The Effect of Storage Temperature (4°C, 15°C and 25°C) on The Shelf Life of Whole Marine Fish (*Rastrelliger kanagurta*)

Sami A. Humaid, Mamdoh T. Jamal

Faculty of Marine Science, King Abdulaziz University, P.O.Box 80207 Jeddah 21589, Saudi Arabia

Abstract: *Organoleptical and microbiological shelf-life evaluations of whole Indian mackerel (Rastrelliger kanagurta) were monitored during storage at 4°C for 144 hours, 15°C for 72 hours and at 25°C for 24 hours. The Organoleptical shelf life of Indian mackerel was found to be 151 hours at 4°C, 59.7 hours at 15°C and 19.4 hours at 25°C. Bacteria of Indian mackerel were dramatically grown at 4°C, 15°C and 25°C exceeding the bacterial spoilage limit of fish (7 log cfu/g) that limited by International Commission on Microbiological Specifications for Foods, and reached to about, 7.83±0.23, 8.43±0.17 and 8.25±.51 log cfu/g, respectively. Microbiological shelf-life of Indian mackerel was found to be 118.2 hours at 4°C, 44.2 hours at 15°C and 15.6 hours at 25°C. Microbiological shelf life in studied fish was significantly shorter than the Organoleptical shelf life in the same fish. In this study, each of quality parameters was varied significantly (P<0.05) among storage temperatures.*

Key words: *Organoleptical evaluation; microbiological; shelf-life; Indian mackerel; storage temperature; quality.*

I.   Introduction

Globally, there is an increasing demand for fish as food [1]. Fish is a good source for a digestible animal protein, healthful polyunsaturated fatty acids, minerals and vitamins that support the biochemical processes of the human body [2]. However, it has a high perishability more than other foods. As soon as the fish dies, spoilage begins faster in tropical countries [3]. High ambient temperature of some countries supports fish spoiling between 6-12 hours after death which causes reduction for fish quality [3,4]. Fish can be kept for a short period therefore it must be eaten immediately after catching or using preservation methods like chilling, freezing, canning, smoking and drying to delay fish spoilage and to extend the shelf life to be fit for human consumption for long period [5]. Therefore handling should be hygienically and under controlled temperature, otherwise spoilage might increase very rapidly [6]. A lot of studies have investigated the relation between storage temperature, time and changes of fish quality [7]. The quality changes are strongly influenced by many factors, the most important one is temperature. If fresh fish is not immediately stored at appropriate conditions and exposed to ambient temperature even for short periods of time, this possibly causes a serious deterioration in fish quality. The tropical climate of Jeddah with an average temperature ranging between 25-40C° is suitable for spoiling fish rapidly. In fishing vessels and/or fish-processing facilities, fish might be exposed to inappropriate hygienic procedures during handling which possibly lead to increase the loss in freshness of fish. Considering the importance of keeping fish quality high, this study was designed to study the effect of different storage temperatures (4°C, 15°C and at 25°C) on fish quality.

II.   Materials and methods

2.1. Raw material and experimental design

Newly caught Indian mackerel (*Rastrelliger kanagurta*) fish were obtained from Jeddah Fish Landing Center. Samples were placed into a portable iced cool boxes and delivered to the Faculty of Marine Science Laboratories of the King Abdulaziz University in Abhor-Jeddah within approximately 1-2 hours. Samples were divided into 3 groups and stored at different storage temperatures 4°C, 15°C, and 25°C.

The analysis included, organoleptic characteristics and bacterial counts. Samples were monitored periodically during storage conditions. Samples stored at 4°C were determined every 48 hours, at 15°C samples were determined every 24 hours and samples stored at 25°C were determined every 6 hours. The first determinations were taken as soon as samples delivered to the lab as time zero.

