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Biochemical degradation of 22 fish species due to microbial spoilage in major fish markets of bhopal

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ABSTRACT

Microbial spoilage of fish and its biochemical changes has been reviewed. Naturally fishes were dominantly contaminated by gram positive and gram negative bacteria's such as pseudomonas, aeromonas, salmonella, shigella, vibrio spp. Growth of these microorganisms in fish caused spoilage of the fish. Microorganisms predominate spoilage of cold storage fish were pseudomonas and aeromonas. Soon after fish death, enzymatic reaction precedes which uses glycogen as energy sources and stop as the glycogen depleted from the tissue. This process named as "rigor mortis". Fish spoilage start at the end of rigor mortis. So, fish spoilage could be delayed by retaining flesh glycogen through reduction in energy consumption. It can be done by preventing vigorous moving of fish during hauling/catching. Delay in spoilage was also due to lactic acid production resulted from glycolyis, which reduced fish flesh pH, so that inhibit microbial growth. Immediately after rigor mortis the

flesh pH increase to normal pH, caused contaminating microorganisms grow, degrade NPN protein such as trimethylamine oxide (TMAO) to trimethylamine TMA. Later stage of spoilage contaminating microorganisms degrade protein compound result in compound that exert putrid smell such as H2S, methyl mercaptan and dimetyhyl sulfide.

Keywords: Degradation, Rigor Mortis, Fish Spoilage

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INTRODUCTION

Fish is widely recognized as a very good source of protein in the human diet. It provides-14% of the world's animal protein intake (Pedrosa-Menabrito and Regenstein, 1988). This protein is high in essential amino acids, and has a good amino acid structure. The fish is also a good source of mineral and vitamin. About 80% of weight of fresh fish consist of water, 18% of protein, carbohydrate content of 1% and lipid content of 1% (Hobbs, 1983). The microorganisms of normal fish are present in the gut cavity and on all the outer surfaces including the gills. It is generally accepted that little or no bacterial activity occurs until the period of "rigor mortis" is passed. However, fresh fish is extremely perishable due to its high pH, normally the pH is 6.4-6.6 due to low reserve of glycogen in the flesh (Buckle, et. al. 1978) and generally spoils within 12-24 hours if not stored under refrigeration or at temperature less than 5^{o}C (Adams 1986), since it provides a favorable medium (high water activity, neutral pH and high level of soluble nutrients) for the growth of spoilage microorganisms (ICMSF, 1980).

The spoilage fish is the result of a series of change brought about in the fish tissue immediately post mortem by the action of endogenous enzymes (autolysis), microorganisms, primarily bacteria and chemical reactions such as oxidation of lipids (Reay and Shewan, 1949; Hobbs, 1983). These actions result in undesirable alteration in flavor, odour and appearance of the fish, which lead to objectionable quality of fish of product. The spoilage pattern of fish depends on initial bacterial flora, the flora acquired during handling and processing, the condition to which the fish has been subjected during storage and

processing, and on the chemical composition of the fish (Shewan and Hobbs, l967; ICMSF, 1980). Although the flesh and internal organs of freshly caught, healthy fish are generally sterile, it is known that, the skin, gills and in the intestine of freshly fish contain high bacterial loads (ICMSF, 1980; Burgess, et al. 1965). The majority of bacteria associated with fresh and spoilage fish come from the environment (Kraft, I992) and belong to the water soil types (Reay and Shewan, l949) which has a lower optimum temperature of generation time than that of normal pathogen. The equipment used such as catch boxes, bins, holds, surfaces, knives and water used for washing gutted fish and cleaning the equipment also become the source of contaminating microflora (Ayres et al. l980; Nickerson and Sinskey, 1972).

MATERIALS AND METHODS

Bhopal, the city of lakes, is situated at 23˚16'N latitude and 77˚26'E longitude. It possesses a number of small and large water bodies, which in addition of promoting aquaculture activities also add to the scenic beauty of the city. However, these water bodies are under great environmental stress due to pollution from various sources. Since last few decades, private entrepreneurs have been using these water bodies for the production of fish. Generally the polyculture of Indian and exotic major carps is being practiced in these water bodies. Incidences of various health hazards have been observed in these fishes. Fishes, from these water bodies, with high microbial load reach the market where the prevailing improper handling and unhygienic conditions make them unfit for human consumption. Following four major fish markets of Bhopal were selected for present study.

Itwara fish market

Itwara fish market is situated in the center of old city. It is housed in a building constructed by Municipal Corporation, about 4 km away from Bhopal railway station. Only freshwater fishes are marketed here. This fish market is run under the control of Bhopal Municipal Corporation. The condition of this market is extremely poor and unhygienic. The chicken market, situated adjacent to the fish market, aggravates this condition more. Bacterial contamination is most prevalent in this market.

