastacembelus pancalus* and lowest APC was 6.4×10^3 cfu/g of *Puntius* sp. (Table 1 and Fig.1).

Table 1: The highest and lowest value of APC of traditional and experimental dried fish products in different temperatures and relative humidiy

Temperature (°C)	Relative humidity (%)	Traditional dried fishes (APC) (cfu/g)		Experimental dried fishes APC (cfu/g)	
		Highest value	Lowest value	Highest value	Lowest value
28	78	1.42×10^5 (*Chanda* sp.)	4.44×10^4 (*Channa punctatus*)	1.42×10^5 (*Mastacembelus pancalus*)	6.4×10^3 (*Puntius* sp.)
36	78	5.9×10^5 (*Chanda* sp.)	3.6×10^3 (*Channa punctatus*)	9.4×10^4 (*Mastacembelus pancalus*)	5.2×10^2 (*Puntius* sp.)
33	86	7.8×10^5 (*Mastacembelus pancalus*)	2.72×10^4 (*Channa punctatus*)	8.29×10^4 (*Mastacembelus pancalus*)	3.2×10^3 (*Puntius* sp.)

On 4th month, the temperature and relative humidity were 36°C and 78% while the highest bacterial load of traditional dried fish was 5.9×10^5 cfu/g in *Chanda* sp. and the lowest APC was 3.6×10^3 cfu/g in *Channa punctatus* whereas in experimental dried fishes the highest APC was 9.4×10^4cfu/g in *Mastacembelus pancalus* and lowest APC was 5.2×10^2cfu/g in *Puntius* sp. (Table 1 and Fig.1).

On 6th month, in temperature 33°C and relative humidity 86%, the highest bacterial load of traditional dried fish was 7.8×10^5 cfu/g in *Mastacembelus pancalus* and the lowest was 2.72×10^4 cfu/g in *Channa punctatus* whereas in experimental dried fishes the highest APC was 8.29×10^4 cfu/g in *Mastacembelus pancalus* and the lowest was 3.2×10^3 cfu/g in *Puntius* sp. (Table1 and Fig. 1).The study reveals that drying methods affect bacterial load count and mean of which are significantly ($P<0.05$) different (Table 2). Number of total bacteria is one of the main factors which affect the quality and shelf-life of perishable food, particularly dried fish. According to IS 14950 [13] the limit for Total Plate Count (TPC) is 1×10^5 cfu/g in the dried products. Surendran [27] reported that at 37°C in fresh fish the acceptable limit is 5×10^5/g but for cooked or dried fish, the permissible limit is 1×10^5/g. Sanjeev [25] worked on Cochin market and found that bacterial count in dried fishes was less than 10^7g^{-1}.

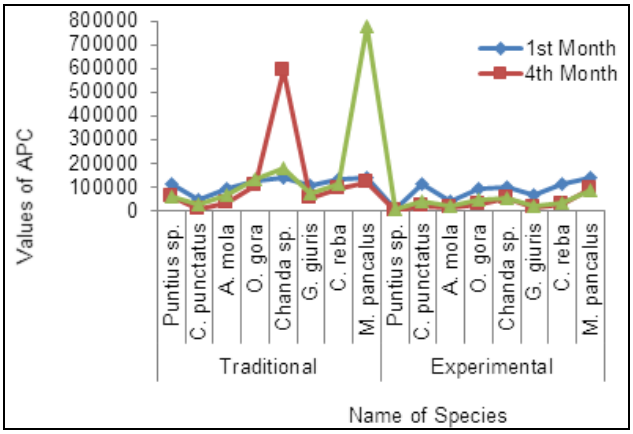

Fig 1: Bacterial load of traditionally and experimentally prepared dried fish during storage period

Table 2: APC of bacteria, TVB-N and TMA of different dried fishes prepared by traditional and experimental method