2.2. Organoleptic analysis

The organoleptic evaluation score sheets for raw fish following Torry schemes method [8]. Torry schemes collated by Archer [9] shown in (table 1) were used for evaluating the organoleptic characteristics of samples in the present study. The organoleptic characteristics included general appearances (gills, eyes, skin, and belly), odour and texture. The Torry schemes were developed at Torry Research Station in the United Kingdom using a gradation from 10 (for high freshness fish) to 3 (for spoiled fish). Scores below a point of 3 are

considered unnecessary because the fish at score 3 becomes unfit for human consumption [8]. Each evaluation of organoleptic changes in fish characteristics was carried out by six trained panelists from Marine Science Faculty. The average of the scores given from the panelists represented as the general organoleptic quality of the fish. The scores were given 10–9 (excellent freshness) and 4 (limit of rejection).

2.3. Microbiological analysis

Direct viable count (DVC) method was used to measure total bacterial count (TBC) of normal flora in flesh tissue of the studied fish according to Hakkimane and Rathod [10]. The TBC has been estimated by surface inoculation of 0.1 ml of six dilutions using digital pipette on Marine Agar (2216 oxoid) plates, in triplicate, and incubation at 25°C for 48 hours. The developed colonies of bacteria ranged of 30-300 were counted and calculated per gram and expressed as colony forming unit per gram (CFU/g).

$$\text{CFU/g} = \frac{\text{Number of colonies (average of three replicates)}}{\text{Amount plated x dilution}}$$

2.4. Data analysis

Minimum, maximum, mean, standard deviation and standard error of mean were used to describe data. The values were expressed as mean ±SD. Data obtained were analyzed by using t-test, one-way Analysis of Variance (ANOVA) and Post-hoc multiple comparisons conducted by using Least Significant Difference (LSD), $P < 0.05$ was considered as significant value. Simple linear regression model was used. Statistical analysis was performed by using statistical package IBM SPSS software, version 21, and Microsoft Excel, version 2007.

Table 1. Scale sheet employed for organoleptic evaluation of Indian mackerel scoring.

| Score	Appearance			Gill Odour
	Skin and Body	**Eyes**	**Gills**	
10 **9**	Firm body with silky smooth skin. Lateral line and reticulations on upper surface well defined. Body colours iridescent with strong royal blue and turquoise colours on upper surface. Blue and violet on ventral surface with silvery sheen. Passing into rigor or in rigor.	Bulging convex eye with protruding lens. Shiny jet-black pupil with metallic brown iris. Eye-cap water clear	Uniformly dark red with free blood and water-clear slime present	Weak, delicate odours, cloying sweet, sharp, pepper, seaweed, blood
8 **7**	Loss of colour definition. Some blood stains apparent. Passing out, or out of rigor	Convex eye lens plane with cornea. Pupil less shiny, iris green/ blue. Slight clouding of eye cap	Dark purple/maroon with paler edge. Congealed blood present with opaque slime	More definite odours as above, also fragrant, fresh grass, fruity, metallic, shellfish
6	Colours of dorsal surface paler, reticulations grey, ventral surface white with golden tinge. Patchy iridescence			Dull muddy odours, musty, mousy, malty, cardboard, linseed oil, cod liver oil, biscuits, blood
5	Washed-out colours, definite golden tinge to skin, patchy iridescence. Body soft with red/brown slime oozing from gill covers. Skin wrinkles on flexing	Flattening of eye but still convex. Pupil wrinkled with slight clouding of lens. Iris silvery and starting to wrinkle. Yellowing of eye cap	Loss of colour with red/brown slime	Stale odours as above.
4	Fish limp and floppy with distinct ice marks. Washed-out colours with mottling or golden tinge	Eyeball plane with eye socket. Cloudy lens with silvery iris showing black specks	Browning of gills, patchy bleaching, increased slime and red/brown slime oozing from gill cover	Mixture of odours from above and below
3	Little distinction between upper and lower surfaces. Body very soft. Skin very wrinkled with distinct ice marks	Concave or flattening eye with cloudy pupil	Marked bleaching and browning of gills covered in black slime	Sweet-rotten odours, oil sweet-rotten fruit(grapefruit), old grass cuttings, sickly sour

