

Original Research Article

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Quality Assessment and Comparison of Three Different Types of Fish Retail Outlets of Navi Mumbai, Maharashtra, India

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ABSTRACT

In India, fish consumers prefer three types of retail markets viz., local dry fish market, local fresh fish market and supermarket wherein no stringent food safety measures are followed and monitored. Hence, the present study was planned to analyze food safety parameters for different outlets by collecting equal numbers of fish samples from all three markets of Navi Mumbai region. Proximate composition, biochemical and microbiological parameters were evaluated. Antibioqram for the isolated *Staphylococcus aureus* and *Escherichia coli* from these fishes was studied. In dry fish market, most of the fish had higher total volatile basic nitrogen (TVB-N) and formaldehyde than permissible level. In present evaluation, formaldehyde content of fishes from different markets exhibited a higher percentage (1.03-1.93 mg%) than the recommended value. Local retail fresh fish market samples had higher levels of Aerobic Plate Count (APC), *E. coli* and *S. aureus*. One sample from dried fish market exceeded the permissible limit of APC (2,36,000 cfu/g). Supermarket samples contained all the microbial and biochemical levels within the limit but exhibited higher formaldehyde content. Multiple drug resistant bacteria also existed in both local and supermarket samples. Monitoring authorities of India need to monitor the quality of fish in different markets at regular interval and strict action may be taken for seafood safety.

Keywords

Supermarket fish, Dry fish, Biochemical quality, Microbiological quality, Formaldehyde

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Introduction

Fish is nourishment food in human diet supplying proteins, lipids, essential amino acids, vitamins, minerals and other nutrients. Hence, it has been attracted human consumption due to its rich source of vital nutritional components. Since, the fishes are highly perishable commodity various

preservation techniques such as chilling, freezing, canning, salting drying, etc. were followed to for the fish preservation. Among the preservation techniques, sun drying is important conventional and cheaper method of fish keeping. Approximately 20% of marine fish catch by artisanal fishing operations is subjected to sun drying operations and sold in local markets (Mukharjee *et al.*, 1990).

External appearance and sanitary quality of fresh and sun dried fish products in domestic market does not meet the standard for human consumption as a result of improper handling practices, unhygienic processing leading to contamination causing spoilage. Stringent quality control tests are available for fish and fishery products meant for export, no such control exists for fish available in markets resulting in availability of poor quality and contaminated fish in markets which pose a serious health hazards to the consumers.

Formaldehyde is chemical having a potential antimicrobial activity but, it is highly carcinogenic in nature. Numerous reports are available about the addition of formaldehyde to the fish to retain the freshness of the fish (Bianchi *et al.*, 2007; Noordiana *et al.*, 2011; Joshi *et al.*, 2015). But, small amount formaldehyde has been produced from the fish and shell fishes due to metabolism. Numerous, reports are available regarding the retail market quality of fishes (Iyer *et al.*, 1986).

But, there no reports are available about the comparison of all three markets of Navi Mumbai region. Hence, this study was taken up for quality evaluation of fry fish, local market fresh fish and super market fish from Navi Mumbai.

Materials and methods

Raw material

Five numbers of different varieties of fish were collected from each market of dry fish market, local retail outlet and super market of Navi Mumbai region. Dried fish samples were brought to the laboratory in aseptic condition by keeping them in polythene bags whereas, fresh fish samples from local retail outlet and super market were brought to laboratory by keeping them in ice in 1: 1 ratio. All the fish samples were analyzed immediately.

Proximate composition and biochemical analyses

Proximate composition analysis containing moisture, crude protein, crude fat and ash content as well as salt content, peroxide values and formaldehyde content were evaluated as per the AOAC (2005). The moisture content of meat was estimated by hot air oven method. The crude protein content of the meat was determined by estimating total nitrogen content by Kjeldahl method. The crude fat content of meat was estimated by Soxhlet extraction method whereas ash content was calculated by keeping the samples in muffle furnace at 550 ± 10 °C for 5 - 6 hrs. The crucibles were removed and cooled in desiccator and weighed. Ash content was calculated from the weight difference of crucible. Tri-methyl amine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) values were estimated by Conway micro diffusion method (Beatty and Gibbons, 1937). A method described by Tarladgis *et al.*, (1960) was followed for determination of thiobarbituric acid (TBA) values.

