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Comparative study of quality changes in physicochemical and sensory characteristics of iced and refrigerated chilled store Indian Mackerel (*Rastrelliger kanagurta*)

BG Chudasama, TH Dave and DV Bhola

Abstract
The study was carried out to evaluate the quality of Indian Mackerel based on its biochemical characteristics like total volatile base nitrogen test (TVB-N) and trimethylamine nitrogen test (TMA-N). The quality assessments of fish were observed with different Quality Index Method. The quality changes of Indian Mackerel was evaluated as ice chilled storage and refrigerated chilled storage for the storage stability at temperature 2-4 ºC. Changes in the quality of iced and refrigerated stored fish were examined at different time intervals as 1 day, 3 days, 5 days and up to 7 days. The result indicated that the value of TMA-N and TVB-N increase rapidly ($p<0.05$) in iced storage fish compared to refrigerated fish and on the 5th day reached to its permissible limit. The pH values of fish stored in iced were significantly ($p<0.05$) increased during storage compared to refrigerated fish. In sensorial evaluation after 5 days of storage, fish stored in refrigerated condition; was found excellent quality whereas fish stored at iced condition was observed with degraded quality during the study period at the chilled temperature. Thus, the study reveals that spoilage occurs in iced fish rapidly with storage time, while refrigerated fish remain acceptable quality at the end of the 7th day according to chemical, physical and sensory evaluation.

Keywords: TMA-N, TVB-N, Chilled storage, Indian Mackerel

Introduction
The Indian Mackerel, *Rastrelliger kanagurta* is one of the most important pelagic fish resources of India in the context of national food security. The resource captured 2.49 lakh tonnes which contributed 6.9% of the total marine fish production in India during 2016-2017 [5].
Fish quality is a complex concept involving a whole range of factors, which for the consumer include safety, nutritional quality, availability, convenience and integrity, freshness, eating quality and the obvious physical attributes of the species, size, and product type. Information about handling, processing and storage techniques, including time and temperature histories that can affect the freshness and quality of the products are very important for the partners in the chain [1]. The quality of fish can be estimated by sensory tests, microbial methods or by chemical methods such as measuring volatile compounds, lipid oxidation, determination of ATP breakdown products and the formation of biogenic amines [8]. Total volatile basic nitrogen (TVB-N) is important characteristics for the assessment of quality in seafood products and appears as the most common chemical indicators of marine fish spoilage [2, 19]. The European Union directive on fish hygiene specifies that if the organoleptic examination reveals any doubt as to the freshness of the fish, inspectors must use TVB-N as a chemical check [4].
Total volatile bases nitrogen (TVB-N) is a group of biogenic amines formed in non-fermented food products during storage [9]. The combined total amount of ammonia (NH_3), dimethylamine (DMA) and trimethylamine (TMA) in fish is called the total volatile base nitrogen (TVB-N) content of the fish and is commonly used as an estimate of spoilage and has been widely used as an index for the freshness of fish [19].
The increase in the amount of TVB-N parallel with the increase in TMA-N during spoilage. As the activity of spoilage bacteria increases after the death of a fish, a subsequent increase in the reduction of TMAO to TMA-N [20]. The source of DMA and TMA in fresh and processed fishery products is trimethylamine oxide (TMAO). They are believed to use it to increase osmotic concentration and thus depress the freezing point of body fluids [4].

Post-mortem degradation of TMAO and the subsequent accumulation of volatile amines play a large role in the quality loss of the fish products due to the objectionable odors associated with the degradation products. The formation of TMA from the reduction of TMAO is caused by bacterial degradation. Dimethylamine formation is produced during frozen storage and does not have a pungent odor compared to TMA-N and ammonia, and is not linked to bacteria degradation [19].

It is necessary to use the chemical methods when the purpose is to adequately define and access the fish quality, and ensure that those results show good agreement with the objective tests. Thus, the objectives of this study were to investigate changes in the quality of Indian mackerel fish during storage in iced chilled storage and refrigerated chilled storage over time-based chemical analysis in terms of TMA-N and TVB-N content and determine storage stability.

