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Microbiological, Biochemical, Sensory and Quality characteristics of Rohu (Labeo rohita) Marketed at Battala Fish market (Agartala, Tripura) and Highlight on the Hygiene Status of the Market

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Abstract

In the present study the quality of rohu (*Labeo rohita*) i.e., brought from other states (ROS) and locally produced in Tripura (RTR) and marketed at Battala (Agartala) fish market was examined in terms of microbiological, biochemical and sensory quality. In addition the microbiological quality of washing water, table tops and ice used in processing of fish in the market was also studied. Higher counts of TPC and pathogenic bacteria were found in all the samples of fish and processing assets. Biochemical and sensory analysis result reveals superior quality of RTR than ROS.

Keywords: Rohu (*Labeo rohita*), icing, microbiological quality, biochemical quality, sensory quality

Contents

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1. Introduction

Tripura is the second smallest hilly and landlocked state of the north-eastern region of India. The per capita consumption of fish in Tripura is 13 kg which is highest among all the states of India whereas national average is calculated to be 9 kg per year per person. The total fish production in the state is 32830mt whereas total demand for fish is estimated to be 43280mt, the deficiency is 10450mt (Barman and Mandal, 2008). Due to this gap between demand and supply of fish large quantity of fish is brought from other states of India to Tripura mainly from Andhra Pradesh, West Bengal, Bihar, Madhya Pradesh and Bangladesh.

A preliminary survey revealed that the total quantity of fish brought daily from other states of India to Tripura is about 1200-1500kg out of which more than 50% is rohu. The transportation of fish from other states of India to reach Tripura takes 7 – 11 days. After reaching Tripura the fish are unloaded first at Battala Fish market (major fish market situated in Agartala, the capital of Tripura) from where fish are distributed to the various retail markets of Tripura. Apart from this rohu *(L. rohita)* which are locally cultured and captured in the various region of the state

are also brought in un-iced condition to the Battala fish retail market which generally got sold within a day because of its high demand. There are various factors that determine the sensory, biochemical and microbiological quality of the fish during transportation and distribution. Fish quality deteriorates rapidly during storage and transportation. Moreover, many of the local fish handlers are unaware of the basic rules needed to safeguard quality and safety of the sea-foods. Hence unsanitary practices are common in market place (Vieira and Vieira, 1989).

It is reported that most iced fish in the major marketing centers' of India are of substandard quality (Nair et al., 1974; Govindan, 1985). The present study was undertaken to analyze the microbiological, biochemical and sensory quality of iced rohu (*Labeo rohita*) coming from other states to Tripura (ROS) comparing it with locally produced rohu (RTR). The microbiological quality of washing water, table tops and ice was also examined to have an idea about the hygienic status of the market. The results are expected to give more insight into the condition and duration of transportation which suits human consumption (Jeyasekaran and Ayyappan, 2003).

2. Materials and Method

Collection of Labeo rohita samples: Three to four samples of iced rohu (*L. rohita*) weighing about 1-1.5 kg were collected from different ice boxes randomly from Battala (Agartala) wholesale fish market where fish coming from other states are first unloaded. Similarly, 3-4 samples of locally produced rohu were also collected. Sampling was done fortnightly for six months, from 15th September to 2nd March 2010 - 11. The sample was collected in aseptic condition in sterilized plastic bags. The sample was carried to the College laboratory in ice boxes in properly insulated condition.

Sampling method: After reaching to laboratory the sensory analysis of the fish was carried out and samples for microbiological and biochemical analysis were taken from the different parts of the fish in aseptic condition. *Microbiological assessment:* Microbiological analysis carried out in this study included total plate count (TPC), Psychrophilic bacteria count, *Staphylococcus* sp. count, *Escherichia coli* count, *Salmonella* sp. count and *Vibrio* sp. count. Media used for microbiological analysis were obtained from Hi-Media, Mumbai, India. Total plate count (TPC) from muscle, gill, intestine and skin of the fish and Psychrophilic bacteria count were done as per APHA (2001). *Staphylococcus* and *E. coli* count was done by following the method given by Varma (2002). *Salmonella* and Vibrio count was done by following the method described by Manik (1992).

Biochemical assessment: Total moisture, ash, protein, fat and free fatty acid (FFA) content was determined by the standard method given by AOAC (1995). pH was measured using a digital pH meter AOAC (1995). TVBN concentration was estimated by using the method given by Conway (1947). PV was estimated by method given by Jacob (1958).