III. Results and Discussion

3.1 Organoleptic analysis

The first evaluation of organoleptic changes was carried out at time zero. The quality scores of organoleptic evaluation of Indian mackerel, were given 9.6±0.547 as an excellent quality. Figure 1 shows the changes of quality scores of Indian mackerel at 4°C, 15°C and 25°C. At 4°C for 48 hours quality scores were 8.4±0.547, after 96 hours at the same storage conditions, quality scores of Indian mackerel were 6.4±1.140 and after 144 hours quality scores were 4±1.224. Changes of quality scores of fish stored at 15°C. Quality scores of Indian mackerel after 24 hours of storage were 7.2±0.836, at 48 hours quality scores of Indian mackerel, reached to 4.6±1.341, and after 72 hours of storage at 15°C Indian mackerel exceeded the acceptable limit with quality scores 3.2±0.447. After six hours of storing at 25°C the quality scores of Indian mackerel were 7.8±0.836, after 12 hours quality scores significantly decreased to 5±0.707 and the quality scores were 3.2±0.447, exceeded the limit of acceptable after 24 hours of storage.
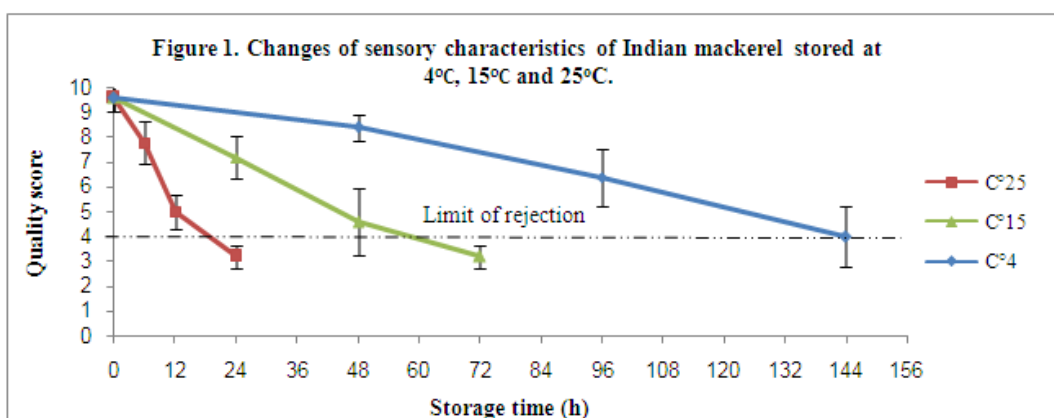
Figure 1. Changes of sensory characteristics of Indian mackerel stored at 4°C, 15°C and 25°C.

The shelf-life of Indian mackerel stored in the three different storage temperatures (4°C, 15°C and 25°C) was as follows (fig. 2); during storage at 4°C samples were found to be organoleptically acceptable up to about 151.02 hours. While storing at 15°C, fish samples were organoleptically acceptable up to about 59.69 hours for Indian mackerel, and fish samples stored at 25°C were found to be organoleptically acceptable for human consumption when they were stored for a period about 19.37 hours for Indian mackerel.

Figure 2. Organoleptical shelf-life (hours) of Indian mackerel during 4°C, 15°C and 25°C.
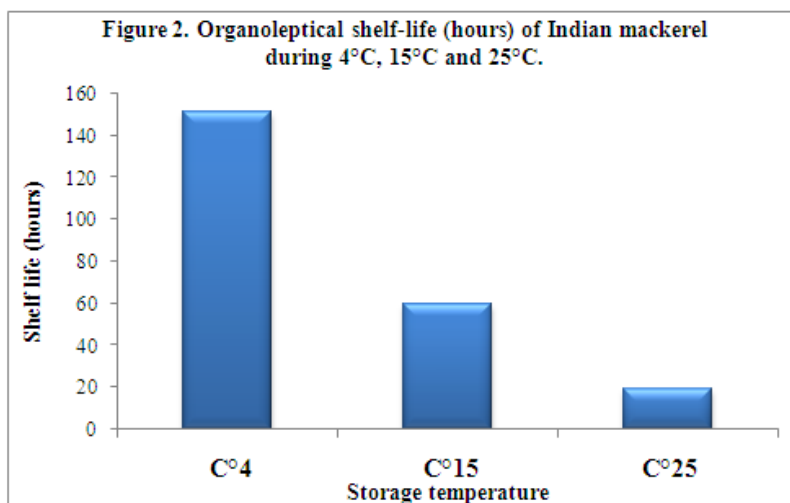
3.2 Microbiological analysis

