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Influence of different storage conditions on *Clarias batrachus* (Linnaeus, 1758) with special reference to Total volatile base nitrogen (TVBN)

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Abstract

Fish are one of the highly demanded foods due to their nutritional and medicinal benefits. But, their susceptibility to get spoiled quickly after harvesting and killing makes them highly perishable in nature. Many preservation and storage techniques, being traditional as well as advanced, are being adopted to increase the shelf life of the fish and fish products. However, the efficiency of the storage techniques varies depending upon the method adopted, the type of fish being stored, environmental condition and the duration of storage. In the present study, an effort has been made to investigate the effects of 4 different storage conditions on the quality of stored fish. The extent of damage was measured in terms of level of Total volatile base nitrogen (TVBN) in muscle of fish. Experimental fish, *Clarias batrachus* were procured from local market in Chapra, bihar. Fresh brought fish were disinfected and washed thoroughly under tap water and their muscle was assessed for level of TVBN and the data obtained was referred to as day "0" of storage. Further, the fish were divided into 4 groups; Group 1: whole fresh fish stored in chilled condition, Group II: whole fresh fish stored in super- chilled condition, Group III: Potassium sorbate treated fish stored in chilled condition and Group IV: Potassium sorbate treated fish stored in super- chilled condition. Muscle content of TVBN was measured at day 4, 8, 12, 16 and 20 days of storage. An increasing trend in TVBN level was observed in all the group on successive days of storage, both in summer as well as in winter season. However, the damage was lesser in winter. Fish stored in chemically treated and super- chilled condition showed lesser increase in the level of TVBN, advocating for its comparatively greater efficacy in increasing the shelf life and maintaining the good quality of stored fish.

Key words : *Clarias*, Potassium sorbate, TVBN, Super- chilled, perishable**,** shelf- life.

List of abbreviation :

$C_6H_7KO_2$: Potassium sorbate

DMA: Dimethylamine

HCl: Hydrochloric acid

HDL: High- density lipoprotein

$KMnO_4$: Potassium permanganate

NaOH: Sodium hydroxide

NH_3: Ammonia

PUFA: Poly unsaturated fatty acid

TCA: Trichloroacetic acid

TMA: Trimethylamine

TVBN: Total volatile base nitrogen

Fish is known to have commercial as well as medicinal values due to presence of various essential amino acids, fats, mineral, vitamins and other nitrogenous compounds. They have been reported to prevent cardio-vascular and other deadly diseases. They are good sources of antimicrobial peptides which provide protection against dreadful pathogens. Fish are economically important commodity providing employment and foreign exchange [2]. However, they are highly perishable in nature and tend to spoil really fast. Spoilage can lead to deterioration of texture, color, appearance, aroma and flavour. Spoiled fish not only lack nutritional benefit but also can risk health of the consumer[1]. Many storage and preservative techniques are being adopted to increase the shelf life of fish and maintain its good quality for a longer duration, namely, chilling, super chilling, freezing, non- thermal control, high pressure treatments, irradiation, packaging technologies, etc.[10,12,18]. Ice provides one such storage condition by maintaining the temperature of fish close to chilling temperature[1]. The quality of fish during different storage conditions can be determined by assessment of proximate composition, nutritional profiles, sensory/ organoleptic characteristics and microbial load[16]. Post mortem enzymatic autolysis of muscles, oxidative reactions and microbial growth are some of the important factors responsible for deterioration of the stored fish[12].

Degradation of protein and non-protein nitrogen components following activity of fish spoilage bacteria and endogenous enzymes can lead to accumulation of ammonia (NH_3), Dimethylamine (DMA) and trimethylamine (TMA), collectively known as, total volatile base nitrogen (TVBN)[3]. TMA is primarily produced by bacteriological actions and decomposition of TMA- N oxides, whereas DMA is derived from action of autolytic enzymes. Deamination of amino acids give rise to ammonia[18]. TVBN is considered as an important indicator to measure freshness of meat and has been used as a parameter to measure quality of seafood[19]. Fish are considered as spoiled when the value of TVBN exceeds 0.30 g/kg (= 30 mg/100g)[8]. These volatile amines are toxic and responsible for characteristic fishy odor and flavour[14]. The content of TVBN increases with increasing time of storage and is accompanied by other biomarkers of spoilage of meat, namely, changes in sensory acceptability and microbial load[4].

Clarias batrachus, commonly known as Walking catfish, are native to Southeast Asia and are integral part of diet as well as

traditional medicine for local people of Bihar[11]. Nutritional profiling of the fish advocated its richness in good cholesterol (HDL), polyunsaturated fatty acids (PUFA), essential amino acids and minerals such as, Zinc, Calcium, Iron and many other nutrients[6]. In this experiment, an attempt has been made to investigate the efficacy of different storage conditions in maintenance of good quality of *C. batrachus* for the given duration by assessing the level of TVBN in muscle of the fish.