Sensory analysis: Sensory analysis was done by following the score sheet given by Shewan et al. (1953). Sensory studies were carried out by expert panel of 10 judges. *Statistical analysis:* Statistical analysis of data was performed using SPSS-16.0 (SPSS Inc., Chicago, IL, USA). Graphs were made in Micro-Soft Excel 2003 and 2007. Results were presented as mean ± standard error. Comparisons of mean values were determined by one way ANOVA and independent t-test. A probability level of 0.05 was used to find out the significance in all cases.

3. Results and Discussion

Microbial assessment

Total plate count: TPC of muscle, intestine and skin of ROS and RTR were not significantly different ($p>0.5$) (Fig.1). According to ICMSF (1986) the recommended limit of TPC in muscle for good quality fish is 5×10^5 CFU g $^{-1}$ and counts at or above 10^7 CFU g^{-1} is regarded as unacceptable quality. The normal range of TPC in the intestine of fresh water fish had been reported as to be 10^3-10^9 by Shewan (1962). So the intestinal bacterial count in both the fishes did not show a sign of major spoilage (Fig. 1). TPC of skin for both the fishes were found to be within the normal range i.e., 10^2-10^7 (Shewan, 1962). TPC of gill was found significantly higher ($p<0.05$) in ROS in comparison to RTR (Fig 1). The count was within the normal range which is, i.e., 10^3-10^9 according to Shewan, (1962). The gill is an ideal site for microbial growth (Russel and Fuller, 1979). Shewan and Murray (1979) reported that in case of chilled fish only few bacteria invade the muscle at the late stage of storage. Thus, as far as TPC is concerned both ROS and RTR were acceptable. This study supports the findings of Nair et al. (1971) in *Cirrhinus mrigala*; Lilabati and Vishwanath (1999) in *Labeo gonius*.

Psychrophilic bacteria count: The count of psychrophilic bacteria were significantly higher ($p<0.05$) in ROS than in RTR (Fig 2). Suhendan et al. (2007) studied psychrophilic bacteria in fish and concluded that the estimation of the psychrophilic microorganisms could be a better tool for shelf-life estimation of chilled fish than that of

mesophilic bacteria. The higher count of psychrophilic bacteria in ROS in comparison to RTR in the present study supports the finding of Cakli et al. (2007) in sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). This may be due to the prolonged storage of ROS in ice where psychrophilic bacteria proliferated slowly and dominated the mesophilic bacteria, as low temperature favoured their growth (Lakshmanan et al. 2002).

Staphylococcus spp. *count:* The *Staphylococcus* spp. Count was not significantly different ($p>0.05$) in ROS and RTR (Fig. 2). However the count was significantly different within the samples. The natural habitat for *Staphylococcus* spp. being the skin and mucus membranes of animals and men, it does not multiply in fish and its presence in fish indicates post harvest contamination (Huss, 1994). Presence of *Staphylococcus* spp. in high numbers indicates unhygienic sanitary condition of the market. The incidence of *Staphylococcus* sp. in the fishery products within limits is not a serious problem. However, careless handling during processing results in the multiplication of the organism, which may lead to food poisoning (Lilabati and Vishwanath, 1999). High counts of *Staphylococcus* spp. have also been recorded by Ali et al. (1992) in *Labeo rohita*; Jeyasekaran and Ayyappan (2003) in *L. rohita* stored at two different temperature; Kumari et al. (2001) in *L. rohita* available at Patna market.

Escherichia coli count: The *E. coli* count in all the samples were significantly different ($P<0.05$) in ROS and RTR. ICMFS (1974) recommend the limit of *E. coli* >100 g^{-1} of fish. As the natural habited of *E. coli* is the gastrointestinal tract of warm blooded animal it indicates the post harvest contamination of fish particularly from the faecal matter. The variations of *E. coli* in ROS and RTR may be due to higher storage time in ROS than in RTR and unhygienic handling. It is worth to note that RTR is normally transported live in clean and aerated water and handled with care for competitive higher price; contrastingly iced ROS is transported in repeatedly used thermocole (polystyrene) boxes. This observation resembles with the findings of Sudhi (2002) in fish of Kochi market (Kerala); Nair and Nair (1988) in *Labeo rohita* and *L. calbasu*. The presence of coliforms, especially *E. coli*, is an indication of faecal pollution and is considered as an indication of the presence of other pathogenic bacteria. *E. coli* counts more than 100 per gram, can be considered as heavily contaminated with faecal matter. The poor quality of ice used adds to the contamination (Sudhi, 2002).