Microbiological analyses

A method described in FAO (1992) was used for determination of Aerobic Plate Count (APC) and *Staphylococcus aureus*, *Escherichia coli* and Enterobacteriaceae counts were determined as per the method described in BAM (2002). A method given by Koutsoumanis and Nychas (1999) was utilized for determination of *faecal streptococci*.

Antibiogram was carried out as per the standard disk diffusion assay with 0.5 McFarland standard and break points interpretation were estimated with the help of CLSI manual (CLSI, 2012; 2014). All the analyses for biochemical quality parameters were done in triplicate and the results are expressed as mean \pm standard deviations.

Results and Discussion

Proximate composition and biochemical quality

Dried fish

Proximate composition and biochemical quality of dried fish and prawns used in the study is presented in table 1. Moisture content varied from 15.22-20.49%. Joseph *et al.*, (1986) have recorded a minimum of 17% moisture along Tamilnadu coast and 23.7% along Maharashtra coast. As per the recommendation of IS 14950:2001, moisture limit for the dried Bombay duck is 15%. Similarly for the dried prawns is 20%. MPEDA has specified 35% as the maximum moisture level for dried ribbon fish (MPEDA, 2002). All the samples had a protein content of 65.13-78.67%. Solanki and Sankar (1988) have recorded a protein content of 61% and a lipid content of 9.20 mg% in salted and dried sole. Ash content varied from 11.26-14.82%. The higher values of ash content in dry and wet salted fish may be attributed to the salt content. The ash content in salted products can be as high as 30% in heavy salted fish. TVB-N values varied between 47.60-196.01 mg%. High TVBN value of commercial samples has been reported by Vijayan and Surendran (2012). Connell (1980) has suggested a limit of 200 mg% TVBN for salted and dried fish. Joseph *et al.*, (1986) have reported TVBN content up to 72.7% in salted anchovy and 105.0% in salted sole. The recommended level of the TMA-N value for human consumption is 10-15 mg/100 g. Dried prawns had TMA-N value within the acceptable limit. However, all the fish samples had beyond the limit (22.40-36.41 mg%). PV usually measures initiation phase of oxidative spoilage which is non sensory method for rancidity evaluation. A peroxide value of more than 20 meq O₂/kg oil for fish usually gives bad smell and rancid taste (Reza, 2006). In this study, PV values for

fish were within acceptable limit (1.80- 14.53 meq O₂/Kg). All the samples had acceptable level of TBA values (0.16-0.77 mg malonaldehyde/kg). Formaldehyde finds its usage as a food and fish preservative and as a disinfectant for food containers. US Environmental Protection Agency (EPA) suggested 0.2 mg/kg as oral reference dose for formaldehyde. Food act and food regulation authorities from Malaysia in 1985 suggested maximum permissible limit of formaldehyde as 5 mg/kg in fish and fishery products. In present evaluation, formaldehyde content of fishes from different markets exhibited a higher percentage (1.03-1.93 mg %) than the recommended value.

Local market and super market fish

Proximate composition and biochemical quality of fresh fish of local and super market is depicted in table 2 and 3. Most of the fishes had moisture content in the range of 72.11-79.47%. Bombay duck had higher moisture of 89.80%. Protein content ranged from 13.87-24.96%. Results suggested that fish from different markets had good quantity of proteins, making them suitable as a part of food for health. Seafood protein content ranges from 17 to 21% depending on the fish species (Sriket *et al.*, 2007). All samples had pH range of 6.36-7.35. As per Mendes (2005), the fresh fish exhibits TVB-N value of < 20 mg N/ 100 g. Moreover, values up to < 30 mg N/100 g are considered as acceptable. However, values beyond 40 mg N/100 g sample are considered as unsuitable for human consumption. Accordingly, none of the sample from retail market and super market crossed the rejection limit. As per the recommendation by Connell (1995), TMA-N values up to 10-15 mg N/100g are suitable for fish consumption. All the samples exhibited values within the acceptable limits. PV and TBA are established for determination of lipid oxidation in seafood. Romeu-Nadal *et al.*, (2006) reported

that Pacific white shrimp during ice storage exhibited PV values within the acceptability limit for fats and oils (10 meqO₂/ kg). Connell (1995) revealed that TBA beyond the limit of 1-2mg of MDA/kg lead to development of undesirable odour. Values indicating lipid oxidation (PV and TBA) were found to be below the rejection level. Formaldehyde content in Bombay duck had higher level (1.32 mg%) than other fishes (0.01-0.50 mg%) collected from local fish market. Formaldehyde content of super market fish samples ranged between 0.55 to 0.75 mg% whereas the local fish market samples showed formaldehyde content in the range of 0.01-1.32 mg%.

