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Biochemical and Microbiological Quality of Ice Stored Catfish *Wallago attu* of the Imphal Market

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The biochemical and microbiological quality of ice stored *Wallago attu* sold in the Imphal market of Manipur, India were studied. TVBN and FFA values were high at 12.00±1.73 mg 100g^{-1} and 5.50±0.29% oleic acid, respectively. TBA value was 0.73 mg of malondaldehyde kg^{-1} of sample. Salmonella and *Escherichia coli* were not detected. However, presence of *Staphylococcus aureus*, faecal Streptococci, coliforms and a high content of bacteria (10^8 g^{-1}) and fungi (10^3 g^{-1}) indicated that the fishes were probably from polluted waters and/or proper sanitary care was not taken after capture. Presence of psychrophiles viz., *Pseudomonas* and *Flavobacterium* in great numbers indicated probability of the incidence of spoilage in the fish.

Key words : Iced storage, fish quality, *Wallago attu*, Imphal market, India

Fishes are prone to contamination at various stages of processing. There are reports of contamination by Streptococci (Gopakumar *et al.*, 1983), Salmonella (Iyer and Shrivastava, 1989), coagulase positive *Staphylococcus aureus* (Sanjeev and Iyer, 1988; Iyer and Shrivastava, 1988) in the fishery products, the sources of which were traced to fish handlers and surroundings. Microorganisms contribute to spoilage either directly by their growth or indirectly by secreting enzymes which exert a disintegrating action on the host tissue (Bramstedt and Auerach, 1962).

Freshwater catfish *Wallago attu* is an important item in community feasts in Manipur, India, in addition to its use for household consumption. As the fish production in this state is not sufficient to meet the demand, a large quantity of ice preserved *W. attu* is brought from other states such as Bihar and West Bengal and sold in the Imphal market. According to Govindan (1985), it takes 1-2 days for iced fishes to reach Calcutta, from where another 4-5 days are required to reach different marketing centres of north eastern states. The fishes reach Imphal in bamboo or plywood boxes lined with leaves and gunny bag, packed along with ice in the ratio of 2-3:1 by volume. Sawdust or paddy husk is also incorporated with ice for insulation. The fishes are transferred to new plywood boxes after reaching the market and fresh ice is added. Fishes are retailed as whole pieces. No attempt has so far been made to assess the quality of ice preserved consignments of fishes reaching the state. This paper reports the biochemical and microbial quality of ice preserved *Wallago attu* sold in the Imphal market of Manipur.

Materials and Methods

Three fishes, weighing 1.5 to 2 kg were selected randomly, each from different iced boxes and brought to the laboratory in aseptic condition. Such samplings were done weekly for three months, from February to April, 1992. For biochemical

estimations, 50 g muscle of the fish was sampled randomly from different parts of the fishes. Non protein nitrogen (NPN) and total nitrogen (TN) were determined by the methods of AOAC (1975). Total volatile base nitrogen (TVBN) was determined by Conway microdiffusion method (Morris, 1959), thiobarbituric acid (TBA) value by colorimetric estimation of malonaldehyde (Sinhuber and Yu, 1958) and free fatty acids (FFA) value by titration with NaOH (Morris, 1959).

For enumeration of bacteria, 50 g of sample in case of gill, intestine and muscle and 10 cm^2 in case of skin were macerated in peptonized (0.1%) distilled water to obtain decimal dilutions. Further dilutions were made with the same diluent. Total viable bacteria were enumerated on plate count agar at ambient temperature (28±2°C). For identification of bacteria, colonies from plate count agar were picked, purified and identified up to generic level (Buchanan and Gibbons, 1974). Cultures in doubt were sent to Central Research Institute, Kasauli, India and International Mycological Institute, Kew, UK, for confirmation. For detection of *Escherichia coli*, Salmonella, *Staphylococcus aureus*, faecal Streptococci and most probable number (MPN) count of coliforms, the methods of APHA (1976) were followed.

For fungal count, serial dilutions of samples were made in sterile distilled water, plated on potato dextrose agar (PDA, pH 3.5) and incubated at ambient temperature (28±2°C). Fungal colonies on PDA were picked, stained with cotton blue in lactophenol and identified based on Gilman (1957) and Ellis (1971, 1976). The results of biochemical analyses are shown as mean ±SD of 12 samples whereas the counts of microbial colony forming unit (cfu) are given in ranges for the 12 samples.

Results and Discussion

The results of biochemical analyses are presented in Table 1. The TBA value of 0.73 mg of malonaldehyde kg^{-1} of sample indicated that peroxidation of lipid had not taken place. TBA values of less than 3 mg malonaldehyde kg^{-1} of sample is considered to indicate good quality in fishery products (Sinhuber and Yu, 1958). However, the value of FFA (5.5% oleic acid) shows that there might have been certain degree of lipid hydrolysis. The values of FFA in fresh lantern fish (Gopakumar *et al.*, 1983) and common murrel (Perigreen *et al.*, 1987) were found to be 2.0 and 3.4% oleic acid, respectively. NPN values seemed to be acceptable, since the values coincided with those of fresh and iced *W. attu* reported by Devadasan *et al.* (1978). The mean TVBN value (12 mg $100g^{-1}$) was within the acceptable limit of < 30 mg $100g^{-1}$.