Collection of experimental animals :

Healthy individuals of *Clarias batrachus* (Siluriformes) weighing about 130-150 g were purchased from local fish farmers in Chapra, Bihar. For summer studies, fish were collected in the months of April to June, whereas for winter studies, fish were collected in the months of December to February. The fish were disinfected using $KMnO_4$ solution and sacrificed with a blow on the head and immediately transferred to tea- chest boxes internally insulated by thermocol sheets.

Storage conditions :

4 different storage conditions were used. Fish were stored as fresh whole fish without any preservative and with a preservative. Temperature dependent storage conditions included chilled and super- chilled conditions. For chilled condition, the fish were arranged in layers alternating with ice. To maintain a super- chilled condition 375 g of salt were mixed with 20 Kg of ice for 12 Kg of fish and fish were stacked in layers alternating with ice. For chemically treated fish, the ice was prepared using water containing 5 % Potassium Sorbate ($C_6H_7KO_2$) solution.

Grouping of the animal :

The fish were randomly divided into 4 groups based on the storage conditions employed. Grouping of animals were done as per previously published report[15].

Group I Whole fresh fish Chilled condition	**Group II** Whole fresh fish Super-chilled condition
Group III Chemically treated fish Chilled condition	**Group IV** Chemically treated fishSuper- chilled condition

Determination of total volatile base nitrogen (TVBN) :

Muscle of fish from each group were analysed for level of total volatile base nitrogen on 4th, 8th, 12th, 16th and 20th day of storage. TVBN was estimated in freshly brought fish prior to exposure to different storage conditions and was considered as control, represented as day "0" of exposure. For estimation of TVBN, 10 g of muscle was homogenized using 30 ml of 5 % TCA and filtered. 5 ml of clear filtrate was added with 5 ml of 2 N Sodium hydroxide and distilled with 15 ml of 0.1 N HCl and 0.1 ml of Rosolie indicator. Further, excess of acid in the receiving flask was titrated using 0.01 NaOH solution until the appearance of pink end point. A blank was used for comparison. Amount of TVBN was calculated using following formula,

TVBN (mg/100g) = N*(A-B)*14*(30-w)/5

Where, N: Normality of NaOH Standard solution

W: Water content in the muscle

A: Volume of NaOH used for blank titration
B: Volume of NaOH used for muscle sample titration

The experiment was conducted in triplicate and data obtained were expressed as Mean ± Standard error. Data were statistically analysed using Two- way ANOVA and differences were considered significant if $p<0.05$ or $p<0.01$.

Effects of different storage conditions on muscle content of total volatile base nitrogen have been studied in summer (Table-1) as well as winter (Table-2) season. The study has been done on different days of storage and efficacy of storage conditions in prevention of spoilage has been expressed in terms of per cent change from day "0" to day "20" of the storage. Muscle content of TVBN in control group fish was comparatively higher in summer season than that in winter. The level of TVBN gradually increased with increasing days of storage in summer as well in winter season. The same trend of elevation has been observed in all the four groups of fish kept under different storage conditions. However, the extent of elevation varied among groups. While comparing the seasonal variability, the per cent increase in muscle content of TVBN was greater in summer season, showing susceptibility of early spoilage. The fish stored as fresh whole condition (Group I & II) showed significantly greater percentage of increase in TVBN, when compared with chemical treated fish (Group III & IV) (Fig. 1 & 2). On the other hand, fish stored in super- chilled condition showed lesser content of TVBN in muscle than that in chilled condition. The extent of day dependent increase in the level of TVBN can be observed in following order, Group I> II>III>IV. During winter season, there was not much variability in the fish of group II, III & IV (Fig. 2). In most of the groups, TVBN values are below the maximum value of 30 mg/100 g flesh.

Table-1. Muscle content of TVBN on successive days of storage under different storage conditions in summer season

Days of storage	Total Volatile Base Nitrogen (mg/100g) in muscle during summer season			
	Groups			
	I	II	III	IV
0	18.10 ± 1.11	18.10 ± 1.11	18.10 ± 1.11	18.10 ± 1.11
4	19.20 ± 1.20	18.78 ± 1.13	19.15 ± 1.05	18.44 ± 1.10
8	21.32 ± 1.16	19.38 ± 1.17	19.50 ± 1.10	19.75 ± 1.13
12	24.00 ± 1.14*	22.56 ± 1.18	21.27 ± 1.22	21.05 ± 1.15
16	30.74 ± 1.22**	24.28 ± 1.16*	23.94 ± 1.16*	23.50 ± 1.11*
20	36.75 ± 1.21**	30.45 ± 1.16**	27.40 ± 1.18**	27.10 ± 1.18**
% change on day "20" from day "0"	(+) 103.04 %	(+) 68.23 %	(+) 51.38 %	(+) 49.72 %

Table-2. Muscle content of TVBN on successive days of storage under different storage conditions in winter season

