

Bacterial Profile of Fresh and Spoiled Fish Mince from *Johnius dussumieri* at Refrigerated Storage

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Fish mince from *Johnius dussumieri* was examined for the changes in the bacteriological profile in fresh and spoiled condition at refrigerated storage ($4\pm 1^{\circ}\text{C}$). The percentage composition of bacterial flora was found to vary with fresh fish, fresh mince and spoiled mince. *Acinetobacter* and *Aeromonas* which were dominant in fresh fish decreased drastically upon mincing, washing and storage. In fresh mince 71.0% of the bacterial population comprised of gram positive group of which *Micrococcus* was the dominant. Flora of the spoiled mince was dominated by gram negative group (80.0%) comprising mainly of *Vibrio* followed by *Pseudomonas*.

Minced fish has been an important ingredient for the production of variety of seafood products in many countries. It offers several advantages such as better yield, easiness in incorporation of stabilizers, flexibility in product preparation and suitability for blending. Moreover, underutilized trash fish could be made use of as food rather than feed. Maceration of tissues during the process of mincing leads to an increased rate of chemical reactions making the minced fish a better medium for bacterial growth than the intact raw materials. The present study was carried out to investigate the effect of mincing and storage on the bacteriological quality of fish mince from *Johnius dussumieri* and the changes in the bacterial profile of fresh and spoiled fish mince at refrigerated storage ($4\pm 1^{\circ}\text{C}$).

Materials and Methods

Fresh sciaenid, *Johnius dussumieri*, procured from Tuticorin Fisheries Harbour was washed thoroughly and kept overnight in refrigerator. The fish were then headed and eviscerated, washed and filleted. The meat was removed manually from the fillets, chopped and thoroughly washed. About 200 g each of chopped meat was placed in glass beakers, covered loosely with aluminium foil and stored in a

refrigerator ($4\pm 1^{\circ}\text{C}$). Samples were drawn and analysed for biochemical and bacteriological changes on alternate days.

Moisture, total nitrogen, fat and ash were determined according to methods of AOAC (1975). Total volatile base nitrogen (TVBN) and trimethyl amine nitrogen (TMAN) were determined by Conway micro diffusion method (Beatty & Gibbons, 1937). Total Plate Count (TPC) on Plate Count Agar and Proteolytic Count (PC) on Skimmed Milk Agar were estimated as described in APHA (1976). Bacterial cultures were isolated randomly from fresh fish and fish mince on the zero and eleventh day of storage, purified and maintained on nutrient agar slants. All the isolates were identified upto generic level based on their morphological and biochemical characteristics as per the scheme described by Lochavallier *et al.* (1980) and Surendran & Gopakumar (1981).

Results and Discussion

The raw material characteristics of *Johnius dussumieri* are presented in Table 1. There was much variations in proximate composition and Total Plate Count between fresh fish and fish mince. Filleting, chopping and washing increased the bacterial count by more than one log unit. A similar

change in the composition and quantity of bacterial flora in filleting of fish (Gillespie & Macrae, 1975), in mechanical deboning of fish (Raccach & Baker, 1978) and in fish mince (Blackwood, 1973) had been reported.

Table 1. *Raw material characteristics*

Parameters	Fresh fish	Washed Fish mince
Moisture, %	73.510	82.225
Protein, %	17.625	12.940
Fat, %	7.260	3.380
Ash, %	1.141	0.670
Total Plate Count g ⁻¹	1.51x10 ⁵	3.50x10 ⁶

The biochemical and bacteriological changes in the fish mince during storage are presented in Table 2. The initial TPC and PC of the fish mince were of the order of 3.60x10⁶ and 1.0x10⁴/g respectively. Proteolytic count increased to 2.60x10⁶/g in 24 h. Over a period of 11 days, the TPC and PC changed to 6.05x10⁹ and 4.10x10⁹/g, an increase of more than 3.0 and 5.0 log units, respectively. The TMAN reached a value 5.51 mg/100g in one day storage from an initial value of 1.38 mg/100 g while TVBN increased to 31.79 mg/100g in 5 days storage. However, fish mince showed no

sign of spoilage upto 5 days inspite of high TMAN and TVBN values.