2. Materials and Methods

2.1. Sample collection and preparation

Fresh Indian mackerel (*Rastrelliger kanagurta*) were collected from the fishing harbour of Veraval, India. The average length and weight of fish were 18.36 ± 0.30 cm and 155.50 ± 18.09 grams, respectively. The fish were immediately iced (1:1 ratio of fish: ice) and transported in an insulated container to the department laboratory in chilled condition. The material were immediately washed in chilled water and stored different chilling temperature at 2- 4°C. The material was divided into two batches, 1st batch was iced stored whole fish and the 2nd batch was refrigerated chilled stored whole fish (Bluestar Refrigeration Equipment Co. Ltd.) at 2- 4°C in the same form. Chilled store samples of fresh whole Indian mackerel were analyzed at every alternate day for physicochemical and sensory analysis. Total duration of chilled storage in both the condition was 7-8 days.

2.2 Biochemical Analysis

2.2.1 Determination of proximate composition

Proximate composition analyses were performed according to AOAC [3] procedures. Water content was determined by drying samples at 105±2 °C until a constant weight was obtained. Wet samples were used for determination of crude fat, protein and mineral contents. Crude fat was measured by solvent extraction method in a soxhlet system where n-hexane was used as solvent. Crude protein content was calculated by using nitrogen content obtained by Kjeldahl method. A conversion factor of 6.25 was used for calculation of protein content. Total mineral content was determined by incinerating samples at 550±10 °C for 5 hours. The weight of the residual ash, expressed as a percentage of the wet sample weight, was taken as the total inorganic residue. All chemicals used were in analytical grade and obtained from Merck (Darmstadt, Germany), Fischer (USA).

2.2.2 Determination of pH

Samples were prepared using a tissue homogenizer [18]. The pH level was monitored using a digital pH-meter (EUTECH Waterproof Multi Parameter, Singapore, Model - PCD-650) in triplicate.

2.2.3 Determination of TVB-N and TMA-N

Levels of TVB-N and TMA-N were determined according to the standard procedure [16] by using Conway micro diffusion unit. The extracts were prepared by mixing 2 g of the sample with 4% TCA in a 50 mL beaker and were homogenized properly. It was left for 30 min at room temperature with occasional grinding and was filtered and the filtrate was labeled and stored. Three thoroughly cleaned Conway units were taken and the edge of the outer ring of each unit was sealed using a sealing agent (Silicon grease). Using a micropipette (Eppendorf, India), 1 mL of boric acid solution was added to the inner ring of each unit. Into the outer ring of each unit, 1 mL of the sample extract was added. One milliliter of saturated K_2CO_3 solution was carefully pipetted into the outer ring of each unit and closed with a clip. The solutions in the units were then mixed gently, to prevent any solution mixing from one ring to the other. The units were placed in an incubator at 37°C for 60 min. Then the covers of the units were removed and the inner ring solution (a green color) was titrated with 0.02 N HCl using a burette until the green color solution turned to pink. An average titrate volume of HCl was found from the results of three titrations for each sample. For each sample, the TVB-N values were calculated. A blank test was also carried out using 1 mL of 1% TCA, instead of sample extract. Trimethylamine in the sample was also determined by the Conway technique, which is similar to TVB-N determination except that prior to the addition of potassium carbonate, 1 mL of 10% neutralized formalin was pipetted into the extract with ammonia and this allows only the TMA-N to diffuse over the unit.

$$\text{TVB-N (mg/100g)} = \frac{(Vs - Vb) \times 0.14 \times \text{Volume of extract} \times 100}{\text{Vol. of sample taken} \times \text{weight of sample}}$$

$$\text{TMA-N (mg /100g)} = \frac{(Vs - Vb) \times 0.14 \times \text{Volume of extract} \times 100}{\text{Vol. of sample taken} \times \text{weight of sample}}$$

Where,

Vs = Titrate value of 0.01 N NaOH for the sample (ml)

Vb = Titrate value of 0.01 N NaOH for the blank (ml)

2.3 Sensory analysis

Sensory characteristics i.e. appearance, color, odor and overall acceptability were evaluated by a trained panel of 5 members using 9-point hedonic scale according to standard procedure [14] as Like extremely (9), Like very much (8), Like moderately (7), Like slightly (6), Neither like nor dislike (5), Dislike slightly (4), Dislike moderately (3), Dislike very much (2), Dislike extremely (1). The limit of acceptability was 4 for all the samples. High score indicated good quality and vice versa.