Bacteria were grown dramatically at all storage temperatures (table 1, fig. 3). The initial TBC (time zero) of Indian mackerel was 2.41±0.23 log cfu/g. During storage of 4°C TBC in fish samples after 48 hours was 4.88±0.21 log cfu/g, after 96 was 6.14±0.19 log cfu/g and in 144 hours of storage TBC was 7.83±0.24 log cfu/g. The numbers of bacterial colonies that proliferated in each period of storage were as followed; in the first 48 hours were about 2.47±0.26 log cfu/g, in the next 48 hours were 1.26±0.28 log cfu/g, and in the last 48 hours were 1.69±0.26 log cfu/g (fig. 4a). At 15°C for 24 hours TBC was 6.36±0.44 log cfu/g, after 48 hours at 15°C TBC was 8.09±0.1915 log cfu/g, and after 72 hours TBC was 8.43±0.176 log cfu/g in Indian mackerel. Micro

flora that proliferated in fish samples stored at 15°C during storage period (0-24, 24-48 and 48-72 hours) shown in (fig. 4b). The bacterial flora proliferation was 3.95 ± 0.589 log cfu/g during the period 0-24 hours, at the period of 24-48 hours micro flora proliferation was 1.73 ± 0.442 log cfu/g, and 0.34 ± 0.353 log cfu/g during the period of 12-24 hours. And at 25°C, TBC in fish samples in 6 hours of storage increased greatly to 5.11 ± 0.63 log cfu/g, after 12 hours of storage TBC reached to 7.44 ± 0.09 log cfu/g, TBC of Indian mackerel in 24 hours of storage was 8.25 ± 0.51 log cfu/g. The numbers of bacterial colonies that proliferated in the each period of storage were as followed; in the first 6 hours were about 2.7 ± 0.73 log cfu/g, in the next 6 hours were 2.33 ± 0.53 log cfu/g and in the period between 12-24 hours were 0.81 ± 0.50 log cfu/g (fig. 4c).

Table 1. TBC log cfu/g of normal flora in flesh tissue of Indian mackerel during storage in three different temperatures.

4°C		15°C		25°C	
Storage time	TBC	Storage time	TBC	Storage time	TBC
0	$2.41\pm0.23^{a*}$	0	2.41 ± 0.23^{a}	0	2.41 ± 0.23^{a}
48	4.88 ± 0.21^{b}	24	6.36 ± 0.44^{b}	6	5.11 ± 0.63^{b}
96	6.14 ± 0.19^{c}	48	8.09 ± 0.19^{c}	12	7.44 ± 0.09^{c}
144	7.83 ± 0.24^{d}	72	8.43 ± 0.18^{c}	18	ND
192	ND**	96	ND	24	8.25 ± 0.51^{d}
Total	5.32 ± 0.22	Total	6.32 ± 0.26	Total	5.80 ± 0.37

