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Bacteriological quality and heavy metals in edible meat portion of Japanese threadfin bream (*Nemipterus japonicus*)

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One hundred and fifty samples of Japanese threadfin bream (*Nemipterus japonicus*) were screened for bacteriological quality and for content of heavy metals. Out of 150 samples, 40.7% had the count above the stipulated limits for TVB (5.7 log cfu/g.) The samples harboured 28.7% of faecal coliforms which were present in the range of 2-3 log cfu/g, while 32 and 6% of the samples contained 3-4 and ≥5 log cfu/g of the sample. Fecal *Streptococci* was present in 40% at 2-3-log cfu/g and 29.3% at 3-4 log cfu/g. The samples contained coagulase positive *Staphylococci*, *E. coli*, *Salmonella* and *V. cholerae* (Non 01) at 14.7, 44, 10 and 14%, respectively. The samples showed a significant decrease in TVB (3-4 log cfu/g of the sample), faecal coliforms and faecal *Streptococci* during 2 months storage at −18±1°C. Pathogenic bacteria were also not present after the 2 months storage. The samples were free from hazardous levels of heavy metals, like Cd, Cu, Hg and Pb.

Keywords: Threadfin bream, *Nemipterus japonicus*, Bacteria, Pathogens, Heavy metals

One of the important species of threadfin breams caught in Southeast Asian region is *N. japonicus*. Because of white colour, smooth texture, and strong gel forming ability of the fish meat, threadfin bream is also widely used as raw material for Japanese "kamaboko" and surimi crabsticks (kani-kama) (Morrissey 2000). In the studies on storage behaviour of frozen fish fingers from croaker and perches, a decrease in the bacterial counts in all fish fingers was seen throughout storage (Lakshminatha Reddy et al 1992). Freeze-dried surimi powder from *N. japonicus* had superior nutritional properties to oven-dried surimi (Huda et al 2000). Threadfin bream was found to be the best source for surimi powder production, followed by purple-spotted bigeye and lizardfish (Huda et al 2001). These studies indicated that *N. japonicus* is a good candidate species for surimi development.

Several common types of seafood bacteria can be present and are retained during surimi processing (Himelbloom et al 2000). The microbiological quality analyses of product belonging to specific variety of fish and the data generated are useful to understand the inherent lacunae associated with the same. This will further help to identify and attend to problems of quality assurance and up-gradation. Analyses of fish for toxic metals such as Pb, Cd, Cu and Hg are important from a public point of view (Prasad et al 1990). Information on the concentration levels of havy metels in this particular variety of fish is scanty. Keeping in view these points the present study was undertaken to assess the bacteriological and chemical quality of one single variety of fish intended for surimi.

Materials and methods

Each sample of frozen fish meat represent one truckload and all the samples belong to single variety of fish, i.e., Japanese threadfin bream (*Nemipterus japonicus*). Individual sample was collected from different frozen fish meat blocks (from one truck), selected at random and from each block the frozen fish meat was drawn from 4 sides and from middle portion in sterile conditions. The composite represents one of the triplicates. Depending on the date of processing all the truckloads were divided into 10 batches. The sampling was planned to compare the bacteriological quality within and between the batches. All the samples were brought to the laboratory in sterile containers containing ice for immediate analyses.

The water and ice samples used for processing were collected at every batch in sterile condition for bacteriological analyses. For water samples, additional sets of plates were incubated at 22°C for 4 days. Faecal coliforms and *E. coli* were determined by Most Probable Number (MPN) 5 tube method employing MacConkey broth in single and double strength wherever necessary.

Bacteriological analyses: Suitable dilutions of the fish meat samples in 0.1% sterile peptone water were surface plated on plate count agar, violet bile salt glucose agar, Kanamycin Aesculin Azide agar and Baird Parker agar for the enumeration of TVB, faecal coliforms, faecal *Streptococci* and *Staphylococci* according to the standard procedures (ICMSF 1978, Mossel et al 1978). Inoculated plates were incubated at 37°C for 24 h for TVB and faecal coliforms, 30 h for *Staphylococci* and 48 h for faecal *Streptococci*. In case of faecal *Streptococci* the incubation period was extended to 96 h till the very small colonies grew to a larger size for the count. Further, suspected colonies of *Staphylococcus aureus* were confirmed by coagulase positive test using rabbit plasma (Sanjeev and Surendran 1996).

Salmonella: The frozen fish samples of 25 g each were weighed aseptically, triturated thoroughly with 225 ml of pre-enrichment in buffered peptone water, pH 7.2±0.20 (USFDA 1995) and the suspension was incubated at 37°C for 24 h. The incubation period was doubled (48 h) when the sampling was carried out with frozen stored samples, to facilitate the growth of injured cells. Suspected colonies from the selective media plates were picked up, and streaked on pre-poured MacConkey agar plates. From MacConkey agar, transparent and colourless colonies were taken on to nutrient agar slopes (USFDA 1984) for further identification.