Microbiological quality of fish

Dried fish

In India, considerable amount of fishes are dried and handled in unhygienic way; hence in addition to the recommended microbial parameters, Faecal streptococci (FS) and Enterobacteriaceae (EB) were tested to analyze quality of the dry fishes. As per the Indian standard i.e., IS 14950:2001, the upper limit of the APC of dry fish is 1,00,000 cfu

g⁻¹. In the present study, among the five samples of dried fish, one sample had exceeded the permissible limit of APC i.e., 2,36,000 cfu.g⁻¹ (Table 4). Even though, there was no visible fungal growth in all sample; fungal count of one sample was more than 1000 cfu.g⁻¹. Presence of mycotoxins producing fungi in dry fishes also reported in Indian dry fishes (Sivaraman *et al.*, 2016). Consumption of such mycotoxins may give illness to the consumers. Enormous reported are available about the poor microbiological quality of fishes from dry fish markets of India and worldwide (Sulieman *et al.*, 2014; Lilabati *et al.*, 1999; Prakash *et al.*, 2011). It has been observed that higher level of enterobacteria and faecal streptococci (FS) in the collected dry fish samples; it may due the drying of the fish in the unhygienic surface area. There is no limit specified by the Indian or International standard for the level of Enterobacteriaceae and FS in dry fish. But, the elevated level of the Enterobacteriaceae and FS in the dry fish indicated the poor hygienic handling practices. Recently, the pathogenic FS were also reported in the fishes (Visnuvinayagam *et al.*, 2017). So, proper education and training has to be given to the dry fish processors to get a safe and superior quality dry fish.

Table.1 Biochemical quality of dried fish collected from dry fish market

Biochemical parameter	BD MH	BD GUJ	Ribbon fish (<i>Lepturacanthus savala</i>)	Acetes (<i>Acetes sp.</i>)	Kardi (<i>Palaemon tenuipes</i>)
Moisture (%)	19.46±0.02	17.26±0.01	15.22±0.02	19.46±0.02	20.49±0.01
Protein (%)	69.51±0.01	76.45±0.02	80.39±0.01	65.13±0.02	78.67±0.02
Fat (%)	8.78±0.01	1.66±0.03	19.58±0.02	3.12±0.01	7.02±0.01
Ash (%)	11.26±0.01	11.56±0.02	12.57±0.01	14.82±0.02	13.36±0.01
pH (%)	6.7 ± 0.01	6.83±0.01	6.86±0.01	7.44±0.01	7.57±0.01
TVBN (mg %)	196.01 ± 0.01	184.79±0.01	128.81±0.01	47.6±0.01	182.01±0.01
TMA (mg %)	36.41 ± 0.02	36.4±0.02	22.40±0.02	1.39±0.02	8.38±0.02
PV (millieq.O ₂ /kg)	1.86±0.02	3.50±0.03	4.46±0.01	7.28±0.03	14.53±0.01
TBA (mg malonaldehyde/ kg)	0.25±0.01	0.32±0.01	0.77±0.02	0.25±0.01	0.16±0.01
Salt content (%)	6.38±0.05	5.07±0.02	4.79±0.01	3.93±0.02	3.62±0.02
Formaldehyde (mg%)	1.26±0.02	1.17±0.01	1.03±0.01	1.93±0.01	1.31±0.01

Where, BD MH- Bombay duck from Maharashtra; BD GUJ- Bombay duck from Gujarat; n=3