Salmonella sp. Count: Salmonella count was objectionably higher in both ROS and RTR. The count was significantly higher ($p<0.05$) in ROS than RTR (Fig 2). According to ICMSF (1974) *Salmonella* should completely absent in fish and fishery products. The probable sources of *Salmonella* in the present case are the filthy floors of the market, the clogged water in uneven surfaces of the concrete table tops, the fish handlers with poor personal hygiene and untreated water used for washing in both the cases. The higher count of *Salmonella* in ROS than RTR seems to be due to unhygienic handling, improper method of icing, exposure to outer environment such as soil, filthy water and surfaces etc. *Salmonella* and other bacteria may contaminate seafood during processing, and may cross-contaminate products during various stages of preparation (Amagliani et al., 2011). The result of this finding can be compared with the results of Sinha et al. (1991) in marketed rohu (*L. rohita*); Jeyasekaran and Ayyappan (2003) in rohu (*L. rohita*).

Vibrio count: Vibrio was detected in two of the samples in low numbers in ROS and was not detected in any of the samples of RTR. Rohu, being a freshwater fish, might not harbor *Vibrio* spp. *Vibrio* was in undetectable level in water, table-tops and ice samples. The probable reason of this may be due to *Vibrios*, are predominant bacteria of estuarine water (Pand and Nayak, 2001).

Bacterial counts of ice, water and fish storage surfaces: The TPC, *Staphylococcus* count and *E. coli* count were high for the table tops, water and ice. (Fig 3). Hence, it may be concluded that one of the reasons of recorded high bacteria count in fish is due to the cross contamination from table tops, water and ice. The results agree with the findings of Suvaraj et al. (1984) that the market premises and market floor and that water could be major sources of contamination of fish in the fish markets in Mangalore, India.

Biochemical analysis: The proximate composition of ROS and RTR shows that total moisture, ash, protein and fat content is significantly different ($P<0.05$) from each other. Moisture content was found higher in ROS whereas ash, crude protein and fat content found to be higher in RTR (Fig 4). pH and TVBN content was significantly higher ($P<0.05$) in ROS than RTR. On the other hand the PV and FFA value of ROS and RTR did not show significant difference ($p>0.05$) with each other and most of the samples were within the acceptable limit (Fig 4). Ice storage results in the uptake of water by fish (Joseph et al. 1988), this may the reason of significantly higher moisture content in ROS even in the same species of fish. In the present study the ash content in RTR was found significantly higher ($p<0.05$), compared to ash content of ROS. This may be due to leaching of minerals along with melt water during storage in ice (Solanki and Venkataraman 1978). The protein content in the present study was

significantly higher ($p<0.05$) in RTR compared to ROS. The lower values of protein content might be due to dripping or leaching out of water soluble protein fraction from muscle along with melt water (Solanki and Venkataraman 1978). The lipid content of RTR was found significantly higher ($p<0.05$) than ROS, probable reason of this finding may be due to the lipid content of fish is closely related to the feed intake, the variation in the percentage of fat is reflected in the percentage of water since fat and water normally constitute 80% of the fillet Huss (1985). The accumulation of alkaline compounds, such as ammonia mainly derived from microbial action during fish muscle spoilage results in increase of pH value (Ozyurt et al. 2009).

The higher value of pH in ROS may be due to prolonged storage. Total volatile basic nitrogen (TVBN) is mainly composed of ammonia and primary, secondary and tertiary amines (Beatty 1938), resulted from degradation of proteins and non-protein nitrogenous compounds, which is chiefly caused by microbial activity (Ruiz-Capillas and Moral 2005). The level of 30-35 mg TVBN $100g^{-1}$ of fish muscle are generally regarded as the limit of acceptability (Lakshmanan 2005). In the present study TVBN concentration was significantly higher ($p<0.05$) in ROS than RTR. However, TVBN concentration was within the acceptable limit in both ROS and RTR. This may be due to prolonged storage time of ROS in ice, as reported earlier by Civera et al. (1995) in three Sparidae sp; Grigorakis et al.(2003) in sea bream (Sparus aurata); Ababouch et al. (1996) in Sardines; Karungi et al. (2004) in Nile perch. In fish the ash content varies between 0.4 to 2% (Balachandran 2001).

