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Changes in Bacterial and Fungal Quality During Storage of Smoked, *Esomus danricus* of Manipur

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Total plate counts of bacteria (TPC) and fungi (TFC) gradually decreased with the decrease of moisture content in smoked *Esomus danricus* during storage of the fish in bamboo baskets. The counts increased with the increase of humidity of the environment and the moisture content of the fish. Coliforms were not detected after 30 days of storage and *Staphylococcus aureus* and feacal *Streptococci* were not detected after 60 days of storage. *E. coli* and *Salmonella* were not detected in the present analysis. Xerophilic fungi, *viz.*, *Aspergillus candidus, Aspergillus fumigatus, Aspergillus niger, Aspergillus sydowii* and *Penicillium chrysogenum* were observed during storage of the fish. *A. niger* and *A. candidus* were dominant in April, May and June, whereas *Aspergillus flavipes* was dominant in July and August. Growth of fungi was rapid in the rainy season. Smoked *E. danricus* was in unacceptable condition after storage for 120 days.

Key words : *Esomus danricus*, smoked fish, bacteria, fungi, effect of moisture

Smoking has been considered as a technique for preservation of fish and also to impart the smoky flavor since time immemorial. *Esomus danricus* is a small sized fish (4.0-5.0 cm), commonly available in sun-dried or smoked form in the markets of Manipur. Large quantities of this fish are caught from the Loktak lake, 40 km south of Imphal town. The villagers smoke it in traditional Manipuri style and sell at Moirang market, the main fish-marketing centre near the lake. The method for smoking of fish in the state has been reported by Singh *et al.* (1990). The smoked fish are stored in small boxes called 'Ngarubak' made of split bamboos. Determination of microbiological quality of such processed fish from the market as well as the changes in the quality during storage is very important for safeguarding consumer's health and hygiene. This paper reports the changes of total bacterial count (TPC), total fungal count (TFC) and fungal flora of the fish during storage at room temperature for a period of 120 days. Attempts have been made to correlate the data with the moisture content of the fish and also with meteorological parameters.

Materials and Methods

Smoked *E. danricus* was collected from 6 different fish sellers in the month of April, 1997, packed separately and then brought to the laboratory. They were stored separately in the bamboo boxes.

Colour, texture and odour of the fish were judged by a panel of seven judges, which included 2 research scholars, 2 teachers of the department and 3 pepole involved in the business of smoked fish. Colour was recorded based on visual observation and texture, by applying pressure by fingertips. Odour was assessed on a three-point hedonic scale as good, medium and poor. Reconstitution property was assessed as percentage of water imbibed by 100 g of the sample soaked in 400 ml of water for a period of 3.5 h (Valsan, 1975).

Moisture content of the fish was determined by AOAC method (1975). Ambient temperature and humidity were recorded during the entire period of study.

Enumeration of TPC and TFC, most probable number (MPN) of coliform and detection of pathogenic bacteria, viz. *Salmonella, Escherichia coli, Staphylococcus aureus* and faecal *Streptococci* were done as per the procedure of APHA (1976). TPC was enumerated on plate count agar; TFC, on acidified potato dextrose agar (PDA); MPN of coliforms, on brilliant green lactose bile (BGLB) broth; *E. coli* on eosine methylene blue (EMB) agar after enrichment in BGLB broth; *Salmonella,* on brilliant green agar (BGA) after enrichment in selenite cystine broth (SCB); *Staphylococcus aureus,* on Baird Parker agar (BPA) and faecal *Streptococci* on KF Streptococcal agar. The suspected pathogenic bacterial colonies were further tested using the methods of APHA (1976) and Kiss (1984), Fungal colonies on PDA were picked, stained with cotton blue in lactophenol and identified following the methods of Gilman (1957) and Ellis (1971, 1976).

Results and Discussion

The reconstitution properties, pH and sensory evaluation of six samples of smoked *E. danricus* are presented in Table 1. All the samples were slightly acidic and the pH ranged between 6.4 and 6.8. The extent of reconstitution was between 106.0 and 115.0%. Texture of the fish was brittle to crisp and the colour, yellowish black to golden yellow. All the fish samples had a medium smoky odour.

The initial moisture content of the smoked fish was 7.3%. It gradually decreased to 4.1% after 30 days of storage. Then, the value increased gradually to 12.2% on the 90th day. Further storage resulted in decrease of moisture content. Relationship between moisture, meteorological data and micro flora of the fish during storage is shown in Table 2. There was a direct relationship between the microbial counts and humidity and also with moisture content of the sample. During the major part of this investigation humidity was above 70%. Rao *et al.* (1962) reported that fungal growth was a major cause of spoilage at relative humidity levels above 70%. Moisture levels of fish also plays an important role in the spoilage. Lowering of moisture retards the spoilage (Stansby, 1963). According to Kaneko (1976), smoked fish products deteriorate by the growth of mould if the water content is approximately 15%. In the present study the fungal count suddenly increased during the rainy season and fungal growth was visible on the samples.