*. Values are means ± standard deviation (SD) of three determinations of each sampling. Means followed by same letter (s) are not significantly different (P>0.05).
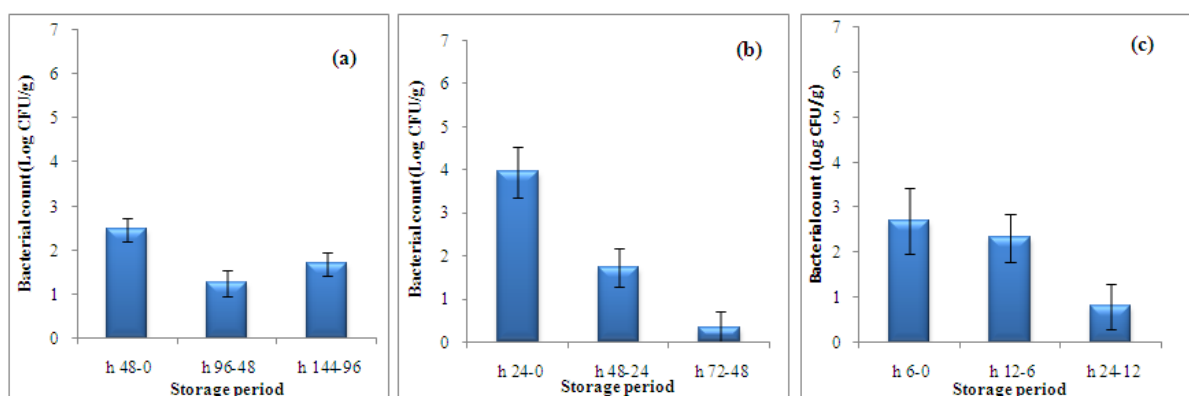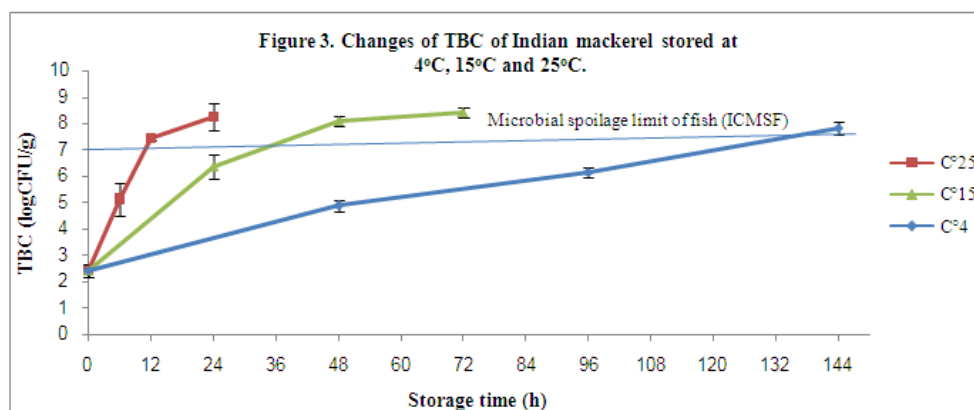**. Not data found.

Figure 4. TBC proliferation (log cfu/g) in Indian Mackerel during storage period of 4°C (a), 15°C (b) and 25°C (c). Each point shown is the mean value of three determinations of each sampling. Error bars represent the standard deviation.

The microbiological shelf-life of Indian mackerel stored in the three different storage temperatures (4°C, 15°C and 25°C) was as follows (fig. 5); during storage at 4°C samples were found to be acceptable up to about 118.2 hours, while storing at 15°C fish samples were acceptable up to about 42.2 hours and fish samples

stored at 25°C were found to be acceptable for human consumption when they were stored for a period about 15.6 hours for Indian mackerel.

Figure 5. Microbiological shelf-life (hours) of Indian mackerel during 4°C, 15°C and 25°C.
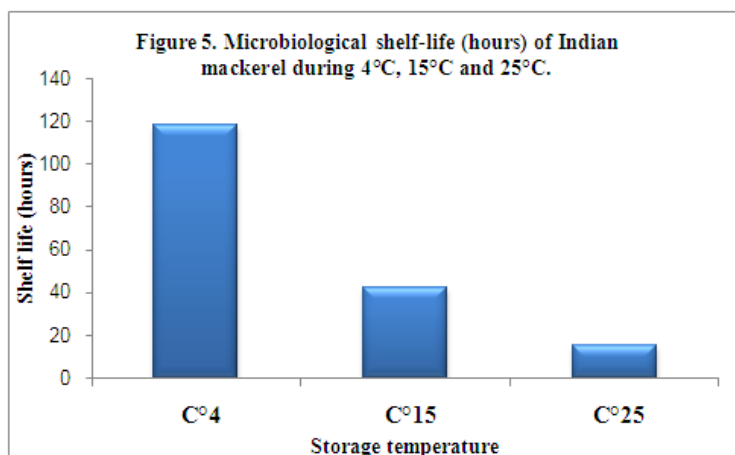
The organoleptical shelf life of Indian mackerel was significantly longer than microbiological shelf life about 21.72%, 29.31% and, 19.58%, during storage at 4°C, 15°C and 25°C, respectively (fig. 6).