Species	Drying method	APC(cfu/g)	TVB-N(mg/100g)	TMA(mg/100g)
Puntius sp.	Traditional	$7.693 \pm 3.12\times10^{4a}$	29.21 ± 18.08^a	7.30 ± 1.18
	Experimental	$3.373 \pm 2.94\times10^{3b}$	20.43 ± 11.35^b	4.98 ± 1.27
Channa punctatus	Traditional	$2.506 \pm 2.10\times10^{4a}$	30.18 ± 21.26^a	6.92 ± 2.29
	Experimental	$5.763 \pm 0.50\times10^{4b}$	31.77 ± 7.92^b	10.58 ± 1.24
Amblypharyngodon mola	Traditional	$6.440 \pm 3.06\times10^{4a}$	23.13 ± 14.32^a	7.31 ± 0.74
	Experimental	$2.370 \pm 1.46\times10^{4b}$	18.58 ± 10.88^b	5.51 ± 1.06
Oxygaster gora	Traditional	$1.220 \pm 0.14\times10^{5a}$	38.08 ± 19.44^a	6.91 ± 1.71
	Experimental	$5.520 \pm 3.55\times10^{4b}$	18.12 ± 10.25^b	5.49 ± 1.02
Chanda sp.	Traditional	$3.030 \pm 2.49\times10^{5a}$	24.22 ± 17.58^a	7.95 ± 1.85
	Experimental	$6.850 \pm 2.70\times10^{4b}$	17.02 ± 13.09^b	5.49 ± 0.51
Glossogobius giuris	Traditional	$7.806 \pm 2.71\times10^{4a}$	28.77 ± 16.74^a	7.49 ± 0.87
	Experimental	$3.376 \pm 2.82\times10^{4b}$	19.02 ± 8.14^b	7.37 ± 1.24
Cirrhinus reba	Traditional	$1.125 \pm 0.20\times10^{5a}$	36.80 ± 27.17^a	8.66 ± 1.50
	Experimental	$5.663 \pm 4.82\times10^{4b}$	24.55 ± 15.76^b	8.85 ± 0.86
Mastacembelus pancalus	Traditional	$3.460 \pm 3.76\times10^{5a}$	32.43 ± 21.50^a	8.70 ± 1.48
	Experimental	$1.063 \pm 0.31\times10^{5b}$	18.02 ± 10.20^b	5.31 ± 0.85

Means with different superscript letters (a and b) of each species within a column are significantly different ($P<0.05$).

As far as experimentally prepared fish is concerned, all the samples had a bacterial count lesser than the standard value and appeared to be of good quality for human consumption till the end of six month storage period whereas, in respect of traditionally processed dried fish, bacterial count was found to exceed standard limit, particularly in *Chanda sp., Cirrhinus reba, Mastacembelus pancalus,* and *Oxygaster gora*. Mansur *et al.* [19] have reported the total bacterial count of some traditionally dried Small Indigenous Species (SIS) ranged from 1.0×10^5 to 1.5×10^6 cfu/g which appreciably similar with the present results. Similar results were observed by Reza [23] for Silver Jewfish, Bombay duck, big eyes Tuna, Chinese pomfret and Ribbon Fish where APC ranged between 1.1×10^5 cfu/g to 6.7×10^4 cfu/g which is almost similar with the present findings (Table 1).

3.2 Chemical quality

The present findings reveal that the highest average value of TVB-N estimated from the results of 1st, 4thand 6th months was observed in *Oxygaster gora* (38.08mg/100g) and the lowest in *Amblypharyngodon mola* (23.13 mg/100g) for traditionally dried fishes whereas in experimentally dried

fishes the highest TVB-N value was 31.77 mg/100g in *Channa punctatus* and the lowest value was 17.02 mg/100g in *Chanda* sp. (Table 2). From the results shown in (Fig. 2) it is apparent that TVB-N value in all species produced both in traditional and experimental methods increased in relation to time of storage. Traditionally dried *Cirrhinus reba* (67.35 mg/100g) and experimentally dried *Glossogobius giuris* (27.25 mg/100g) exhibited highest and lowest TVB-N level respectively on 6thmonth storage period and in case of experimentally prepared *Chanda* sp. this value was 5.90 mg/100g on 1stmonth (Fig. 2).