Primary lipid oxidation was evaluated by means of the PV. The highly labile lipids in fish are susceptible to oxidation and form peroxides (Huss 1985). Connell (1975) has suggested that if the PV is above 10-20 mg %, then the fish will probably smell and taste rancid. The results in the present study show higher level of PV both in ROS and RTR. The PV values in ROS and RTR were not significantly different ($p>0.05$) from each other. The reason behind this may be due to the PV value is comparatively low in samples stored in ice than those kept in ambient temperature (Razaei et al. 2008). Hence even though prolonged storage of ROS the PV value did not showed significant increase than RTR which was generally stored for shorter duration after catch in ambient temperature. The increase in FFA with storage time in all cases is related to hydrolysis of fats (Stansby and Lemon 1941). In the present study higher value of FFA both in ROS and RTR was recorded. The FFA value of ROS and RTR were not significantly differ ($p>0.05$) from each other. It seems therefore lipid hydrolysis is more rapid at the high ambient temperature than during storage in ice (Karungi et al. 2004).

Sensory analysis: Sensory scores obtained from the different parameters i.e.; General appearance, Odour, Texture and Flesh (including belly flap) of RTR were significantly higher ($p<0.05$) then the scores obtained by ROS (Fig 5). The scores of different sensory parameters obtained by RTR were in the range of 7.09 to 7.26 whereas in case of ROS the range was 6.53 to 6.95 out of total score of 10 for each parameter. Though scores obtained by both ROS and RTR were within the acceptable limit, the lower scores of ROS than RTR illustrate that sensory quality decreases as storage time increases but icing definitely lowers the spoilage period (*Chytiri et al.* 2004).

Table 1. T-test of different bacterial counts in *L. rohita* from Other States (ROS) and Tripura (RTR)

Parameters	State		t-Value	P-Value
	ROS	RTR		
TPC-Muscle	$2.2\times10^6\pm0.32\times10^6$	$(1.7\pm0.35)\times10^6$	0.878	>0.05
TPC-Intestine	$(2.0\pm0.26)\times10^7$	$(1.6\pm0.36)\times10^7$	0.846	>0.05
TPC-Gill	$(2.8\pm0.4)\times10^7$	$(1.2\pm0.16)\times10^7$	3.623	< 0.05
TPC-Skin	$(2.5\pm0.36)\times10^6$	$(1.6\pm0.26)\times10^6$	1.937	>0.05
Psychrophillic bacteria count	$(1.0\pm0.14)\times10^7$	$(9.7\pm0.14)\times10^4$	6.973	< 0.05
Staphylococcus count	$(2.6\pm0.43)\times10^5$	$(2.2\pm0.42)\times10^5$	0.569	>0.05
E. coli count	$(5.2\pm0.11)\times10^4$	$(1.5\pm0.26)\times10^4$	3.355	< 0.05
Salmonella count	$(1.9\pm0.17)\times10^4$	$(4.0\pm0.44)\times10^3$	8.464	< 0.05

Table 2. Biochemical parameters examined in *L. rohita* from Other States (ROS) and Tripura (RTR)

Parameters	State		t-Value	P-Value
	ROS	RTR		
Moisture (%)	78.48±0.29	76.94±0.27	3.888	< 0.05
Ash (%)	1.10±.0.03	1.23±0.04	-2.514	< 0.05
Protein (%)	14.97±0.14	16.05±0.11	-5.709	< 0.05
Fat (%)	5.10±0.08	5.36±0.08	-2.213	< 0.05
pH	6.71±0.02	6.54±0.02	7.497	< 0.05
TVBN (mg%)	22.68±0.44	13.75±0.52	23.638	< 0.05
PV (mg%)	11.43±0.35	10.75±0.34	46.661	> 0.05
FFA (mg%)	11.28±0.32	11.18±0.22	16.852	>0.05

Values are mean ± S.E (n=36)

Table 3. T-test for Sensory analysis of *L. rohita* from Other States (ROS) and Tripura (RTR)

Parameters	State		t-Value	P-Value
	ROS	RTR		
General Appearance	6.61±0.06	7.18±0.05	-7.222	<0.05
Odour	6.76±0.05	7.26±0.04	-6.483	<0.05
Texture	6.53±0.07	7.13±0.05	-6.867	<0.05
Flesh (including Belly flap)	6.81±0.05	7.09±0.05	-3.927	<0.05