Figure 6. Organoleptical and microbiological shelf life of Indian mackerel during storage temperatures
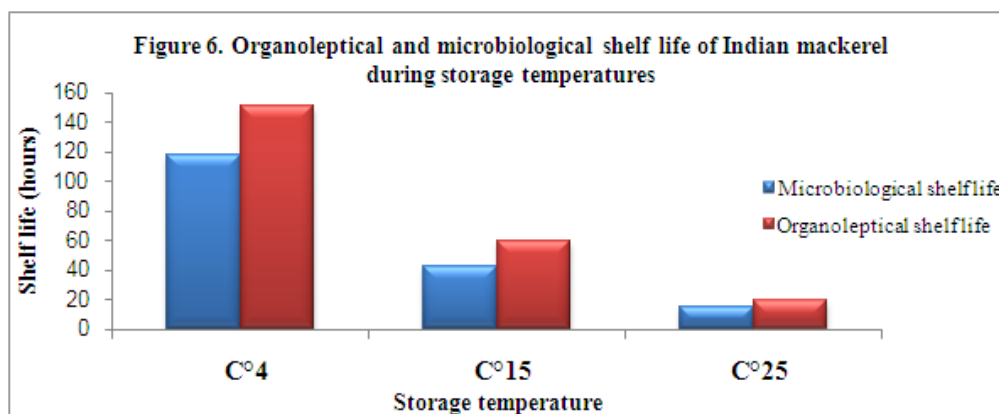
IV.   Discussion
4.1 Organoleptic analysis
The quality scores of organoleptic characteristics of Indian mackerel at time zero were given as an excellent quality. Then quality was deteriorated gradually along the progress of storage period. During storage periods of Indian mackerel, it showed that the lowest quality scores were given to the Indian mackerel stored in 25°C followed 15°C. The Indian mackerel stored at 4°C recorded the highest quality scores, indicating higher quality and slower spoilage rate than in the other two storage temperatures (25°C and 15°C) throughout the storage period. The differences in the quality scores during storage conditions are possibly due to the effect of temperature to the enzymatic and microbial activities in fish samples, whereas low temperatures delayed the enzymatic and microbial activities. Huss [11] indicated similar effects of different storage temperatures in fish. Great differences were observed ($P<0.05$) among the storage periods of each storage temperature. Also, significant differences ($P<0.05$) were found between fish shelf life and their storage temperatures 25°C, 15°C and 4°C. The shelf life of Indian mackerel stored at 4°C were found to be significantly longer than 25°C followed by 15°. Graham *et al*. [12] reported that fish with a shelf life of only about 48 hours at 15°C will keep for 144 hours at 5°C and about 360 hours at 0°C before becoming unacceptable for human consumption.

4.2 Microbiological analysis
In this study, The trends of bacterial growth were significantly different among storage temperatures. Rapid increasing in the rate of the bacterial load was observed in the fish samples that stored at 25°C followed by 15°C, and samples stored at 4°C showed the slowest rates of bacterial growth. These results agreed with Kim *et al*. [13], when they monitored the bacterial growth in mackerel during different storage temperatures (0°C, 4°C, 15°C and 25°C) and they found that bacterial counts increased rapidly at 25°C and reached to about 8 log cfu/g in 48 hours faster than other storage temperatures. Jiang *et al*. [14] also concluded that bacterial

multiplication was accelerated with the increase of the storage temperature. In the same way, Mendens [15] previously stated similar results. Fish bacteria showed a very sensitive to temperature. Elevated temperatures stimulated the fish bacteria to grow and multiply rapidly using the organic compounds of fish as food to obtain the energy. On the other hand, lower temperatures did not totally stop the growth of bacteria but they possibly decreased or slowed down the bacterial growth and the activities that accelerated spoilage of fish [12].