2.4 Statistical analysis

Data obtained from all the tests were analyzed by using one-way analysis of variance (ANOVA) and followed by Duncan multiple range test of the Statistical Package for Social Science version 22.0 (SPSS inc., Chicago, Illinois, U.S.A) [17]. Statistical significance was indicated at the 95% confidence level. Values expressed are means of three determinations ± standard deviation.

3. Results and Discussions

3.1 Physicochemical assessment:

Changes in proximate composition of Indian mackerel during iced and refrigerated storage is summarised in Table - 1. In fresh fish, the moisture, protein and lipid and ash contents were 74.37, 18.46, 4.11 and 1.45 g/100 g sample,

respectively. These values were almost similar to those found in frozen storage Indian mackerel [12]. Chemical composition of fish can be various greatly depending on species, age, sex, environment, season, feed intake and reproduction cycle [15]. There is significant (p<0.05) effect of different chilling methods on biochemical composition of Indian mackerel noticed (Table - 1). Moisture and protein contents showed a decreasing trend in refrigerated fish in comparison to iced fish. The decrease in the moisture content could be attributed to the dehydration, whereas the decrease in protein could be due to the loss of soluble protein in drip loss.

Protein levels showed significant differences (p<0.05) during days of storage and decreased from 18.46 g/100 g in fresh fish to 16.79 g/100 g at 7th days. Significant reductions in protein content were also observed during chilled storage of other fish species such as silver jewfish, Bombay duck and ribbon fish [15] and croaker muscle protein [7]. The loss of protein in fish during chilled storage was possibly due to leaching of water soluble protein fraction from fish muscle.

Changes in lipid content of samples were in the range of 0.57–1.45 g/100 g (p<0.05). These variations can be explained by variation of lipid content from fish to fish even within the same fish depending on sex, age, and feeding. Upon chilling fat decreased in both the methods during the subsequent storage period, which could be attributed to the changes in moisture content.

Table 1: Changes in moisture, lipid, protein, and ash content (%) of *Rastrelliger kanagurtae* during Iced and Refrigerated storage at 2-4 ºC.

Storage days	Moisture		Protein		Lipid		Ash	
	Iced fish	Refrigerated fish	Iced fish	Refrigerated fish	Iced fish	Refrigerated fish	Iced fish	Refrigerated fish
0	74.37±0.19^{d}	74.37±0.19^{d}	18.46±0.18^{a}	18.46±0.18^{a}	4.11±0.12^{bcd}	4.11±0.12^{bcd}	1.45±0.08^{a}	1.45±0.08^{a}
1	77.12±0.11^{c}	73.94±0.13^{e}	17.93±0.07^{c}	18.29±0.13^{a}	3.90±0.06^{e}	4.22±0.14^{abc}	1.40±0.04^{a}	1.40±0.04^{a}
3	77.72±0.20^{b}	73.39±0.26^{f}	17.19±0.13^{cd}	17.90±0.15^{b}	3.95±0.07^{de}	4.28±0.03^{ab}	1.18±0.18^{bc}	1.25±0.25^{ab}
5	78.17±0.07^{a}	72.96±0.10^{g}	17.02±0.19^{de}	17.37±0.16^{c}	4.04±0.06^{cde}	4.30±0.03^{ab}	0.71±0.08^{d}	1.03±0.03^{c}
7	78.41±0.12^{a}	72.35±0.28^{h}	16.79±0.04^{e}	17.17±0.22^{cd}	4.18±0.16^{abc}	4.33±0.14^{a}	0.57±0.09^{d}	0.99±0.08^{c}

^{a,b,c,d,e,f,g,h} Means in the same column followed by different superscripts are significantly different (p<0.05). Mean ± SD, n = 3.

The changes in pH during iced and refrigerated storage fish are presented in Table - 2. The initial pH of Indian mackerel samples was 5.67 and increased to 7.27 at the end of storage. Statistical analysis showed significant differences (p<0.05) between all days of storage. The initial post mortem pH of fish varies with species, catching ground and season [10]. During storage, pH increases slightly because of the formation of alkaline compounds as ammonia compounds and TMA, mainly derived from microbial action. In the present study, increase of pH throughout 7 days of iced storage fish might be attributed to the formation of basic decomposition products, such as ammonia and trimethylamine produced by endogenous enzymes and bacterial spoilage. Similar finding also reported a significant increase in pH of wire-netting reef cod (*Epinephelus merra*) during ice storage [11]. It suggested that pH changes during refrigerated storage of fish differed according to the species of fish and other factors.