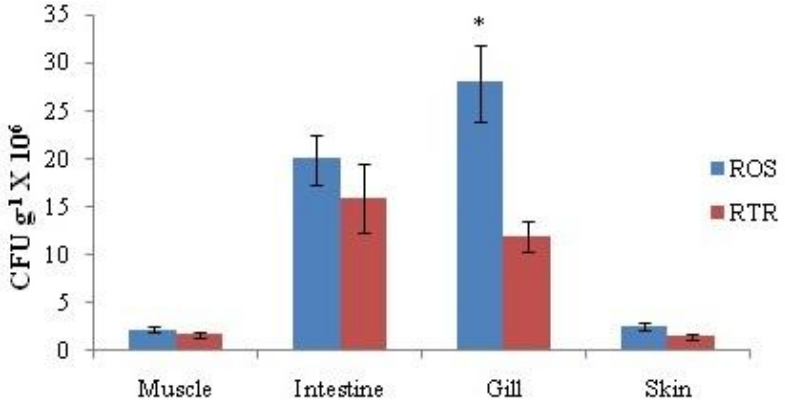

Figure 1. TPC of muscle, intestine, gill and skin

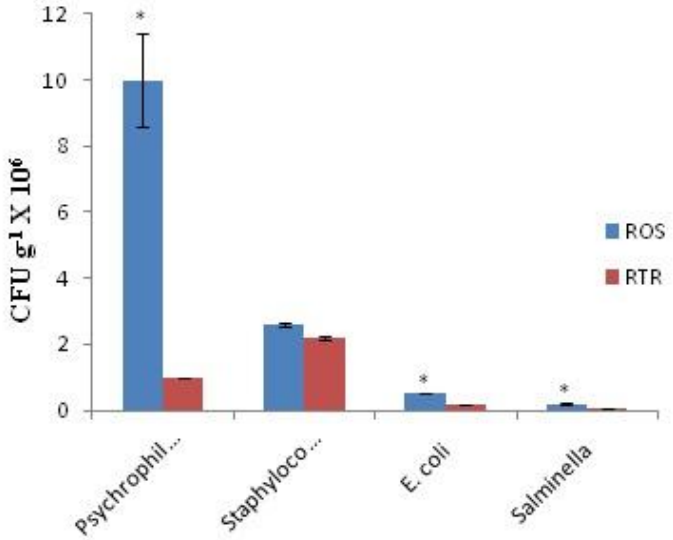

Figure 2. Total pathogenic bacteria count

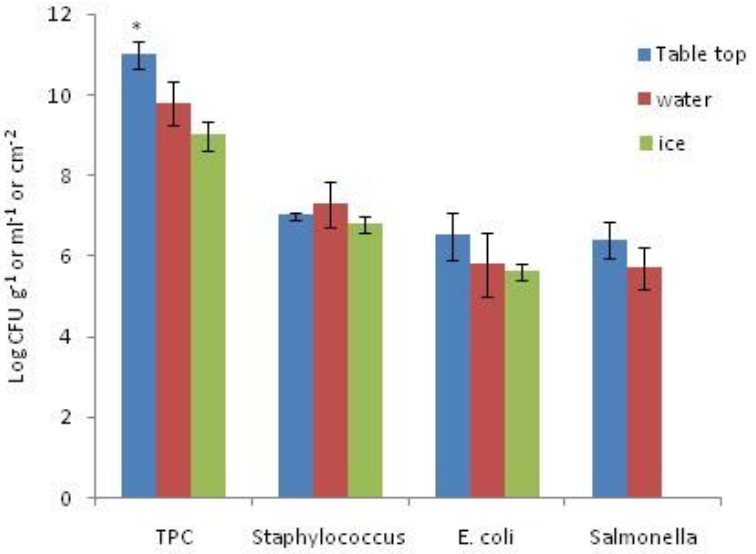

Fig.3. Total Plate Count and the occurrence of *Staphylococcus* spp., *E. coli* and *Salmonella* spp. in Table top (Log CFU cm-2), washing water (Log CFU ml-1) and ice (Log CFUg-1) (Bars with asterisks indicate significant difference (P<0.05), error bars show Mean ± S.E., n=36)