Days of storage	Total Volatile Base Nitrogen (mg/100g) in muscle during winter season			
	Groups			
	I	II	III	IV
0	13.96 ± 0.91	13.96 ± 0.91	13.96 ± 0.91	13.96 ± 0.91
4	14.28 ± 0.95	14.40 ± 0.96	14.52 ± 1.02	14.30 ± 1.00
8	14.76 ± 0.88	15.10 ± 0.91	15.08 ± 0.98	14.85 ± 0.96
12	16.70 ± 1.02	16.00 ± 1.00	16.10 ± 1.00	16.02 ± 1.04
16	18.25 ± 0.92*	17.40 ± 0.98	17.10 ± 1.02	17.00 ± 0.90
20	20.56 ± 1.04**	18.65 ± 1.04*	18.54 ± 1.04*	18.48 ± 0.95*
% change on day "20" from day "0"	(+) 47.28 %	(+) 33.60 %	(+) 32.81 %	(+) 32.38 %

Values were represented as mean ± SE. *p<0.05, **p<0.01. (+) represented an increase from the initial value.

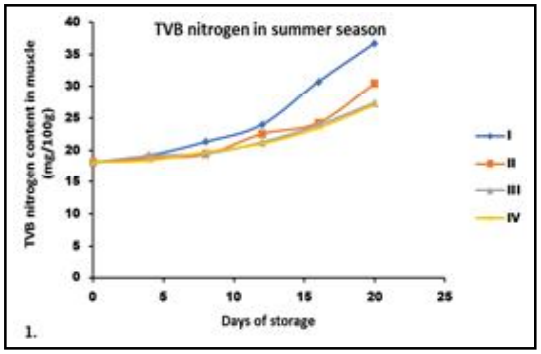

Fig. 1. Time- dependent fluctuations in TVBN level in the muscle of *Clarias batrachus* kept under different storage conditions in summer season.

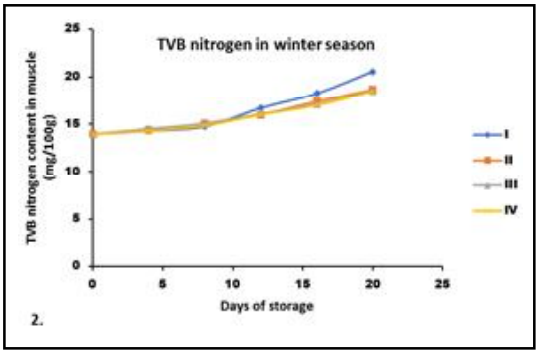

Fig. 2. Time- dependent fluctuations in TVBN level in the muscle of *Clarias batrachus* kept under different storage conditions in winter season.

The rate of spoilage depends upon temperature and chemical environment of the storage condition. Greater amount of TVBN in fish stored in summer season (27.4 to 36.75 mg/100g) advocated for comparatively faster spoilage (Table-1). Lesser quantity of TVBN in fish stored in chemical treated and super-chilled condition showed inhibition of fish spoilage for longer duration (Table-1 & 2). Findings of the present study were supported by the argument that the concentration of TVBN increased during storage at different

temperatures, however the rate of increase slowed with lowering temperature. Increase in TVBN content can be attributed to degradation of protein and non- protein compounds, especially due to microbial activity[19]. In agreement with present findings, a rapid increase was observed in the level of TVBN in pond- raised freshwater fish, Silver perch, stored at 5 ^{o}C, with and with treatment with a preservative, Potassium Sorbate. Whereas, no significant change was observed in Silver perch stored at 0 to 2 ^{o}C temperature[8]. Similar to present findings, a significant increase in the level of TVBN was observed in smoked *hetroclarias* from day "0" to day "28. This increase might be attributed to production of volatile amines due to autolysis of muscle tissues[2]. A significant increase in TVBN content in rainbow trout fish fillets during storage period was reported to be a result of destructive activities of micro- organisms[13]. Similarly, fish meal stored at 80 % relative humidity showed an increasing trend in level of volatile base nitrogen. High relative humidity resulted in proliferation of mold which further accelerated the decomposition of nutrients, especially nitrogenous substances[9]. A similar trend of increase in the level of TVBN in fish stored in different conditions was reported by many researchers[7,17]. Apart from effects on freshness/quality of the food, biogenic amines can be of health concern for consumers also. The type and level of biogenic amines can be linked to cardio- vascular diseases, diabetes, cancer, dietary related illnesses and renal complications[5].

Present study confirmed the idea that level of TVBN can be used as a marker to asses the freshness of the fish under different storage conditions. Increased level of TVBN showed higher degree of spoilage. Further, the efficiency of different storage conditions in inhibition of fish spoilage in terms of lowered level of TVBN can be arranged as, fish stored in chemically treated super- chilled condition> chemically treated chilled condition> whole fresh fish in super- chilled condition> whole fresh fish in chilled condition. Overall, super-chilled condition and use of preservative were more efficient in extending the shelf- life of stored fish. Fish were more susceptible to degradation in summer season than winter. The knowledge of efficiency of different storage conditions, which are easily accessible and cost- friendly, in maintenance of good quality of stored meat, especially highly perishable ones, can be beneficial for the local farmers and suppliers.

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