The changes in the bacterial profile of fresh fish, fresh and spoiled mince are presented in Table 3. The fresh fish and fresh mince had more diverse bacterial flora than the spoiled fish mince. The gram negative bacteria constituted about 67.0% of the total population in fresh fish as against 84.0% reported for sardine (Karthiyani & Iyer, 1967) and 80.0% reported for mackerel (Surendran & Iyer, 1976). *Acinetobacter* and *Aeromonas* were the dominant genera in fresh fish each comprising 24.0% of the total population. In the case of gram positive bacteria, *Staphylococcus* (12.0%) and *Micrococcus* (10.0%) were dominant. The process of filleting and meat removal altered the composition of the bacterial population substantially. The gram positive bacteria constituted about 71.0% of the total population in fresh mince of which *Micrococcus* (26.0%) was dominant followed by *Bacillus* and *Staphylococcus* each constituting 16% which clearly revealed the extent of contamination during handling and processing. There was an increase in *Pseudomonas* (17.0%) and a drastic reduction in *Acinetobacter* and *Aeromonas* groups.

In the case of spoiled mince *Alcaligenes*, *Micrococcus*, *Moraxella* and *Corynebacterium*

Table 2. *Biochemical and Bacteriological characteristics of fish mince*

Storage period, Days	TPC, g ⁻¹	PC, g ⁻¹	TPC/PC	Percentage of proteolytic organisms	TMAN, mg/100 g	TVBN, mg/100g
0	3.60x10 ⁶	1.00x10 ⁴	1.64	61.0	1.38	7.19
1	8.30x10 ⁶	2.50x10 ⁶	1.08	92.0	5.51	17.23
5	1.82x10 ⁷	6.45x10 ⁶	1.07	94.0	20.73	31.79
7	4.30x10 ⁷	2.35x10 ⁷	1.04	96.0	25.92	40.32
9	3.40x10 ⁹	1.76x10 ⁹	1.03	97.0	32.70	76.30
11	5.05x10 ⁹	4.10x10 ⁹	1.02	98.0	36.12	116.52

Table 3. *Bacterial profile of fresh fish, minced fish and spoiled mince*

Micro organisms	Fresh fish	Fish mince	
	%	fresh %	spoiled %
<i>Acinetobacter</i>	24.00	3.00	2.00
<i>Aeromonas</i>	24.00	3.00	7.00
<i>Alcaligenes</i>	2.00	3.00	ND
<i>Arthrobacter</i>	2.00	7.00	9.00
<i>Bacillus</i>	7.00	16.00	9.00
<i>Corynebacterium</i>	2.00	6.00	ND
<i>Enterobacteriaceae</i>	7.00	ND	5.00
<i>Micrococcus</i>	10.00	26.00	ND
<i>Moraxella</i>	2.00	ND	ND
<i>Pseudomonas</i>	3.00	17.00	21.00
<i>Staphylococcus</i>	12.00	16.00	2.00
<i>Vibrio</i>	5.00	3.00	45.00

ND-Not detected

were not encountered. A gradual decrease and in some cases disappearance of originally present constituent genera on storage were reported by Surendran & Gopakumar (1981, 1982), and Gowda & Karunasagar (1985). The incidence of Gram negative bacteria was higher of which *Vibrio* (45%) and *Pseudomonas* (21%) were dominant. Several authors reported the dominance of *Pseudomonas* at the time of spoilage and in spoiled fish (Surendran & Gopakumar, 1981; 1982; Gowda & Karunasagar, 1985). The presence of *Vibrio* sp. as a component of late spoilage flora in ice stored mackerel (Surendran & Gopakumar, 1982) and in tropical white prawn, *Penaeus indicus*, at 4°C (Chandrasekaran *et al.*, 1985) have been reported. The members of *Vibrio* are

reported to be an obligate psychrophile with optimum temperature between 0 and 5°C (Anand & Rudrasetty, 1977) and to produce TMAN from TMAO (Fuji *et al.*, 1977, Gowda & Karunasagar, 1985, Chandrasekaran *et al.*, 1985, 1987). The generic distribution of the most commonly occurring fish spoilage bacteria though extremely variable, follows a definite "generic succession" during spoilage (Castell & Anderson, 1948).

The shelf life of fish mince was reported to be 5 days at 2°C with a corresponding TPC of $1.0\text{--}5.0 \times 10^8/\text{g}$ (Raccach & Baker, 1978). In the present study though the TPC of the fish mince was well within 7.0 log units upto 7th day the sample exceeded the acceptable level of TVBN (30 mg/100g, on 5th day and TMAN (5.0 mg/100g) on 1st day. The sharp increase in TMAN and TVBN values could be mainly due to the proteolytic action of bacteria in fish mince as the ratio between the TPC and PC reached almost unity and 98% of the total population are of proteolytic in nature and to certain extent by TMAO reductase which may play a role in minced products.

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