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Microbiological & Sensory evaluation of a freshwater air breathing fish, *Clarias batrachus* (Linn) during different storage conditions

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Abstract- The total bacterial count in the muscle and skin of the fish, *Clarias batrachus* in fresh condition ("0"day) has been recorded more in summer than the winters months. During both summer and winter months, the fish stored in ice (chilled) conditions showed a gradual increase in TBC number which were recorded maximum on 20th & 25th days respectively, whereas, in superchilled conditions the TBC showed an initial decrease up to 8 days followed by an increase with maximum on 24th & 29th days in summer & winter months respectively. The sensory evaluation i.e. appearance, odour, eyes & gills gradually changed & as per torry's scale, the shelf life of the fish in chilled & superchilled conditions has been recorded to be 18th & 23rd day in summer and on 22nd & 27th day in winter months respectively.

Key words: : Microbiology, *Clarias batrachus*, TBC number, Sensory evaluation

INTRODUCTION

Microbiological activity is relatively more important in temperature range of 0° to 25°C and temperature changes have greater impact on microbial growth than on enzymatic activities.[1,2] Estimation of bacterial number in fish is frequently used to access microbial quality of the product when total viable count reaches $10^6 g^{-1}$(6.0 log cfu/gm^{-1}), the product is assumed to be at or nearing spoilage.[3] Liston (1992)[4] has stated that microflora responsible for spoilage of fresh fish changes with storage conditions. It was reported that, an initial reduction up to 3 days followed by a gradual increase till 9th day followed by a rapid decline up to 12 days of *Pangasiandon* fillets in ice chilled condition,[5] whereas, in *Labeo rohita* stored in ice, observed a gradual increase from "0" day to 21st day.[6] It was also reported in some freshwater fishes like *Labeo rohita, Catla catla, Cirrhinus mrigala* & *Clarias batrachus* and some marine fish like P*ampus argentus, Liza parsia* & *Lates calcifer* from in and around Kolkata, where it was observed TBC in the range 5.97-7.32, 6.23-7.65, 5.04-7.04, 5.39-6.87 log Cfu/g^{-1} and 5.30-6.95, 5.54-7.20 & 5.20-6.96 Cfu/g^{-1} respectively.[7]

Considering the above mentioned fact this study has been taken to determine the Total bacterial plate count (TBC) in the muscle with skin of the antero-dorsal region of Clarias batrachus sensory evaluation of the fish in fresh as well as ice & supercilled storage conditions during summer & winter seasons.

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MATERIALS & METHODS

Sufficient number of living & healthy fish, *Clarias batrachus* (Clariidae:Siluroidae) of 136.5 ± 15.6 & 144.8 ± 13.4 gm weight groups were collected from the local fish

farm during summer & winter months respectively. The fish were killed with a blow on the head & immediately placed in Tea chest boxes internally insulated by thick thermocol sheets of at least 1/2" thickness. At the same time some live fishes were carried in the laboratory in big baskets containing water of the same farm. For chilled storage condition, the fish & ice was layered one by one i.e. the first layer of ice followed by fish in layer & again ice & so on in insulated tea-cheat boxes. For supper chilled storage condition, 375gm of common salt (Tata refined) were mixed with 20 kg of ice for 10-12 kg of fish & arranged in tea-chest as described above during both summer & winter months. The ice in chilled & salt + ice in super chilled storages were changed on every 24 hrs. For total bacterial plate count, required amount of the muscle of antero-dorsal region of the fish were taken. Five observations were made & averages were recorded.

1. To determine total bacterial plate count, at first the following preparations were made:

(i) Buffer Soln.: 34gm $KH_2 PO_4$ + 100 ml distilled. water (Adjusted to pH 7.0).

(ii) Media preparation: Pepton, 30gm + Trypton, 5.0gm + Dextrose, 1.0gm, Agar, 15gm + Distl. water, 1 litre (pH adjusted to 7.0 ± 0.1)

These substances were soaked for 15 minutes in 100ml distilled water by gentle heating and then sterilized with container in Autoclave at 15 lbs pressure (121°C) for 15 minutes. Some petridishes were also sterilized. Now, 15.0 ml of medium was taken in a sterilized petridish and Agar was added to solidify. The petridishes were dried in incubator at 42°C, Keeping them inverted for 20 minutes. For the further experiments, well dried petridishes were used.

