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Effect of refrigerated storage on the biochemical parameters of stored Indian mackerel (*Rastrelliger kanagurta*) in solar operated indigenously developed fish vending unit

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Abstract
The objective of this study was to investigate the effects of refrigerated storage on the biochemical and sensory changes (overall acceptability) of Indian mackerel (*Rastrelliger kanagurta*) fillets during a 8 day period at +3-6 °C by using solar energy as the energy source of the unit. The vending unit consists of 3 different compartments to keep different sized fishes separately and also have the display facility. The samples were collected from the mangalore landing centre in very fresh condition and the evaluation on the biochemical and sensory attributes was done on every alternate day interval upto 8 days. The NPN content of mackerel decreased significantly (p<0.05) to 3.5 mg% and TVBN,TMAN, PV,TBARS and FFA content increased significantly to 33.13 mg%, 8.40 mg%, 21.28 meqO_2/kg of fat, 3.93 mg malonaldehyde/ Kg of meat, 24.01% oleic acid respectively. Whereas, pH reached 6.21 and AAN reached 0.37 mg% on the 8th day but the values did not showed any significant difference with the increase in storage days.

Keywords: Refrigerated; vending unit; biochemical; shelf life; Indian mackerel

1. Introduction

Fish is recognized as being highly perishable, having a relatively short shelf-life [1]. Fish is an important source of high-quality proteins for humans [2]. It is highly susceptible to both microbiological and chemical deterioration, due to its high water activity, neutral pH, relatively large quantities of free amino acids, and presence of autolytic enzymes [3]. Oily fish, such as salmon, mackerel, and sardine, are rich in omega-3 fatty acids. As the significant structural components of the phospholipids of cellular membranes, omega-3 fatty acids, particularly α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are very important for human health [4]. The shelf life of fresh fishery products is usually limited by microbial activities that are influenced mostly by storage temperature [5, 6]. Typical shelf life of fish fillets under icing and refrigerated storage condition varies from 2 to 14 [7].

Freshness is one of the most important aspects of fish, and because of consumer preferences, there is a strong tendency to select very fresh fish. Quality of fish depends on the nature of species and on the handling and storage conditions [8, 9], the changes after catch due to chemical reaction and microbiological spoilage [10]. During handling and storage, quality deterioration of fresh fish occurs rapidly and limits the shelf life of the product. Spoilage of fish and shellfish results from changes caused by three major mechanisms (i) the breakdown of tissue by the fish's own enzymes (autolysis of cells), (ii) growth of microorganisms, and (iii) oxidative reactions. In order to reduce the loss in freshness, different preservative methods, such as flake ice [10], slurry ice [11] and chemical addition [12] have been employed. Temperature control is the most important physical factor in the preservation of seafood, and it is also important for the prediction of the remaining days of the shelf life of a product. It is known that if fresh seafood is properly handled and kept at low temperatures, the bacterial growth is reduced and other spoilage factors are controlled [10]. Refrigeration is a method of lowering the temperature of substances below that of the surrounding in order to preserve or make them suitable for consumption in the nearest future [13].

Indian mackerel is a commercially important fish species in India. It is a pelagic fish exhibiting shoaling behaviour in the Indo-Pacific region, found abundantly between Quilon and Ratnagiri [14], the fresh Mackerel has high market demand. The present study was conducted to determine the shelf life of Indian mackerel stored in indigenously developed solar operated refrigerated fish vending and display unit.

2. Materials and Methods

2.1. Sample Preparation

Fresh Indian mackerel (*Rastrelliger kanagurta*), a fatty marine fish species was used for this study. The fishes were procured in very fresh condition from Mangalore fish landing centre during the month of February 2016, were washed in chilled water and stored in the vending unit. The temperature of the unit was strictly maintained in the range of 3-6 ºC, and the samples were filleted at every alternate day for bio-chemical and sensory analysis. The total period of refrigerated storage was 8 days.