Table.2 Biochemical quality of fishes collected from retail fish market

Biochemical parameter	Bombay duck (<i>Harpadon nehereus</i>)	Seer fish (<i>Scomberomorus commerson</i>)	Sardine (<i>Sardinella longiceps</i>)	Mackerel (<i>Rastrelliger kanagurta</i>)	Tilapia (<i>Oreochromis mossambicus</i>)
Moisture (%)	89.81±0.01	79.48±0.01	77.72±0.01	72.12±0.02	79.12±0.01
Protein (%)	13.87±0.02	18.65±0.02	18.15±0.02	21.35±0.02	18.06±0.02
Fat (%)	0.48±0.01	0.29±0.01	1.00±0.01	3.83±0.01	2.55±0.01
Ash (%)	0.90±0.02	1.25±0.01	1.52±0.03	1.25±0.03	1.16±0.02
pH	7.35± 0.01	7.04±0.02	6.65±0.02	6.36±0.01	6.84±0.01
TVBN (mg%)	2.50±0.01	2.11±0.01	5.58±0.02	5.6±0.01	5.60±0.02
TMA (mg%)	8.41±0.02	7.00±0.03	13.99±0.03	2.8±0.02	4.20±0.03
PV(millieq.O ₂ /Kg)	17.72±0.01	8.06±0.01	6.88±0.03	16.69±0.03	6.57±0.02
TBA (mg malonaldehyde/Kg)	0.46±0.02	0.33±0.03	2.21±0.02	12.66±0.02	0.27±0.01
Formaldehyde (mg %)	1.32±0.01	0.01±0.01	0.49±0.02	0.50±0.01	0.48±0.01

Table.3 Biochemical quality of fishes collected from super market

Biochemical parameter	Snapper (<i>Lutjanus johni</i>)	Catla (<i>Catla catla</i>)	Salmon (<i>Eleutheronema tetradactylum</i>)	Tuna (<i>Euthynnus alletteratus</i>)	Mackerel (<i>Rastrelliger kanagurta</i>)
Moisture (%)	77.29±0.01	78.06±0.02	76.46±0.01	72.01±0.02	77.02±0.01
Protein (%)	22.81±0.02	22.93±0.01	20.72±0.01	24.96±0.01	21.00±0.02
Fat (%)	0.81±0.01	0.82±0.01	1.14±0.02	0.19±0.01	1.55±0.01
Ash (%)	1.40±0.01	1.17±0.02	1.16±0.01	1.33±0.02	1.01±0.02
pH	7.11±0.02	6.83±0.01	6.71±0.01	6.01±0.03	6.86±0.02
TVBN (mg%)	2.80±0.03	4.20±0.01	4.20±0.02	16.80±0.01	11.20±0.01
TMA (mg%)	1.79±0.01	2.20±0.01	2.20±0.01	6.79±0.02	5.18 ±0.01
PV(millieq.O ₂ /kg)	29.50±0.01	3.47±0.02	6.82±0.03	48.72±0.02	8.26±0.01
TBA (mg malonaldehyde/kg)	0.02±0.03	0.10±0.01	0.50±0.02	0.92±0.01	2.77±0.01
Formaldehyde (mg %)	0.56±0.01	0.55±0.01	0.54±0.02	0.75±0.02	0.55±0.01

Table.4 Microbiological quality of dried fish collected from dry fish market

Name of the seafood items	APC (cfu/g)	Enterobacteriaceae	FS	Fungus
BD MH	4.52 x 10 ⁴	0	400	0
BD GUJ	2.8 x 10 ⁴	0	300	800
Ribbon fish (<i>Lepturacanthus savala</i>)	2.36 x 10 ⁵	400	300	0
Jawla (<i>Acetes sp.</i>)	1.8 x 10 ⁴	200	300	800
Kardi (<i>Palaemon tenuipes</i>)	2.16 x 10 ⁴	1200	300	200

Where, BD MH- Bombay duck from Maharashtra; BD GUJ- Bombay duck from Gujarat; FS – *Faecal Streptococci*, n=3

Table.5 Microbiological quality of fishes collected from local retail fish market

Name of the fish	APC (cfu/g)	<i>E. coli</i>	<i>S. aureus</i>	FS
Bombay duck (<i>Harpadon nehereus</i>)	1.80 x 10 ⁵	35	80	6
Seer fish (<i>Scomberomorus commerson</i>)	1.88 x 10 ⁵	27	110	7
Sardine (<i>Sardinella longiceps</i>)	2.62 x 10 ⁵	28	40	8
Mackerel (<i>Rastrelliger kanagurta</i>)	13.8 x 10 ⁵	20	60	5
Tilapia (<i>Oreochromis mossambicus</i>)	1.72 x 10 ⁵	27	70	9