Bittan fish market

It is situated about 12 km away from Bhopal railway station. There is no shelter for this market and the fishes are sold on road side under open sky. This fish market is run under the control of Bhopal Municipal Corporation. Besides freshwater fishes, considerable quantity of marine fishes, crabs, prawns, roasted fishes, sun dried fishes and salted fishes are marketed here. On the periphery of this market is situated chicken market. The condition of this market is also extremely unhygienic.

Piplani fish market

It is situated about 14 km away from Bhopal railway station. This fish market is under the control of BHEL administration. Both freshwater and marine fishes, along with smoked and cured fishes and prawns are marketed here. In this fish market, cemented platforms without any roofing are provided.

Govindpura fish market

This fish market is situated about 7 km away from Bhopal railway station. It also comes under the control of BHEL administration. This market is also provided cemented platforms without any roofing. Both freshwater and marine fishes are marketed here.

BIOCHEMICAL ESTIMATION OF PROTEINS, CARBOHYDRATES AND LIPIDS

Estimation of proteins

Proteins were estimated by the Kjeldahl method. 0.5 g of dried fish sample to which 0.1ml of acid mixture (concentrated sulphuric acid and orthophosphuric acid) and catalyst mixture (K_2SO_4 and Selenium) were added in digestion flask. The mixture was exposed to about 160˚C temperature in order to allow digestion and then distilled with Kjeldahl apparatus by adding 40% NaOH solution. The distillate was collected in saturated boric acid solution containing a mixture of bromocresol green and methyl red as an indicator. Finally, the distillate was titrated with 1.02 N sulphuric acid till a reddish colour appears. The protein content was estimated by using the following formula:

Nitrogen Content (%) = ml of acid added x Normality of acid x 100 x 0.014/ Weight of wet product sample (g)

Protein content (%) = Nitrogen content (%) x 6.2

Estimation of carbohydrates

Total carbohydrates were estimated by the phenol sulphuric acid method. 10mg of dried tissue sample was treated with 2ml of 80% sulphuric acid and was allowed to digest for about 20 - 21 hours at room temperature. 2ml of 5% phenol reagent followed by 5ml of concentrated sulphuric acid were added to the digested sample and was allowed to cool at room temperature. Absorbency was measured at 490nm in UV Spectrophotometer.

Estimation of lipids

Lipids were estimated by the Soxhlet method. Clean and dried thimble containing about 5g of dried sample was covered with fat free cotton at the bottom and top was placed in the extraction chamber. The sample was lyophilized before solvent extraction for 6 hours by Soxhlet apparatus using ethyl acetate. Finally, the fat content was estimated by using the following formula:

Fat g/100g fresh sample = Wf x (100 – Moisture %)/WD

Weight of fat (Wf) = Wa – Wb Where: Wa = Weight of extraction flask after extraction

Wb = Weight of extraction flask before extraction WD = Dried sample obtained after determination of moisture.

OBSERVATIONS

Fish is highly perishable food and susceptible to faster postmortem deterioration. Deterioration in quality of fish food is attributed to highly sensitive proteins and fats present in fish. The major deteriorative processes that affect the texture, colour and flavour of fish food are microbial spoilage and autolysis. The loss of quality depends directly on nature of fish species and on the handling and storage conditions. The rate at which microbial and autolytic spoilage occurs vary according to the species of fish, area of catch, method of catch and, above all processing and storage temperature. Once the fish is caught, on-board storage conditions exert a strong effect on the quality of manufactured fish products and accordingly, on their commercial value. Many factors such as type of species, size of fish, temperature of the storage, physical conditions, and methods of catching and handling can

affect the shelf life of fish during storage. The quality of fish in general decreases after death due to chemical reactions (changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine) and microbiological spoilage.

During present investigation carried out on four major fish markets of Bhopal, twelve species of bacteria viz. Aeromonas hydrophila, Pseudomonas fluorescens, Nocardia sp., Streptococcus iniae, Staphylococcus aureus, Shigella sp., Salmonella sp., Listeria monocytogenes, Vibrio parahaemolyticus, Clostridium perfringens, Escherichia coli, Fecal coliforms and three species of fungi viz. Aspergillus niger, Aspergillus fumigatus and Penicillium notatum have been isolated and identified.

Biochemical estimation of fishes

The variations in the levels of proteins, carbohydrates and lipids in 22 fishes collected from four fish markets of Bhopal infested with micro-organisms were observed.