2.2. Biochemical Analysis

2.2.1. Changes in pH

The pH of the fish was measured using a pH meter in the temperature range of 27-29 ºC as the method described by Suzuki [15], 5 g of sample was macerated with 45 ml of distilled water and pH was measured. Prior to pH measurement of the sample pH meter was calibrated with a standard buffer solution of pH 4.2 and 9.2 prepared using buffer capsules.

2.2.2. Changes in nitrogenous compound

2.2.2.1. Total volatile base nitrogen (TVB-N)

Total Volatile Base Nitrogen (TVB-N) of fresh and refrigerated stored sample was determined by Conway micro diffusion method [16]. 10 g of the sample was macerated with 10 ml of 20% tri-chloro acetic acid (TCA) solution using pestle and mortar. The slurry was filtered through coarse filter paper and the filtrate was made upto 100ml by adding distilled water in a volumetric flask. 2ml of boric acid containing mixed indicator (0.066% methyl red and 0.066% bromo cresol green in alcohol in the ratio of 1:1) into the inner chamber and 1 ml of sample into the outer chamber followed by addition of 1 ml of saturated K_2CO_3 solution in the outer chamber of Conway diffusion unit. The grease was applied on the rims of the unit to make air tight. Solution was kept overnight at room temperature. Then the inner chamber content was titrated against 0.02 N Sulphuric acid. A blank was conducted using 2% TCA solution instead of sample. TVB-N calculated using following formula and expressed in mg %.

$$\text{TVB-N (mg \%)} = \frac{14 \times N \times (X-Y) \times 100 \times 100}{S}$$ Where,

N = Normality of sulphuric acid
X = ml of sulphuric acid required for titration of sample
Y = ml of sulphuric acid required for the titration of blank
S = Weight of sample in gm

2.2.2.2. Tri-methyl amine Nitrogen (TMA-N)

Tri-methyl amine Nitrogen (TMA-N) of fresh refrigerated stored sample was determined by Conway micro diffusion method [16]. 10 g of the sample was macerated with 10 ml of 20% tri-chloro acetic acid (TCA) solution using pestle and mortar. The slurry was filtered through coarse filter paper and the filtrate was made upto 100ml by adding distilled water in a volumetric flask. 2ml of boric acid containing mixed indicator (0.066% methyl red and 0.066% bromo cresol green in alcohol in the ratio of 1:1) into the inner chamber and 1 ml of sample into the outer chamber followed by addition of 1 ml of saturated K_2CO_3 solution in the outer chamber of Conway diffusion unit. The unit was covered with glass covering with the application of grease on the rims of the unit, leaving a small gap for adding the formaldehyde in the outer chamber of the unit. The content of outer chamber were mixed gently by rotation. Solution was kept overnight at room temperature. Then the inner chamber content was titrated against 0.02 N Sulphuric acid. A blank was conducted using 2% TCA solution instead of sample. TMA-N calculated using following formula and expressed in mg %.

$$\text{TMA-N (mg \%)} = \frac{14 \times N \times (X-Y) \times 100 \times 100}{S}$$

Where,

N = Normality of sulphuric acid
X = ml of sulphuric acid required for titration of sample
Y = ml of sulphuric acid required for the titration of blank
S = Weight of sample in gm