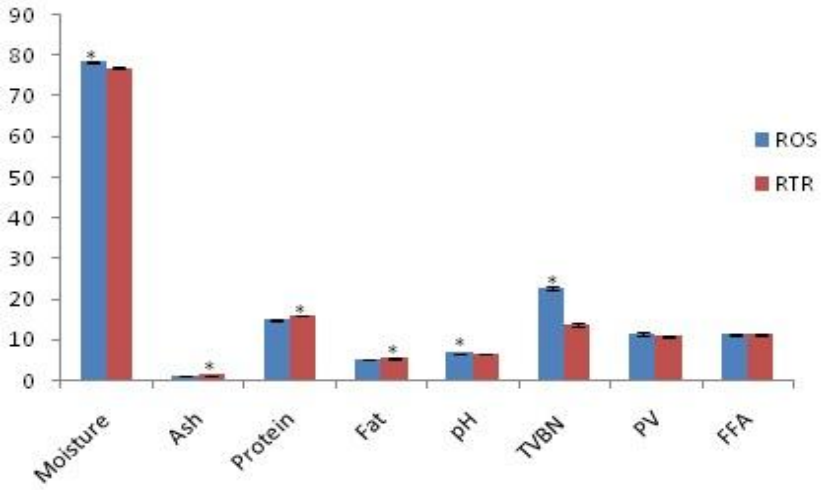

Figure 4. Proximate composition and spoilage indices of ROS and RTR

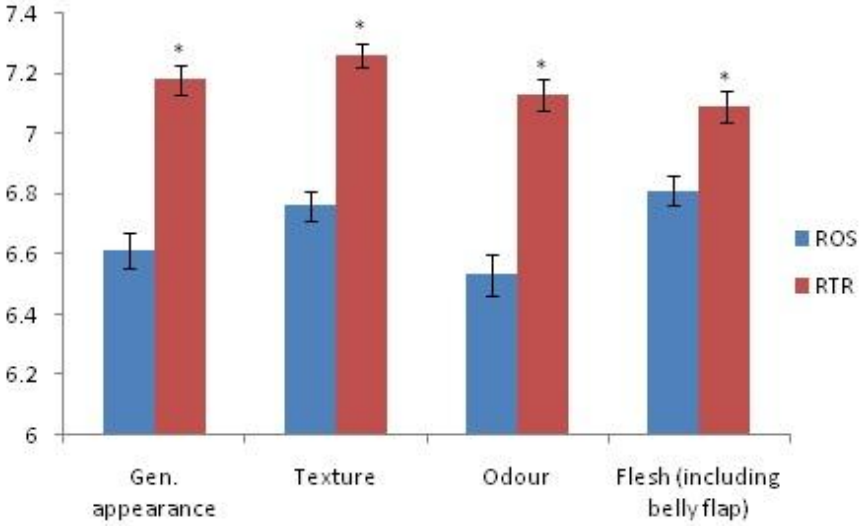

Figure 5. Sensory analysis result of ROS and RTR

4. Conclusion

The findings of the present study revealed that the microbiological quality of both ROS and RTR were poor. Though TPC was found within the acceptable limit in both ROS and RTR, the count was higher. Higher counts of pathogenic bacteria in all samples of fish and processing premises are highly objectionable and it indicates the poor sanitary condition of the market and lack of awareness about handling of seafood. The biochemical and sensory quality of ROS was found inferior than RTR. Although different spoilage indices like TVBN, PV and FFA value was much higher in ROS compared to RTR but it was within the acceptable limit, the higher values of these parameters in ROS is a clear sign of prolonged storage of rohu in ice as transportation time of ROS is much longer. Based on these findings certain management measures can be suggested for maintaining better keeping quality of fish; 1. Use of good quality ice made from potable water and proper packaging with recommended proportions of ice and fish 1:2 (fish to ice). 2. Chlorination of water use for washing of fish at 5 ppm level and for premises table tops, surfaces of equipments at 10 ppm level. 3. Construction of proper drainage systems in the auction centres and retail markets. 4. Organization of awareness camps and training programmes among the fish handlers about seafood handling, maintenance of good personal hygiene etc. 5.

Government may impose strict rules and regulations for adopting sound measures for maintaining hygiene at fish markets. 6. Construction of proper infrastructure facilities in the fish market places such as; concrete road and floor, covered drain, chlorinated water supply system, toilet and bathrooms, exhaust fan in the buildings, air curtains in the doors, foot dips before entering the fish markets. 7. Reduction of transportation time by using fast moving vehicles and removing bottle-nets of customs and duties. 8. Use of refrigerated vans instead of insulated trucks. As fish is a readily perishable food commodity, mulnutrition and food borne diseases are very common in developing countries these findings may able to set a basis for future study about the source of deadly food toxin causing pathogens, may create an awareness about maintenance of hygienic condition in fish market places and can set a measure about shelf life period of freshwater fish stored in iced and un-iced condition.