Table no 1. Showing variation in carbohydrates, proteins and Lipid levels of fishes

Clarias batrachus	
Carbohydrates	**76.74 mgdl-1**
Total proteins	6.72 gdl-1
Lipids	4.22 mgdl-1
Glycogen	10.67 mgdl-1

Oreochromis mossambica	
Carbohydrates	**60.32 mgdl-1**
Total proteins	3.06 gdl-1
Lipids	0.64 mgdl-1
Glycogen	8.51 mgdl-1

Nemipterus japonicus	
Carbohydrates	**56.0 mgdl-1**
Total proteins	13.4 gdl-1
Lipids	0.33 mgdl-1
Glycogen	7.2 mgdl-1

Megalaspis cordyla

Carbohydrates	**50.0 mgdl-1**
Total proteins	12.0 gdl-1
Lipids	0.22 mgdl-1
Glycogen	6.0 mgdl-1

Carangoides malabaricus

Carbohydrates	**50.0 mgdl-1**
Total proteins	12.0 gdl-1
Lipids	0.27 mgdl-1
Glycogen	6.0 mgdl-1

Hemirhamphus far

Carbohydrates	**60.0 mgdl-1**
Total proteins	22.0 gdl-1
Lipids	0.22 mgdl-1
Glycogen	5.2 mgdl-1

Mastacembelus armatus

Carbohydrates	**65.0 mgdl-1**
Total proteins	25.0 gdl-1
Lipids	0.35 mgdl-1
Glycogen	3.2 mgdl-1

Rohtee cotio

Carbohydrates	**42.0 mgdl-1**
Total proteins	13.0 gdl-1
Lipids	0.53 mgdl-1
Glycogen	2.2 mgdl-1

Rita rita

Carbohydrates	**55.0 mgdl-1**
Total proteins	20.0 gdl-1
Lipids	0.27 mgdl-1
Glycogen	1.2 mgdl-1

Notopterus notopterus

Carbohydrates	**51.0 mgdl-1**
Total proteins	20.0 gdl-1
Lipids	0.22 mgdl-1

Glycogen	1.2 mgdl-1

Labeo rohita

Carbohydrates	**65.0 mgdl-1**
Total proteins	18.0 gdl-1
Lipids	0.29 mgdl-1
Glycogen	1.2 mgdl-1

Aorichthys seenghala

Carbohydrates	**45.0 mgdl-1**
Total proteins	24.3gdl-1
Lipids	0.65 mgdl-1
Glycogen	1.3 mgdl-1

Labeo gonius

Carbohydrates	**67.0 mgdl-1**
Total proteins	29.8gdl-1
Lipids	0.93 mgdl-1
Glycogen	1.8 mgdl-1

Xenentodon cancila

Carbohydrates	**35.0 mgdl-1**
Total proteins	10.0 gdl-1
Lipids	0.27 mgdl-1
Glycogen	1.8 mgdl-1

Pampus chinensis

Carbohydrates	**79.0 mgdl-1**
Total proteins	29.0 gdl-1
Lipids	0.56 mgdl-1
Glycogen	1.0 mgdl-1

Channa striatus

Carbohydrates	**60.0 mgdl-1**
Total proteins	12.0 gdl-1
Lipids	0.93 mgdl-1
Glycogen	1.5 mgdl-1

Sardinella sirm

Carbohydrates	**47.0 mgdl-1**
Total proteins	12.0 gdl-1

Lipids	0.47 mgdl-1
Glycogen	1.8 mgdl-1

Puntius sarana

Carbohydrates	**65.0 mgdl-1**
Total proteins	22.0 gdl-1
Lipids	0.28 mgdl-1
Glycogen	1.8 mgdl-1

Harpodon nehereus

Carbohydrates	**52.0 mgdl-1**
Total proteins	25.6gdl-1
Lipids	0.47 mgdl-1
Glycogen	1.7 mgdl-1

Chanda nama

Carbohydrates	**32..0mgdl-1**
Total proteins	7.6 gdl-1
Lipids	0.64 mgdl-1
Glycogen	1.0 mgdl-1

Eutropiichthys vacha

Carbohydrates	**54.0 mgdl-1**
Total proteins	32.6gdl-1
Lipids	0.43 mgdl-1
Glycogen	1.5 mgdl-1

Hilsa toil

Carbohydrates	**59.0 mgdl-1**
Total proteins	33.7 gdl-1
Lipids	0.37 mgdl-1
Glycogen	4.2 mgdl-1

DISCUSSION

Fish flesh is composed of protein, fats, carbohydrate, water and amino acids compounds such as triniethylamine oxide (TMAO), urea, taurine, creatine, anserine, free amino acids and trace glucose etc. The amount of this substrate vary with fish species. In chilled/iced fish the