Table 2: Changes in pH of *Rastrelliger kanagurtae* during Iced and Refrigerated storage at 2-4 ºC.

Storage days	Iced fish	Refrigerated fish
0	5.67±0.15^{e}	5.67±0.15^{e}
1	6.07±0.25^{cde}	6.03±0.15^{de}
3	6.50±0.30^{bc}	6.27±0.25^{bcd}
5	7.00±0.40^{a}	6.37±0.21^{bcd}
7	7.27±0.21^{a}	6.53±0.25^{b}

^{a,b,c,d,e} Means in the same column followed by different superscripts are significantly different (p<0.05). Mean ± SD, n = 3.

3.2 Changes in total volatile base nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) levels have been recognized as useful indicators of seafood spoilage; under EU directive 95/149/EEC, the European Commission has specified that, the TVB-N level can be used, if sensory methods raise doubts about the freshness of seafood species. Although, Total volatile nitrogen content (TVB-N) of fish is an indicator of raw material freshness. The level of TVB-N in freshly caught fish is generally between 5 and 20 mg N_2 per 100 g muscle. However, the levels of 30 to 35 mg N_2 per 100 g muscle are considered the limit of acceptability [6, 10]. In the present investigation changes in TVB-N content during iced and refrigerated stored Indian mackerel are shown in Table - 3.

The average of initial TVB-N content (mg/100 g fillet) for control sample (day 0) was 3.8 mg/100 g while TVB-N values of samples stored in ice increased to 32.9±1.45 mg/100 g on the 5th day and to 41.3±1.47 mg/100 g on the 7th day significantly (p<0.05). The results of the present study are in agreement with other findings [12, 13], as they used sardine for analysis during refrigerated storage, TVB-N increased with storage time. However, the refrigerated samples were all in good condition and the TVB-N values remained 25.9 ±0.61 mg/100g even after 7th days of storage. These significantly lower TVB-N levels were attributed to the inhibition of growth of microorganisms and the muscle autolytic reactions. In the present study, TVB-N value was 41.3 mg N/100 g when the iced stored fish was rejected by panel lists after 7 days of storage. However, a level of 30–35 mg TVB-N/100 g of fish muscle is usually regarded as spoiled. Results showed that the TVB-N level of more than 35 mg/100 g could be regarded as the limit of beyond acceptability for iced stored fish. The results also signified that TVB-N could be used as an indicator of chilled stored Indian mackerel quality.

Table 3: Changes in TVB-N of *Rastrelliger kanagurtae* during iced and refrigerated storage at 2-4 ºC.

Storage days	Iced fish	Refrigerated fish
0	3.8±0.18^{g}	3.8±0.18^{g}
1	8.4±0.25^{f}	4.9±0.25^{g}
3	22.4±1.55^{d}	9.2±0.35^{f}
5	32.5±1.45^{b}	18.6±1.17^{e}
7	41.3±1.47^{a}	25.9±0.61^{c}

^{a,b,c,d,e,f,g} Means in the same column followed by different superscripts are significantly different (p<0.05). Mean ± SD, n = 3.

3.3 Changes in trim ethylamine nitrogen (TMA-N):

Trim ethylamine oxide (TMAO) is the compound which occurs naturally in most of the marine fishes responsible for their characteristic fishy odour and flavor. Trimethylamine is the bacteriologically degraded product from trim ethylamine

oxide (TMA-O). Trimethylamine (TMA) level in fish is an important factor in the subjective evaluation of fish quality because of its close association with fish spoilage. Fish with a level beyond 10-15 mg TMA-N/100g fish are considered as spoiled. TMA-N production in fish and fishery products is due to enzymatic and microbial degradation of protein and non-protein nitrogenous compounds. The increase in TMA-N levels may result from domination of free amino acid, oxidation of amines and degradation of nucleotides by autolytic enzymes and microbial activity. The initial TMA content of samples used in this study was 1.11±0.19 mg/100 g, which increased in all samples during the storage period. Significantly (p<0.05) higher values of TMA were observed in ice chilled fish samples than in refrigerated chilled stored samples. TMA contents increased to 11.20±0.46 mg/100 g for samples stored in ice on the 5th day. Whereas, refrigerated stored fish samples goes up-to 9.80±0.23 mg/100 g on the 7th day storage (Table - 4).