2.2.2.3. Alpha amino nitrogen (AAN)

The alpha amino nitrogen (AAN) in the samples was estimated by the method described by Pope and Stevens [17]. 20 ml of TCA extract was taken into a 100ml volumetric flask. Few drops of thymolpthalein indicator were added and the extract was made alkaline by adding normal NaOH, till a distinct blue colour appeared, then 1 part by volume of $CuCl_2$ was mixed with 2 parts by volume of tri-sodium phosphate and 2 parts by volume of borate buffer. The solution was mixed well and 60 ml of this suspension was added to alkaline solution in a standard flask containing the extract, the volume was made upto 100 ml with distilled water and after shaking it was allowed to stand for 30 min and then filtered. 10 ml of the filtrate was pipetted out into a conical flask and 0.5 ml of glacial acetic acid (CH_3COOH) was added followed by addition of 1.0 g of potassium iodide. The librated iodine was titrated against N/100 sodium thiosulphate using starch as an indicator. When a yellow solution of iodine became faint yellow, few drops of saturated starch solution were added and titration was continued till blue colour completely disappears. The AAN was calculated using the following formula and expressed as mg % of the sample.
1 ml of N/100 $Na_2S_2O_3$ = 0.28 mg of AAN

$$\text{AAN (mg \%)} = \frac{28 \times N \times V4 \times V1 \times 100}{V3 \times V2 \times W} \times 100$$ Where,

V_1 = Total volume of TCA extract
V_2 = Volume of TCA extract taken for estimation
V_3 = Filtered extract taken for titration
V_4 = Volume of $Na_2S_2O_3$
W = Weight of sample

2.2.2.4. Non-protein nitrogen (NPN)

The non-protein nitrogen (NPN) content of the samples was determined by Kjeldhal method [18]. 10 g of sample was digested with 10 ml of 20% tri-chloro acetic acid (TCA) solution using pestle and mortar. The slurry was filtered through coarse filter paper, the filtrate was made upto 100 ml with distilled water. 10 ml of aliquot was digested with 10 ml of concentrated sulphuric acid and a pinch of digestion mixture [K_2SO_4 (10): $CuSO_4$ (1): SeO_2 (0.25)] in a 250 ml

digestion flask. Few glass beads were added to the digestion flask to avoid bumping. The contents in the digestion flask were heated in the digestion chamber. Digestion was continued till a colourless solution was obtained. After cooling the volume was made by adding distilled water in 100 ml volumetric flask. 2 ml of aliquot was taken and distilled in the Kjeldhal distillation unit with 10 ml of 40% sodium hydroxide solution. The librated ammonia was absorbed in 10 ml of 2% boric acid solution containing mixed indicator (0.1% methyl red and 0.1% bromocresol green in 1:5 ratio dissolved in 95% ethyl alcohol) till the colour of boric acid solution turned to green. This was titrated against 0.02 N standard sulphuric acid until the pink colour was developed. Non-protein nitrogen content was calculated by multiplying total nitrogen content by 6.25 and expressed as percentage weight of meat.

$$\text{Total nitrogen (\%)} = \frac{28\times N\times V4\times V1\times 100}{V3\times V2\times W}$$

Where,
N = Normality of H_2SO_4
X = ml of standard H_2SO_4 required for titration of sample
V_1 = Aliquot (ml) of digested extract taken for distillation
V_2 = Aliquot (ml) of TCA extract taken for digestion
V_3 = Total volume of TCA extract
W = Weight (g) of sample

2.2.3. Lipid oxidation analysis

2.2.3.1. Peroxide value (PV)

The peroxide value (PV) of fish sample was determined following the method of Jacob (1958) [19] iodometrically. 10 g of sample was macerated with anhydrous sodium sulphate using pestle and mortar to make sample free from moisture and then transferred to a conical flask, 60 ml of chloroform was added and kept for 15 min. The slurry was filtered and 10 ml of filtrate was taken in conical flask, dried on water bath to evaporate the chloroform. 10 ml of chloroform extract was taken in an iodine flask and a pinch of KI was added. The content was shaken well for 1 min and then allowed to stand in dark for 30 min. Then about50 ml of distilled water was added by washing the stopper and sides of the flask, and 15 ml acetic acid was added. The content in the flask was titrated against 0.01 N sodium thiosulphate solution using starch as indicator with vigorous shaking till the complete disappearance of the blue colour. A blank was also done simultaneously without sample. The peroxide value of an oil or fat is the amount of peroxides expressed as meq O_2/Kg of fat by using following formula:

$$\text{Peroxide (meq } O_2\text{/Kg of fat)} = \frac{28\times N\times V4\times V1\times 100}{V3\times V2\times W}$$ Where,