Pseudomonas, predominating groups rapidly metabolized most amino acids, dipeptides found in the non protein nitrogen. This action result in ammonia and volatile fatty acids (NPN) fraction of the (Liston, I982). This process occurs in the early stage of spoilage. As spoilage of fish proceeds, there is a gradual change in the physical, microbiological and organoleptic. In raw fish undergoing spoilage. There is a characteristics sequence of odour changes as the following (Clucas, l98l). TMA associated with fishy odour spoilage is part of spoilage pattern of many fish species. When TMA reacts with fat in the muscle of the fish, a characteristic fishy odor of low quality fish is produced. The odor will appear when the levels of TMA is about 4-6 mgN/ 100 ml of muscle extract and it will be definitely smell at the level l0 mgN/100 ml of the muscle extract (Pedrosa-menaberto and Regenstein, 1988). In fillet of fish which is stored at refrigerated and undergo bacterial spoilage, TMA content will increase as the number of bacteria increase (Nickerson and Sinskey, 1972) and the fillets eventually take on trimethylamine-like odor prior to the development of ammoniacal and putridiodors. Further they stated that Pseudomonas putrfaciens has responsible for TMA production.

Proteolysis

Following the phase of TMAO reduction, during which there was no appreciable change in amino-nitrogen, deamination process which result in the formation of ammonia. In anaerobic storage condition on boards fish may develop an odor which has been describe as bilgy. This odor is especially offensive odor usually reminiscent of some hydrogen sulfide mixed with other odors. In anaerobic condition (when oxygen is depleted) TMAO can be used by spoilage bacteria as an electron acceptor or oxidizing agent, thus stimulating bacterial growth.

Spoilage of Fat

Fish contain fats which is proportionately high in unsaturated fatty acids which are subject to attack by atmospheric oxygen leading to deteriorative changes especially in fatty fish. The fat oxidation causes changes in flavor, colour and possibly texture of the fish flesh associated with rancidity (Hobbs, 1982). ln addition to lipid oxidation, fish fat can also undergo hydrolysis due to lipolytic activity of fish tissue, but it can be promoted by bacterial lipases during fish spoilage. These types of spoilage generally happen in the later stage of spoilage and it is usually occur at chilling and freezing temperatures (ICMSF, l980).

Biochemical examination of fishes

Infested with various pathogenic micro-organisms exhibited following variation in the levels of proteins, carbohydrates, and lipids. In Clarias batrachus, total proteins, carbohydrates and lipids recorded are (6.72 gdl-1), (76.74 mgdl-1) and (4.22 mgdl-1), respectively. In Oreochromis mossambica, total proteins, carbohydrates and lipids recorded are (3.06 gdl-1), (60.32 4mgdl-) 0.64 mgdl-1), respectively. In Nemipterus japonicus, total proteins, carbohydrates and lipids recorded are (13.4 gdl-1), (50.04 mgdl-1) and lipids (0.33 mgdl-1), respectively. In Megalaspis cordyla, total proteins, carbohydrates and lipids recorded are (12.0 gdl-1), (56.04 mgdl-1) and (0.23 mgdl-1), respectively. In Carangoides malabaricus, total proteins, carbohydrates and lipids recorded are (12.3 gdl-1), (51.0 mgdl-1) and (0. 27 mgdl-1), respectively. In Hemirhamphus far, total proteins, carbohydrates and lipids recorded are (22.6 gdl-1), (60.0 mgdl-1) and (0. 43 mgdl-1), respectively. In Mastacembelus armatus, total proteins, carbohydrates and lipids recorded are (25.8 gdl-1), (60.0 mgdl-1) and (0.2 mgdl-1), respectively. In Rohtee cotio, total proteins, carbohydrates and lipids recorded are (13.2 gdl-1), (42.0 mgdl-1) and (0. 53 mgdl-1), respectively. In Rita rita, total proteins, carbohydrates and lipids recorded are (20.0 gdl-1), (55.0 mgdl-1) and (0.27 mgdl-1), respectively. In Notopterus notopterus, total proteins, carbohydrates and lipids recorded are (20.1 gdl-1), (51.0 mgdl-) and (0. 20 mgdl-1), respectively. In Labeo rohita, total proteins, carbohydrates and lipids recorded are (18.4 gdl-1), (65.0 mgdl-1) and (0. 29 mgdl-1), respectively. In Aorichthys seenghala, total proteins, carbohydrates and lipids recorded are (24.3 gdl-1), (45.0 mgdl-1) and (0. 65 mgdl-1), respectively. In Labeo gonius, total proteins, carbohydrates and lipids recorded are (29.8 gdl-1), (67.0 mgdl-1) and lipids (0. 93 mgdl-1), respectively. In Xenentodon cancila, total proteins, carbohydrates and lipids recorded are (10.3gdl-1), (67.0 mgdl-1) and (0.27 mgdl-1), respectively. In Pampus chinensis, total proteins, carbohydrates and lipids recorded are (29.0gdl-1), (79.0 mgdl-1) and (0.56 mgdl-1), respectively. In Channa striatus, total proteins, carbohydrates and lipids recorded are (12.0gdl-1), (60.0mgdl-1) and (0.93 mgdl-1), respectively. In Sardinella sirm, total proteins, carbohydrates and lipids recorded are (12.6 gdl-1), (47.0 mgdl-1) and (0. 47 mgdl-1), respectively. In Puntius sarana, total proteins, carbohydrates and lipids recorded are (22.6 gdl-1), (65.0 mgdl-1) and (0. 28 mgdl-1), respectively. In Harpodon nehereus, total proteins, carbohydrates and lipids recorded are (25.6 gdl-1), (52.0 mgdl-) and (0.47 mgdl-1), respectively. In Chanda nama, total proteins, carbohydrates and lipids recorded are (7.6 gdl-