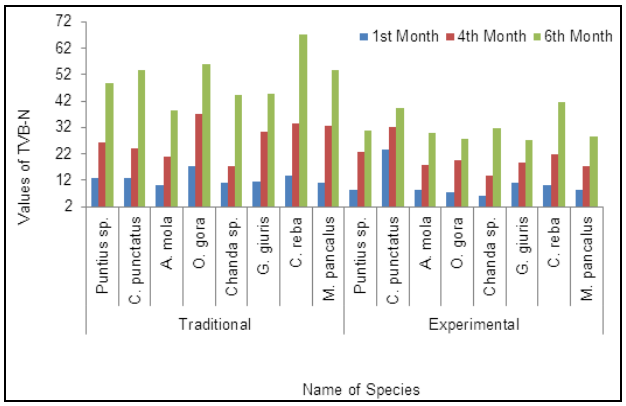

Fig 2: TVB-N of traditionally and experimentally prepared dried fish during storage period

Reza [24] observed that the TVB-N content were 3.5 to 25.2, 1.9 to 8.9, 2.5 to 15.2, 3.6 to 15.6 and 5.3 to 19 mg/100g for silver jaw fish and chinese pomfret ranged from 16.56 to 44.83 mg/100g, which is similar to the present findings. The acceptable level of TVB-N in fishes is 35-40 mg/100g Kimura and Kiamukura [15]. However, the findings of this study show that the TVB-N content obtained from traditionally dried fishes were very close to the upper limit of acceptable range and in some cases the values were found to cross the level of acceptability over time indicating the fish unsuitable for consumption. On the contrary, in terms of experimentally dried fish TVB-N values were observed to belong in the acceptable range on 6th month reflecting good quality with desired shelf-life. Average TVB-N contents for experimentally and traditionally produced dried fish samples were significantly different (P<0.05) from each other (Table 2).

In a study Yusuf [29] reported that TMA values indicate freshness of the fish and the production of TMA is dependent on the bacterial activity as well as from endogenous enzyme. The lowest average TMA value was 6.91mg/100g in *Oxygaster gora* and the highest value was observed 8.70 mg/100g in *Mastacembelus pancalus* as far as traditionally prepared dried fish is concerned whereas in case of experimental dried fishes, the lowest TMA was 4.98mg/100g in *Puntius* sp. and the highest was 10.58 mg/100g *Channa punctatus* (Table 2 and Fig. 3).