V = Vol. of sodium thiosulphate used for sample
X = Vol. of sodium thiosulphate used for blank
N = Normality of sodium thiosulphate
W = Weight of sample

2.2.3.2. Thiobarbituric acid-reactive substance (TBARS)

The thiobarbituric acid reactive substance (TBARS) of fish samples was determined by the method of Raghavan and Hultin (2005) [20]. 6 g of fish meat was mixed with 18 ml of 7.5% TCA and homogenised at 10,000 rpm for 10 min using centrifuge. The supernatant was filtered by using Whatman no. 4 filter paper. From this 2 ml of filtrate was mixed with 2 ml of 0.02 MTBA solution in culture tubes and kept in water bath for 40 min and cooled to room temperature. The pink colour developed was measured by reading the absorbance at 530 nm. The TBARS was calculated using the following formula and expressed as mg of malonaldehyde/ kg of sample.

$$\text{TBARS (mg malonaldehyde/ kg of sample)} = \frac{28\times N\times V4\times V1\times 100}{V3\times V2\times W}$$ Where,

A = Absorbance at 530 nm
72.03= Molecular weight of malonaldehyde
TVE = Total volume of extract
V = Volume of reactant (TBA + TCA extract)
VE = Volume of extract added to TBA
W = Weight of sample

2.2.3.3. Free fatty acids (FFA)

The free fatty acids (FFA) was determined by the method of Dyer and Morton (1956) [21]. 10 g of sample was macerated with anhydrous sodium sulphate to make sample free from moisture using pestle and mortar, then transferred to a conical flask. 60 ml of chloroform was added and kept for 15 min. The slurry was filtered and 20 ml of filtrate was taken in conical flask, dried on water bath to evaporate the chloroform. Weight of the fat was taken after evaporating off the chloroform. To this flask 10 ml of neutral alcohol was added and warmed to dissolve the fat. The content was titrated by 0.02 N Noah using phenolphthalein as indicator, shaken vigorously till the appearance of faint pink colour indicating the end point of titration. The colour must persist for atleast 30 min. FFA was calculated by using the following formula:

$$\text{FFA (\% Oleic acid)} = \frac{28\times N\times V4\times V1\times 100}{V3\times V2\times W}\times 100$$ Where,

N = Normality of NaOH used for titration

2.3. Sensory analysis

Sensory characteristics and overall acceptability of fish were assessed by a panel of six members on the basis of ten point scale on each sampling [22] with little modification. Sensory characteristics study includes general appearance, odour, and texture of fish. The scores were given in the decreasing order scale with 10-9 excellent, 8-7 good, 6-5 fair and acceptable, 4-3 poor, and 2-1 very poor. The mean of the score given by panel represented the overall sensory quality of fish.

2.4. Statistical analysis

The data obtained from sensory and biochemical analysis was further analyzed by using Statistical Package for Social Science (SPSS, version 21.00). Analysis of variance (One way-ANOVA) was performed and the significance of difference was defined at $p<0.05$.

3. Results and discussions

3.1. Changes in pH

The changes in the pH value of mackerel is presented on the Fig. 1 and showed no significant difference ($p>0.05$). The pH on the initial day recorded was 6.21 and increased slightly on 4th d, then again decreased to 6.21 on 8th day. Much variation was not observed during the storage period. Similarly [23] reported no major changes in the initial pH (6.6) of fish muscle up to day 6 of storage. According to [24], the initial pH in farmed tilapia was 6.66, which decreased slightly ($p<0.05$) to 6.42 during storage at 4 °C. The post-mortem pH, [25] is the most significant factor influencing the texture of the meat and the degree of “gaping”.