5. References

1. Ababouch, L. H. Souibri, L. Rhaliby, K. Ouahdi, O. Bttal, M., Busta, F. F. Quality changes in sardines (*Sardina pilchardus*) stored in ice and ambient temperature. *Food Microbiology*, **1996**, 13: 123-132.
2. Ali, A. Karunasagar, I. Karunasagar, I., Dardignac, J., Bacteriological changes during iced storage of the tropical fresh water carp *Labeo rohita*. *Fisheries Research*, **1992**, 13(2): 189-197.
3. Amagliani G., Brandi, G., Schiavano G F. (**2011**). Incidence and role of *Salmonella* in seafood safety. *Food Research International* (In press).
4. AOAC. (**1995**). Official methods of analysis. Association of official analytical chemists (16th eds). Washington, D. C.
5. APHA. (**2001**). Compendium of methods for the microbiological examination of foods. pp 25-35. *In* Speck, M. L. (Eds). American public health association, Washington D. C.
6. Balachandran, K. K. (**2001**). On board handling and preservation, pp. 57-76. *In* Post harvest technology of fish and fish products. Daya publishing house, New Delhi, India.
7. Barman. D, Mandal. S. C. (**2008)**. Achieving self sufficiency in fish production in Tripura state of India – some policies and suggestions. Aquafind.
8. Beatty, S. (**1938**). Studies of fish spoilage. II. The origin of Trimethylamine produced during the spoilage of cod muscle press juice. *Journal of Fisheries Research* 4: 63.
9. Cakli, S. Kilinc, B. Cadun, A. Dincer, T., Tolasa, S., Quality differences of whole ungutted sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) while stored in ice. *Food Control*, **2007,** 18(5): 391-397.
10. Chytiri, S. Chouliara, I. Savvaidis, I. N., Kontominas, M. G., Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiology*, **2004**, 21(2): 157-165.
11. Civera, T. Turi, R. S. Parisi, E., Fazio, G., Further investigation on total volatile basic nitrogen and trimethylamine in some Mediterranean teleosteans during cold storage. *Sciences Des Aliments*, **1995**, 15: 179-186.
12. Connell, J.J. (**1975**). Control of fish quality, 1st edition. Fishing News Books Limited, London, Great Britain.
13. Conway, E. J. Microdiffusion analysis and volumetric error (4th Eds). Van Nostrad Co. Inc., Newyork. **1947**, 159-172.
14. Govindan, T. K. (**1985**). Handling, preservation and transportation of fresh fish. pp 44-76. *In* Fish Processing Technology. Oxford and IBH Publishing Co., Oxford.