Table 4: Changes in TMA-N of *Rastrelliger kanagurtae* during Iced and Refrigerated storage at 2-4 ºC.

Storage days	Iced fish	Refrigerated fish
0	1.11±0.19^{g}	1.11±0.19^{g}
1	1.47±0.16^{g}	1.21±0.09^{g}
3	5.66±0.22^{e}	2.86±0.25^{f}
5	11.20±0.46^{b}	6.40±0.11^{d}
7	14.73±0.15^{a}	9.80±0.23^{c}

^{a,b,c,d,e,f,g} Means in the same column followed by different superscripts are significantly different (p<0.05). Mean ± SD, n = 3.

3.4 Changes in sensory score

The sensory scores decreased progressively with time in both the iced and refrigerated fish samples. The overall quality of fish was comprised of both wholesomeness and sensory acceptability by the consumer. In the present investigation sensory attributes of iced and refrigeration during storage studies were depicted in Table - 5. This decreasing score indicated the loss of freshness in both the samples. However, the panelists scored high and within acceptable limit for refrigerated fish samples compared to iced store fish samples. The present study indicated that iced sample observe to be spoiled on 5th days of storage. While refrigerated fish remain good in quality scores at the end of 7th days of storage significantly (p<0.05).

Table 5: Changes in sensory quality of *Rastrelliger kanagurta* during Iced and Refrigerated storage at 2-4 ºC

Treatments	Storage Days	Sensory quality attributes			
		Appearance	Color	Odor	Overall acceptability
Iced fish	0	7.2±0.45^{a}	7.2±0.45^{a}	7.2±0.45^{a}	7.2±0.45^{a}
	1	7.0±0.00^{a}	6.8±0.45^{bc}	7.0±0.00^{bc}	6.8±0.00^{abc}
	3	6.4±0.55^{b}	6.2±0.45^{bc}	6.4±0.55^{c}	6.0±1.00^{c}
	5	5.0±0.82^{c}	5.3±1.26^{d}	5.0±0.82^{d}	5.0±0.82^{d}
	7	4.4±0.55^{c}	5.0±0.71^{d}	4.4±0.55^{d}	4.4±0.00^{e}
Refrigerated fish	0	7.2±0.45^{a}	7.2±0.45^{a}	7.2±0.45^{a}	7.2±0.45^{a}
	1	7.2±0.45^{ab}	7.0±0.17^{ab}	7.2±0.45^{ab}	7.0±0.71^{ab}
	3	7.0±0.00^{ab}	7.0±0.00^{abc}	7.0±0.00^{abc}	7.0±0.71^{ab}
	5	6.8±0.45^{ab}	6.4±0.55^{abc}	6.8±0.45^{bc}	6.6±0.89^{abc}
	7	6.4±0.55^{b}	6.2±0.45^{c}	6.4±0.55^{c}	6.2±0.84^{bc}

^{a,b,c,d,e} Means in the same column followed by different superscripts are significantly different (p<0.05). Mean ± SD, n = 5.

4. Conclusion

The biochemical and sensory characteristics are determined to assess the quality and acceptability of Indian Mackerel fish during storage in iced and refrigerated condition. TVB-N and TMA increased in all samples and observed to a good time-dependent relationship. Fish stored in refrigerated condition found better compared to iced fish, it is due to the temperature fluctuation is less in refrigerated condition compared to iced store fish. Hence, the time elapses the degradation of the quality of iced fish was also increase due postmortem changes in the initial quality. The maximum freshness and minimal TVB-N and TMA content was observed in Indian Mackerel fish stored for a period between 0-3 days in both the storage system. Although, the quality of fishes was inversely proportional to time and temperature was observed. Hence, the present findings suggest that maintaining of time and temperature during processing and storage is useful to maintain uniform freshness and quality of Indian Mackerel for longer period under chilled condition.

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