The slant cultures on nutrient agar slopes, obtained as above, were subjected to morphological, biochemical, fermentation and serotyping tests (USFDA 1984).

Vibrio cholerae: Fish meat samples were screened for *Vibrio cholerae* by the method of Madden et al (1984) and the Tokyo quarantine method (Prasad and Rao 1994). Identification of the suspected *Vibrio cholerae* isolates was carried out employing standard methods (USFDA 1984).

The dehydrated media and reagents employed in the present study were of Hi Media (Mumbai, India) make and chemicals were Qualigens (India) make. The serological identification of suspected *Salmonella* and *V. cholerae* isolates was carried out by using the sera obtained from Central Research Institute, Kasauli (HP), India.

Storage: After drawing the samples for analyses of different quality parameters the same batches were kept in frozen storage at -18±1°C for further studies.

Heavy metals: Fish samples of different batches, at random were selected for heavy metal analyses. After bringing samples to the laboratory, extractions were carried out with edible meat portion according to wet digestion method of AOAC (1975). The extracts were analyzed for Cd, Pb and Cu using Atomic Absorption Spectrophotometer (GBC Model 902, Australia). The total Hg in the digested extracts was determined by Cold Vapor Atomic Absorption Spectrophotometer (Mercury analyzer- Model MA 5800 A ECIL, India).

The statistical analyses of the data were carried by standard methods (Visweswara Rao 1996). Sampling was done in triplicates.

Results and discussion

The TVB counts varied from 5.4 to 6.4 log cfu/g of the sample (Table 1). Among the samples, 40.7% had the count above the stipulated limit of 5.7 log cfu/g. Variation is seen in the number of TVB between batches ($p<0.05$) but not between the samples ($p>0.05$).

Out of 150 truckloads screened, 82% of the samples were positive for coliforms (Table 1). Among the samples 28.7% showed faecal coliforms in the range of 2-3 log cfu/g, while 32 and 6% of the samples contained 3-4 and ≥5 log cfu/g of sample. Out of 150 samples screened 13% of them did not harbour faecal *Streptococci* and 53% of the samples had counts below 3 log cfu/g (Table 1). The occurrence of these bacteria is seen in 40% at 2-3 log cfu/g and 29.3% at 3-4 log cfu/g. This reveals that though majority of the samples harboured these bacteria, the number was less. Significant variation was observed in the occurrence of these groups of bacteria between the batches at $p<0.05$ but not between the samples. Seasonality played an important role in surimi made from 3 different varieties of fish in Mexico resulting in higher microbial loads in the products prepared in warm temperatures of summer (Hernandez et al 1997). The present study was carried out in tropical conditions, during summer and the same could be the reason for high initial bacterial counts prior to frozen storage.

Table 1. Microbiological quality of meat from *Nemipterus japonicus*

Batch No.	Total viable bacteria, log cfu/g	Faecal coli-forms, log cfu/g	Faecal *Strepto-cocci*, log cfu/g	*Coagulase +ve Staphylococci	*E. *coli*	*Salmo-nella	*V.cholerae* (Non 01)
1	6.4±0.79	5.5±0.93	4.1±1.14	26.7	80.0	ND	ND
2	6.1±0.54	3.1±0.47	2.2±0.93	33.3	53.0	6.6	ND
3	5.6±0.79	3.1±0.94	4.1±1.06	26.7	13.3	ND	6.6
4	5.7±0.39	3.0±0.52	3.1±0.50	ND	26.7	ND	ND
5	5.7±0.49	2.7±0.39	3.7±0.91	ND	13.3	ND	ND
6	5.4±0.59	2.9±0.48	3.2±0.53	ND	80.0	ND	13.3
7	5.5±0.87	3.4±1.59	3.4±0.46	6.7	20.0	ND	ND
8	5.4±0.98	4.3±0.85	3.5±0.57	6.7	33.3	26.7	20.0
9	5.7±0.82	3.8±0.55	3.6±0.66	20.0	60.0	53.3	13.0
10	5.9±0.34	4.7±0.84	3.8±0.67	46.7	53.3	13.3	80.0

Each batch represents 15 truck loads of fish and from each truck load three samples were drawn (n=3). * Occurrence in%. ND: Not detected

The ratio of occurrence of faecal coliforms and faecal *Streptococci* in the present study varied from 0.76 to 1.39 (Table 1). The ratio indicated that the source of these bacteria was not necessarily of human origin (Prasad and Seenayya 1998). Different steps involved in surimi processing offer congenial conditions for growth and contamination of bacteria. Difficult to clean equipment also perpetuates the retention of contaminated fish particles (Himelbloom et al 2000).