APC: Aerobic Plate Count, FS: faecal streptococci count

Table.6 Microbiological quality of fishes collected from super market

Name of the fish	APC (cfu/g)	<i>E. coli</i>	<i>S. aureus</i>	FS
Snapper(<i>Lutjanus johni</i>)	1.08 x10 ⁵	3.6	60	2
Catla (<i>Catla catla</i>)	7.2 x10 ⁴	0	56	0
Salmon (<i>Eleutheronema tetradactylum</i>)	3.04 x10 ⁵	20	40	8
Tuna (<i>Euthynnus alletteratus</i>)	9.0 x10 ⁴	28	84	3
Indian Mackerel (<i>Rastrelliger kanagurta</i>)	6.4 x10 ⁴	20	34	6

APC: Aerobic Plate Count, FS: faecal streptococci count

Table.7 Antibiogram for *E. coli* of fish samples collected from local fish and super market

Name of the Antibiotics		Local fish market <i>E. coli</i> antibiogram			Super Market <i>E. coli</i> antibiogram		
		No. of Isolates	Resistant	Intermediate resistant	No. of Isolates	Resistant	Intermediate resistant
Amikacin	AK	15	0	0	6	0	0
Augmentin	AMC	15	5	1	6	0	1
Aztreonam	AT	15	0	0	6	0	0
Cefoxitin	CX	15	0	1	6	0	0
Cefpodoxime	CPD	15	0	0	6	0	0
Ceftazidime	CAZ	15	0	0	6	0	0
Ceftriaxone	CTR	15	0	0	6	0	0
Ciprofloxacin	CIP	15	0	0	6	0	0
Co-trimazole	COT	15	1	0	6	0	0
Colistin	CL	15	0	5	6	0	2
Gatifloxacin	GAT	15	0	0	6	0	0
Gentamicin	GEN	15	0	0	6	0	0
Imipenam	IMP	15	0	0	6	0	0
Levofloxacin	LE	15	0	0	6	0	0
Moxifloxacin	MO	15	0	0	6	0	0
Nalidixic acid	NA	15	0	0	6	0	0
Nitrofurantoin	NIT	15	0	0	6	0	0
Norfloxacin	NX	15	0	0	6	0	0
Ofloxacin	OF	15	0	0	6	0	0
Tobromycin	TOB	15	0	0	6	0	0

Table.8 Antibiogram for *S. aureus* of fish samples collected from local fish market and super market

Name of the Antibiotics		Local fish market <i>S. aureus</i> antibiogram			Super Market <i>S. aureus</i> Antibiogram		
		No. of Isolates	Resistant	Intermediate resistant	No. of Isolates	Resistant	Intermediate resistant
Erythromycin	E	18	0	0	13	1	0
Azithromycin	AZM	18	2	0	13	8	0
Clarithromycin	CLR	18	1	1	13	0	0
Penicillin G	P	18	1	0	13	1	1
Ampicillin	AMP	18	1	0	13	1	0
Amoxyclav	AMC	18	1	0	13	0	0
Oxacillin	OX	18	3	0	13	6	0
Methicillin	MET	18	4	1	13	5	0
Co-TrimoxazolCoe	COT	18	0	0	13	0	0
Gentamicin	GEN	18	0	0	13	0	0
Amikacin	AK	18	0	0	13	0	0
Ofloxacin	OF	18	0	0	13	2	1
Tetracyclin	TET	18	1	0	13	0	0
Cephalothin	CEP	18	1	0	13	0	0
Novobiocin	NV	18	0	0	13	0	0
Vancomycin	VA	18	2	0	13	0	0
Teicoplanin	TE	18	7	0	13	7	0
Linezolid	LZ	18	1	0	13	0	0
Chloramphenicol	C	18	1	0	13	0	0
Clindamycin	CD	18	1	0	13	1	0