3.2. Changes in nitrogenous compounds

3.2.1. Total volatile base nitrogen (TVBN)

The total volatile base nitrogen (TVBN) of mackerel stored in refrigerated condition is shown in Fig 2. It showed significant difference ($p<0.05$), on the initial day the TVB-N value observed in mackerel was 15.87 mg %, it increased up to 6th d (33.13 mg %), then it decreased to 15.86 mg % on the 8th day. [26] Ozogul *et al*. (2011), reported similar fluctuating trend in common sole (*Solea solea*) during chilled storage.

Increase in TVBN with length of storage is mainly attributed to the production of ammonia [27]. Ammonia is usually formed by bacterial deamination of amino acids as well as generated by the autolytic breakdown of adenosine monophosphate (AMP) [28].

3.2.2. Tri-methylamine nitrogen (TMA-N)

Tri-methylamine nitrogen (TMA-N) values of mackerel under refrigerated conditions is represented Fig. 3, showing the significant difference ($p<0.05$). It also followed the similar pattern like changes in TVBN content. On the initial day the TMA-N value calculated for mackerel was 2.8 mg %, it increased upto 8.40 mg % on 6th day and then decreased to 3.73 mg %. Similarly the TMA-N value in tilapia increased significantly ($p<0.05$) initially from 0.65 mg % to 15.96 mg % on 5th d stored at 4 °C [24]. The acceptability of fish for 5 d and even more was reported [29] during refrigerated storage.

3.2.3. Alpha amino nitrogen (AAN)

The alpha amino nitrogen (AAN) content is represented in the Fig. 4, it showed that the AAN value showed no significant changes with the increase in the storage days. At the initial day the AAN value recorded was 0.61 mg % and it reached 0.37 mg % on the 8th day. A similar observation was reported in chilled murrel and milk fish [30].

3.2.4. Changes in non-protein nitrogenous (NPN)

The non-protein nitrogenous (NPN) content recorded on the initial day was 1.05 mg %. It decreased significantly ($p<0.05$) and reached 0.35 mg % on the 8th d of storage period, which is represented in Fig 5. A decrease in NPN contents in ice stored *Penaeus indicus* for 18 days was observed and the decrease was attributed to protein hydrolysis by bacterial enzymes [31]. Results obtained in the study conducted by [32] showed gradual decrease in NPN of iced black tiger shrimp. During ice and chilled storage NPN fraction leached out including a major portion of the water soluble amino acids. The loss of amino acids affects the taste and results in bitterness of meat.

3.3. Changes in lipid content

3.3.1. Peroxide value (PV)

The PV values showed significant difference ($p<0.05$) and are represented in the Fig 6. The value calculated on the initial day was 9.90 meq O_2/kg of fat. The PV value first increased with the increase in storage period and decreased on the 8th day. On the 8th d the PV value calculated for mackerel was 21.28 meq O_2/kg of fat [33]. The PV of fish finger increased from 10.73 to 17.8 meqO_2/kg of fat till the end of 5th d of storage and then subsequently decreased to 10.4 meqO2/kg of fat at the end of 15 d of storage period. Similar results were reported [34] in chilled steaks of Silver carp where the PV value increased upto 7th d and thereafter showing a decreasing trend. Hydroperoxide formed as primary oxidation products at higher levels might undergo the decomposition into secondary oxidation products.

3.3.2. Thiobarbituric acid reactive substance (TBARS)

TBARS value showed significant difference ($p<0.05$) during the storage study period, represented in Fig 7. The initial TBARS value calculated was 0.25 mg malonaldehyde/kg of meat, it increased upto 6th d, and then on 8th d it decreased to 2.24 mg malonaldehyde/kg of meat [34]. Study reported that TBA values first increased with storage period and then showed a significantly low ($p<0.05$) TBA values on the final day of storage probably due to the losses in secondary oxidation products formed, particularly the low molecular weight volatile compounds. That decline could be attributed to production of secondary lipid oxidation products from MDA, this phenomena was also observed by [35].