15. Grigorakis, K. Taylor, K. D. A., Alexis, M.N. (**2003**). Seasonal patterns of spoilage of ice-stored cultured gilthead sea bream (*Sparus aurata*). *Food Chemistry,* 81(2): 263-268.
16. Huss, H. H. (**1994)**. Assurance of seafood quality, FAO, Fisheries technical paper, 334: 59, Rome.
17. Huss, H.H., (**1985**). Fresh fish quality and quality changes. FAO Fisheries series, 29: 132 Rome.
18. International Commission on Microbiological Specification for food (ICMSF), (**1986**), 2nd edition. Microbiological analysis: principles and specific applications. University of Toronto press, Newyork, USA.
19. International Commission on Microbiological Specification for food (ICMSF), (**1974**). Sampling for microbiological analysis: principles and specific applications. University of Toronto press, Newyork, USA.
20. Jacob, M. B. (**1958)**. The chemical analysis of foods and food products. Kreiger publishing co. Inc. New York, USA. 393-394.
21. Jeyasekaran, G., Ayyappan, S. (**2003**). Microbiological quality of farm-reared freshwater fish, rohu (*Labeo rohita*). *Indian Journal of Fish*, 50(4): 455-459.
22. Joseph, J. Surendran, P. K. and Perigreen, P. A. (**1988**). Studies on ice storage of cultured rohu (*Labeo rohita*). *Fishery Technology*. 25(2): 105-109.
23. Karungi, C. Byaruhanga, Y. B., Muyonga, J. H. (**2004**). Effect of preicing duration on quality deterioration of iced Nile perch (*Lates niloticus*). *Food Chemistry*, 85: 13-17.
24. Kumari, S. Prasad, B. N. Kumari. G, Quasim, A. Basant, K., Singh, K. (**2001**). Microbiological quality of fish, rohu marketed in Patna and its public health significance. *Journal of Food Science and Technology*, 38(6): 607-608.
25. Lakshmanan, P. T. (**2000**). Fish spoilage and quality assessment, pp 26-40. *In* Iyer, T. S. G. Kandoran, M. K. Thomas, M. and Mathew, P. T. (Eds). *Quality assurance in seafood processing*. Society of Fisheries Technologists, Cochin, India.
26. Lakshmanan, R. Jeya Shakila, R., Jeyasekaran, G. (**2002**). Survival of amine forming bacteria during the ice storage of fish and shrimp. *Food microbiology*, 19: 617-625.
27. Lilabati, H., Vishwanath, W. (**1999**). Biochemical and microbiological quality of *Labeo gonius* stored in ice. *Fishery technology*, 36(1): 24-27.
28. Manik, B. S. (**1992**). A laboratory manual on veterinary bacteriology, mycology and immunology. CBS publishers and distribution, New Delhi, India.
29. Nair, K. K. S., Nair, R. B. (**1988**). Bacteriological quality of freshwater fish from Krishnarajendra sagar reservoir. *Fishery Technology*, 25: 79-80.
30. Nair, R. B. Taramani, P. K., Lahiry, N. C. (**1971**). Studies on chilled storage of fresh water fish. *Journal of food science and technology*, 8: 53-56.
31. Nair, R. B. Tharamari, P. K., Lahiry, N. L, Studies on chilled storage of fresh water fish. II. Factors affecting quality. *Journal of Food Science and Technology*, **1974**,11(1): 118-122.
32. Ozyurt, G. Kuley, E. Ozkutuk, S., Ozogul, F, Sensory, microbiological and chemical assessment of the freshness of red mullet (*Mullus barbatus*) and goldband goatfish (*Upeneus moluccensis*) during storage in ice. *Food Chemistry*, **2009**, 114 (2): 505-510.
33. Panda, S. K. Nayak, B. B. (**2001**). Analysis of seafood for *Vibrios* pp. 192-195. *In* Quality management in export of seafood products. Central institute fisheries education publication, Mumbai, India.
34. Razaei, M. Hosseini, S. F. Langrudi, H. E. Safari, R., Hosseini, S. V. (**2008**). Effect of delayed icing on quality changes of iced rainbow trout (*Onchorynchus mykiss*). *Food Chemistry*, 106: 1161-1165.
35. Ruiz-Capillas, C. Moral, A. (**2005**). Sensory and biochemical aspects of quality of whole bigeye tuna (*Thannus obesus*) during bulk storage in controlled atmospheres. *Food Chemistry*, 89(3): 347-354.
36. Russel, A. D., Fuller, R. (**1979**). Cold tolerant spoilage and their environment. Academic press, UK pp 117.
37. Shewan, J. M. (**1962**). The bacteriology of fresh and spoiling fish and some related chemical changes, pp. 167-193. *In* Hawthorn, J. and Leitch, J. M., *Recent advances in food science*. Butterworths, London.
38. Shewan, J. M., Murray, C. K. (**1979**). The microbial spoilage of fish with special reference to the role of psychrophiles, pp 36-117. *In* Russell, A. D., Fuller, R., *Cold tolerant microbes in spoilage and the environment.* Academic press, London.
39. Shewan, J. M. Macintosh, R. G. Tucker, C. G., Ehrenberg, A. S. C. (**1953**). The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. *Journal of science of food and agriculture*, 4: 283-297.
40. Sinha, D. K. Choudhary, S. P., Narayan, K. G. (1991). Microbiological characteristics of marketed rohu (*Labeo rohita*). *Indian Journal of fish*, 38: 69-71.

41. Solanki, K. K., Venkataraman, R. (**1978**). Iced storage characteristics of fresh and brined shark fillets. *Fishery Technology*, 15(1): 7-11.
42. Stansby, M. E., Lemon, J. M. (**1941**). Studies on the handling of fresh mackerel (*Scomber scombrus*).U.S. Fish and Wildlife Service, Research report no 1, pp. 46.
43. Subburaj M., Karunasagar Indrani, Karunasagar I. (**1984**). Incidence of histidine decarboxylating bacteria in fish and market environs. *Food Microbiology* 1:263-267.
44. Sudhi, K. S. (**2002**). Fish in Kochi markets highly contaminated: A study. Online edition of India's National Newspaper. Kerala, India.
45. Varma, P. R. G. (**2002**). Bacterial detection technique pp. 146-147. *In* Iyer, T. S. G. Kandoran, M. K. Thomas, M. Mathew, P. T. (eds). Quality assurance in seafood processing. Central institute of fisheries technology and Society of fisheries technologists publication, Cochin, India.
46. Vieira, R. H. S. F., Vieira, G. H. F. (**1989**). Handling of fish products under refrigeration on fishing boats. pp 3001-3004. *In* Vieira, R. H. S. F., Vieira, G. H. F. (eds). Science and Technology of Fisheries Products: Methods of Preservation and Transportation of Fisheries Products.