Table 1. pH, reconstitution properties and sensory evaluation of smoked *Esomus danricus*

Sample	pH	Reconstitution %	Organoleptic properties		
			Texture	Colour	Smoky odour
E_1	6.51	107.5	Crisp	Yellowish black	Medium
E_2	6.60	115.0	Brittle	Yellowish black	Medium
E_3	6.50	112.8	Brittle	Golden yellow	Medium
E_4	6.82	110.6	Brittle	Blackish yellow	Medium
E_5	6.66	112.0	Crisp	Blackish yellow	Medium
E_6	6.42	106.0	Crisp	Blackish yellow	Medium

The values are mean of 6 observations for each sample

Table 2. Changes in bacterial and fungal population and moisture levels during storage of *Esomus danricus* (Mean ± SD of 16 observations for each sample)

Days	Moisture	Meterological data*		Visible fungal colony	TPC log cfu g^{-1}	TFC log cfu g^{-1}	*Staphylo-coccus aureus* log cfu g^{-1}	Feacal *Strepto-cocci* log cfu g^{-1}	MPN of Coliform log cfu g^{-1}
		Temp. (°C)	Humidity (%)						
0	7.34±2.62	19.95	73.5	Nil	5.65 ±0.7	2.78 ±0.44	3.00 ±0.50	2.48 ±0.32	2.66 ±0.20
15	3.15±1.50	22.32 ±2.43	71.60±6.90	Nil	4.30 ±0.21	2.60 ±0.20	2.36 ±0.13	2.10 ±0.27	2.38 ±0.15
30	4.12±0.70	24.46 ±1.74	66.32±13.3	Nil	4.00 ±0.40	2.43 ±0.12	2.32 ±0.10	2.00 ±0.13	Nil
45	9.43±1.50	25.30 ±1.50	76.50±10.5	Nil	3.32 ±0.18	1.60 ±0.18	1.70 ±0.15	1.90 ±0.10	Nil
60	9.60±0.50	26.10 ±1.20	85.70±6.90	Nil	3.38 ±0.18	1.70 ±0.20	Nil	Nil	Nil
75	10.72±0.20	26.12 ±1.22	86.30±4.85	Nil	3.47 ±0.20	1.85 ±0.50	Nil	Nil	Nil
90	12.15±1.00	25.85 ±1.30	86.60±5.40	+	3.48 ±0.12	2.00 ±0.32	Nil	Nil	Nil
105	10.95±0.90	26.49 ±0.75	82.07±5.20	+	3.63 ±0.35	3.18 ±0.24	Nil	Nil	Nil
120	8.68±1.00	25.85 +0.68	78.09±4.78	++	4.54 ±0.54	3.68 ±0.37	Nil	Nil	Nil

+ a few scattered colonies, ++ many isolated colonies,
* Temperature and Humidity were recorded in the laboratory

The initial load of *Staphylococcus aureus* and faecal *Streptococci* gradually decreased during storage and after 60 days, they were absent in the samples. Coliform, disappeared after 30 days of storage. The chemical compounds deposited on the fish during smoking may be responsible for the decline in the population of bacteria. In the present study *E. coli* and *Salmonella* were not detected in any of the smoked fish analysed.

Table 3. Fungi isolated during the storage of smoked Esomus danricus

Days	Fungi
0	**A. niger, Mucor, Penicillium* sps., Sterlie mycellium
15	**A. niger, Curvularia sengalesis, Penicillium, Phomopsis vexans*
30	**A. niger, Penicillium* sps., *A. candidus*
45	**A. niger, Penicillium* sps., *A. candicus*
60	**A. niger, A. candidus, Penicillium* sps.
75	*A. niger, A. canchidus,* white sterile mycelia
90	*Penicillium* sps., **A. flavipes, Trichoderma logibranchiatum, A. candidus*
105	*A. candidus, *P. funiculosum, P. rubrum, P. chrysogenum, A. flevipes*
120	**A. flevipes, Penicillium sps., A. nidulans*

* - Dominant fungi

Fungi isolated during storage of smoked *E. danricus* are listed in Table 3. Moulds are one of the important causes of spoilage of any kind of food. The most common and obvious causes of spoilage are *Penicillia* and *Aspergilli* (Frazier & Westhoff, 1978). In the present analysis xerophilic fungi like *Aspergillus candidus, Aspergillus fumigatus, Aspergillus niger, Aspergillus sydowii* and *Penicillium chrysogenum* were observed. Pitt (1975) pointed out that all the xerophiles i.e., *Aspergillus* (including *Eurotium* and *Emericella*) and *Penicillium*, were mycotoxic.

In April, May and June, the dominant fungi was *A. niger.* On further storage there was a decline in its population and was not detected during the final stage. The successor species was *Aspergillus flavipes.* At the stage when the sample was unacceptable, the fungi observed were *A. flavipes, Penicillium* sp. and *Aspergillus nidulans.*

From the results of the present study it is concluded that the conditions under which smoked *E. danricus* is stored may lead to the spoilage of the fish by bacterial and fungal attack. A low moisture level of the product and proper storage conditions will help to improve the quality.

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