Now, 10.0 mg muscle with skin was taken in a pre-weighed sterilized petridish & blended for 2 minutes at 1000 r.p.m in a sterilized homogeniser with working sterilized buffer (1.25 ml of stock buffer made up to 100 ml with distilled water in the ratio of 1:9 (buffer). A series of dilution of sample suspension i.e. 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5},…….10^{-8}, were prepared with buffer solution and 1.0 ml of each selected dilution were poured in separate petridish containing 9.0 ml of buffer solution media in duplicate. The media was spread by rotating & tilting the petridishes and thereafter the petridishes were incubated at 37±1°C in an incubator in inverted position for 48 hrs. Colony counting was done manually. Plates were showing the counts between 30-300 were enumerated. The total plate count was expressed in colony forming unit. (Cfu/gm) of fish muscle [10,000 Cfu/gm].

TPC (Cfu/gm) = No. of colonies × Dilution/wt. of muscle sample.

2. Sensory evaluation: The sensory evaluation i.e. changes in fish, appearance, odour, eyes & gills in fresh as well as Chilled & Superchilled storage conditions were evaluated on selected days, for this, a panel of five persons were selected who carried out individually as per Torry's scale of 10-0 point scale & average were recorded. The score up to 5 are border line & below becomes spoiled or unacceptable for consumption respectively.

RESULTS

The total bacterial plate count as Cuf/gm in the muscle with skin of the fish, *Clarias batrachus* in fresh conditions (i.e. on "0"day) has been recorded to be 1.26×10^2 & 6.70×10^1 Cuf/gm during summer & winter months respectively (Table-1). During both summer & winter months, the TPC were gradually increased in chilled (ice) condition, whereas, in super chilled condition, it decreased initially followed by a gradual increase, In summer months, the TPC gradually increased from 1.26×10^2 Cuf/gm on "0" day to the maximum increase up to 4.23×10^6 Cuf/gm on 20th day or flag day, whereas, in super chilled condition, it gradually decreased up to 8 days (5.80×10^1 Cuf/gm) followed by an increase which was maximum on 24th day (2.76×10^6 Cuf/gm). Similarly, during winter months, the fresh fish ("0"day). Contained 6.70×10^1 Cuf/gm which gradually increased in Chilled storage conditions up to maximum 1.11×10^6 Cuf/gm on 25th day. In Super chilled storage condition, an initial decrease has been observed up to 8th day (4.14×10 Cuf/gm) followed by an increase which has been recorded maximum on 29th day (1.3×10^6 Cuf/gm). The initial decrease may be due to leaching & cold shock as a result, number of mesophilic bacteria decreased and the increase in TPC may be due to increase in phychrophilic bacteria while maximum increase during flag days may be related to the spoilage of fish.

The sensory evaluation on the basis of Torry's scale has been summarized on Table 2 & 3. Accordingly for the raw fish, appearance, odour, eyes & gills were taken in to account. The average of the points given by five penalist individually in 10^{-1} points hedonic scale were considered for which 10 marks were given for excellent, 9 - very good; 8 - good; 7 - slightly good; 6 - above border live, 5

Table 1- Average changes in Total bacterial plate count (in Cuf/gm) in the muscle + skin of *Clarias batrachus* at selected storage days in chilled & supper chilled conditions during summer & winter months.