Table 1. Biochemical analyses of iced *Wallago attu*

Moisture, %	80.47±0.53
TVBN, mg $100g^{-1}$	12.00±1.73
FFA, % oleic acid	5.50±0.30
TBA, mg of malonaldehyde kg^{-1}	0.73±0.10
NPN, %	0.47±0.01

Total counts of fungi (TFC), bacteria (TBC) and coliforms are presented in Table 2. Visual examination of the fish and tissue did not reveal fungal growth. However, detection of moulds in PDA indicate contamination by fungal spores/propagules. These might have come either from packing materials such as saw dust, leaves or boxes or from the market where it is sold. It is also probable that the landing and initial packing sites are contaminated with the fungi. However, no definite conclusion could be drawn as analyses of such environments were not conducted. The genera of fungi in Table 3 are common moulds found in nutrient rich substrates.

Total viable bacteria count was high, reaching up to 10^8 g^{-1}; the highest being in the gills. It has been confirmed by using histological techniques that gills offer an ideal surface for microbial growth (Russel & Fuller, 1979). Microflora of the fish reflect to a certain degree, the microflora of the environment (Graikoski, 1973). Though total counts of bacteria, have been used to assess sanitary quality and organoleptic acceptability of various food products, it is a poor index of safety since spoilage of perishable food is seldom caused by pathogenic organisms (Silliker, 1963).

Though number of coliforms (Table 2) on the fish is low, which may due to cold shock (Perigreen *et al.*, 1987), their presence is indicative of sewage contamination and hence the probable presence of enteric pathogens. *E. coli* and Salmonella were not detected in any of the samples. During iced storage for 3-6 days, *E. coli* may totally vanish (Perigreen *et al.*, 1987). Although the less resistant *E. coli* was absent, the presence of highly resistant faecal Streptococci indicated that fish had faecal contamination. Staphylococcus (10^2-10^3 g^{-1}) was also detected. The incidence of this organism in fishery products in a few numbers is not a serious problem, but food poisoning outbreaks may happen if the product is handled carelessly during processing which results in the multiplication of the organism.

Eight probable genera of bacteria were identified while some could not be identified (Table 4). Among those identified, six were psychrophiles. *Micrococcus*, *Aeromonas* and *Pseudomonas* were found in

Table 2. Counts of bacteria and fungi in different tissues of *Wallago attu* (log cfu cm^{-2} in skin; log cfu g^{-1} in other tissues)

Tissue	Total fungal count	Total bacterial count	Coliforms	Faecal Streptococci	Staphylococci
Skin	2.47-3.47	6.85-7.53	1.14-1.96	3.60-4.79	4.17-4.77
Gill	2.84-3.77	8.38-8.95	1.36-2.66	2.00-4.77	3.07-5.04
Intestine	2.00-3.47	7.62-8.56	1.66-2.46	2.84-3.46	3.69-4.30
Muscle	0.00-1.30	6.10-6.41	0.30-1.36	2.00-2.07	2.47-3.07

Table 3. Fungal flora of iced *Wallago attu* (Percentage of total fungal flora)

Fungi	Gill	Skin	Intestine	Muscle
Aspergillus	0.00-25.00	ND	0.00-50.00	0.00-100.00
Cladosporium	0.00-16.67	20.00-23.53	0.00-16.67	ND
Fusarium	ND	0.00-5.88	ND	ND
Geotrichum	0.00-100.00	60.00-100.00	0.00-28.57	ND
Mucor	0.00-8.35	ND	ND	ND
Penicillium	8.33-50.00	5.88-100.00	0.00-100.00	0.00-100.00
Rhizopus	0.00-12.50	ND	0.00-100.00	ND
Sterile mycelium	25.00-75.00	33.33-100.00	0.00-100.00	ND

ND = Not detected

Table 4. Bacterial flora in iced *Wallago attu* (Percentage of total bacterial flora)

	Gill	Skin	Intestine	Muscle
Acinetobacter	10.00-16.19	10.07-9.12	11.89-15.48	4.23-11.54
Aeromonas	8.64-9.58	8.04-9.41	11.08-12.26	7.69-8.85
Bacillus macerans	0.00-4.58	1.02-7.06	ND	ND
Flavobacterium	7.55-16.25	2.22-13.53	2.38-14.05	3.08-15.00
Micrococcus	15.83-26.26	11.91-14.12	12.43-19.05	12.69-15.38
Moraxella	0.00-5.36	2.80-6.18	4.76-7.57	6.54-7.69
Pseudomonas	14.17-15.32	2.33-15.59	5.24-15.14	16.92-18.46
Vibrio	5.36-8.33	9.12-42.35	6.22-12.26	5.38-6.92
Unidentified	15.32-21.25	15.88-20.26	21.62-28.57	28.85-30.77

ND = Not detected

higher percentage than the rest. Ali *et al* (1992) reported that *Aeromonas* were the most important spoilers of freshwater fish. There are reports on spoilage of freshwater fish by *Pseudomonas* (Surendran and Gopakumar, 1981). *Bacillus macerans* was isolated from skin and gill of the iced fish. Though this is a plant inhabiting organism (Buchanan and Gibbons, 1974), it is known to have strong hydrolysing property which may be responsible for spoilage of food products.

It may be concluded that the iced *W. attu* sold in the Imphal market is not very fresh and a certain degree of spoilage has set in. Contamination by fungi, bacteria and also the presence of coliforms, *Staphylococcus*, faecal Streptococci are of concern in relation to human health and hygiene. However the absence of *E. coli* and Salmonella is a consolation and the fish may be recommended for human consumption after proper cooking. Attempts should be made to improve the quality by strict vigil during icing and packing. Quality of packing material and selling method in the market also require improvement. Thus, the quality of such fishes could be improved only by establishment of cold storage chain, proper handling, packaging, transportation and retailing facilities.

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