Local fish market and super market fish

As per Indian standard recommendations, the limitation for APC in fish is less than 5,00,000 cfu/g, the same for *E. coli* is 20 cfu/g and for *S. aureus* is 100cfu/g (FSSAI, 2012; IS: 4780 – 1978; ICMSF, 1986). In the present study, among the five samples collected from the retail fish market, one sample contained higher level of the aerobic plate count (APC) than the recommended limit; four samples contained the higher *E. coli* level and one sample contained higher *S. aureus* than the recommended limit (Table 5). In total, all the samples were microbiologically unsuitable for consuming

because of higher APC, *E. coli* and *S. aureus* counts. Numerous reports are available regarding the retail fish market of the fish in India, where they reported on the poor quality of fish and fish infections with bacterial pathogens (Visnuvinayagam, 2015; Visnuvinayagam *et al.*, 2016). Fish samples collected from the super-market had the APC, *E. coli* and *S. aureus* level within the recommended limit with exception of one sample which contained higher level of *E. coli* than the recommended limit (Table 6). It was observed that, super market fish exhibited high level of formalin. It could be a reason for the lesser level of APC in fishes of the supermarket. Addition of formalin either after

catching the fish or after reaching in the super market may be controlled by monitoring authorities. Most of the pathogens in the retail fish market are due to repeated use of contaminated water for fish cleaning. So, fish has to be cleaned in the running water to get a safe fish.

Antibiogram studies

Antibiogram against 20 antibiotics were also analyzed for the *E. coli* (Table 7) and *S. aureus* (Table 8) isolated from the super market and local market. In super market sample, among the six *E. coli* isolates - 2 isolates were resistant to Colistin and one isolate was intermediate resistant to the Augmentin (Amoxicillin and Clavulanic acid). In Local fish market *E. coli* isolates, among the 15 *E. coli* isolates - 5 isolates were resistant to Augmentin and 5 isolates were intermediate resistant to Colistin and one isolates was intermediate resistant to Cefoxitin. In super market sample *S. aureus* isolates - 8, 7, 6, 5 and 1 number of isolates were resistant to Azithromycin, Teicoplanin, Oxacillin, methicillin and Ofloxacin respectively. Likewise, in local fish markets, 7, 4 and 3 number of isolates were found resistant to Teicoplanin, methicillin and Oxacillin respectively. Two strains were resistant to Vancomycin and azithromycin. Antibiotics such as Clarithromycin, penicillin, ampicillin, Amoxycylav, Clindamycin, Chloramphenicol and Linezolid were found resistant to each one strain. Hence, it was observed that the local fish market *E. coli* and *S. aureus* were highly resistant to isolates of the super market. Dutta *et al.*, (2015) reported that most of the *E. coli* isolates are resistant to Erythromycin (72%) similarly, 94% of the *E. coli* isolates were intermediate resistant to Ampicillin. Presence of these types of Multi drug resistant (MDR) bacteria is increasing in most of the fishes sold in the market. Recently, the report of Methicillin resistant

Staphylococcus aureus also reported in Cochin retail fish markets (Visnuvinayagam *et al.*, 2015; Murugadas *et al.*, 2017) and presence of extended-spectrum β -lactamase (ESBL) producing *Escherichia coli* also reported in the Indian retail fish markets (Sivaraman *et al.*, 2017). So, increased increase incidence of highly pathogenic such as MRSA and ESBL indicate the fish market becomes a reservoir of the resistant bacteria which spread infection to handlers and consumers. A person carries such type of bacteria will not respond to any recent antibiotics in future to control the infection. So, to control the spread of pathogenic bacteria in the fish market, in addition to the training on hygienic handling practices of fish to the fish retailer; a proper awareness has to be created to the public and consumer regarding role of fish market and the spread of pathogenic bacteria via fish. Consumer has to purchase the fish from the hygienic fish retail shop only, otherwise the it is difficult to change the situation. It can be concluded that, in dry fish market, microbiological quality parameters of most of the fish samples were within the limit but samples had higher TVB-N value and formaldehyde content than the permissible level. Ironically, the local retail fish market, most of the samples' biochemical levels were within the limit; but samples exhibited higher level of APC, *E. coli* and *S. aureus*. In addition, one sample showed higher formaldehyde content. Even though, super market sample contained all the microbial and biochemical levels within the limit, all the samples had higher formaldehyde content. The lower microbial count in the supermarket fish sample may due to formaldehyde treatment. Multiple drug resistant bacteria also existed in both local and supermarket samples. The monitoring authority of India need to evaluate fish quality for different fish markets at regular interval and strict action has to be taken to produce safe seafood.

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