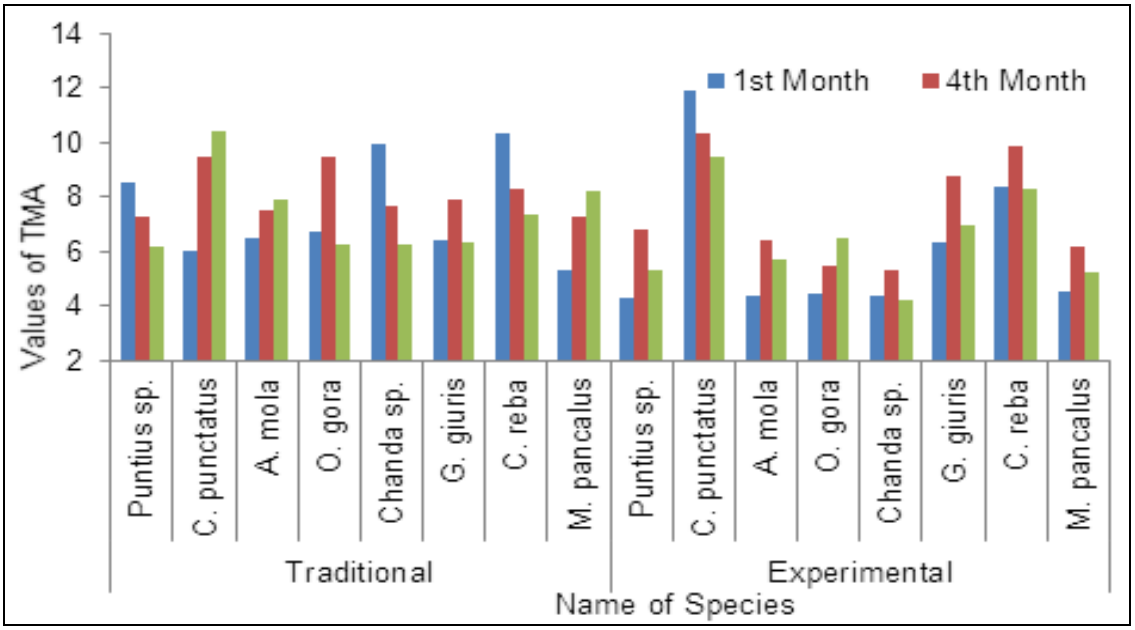

Fig 3: TMA of traditionally and experimentally prepared dried fish during storage period

Connell [7] recommended that the level of TMA value for human consumption is 10-15 mg/100g. Results indicated that TMA values of all the species were within acceptable range. Balachandran [4] suggested that TMA is a product of early stages of spoilage and may be lost during storage The TMA content resulted from dried fish was not found to fluctuate sharply with the time since it exists in little amount in freshwater fish. Average TMA contents for experimentally and traditionally produced dried fish samples were not significantly different (P>0.05) from each other (Table 2).

In the present study no pathogenic bacteria were found in all experimental dried fishes. But in traditional dried fishes two types of bacteria were found. The *Escherichia coli* was found in *Cirrihinus reba* and *Klebsiella sp.* in *Oxygaster gora* (Table 3).

Table 3: Presence of pathogenic bacteria in traditional and experimental dried fishes

Sl. No.	Local Name	Scientific Name	Presence of pathogenic bacteria	
			Traditional dried fishes	Experimental dried fishes
1	Punti	*Puntius sp.*	ND	ND
2	Chanda	*Chanda sp.*	ND	ND
3	Mola	*Amblypharyngodon mola*	ND	ND
4	Chela	*Oxygaster gora*	*Klebsiella sp.*	ND
5	Raikhor	*Cirrhinus reba*	*Escherichia coli*	ND
6	Bele	*Glossogobius giuris*	ND	ND
7	Taki	*Channa punctatus*	ND	ND
8	Guchi	*Mastacembelus pancalus*	ND	ND

* Not Detected

From the present findings, the *E.Coli* obtained from fish sample was cfu 1/10mg. The samples collected from study area showed the presence of *E.Coli* which may be attributed to the use of polluted water from nearby pond or beel for washing and to the unhygienic drying method. In the present study the *Kalebsiella sp.* obtained from fish sample was cfu 1/10mg. The *Klebsiella sp.* contamination took place in traditional dried fish sample also due to using of poor quality water and drying in unhealthy environment. Patterson [21] reported that total plate count and *E.coli* counts were higher in commercially sun dried fish in Tuticorin local market than that of experimentally sun dried fish due to high content of moisture and humidity of environment and unhygienic method of preparation in case of commercial dried fish.

4. Conclusion

The quality aspects of dried fishes produced employing traditional and experimental techniques were assessed through examining bacterial load, TVB-N and TMA with a view to making a prediction on quality and storage duration. In terms of experimentally prepared products, all chemical and bacteriological analyses exhibited satisfactory results and varied significantly compared to the traditionally processed samples. However, traditional and experimental methods showed significant distinction in all parameters with respect to shelf-life and quality. Experimentally dried samples appeared to be safer for health of the consumers due to keeping quality and ensuring hygienic practices. Therefore, it is necessary to adopt hygienic as well as scientific method in fish drying sector in order to get safe products.

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