Days of storage	Summer		Days of storage	Winter	
	Chilled	Supper chilled		Chilled	Super chilled
0	1.26×10^{2}	1.26×10^{2}	0	6.70×10^{1}	6.70×10^{1}
4	4.36×10^{2}	7.74×10^{1}	4	7.28×10^{1}	2.68×10^{1}
8	8.28×10^{2}	5.80×10^{1}	8	9.75×10^{1}	4.14×10^{1}
10	4.44×10^{3}	2.45×10^{2}	10	1.38×10^{2}	9.26×10^{1}
12	7.76×10^{3}	3.95×10^{2}	12	3.48×10^{2}	1.45×10^{2}
14	3.26×10^{4}	5.04×10^{2}	14	6.96×10^{2}	3.39×10^{2}
15	5.75×10^{4}	3.76×10^{3}	16	1.14×10^{3}	5.56×10^{2}
16	7.68×10^{4}	8.95×10^{3}	18	4.58×10^{3}	7.58×10^{2}
17	1.45×10^{5}	2.56×10^{4}	19	8.72×10^{3}	1.33×10^{3}
18	5.10×10^{5}	5.78×10^{4}	20	3.45×10^{4}	3.78×10^{3}
19	9.00×10^{5}	8.65×10^{4}	21	5.17×10^{4}	6.37×10^{3}
20	4.23×10^{6}	9.86×10^{4}	22	8.95×10^{4}	8.68×10^{3}
21	-	1.15×10^{5}	23	2.88×10^{5}	1.15×10^{4}
22	-	4.22×10^{5}	24	6.10×10^{5}	4.32×10^{4}
23	-	8.35×10^{5}	25	1.11×10^{6}	6.08×10^{4}
24	-	2.76×10^{6}	26	-	9.21×10^{4}
25	-	-	27	-	2.68×10^{5}
	-	-	28	-	7.75×10^{5}
	-	-	29	-	1.31×10^{6}
	-	-	30	-	-

Table 2-Sensory evaluation of raw fish, *C. batrachus* during Summer and winter months on different days of Chilled (C) and Super chilled (SC) storage conditions based on Torry's scale. (Av. of five observations).

Days of storage	Storage condition	Appearance		Odour		Eyes		Gills	
		S	W	S	W	S	W	S	W
0	C	10	10	10	10	10	10	10	10
	SC	10	10	10	10	10	10	10	10
4	C	9.5	10	9.0	9.5	8.9	9.7	9.5	10
	SC	10	10	10	10	10	10	10	10
8	C	8.5	9.2	8.2	9.0	7.8	9.3	8.4	9.5
	SC	10	10	10	10	10	10	10	10
12	C	7.3	8.8	7.0	8.5	6.8	8.6	72	8.8
	SC	9.5	10	9.2	9.5	9.5	9.5	96	10
16	C	6.4	9.9	6.2	7.7	6.0	7.8	6.5	7.8
	SC	8.7	9.0	8.5	9.0	8.2	8.7	7.7	8.8
18	C	5.5	7.0	5.0	7.0	5.4	7.2	5.2	7.0
	SC	7.0	7.7	7.6	7.5	7.8	7.5	6.8	8.0
20	C	4.0	6.3	3.8*	6.5	4.5*	6.5	4.5*	6.3
	SC	6.4	7.2	6.5	7.0	6.8	7.0	6.0	6.4
22	C	-	5.7	-	5.9	-	5.5	-	5.5
	SC	5.2	6.5	5.4	6.5	5.0	6.9	5.0	6.7
23	C	-	5.2	-	5.4	-	5.4	-	5.0
	SC	4.5*	6.2	5.0	6.4	4.2*	6.5	3.9*	6.5
24	C	-	3.8*	-	4.5*	-	3.8*	-	4.5*
	SC	-	5.9	-	6.2	-	6.2	-	6.0
25	C	-	-	-	-	-	-	-	-
	SC	-	5.7	-	6.0	-	6.0	-	5.7
26	C	-	-	-	-	-	-	-	-
	SC	-	5.4	-	5.8	-	5.8	-	5.5
27	C	-	-	-	-	-	-	-	-
	SC	-	5.0	-	5.5	-	5.5	-	5.0
28	C	-	-	-	-	-	-	-	-
	SC	-	4.5*	-	4.2*	-	4.5*	-	4.0*
29	C	-	-	-	-	-	-	-	-
	SC	-	-	-	-	-	-	-	-

S = Summer; W = Winter; C = Chilled; SC = Super chilled; '*' = Spoiled

Table 3-Sensory evaluation of fresh *Clarias batrachus* during different days of selected storage conditions during summer & winter (Combined together)

Days of storage	Raw fish (Appearance, odour, eyes & gills)
'0' day	Pre regor state, eyes, convex; good natural smell, bright red gills, slime on the surface & shining body colouration.
1 to 12 days	Followed by later stages of rigor, eyes – convex but slightly opaque, gills – red with some muscus, natural odour, Later on, eyes became shrunken gills show slightto moderate discolouration & dull in body colouration depended on storage conditions.
13 to 20 days	Eyes become shrunken, almost no muscus on body surface, gills show discolouration. Further, eyes are surrounded by a reddish line (circle) gills became almost brown to dark/dirty white colouration, muscle becomes soft & dull in appearance.
21 to 29 days or during flag days indifferent storage conditions	Depended on storage conditions eyes become opaque, shrunken, surround by red colouration; gills are almost dirty white or brown to dark in colour, muscles are soft & pasty and odour gradually decayed.