1), (32.0 mgdl-1) and (0. 64 mgdl-1), respectively. In Eutropiichthys vacha, total proteins, carbohydrates and lipids recorded are (32.6 gdl-1), (54.0 mgdl-1) and (0. 43 mgdl-1), respectively. In Hilsa toli, total proteins, carbohydrates and lipids recorded are (33.7gdl-1), (59.0mgdl-1) and (0. 37 mgdl-1), respectively. These observations are in conformity with those observed by Jaroli and Sharma (2005), Abbas et al. (2008), Nakagawa et al. (1976), Mastsumoto and Obika (1968), Magalhaes et al. (2006), Shenouda (1980), Jones (1957), Choo and Williams (2003), Benjakul et al. (1977), Greene Atanasova (2008) stated that the lower the muscular reserves of glycogen as soon as the fish is caught and further stated that catching methods influence the levels of initial glycogen. Dyer et al. (1947a) and Cutting (1953) stated that there is a considerable loss in protein from the moment of catch to the consumer. Mori and Sato (1975) stated that besides amino acids spoiling bacteria need Carbohydrates, minerals and vitamins for optimal growth. Larssen et al. (1951) found that rise in free fatty acids in fish during spoilage from 1.1% after 24 hours of storage kept at room temperature. He noticed considerable amount of free fatty acids and contain bad odours and off flavours presumably derived from the degradation of fat. Lovern and Wood (1937), Reay et al. (1943), Shewan (1953b, 1955b and 1956), Tarr (1954, 1958) and Ranke et al. (1957) stated about considerable amount of protein depletion.

CONCLUSION

Fish is a very good source of protein in human diet because contains about 18% protein, which is mostly composed of essential amino acids. But fish contain very low glucose, which make them to have high ultimate pH (6.2-6.5). These conditions caused the fish susceptible to microbial spoilage easily. Microbial spoilage of fish is affected by some factors such 2 initial microflora, handling, processing and environment such as temperature, season, handling such as gutting; processing such as chilling, drying and smoking Environment give variation in the type of microorganisms contaminate the fish (initial microflora). In temperate region, psychrophilic and psychrotropic bacteria predominate, while in tropical area the mesophilic gram positive will predominate. During handling staphylococcus will introduce to the fish.

Drying and smoking will changes the microflora of the fish. In drying fish, mould will be the most contaminant if the aW of the product less than 0.75 as no bacteria can grow in this aW.

This also happen in smoking, but in addition to moulds smoked fish also bring about Clostridium species. As the fish die, it will undergo rigor mortis. Microbial growth will not occur unless the rigor mortis ceases. As rigor mortis finish, microorganisms on the surface of the fish grow because the fish support the growth by nutrient availability. The microorganisms grow on the surface of the fish as well as invade the fish flesh through the gills or kidney and also their enzyme invade the fish flesh causing degradation of amino acid result in chemical change, odor. Initially the odor is fresh then become sweetish, later ammoniacal or fishy odor dominates until finally putrefactive odors dominate which appear as a result of microbial spoilage. The fish usually become inedible when the mixture of ammoniacal (Ammonia, TMA) and putrid elements (H_2S) appear. In freeze and chilled stored fish there is also oxidation of fat result in rancidity of the fish.

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DECLARATION OF COMPETING INTEREST

All authors declare no conflicts of interest.

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