Changes in bacterial load during frozen storage: Significant reduction in TVB counts (3-4 log cfu/g) at the end of the storage period (Table 2) was noticed. At the end of 2 months storage at -18±1°C, the counts were below 1 log cfu/g in all the samples. Similar trends were also seen with faecal coliforms and faecal *Streptococci.*

The decreasing pattern of total bacterial counts during frozen storage in the present study is in agreement with the observations made by earlier investigators (Elliot 1987, Himelbloom et al 1991) where the declining tendency of microbial burden in frozen surimi was seen at an average of 39% from the original total bacterial counts of prefrozen surimi. Contrary, a study on surimi showed no change in bacterial load during frozen storage (Hernandez et al 1997). The study on pink perch, *N. japonicus*, showed that the TVB decreased to 2.3 x 10^4/g in FC during storage for 12 weeks (Raju et al 1999). These differences are attributed to the sensitivity of specific bacterial flora to freeze damage or the variety of cryoprotectants and concentrations used in surimi (Himelbloom et al 2000). More than 80% of surimi imported to Italy contained <3 log cycles of TBC, whereas,

Table 2. Changes in microbiological counts (log cfu/g) of meat from *Nemipterus japonicus* during frozen storage at -18±10°C

	Storage period, month	
	0	1
Total viable bacteria	6.4±0.37	2.9±0.33
Faecal coliforms	4.2±1.39	2.0±0.50
Faecal *Streptococci*	4.0±1.2	2.2±0.53

Values are mean ± SD of 15 samples. Each sample represents a truck load of fish. After 2 months storage, the counts were <1.0 log cfu/g for all samples.

fecal coliforms and *Streptococci* were <2 log cfu/g of the sample (Ercolini et al 1995). Out of 150 samples, one showed the presence of *Staph. aureus*. The findings of the present study correlated with these results.

Pathogenic bacteria: The occurrence of coagulase positive *Staphylococci*, *E. coli*, *Salmonella* and *V. cholerae* (Non 01) was 14.7, 44, 10 and 14%, respectively (Table 1). The samples selected for frozen storage were also screened for specific pathogens based on results of initial tests. None of the pathogens were recovered even though the pre-enrichment and enrichment incubation period were enhanced by 24 h to facilitate the growth of bacteria injured due to extreme low temperatures. The possibility is that initial numbers could be far less and subzero temperatures have bactericidal effect on the pathogens. Majority of the tropical fish are known to harbour environmental strains that are sensitive to drastic changes in the temperatures. *Staph. aureus* and *Salmonella* were not found in frozen Mexican surimi (Hernandez et al 1997). It was also observed that pathogenic bacteria were not detected in frozen and refrigerated Spanish surimi (Castillo et al 1995).

Heavy metals: All the samples of fish analyzed contained very low levels of Hg ranging from 15-20 ng/g (Table 3) of wet tissue, which are far below the tolerance limit of either 0.5 or 1 ppm of total Hg. The results of Hg concentrations correlated with the earlier observations (Ramamurthy 1979) that reported very low levels of total Hg ranging from 5-93 ng/g on wet weight basis in different varieties of fish and shell fish from different coastal regions of India. In a study on heavy metals in fish from upper East coast of India, it was reported that total Hg in *N. japonicus* was 5 ng/g of the sample (Prasad et al 1990). The range of concentration of Cd in fish muscle varied from 0.35 - 1.07 µg/g with an average of 0.74 µg/g (Table 3). These levels are in agreement with earlier reports (Prasad et al 1990). Considering the requirement of fish meat as 30 g/day in a balanced diet and the provisional weekly tolerable intake of Cd as indicated by WHO/FAO (1972), the critical concentration in seafood required to be 2.4 µg/g. In the present study except two samples, all others examined showed the concentrations of less than 1 µg/g.

Table 3. Heavy metals in edible meat from *N.japonicus*

Hg, ng/g	0.018±0.002
Cd, ppm	0.75±0.27
Pb, ppm	3.68±1.064
Cu, ppm	1.70±0.97

Each value is a mean ± SD (range) of 8 samples

The Pb concentrations in the fish samples varied from 2.25-5.28 µg/g with an average of 3.60 µg/g (Table 3). Considering a provisional tolerable weekly intake of 3 mg of Pb prescribed by WHO expert committee on food additives, 14.30 µg/g becomes the critical level for Pb concentration in fish muscle. The levels of Cu in the fish samples screened ranged from 0.09 to 3.27 µg/g (Table 3) with an average of 1.70. It was shown that highest concentration of Cu found in the muscle of fish was 3.119 µg/g in *N. japonicus* (Prasad et al 1990). Cu is one of the metals categorized as micronutrient and was reported to be toxic in quantities of 100 mg (McKee and Wolf 1963). One of the important safeguards in the consumption of aquatic foods containing high levels of Cu is the unpalatability of foods containing concentrations of even 5 to 7 ppm (Portman 1970).

Conclusion

Potable water and ice samples wherever fish meat comes in contact were free from hygiene indicator and pathogenic bacteria. This indicated the possible source of contamination was while post-harvest handling and during different stages of processing. Majority of the bacterial counts decreased to a considerable level during frozen storage and the pathogens were also not present. The samples were also free from heavy metal contamination. In view of the industrial development, especially in recent years, continuous monitoring is necessary.

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