Note: All the above mentioned characters are depended on chilled & super chilled storage conditions both during summer & winter months. The changes were first observed in chilled & then super chilled conditions in both summer and winter season.

- border line and below 5 - as unacceptable for human consumption or spoiled. Accordingly, the shelf life of the fish were recorded in different storage conditions has been recorded to be 18th & 23th day in chilled and 22nd & 27th day in super chilled conditions during summer & winter months respectively.

DISCUSSION

There is a limited studies on the microbiological aspect in fresh water fishes. Licciardello *et al.* (1967)[8] have reported that range of spoilage is dependent on the number & type of bacteria. Garg & Stephen (1982)[9] in Kati fish stored in ice, observed a fall in bacterial count during initial stage, which might be due to washing out of surface slime & a gradual rise after 5 days might be due to proliferation of psychrophilic bacterial flora. Joseph *et al.* (1988)[10] in Labeo rohita stored in ice, observed an increase in total bacterial count with the day of storage at room temperature. According to ICMSF (1986)[3], the maximum recommended bacterial counts of marginally acceptable quality fish is $10^7 g^{-1}$. Bhattacharyya & Chaudhuri (1990)[11] in *Clarias batrachus* stored at 37°C, 22°C & ice storages have reported a gradual increase from 9.8×10^1 gm on "0" hrs. to 1.90×10^5, 1.62×10^5 at 6 & 8.5 hrs. at 37°C & 22°C and from 2.26×10^1 /gm on "0" day to 8.31×104 gm on 18th day of iced condition. Reddy *et al.* (1997)[12] has stated that the increase in total plate count was due to utilization of non protein nitrogen (NPN) matters during ice storage. Bao *et al.* (2007)[13] found faster microbial growth in chilled than super chilled in Arctic char under effect of dry ice. Faruk (2018)[6] in *Labeo rohita* observed increased total plate count during low temperature during 0 to 21 days.

In the present study, the total bacterial plate count in the muscle with skin of fresh fish on "0" day during summer & winter were recorded to be 1.26×10^2 Cuf/gm (Log 2.10) and 6.70×10^1 Cuf/gm (Log 1.83) respectively. It was comparatively more during summer than winter season. In chilled condition it gradually increased while super chilled conditions, it decreased initially for few days followed by a gradual increase both during summer winter months. In summer season maximum increase in chilled condition were recorded to be 4.23×10^6 Cuf/gm on 20th day and in super chilled condition it was recorded to be 2.76×10^6 Cuf/gm on 24th day, whereas, during winter months maximum increase up to 1.11×10^6 Cuf/gm on 23rd day in chilled and on 29th day 1.31×10^6 (Cuf/gm) in super chilled conditions. The initial decrease in super chilled conditions may be due to leaching & cold shock as a result, number of mesophilic bacteria decreased and the increase in TBC may be due to increase in psychrophic bacteria.

The maximum increase during flag days may be related to the spoilage of fish.

Garg & Stephen (1982)[9] observed that the sensory evaluation of kati stored in ice showed shelf life of 9 days without appreciable loss of overall quality. Joseph *et al.* (1986)[10] & Kumar (2002)[14] stored in ice stored *Labeo rohita* observed change in appearance, odour, eyes, gills & body muscle which showed gave an indication of the extent of changes of fish condition as gradually the odour decreased & gradually spoiled and weedy odour took and the colour of the gills place, gills colors changed from bright red to pink, bleached, brown & dark coloured. The body muscles gradually became soft.

In the present study, *Clarias batrachus* stored in ice & super chilled conditions during summer & winter months showed the following changes in appearance, odour, eyes, gills & body colouration depended on days storage condition. These are based on Torry's scale. Accordingly, depended on storage conditions both during summer & winter showed the changes, which has been summarized in Table - 2 & 3. Accordingly, the shelf life of the fish during summer and winter months has been recorded to be 18th & 23rd day in chilled; 22nd & 27th day in super chilled conditions respectively.

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