V.   Conclusions

It is known that fish has a short shelf-life after getting caught. Storage in low temperatures considerably delays spoilage occurrence and extends the shelf-life of fish. In this work the Indian mackerel stored in different storage conditions by using three different storage temperatures; 25°C, 15°C and 4°C, revealed that the storage period increased depending on the decompositions occurred by biochemical and bacteriological interaction. Storage temperatures directly effect on shelf-life of fish, where the higher storage temperature caused rapid spoilage than the lower storage temperatures. The storage temperature of 4°C created unfavorable environmental conditions for bacteria which slowed down the bacterial proliferation and reduced the decomposition in fish samples, while storage temperatures of 25°C and 15°C allowed the bacterial to proliferate which reduces the shelf-life of stored fish. Therefore, it is suggested to chill fish rapidly after catch and use low storage temperatures (≤4°C) to extend the shelf-life of fish. For the insurance fish quality, the microbiological indicator is better than organoleptical indicator to determine the quality and freshness of fish and to detect the fish spoilage. Future studies that have been suggested by this study are: studying the effect of storage at vacuum and modified atmospheres on fish quality and comparative study on different quality indicators of fish.

References

[1].   FAO (2012). The State of World Fisheries and Aquaculture 2012, Access date,   May, 14, 2014, from : http://www.fao.org/docrep/016/i2727e/i2727e00.htm

[2].   Alasalvar, C., Miyashita, K., Shahidi, F. and Wanasundara, U. (2011). Handbook of seafood quality, safety and health applications, New Delhi: Blackwell Publishing Ltd; 2011. p.13-29.

[3].   Bhat, R., Alias A. and Paliyath G. (2012). Progress in Food Preservation, 1st edition. New Delhi: Blackwell Publishing Ltd. p. 630. ISBN-10: 0-470-65585-2.

[4].   Frazier, W.C. and Westhoff, D.C. (1988). Food microbiology, 4th edition. McGraw-Hill, New York. USA.

[5].   FDA (2011). Fish and Fishery Products Hazards and Controls Guidance, 4th Edition., p. 467. Washington, DC: Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition Office of Seafood, Washing-ton, D.C. (240) 402-2300.

[6].   Børresen, T. (2008). Improving seafood products for the consumer, Woodhead Publishing Ltd., Cambridge, p 608.

[7].   Burgaard, M.C. (2010). Effect of frozen storage temperature on quality-related changes in fish muscle. PhD Thesis, Technical University of Denmark www.food.dtu.dk.

[8].   Shewan, J. M., Macintosh, R. G., Tucker, C. G. and Ehrenberg, A. S. C. (1953). The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice. Journal of the Science of Food and Agriculture. 4(6): 283–298.

[9].   Archer, M. (2010). Sensory assessment score sheets for fish and shellfish - Torry & QIM. Edinburgh: Seafish. Access date, May, 14, 2014, from http://www.seafish.org/media/Publications/sensory_assessment_scoresheets_14_5_10.pdf

[10].   Hakkimane, Shreedevi S. and Rathod, J.L. (2011). Isolation and enumeration of bacterial flora in false trevally, Lactarius lactarius of Karwar, central west coast of India. NISCAIR-CSIR, India. Indian Journal of Geo-Marine Science. Vol. 40(4), August 2011, pp. 583-586.

[11].   Huss, H.H. (1995). Quality and Quality changes in fresh fish. FAO Fisheries Technical Paper 348. 195 pages. FAO. Rome. Italy.

[12].   Graham, J., Johnston, W.A. and Nicholson, F.J. (1992). Ice in fisheries. FAO Fisheries Technical Paper No. 331. Rome, Italy.

[13].   Kim, S. H., Field, K. G., Chang, D. S., Wei, C. I. and An, H. (2001). Identification of bacteria crucial to histamine accumulation in Pacific mackerel during storage. J. Food Prot. 64, 1556–1564.

[14].   Jiang, Q. Q., Dai, Z. Y., Zhou, T., Wu, J. J., Bu, J. Z. and Zheng, T. L. (2013). Histamine production and bacterial growth in Mackerel (Pneumatophorus Japonicus) during storage, Vol. 37, J. Food Chem. 2:246–253.

[15].   Mendes, R. (1999). Changes in biogenic amines of major Portuguese bluefish species during storage at different temperatures. J. Food Biochem. 23:33-43.