3.3.3. Free fatty acid (FFA)

On the initial day the FFA value calculated for mackerel was 3.49% of oleic acid. The values of FFA showed significant difference ($p<0.05$) during refrigerated storage period and is represented in Fig. 8. The FFA increased significantly with the increase in storage period and it reached 24.01% of oleic acid on the 8th day. Similar results were reported in fish finger [33] and in fish burger [36] during refrigerated storage. Increasing trend of free fatty acid (FFA) was also observed in chilled common sole [26].

3.4. Changes in overall acceptability

The overall acceptability of stored mackerel is represented in Fig 9. According to the results, the overall acceptability of mackerel decreased significantly ($p<0.05$) with the increase in storage days. The initial scores recorded were 9.4, the scores decreased progressively upto 3.8 on the 8th day. [37] Similarly progressively quality loss in sensory scores of silver pomfret and mackerel were observed in the chilled condition.

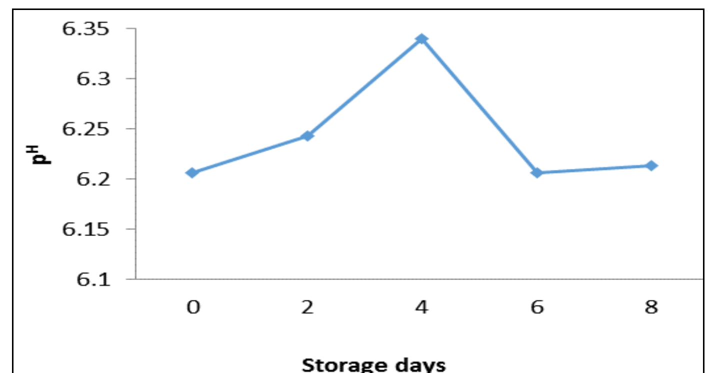

Fig 1: Changes in pH

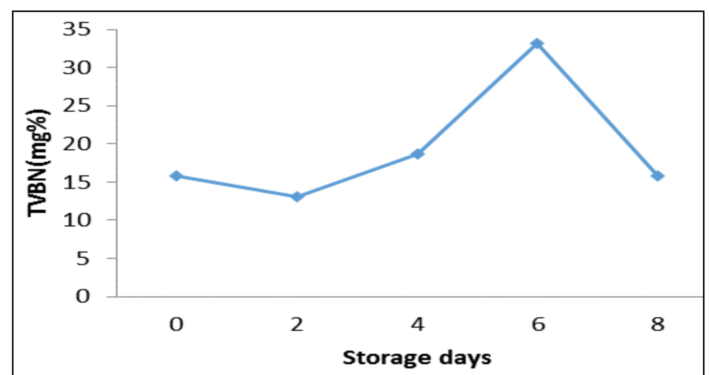

Fig 2: Changes in TVBN content

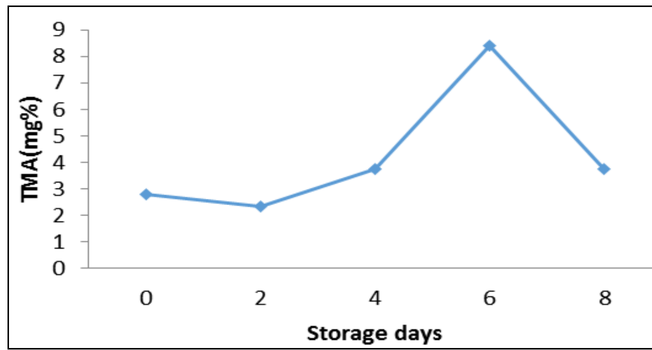

Fig 3: Changes in TMAN content

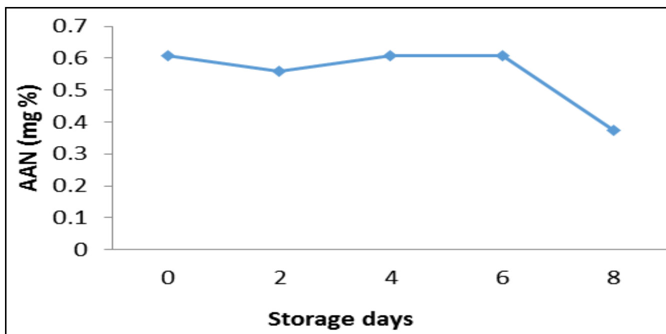

Fig 4: Changes in AAN content

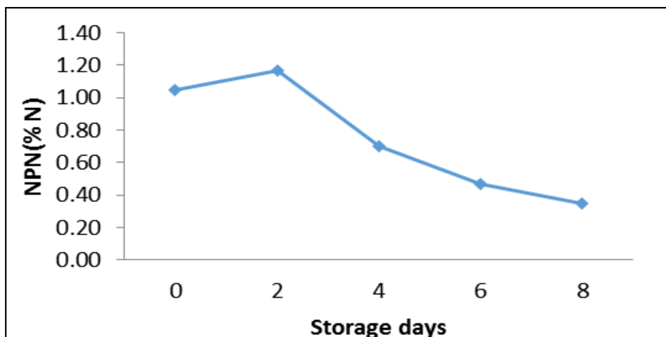

Fig 5: Changes in NPN content

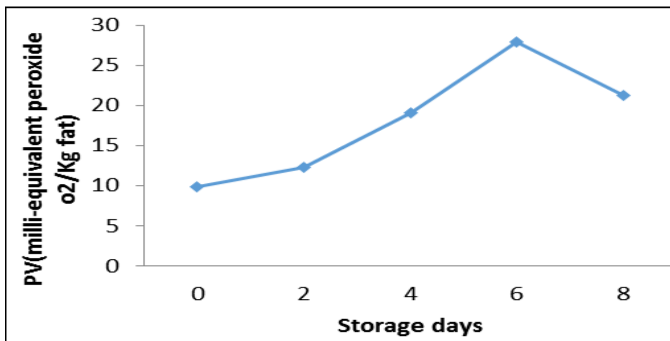

Fig 6: Changes in PV content

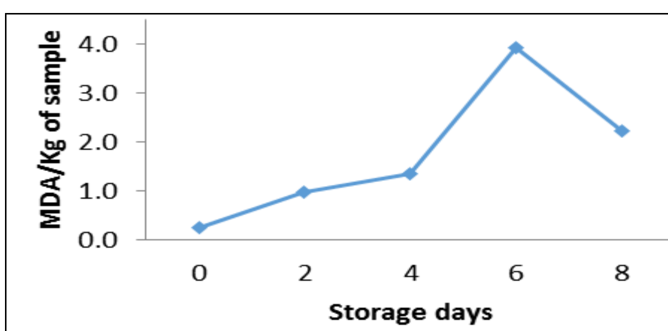

Fig 7: Changes in TBARS content

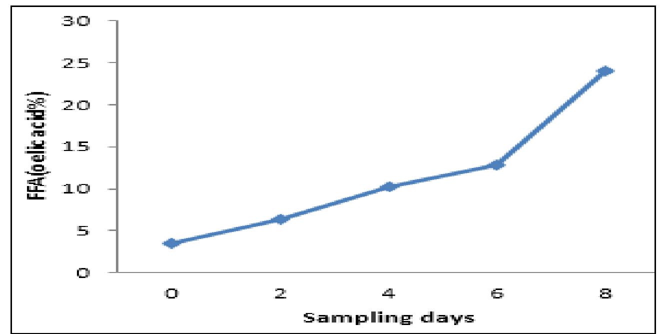

Fig 8: Changes in FFA content

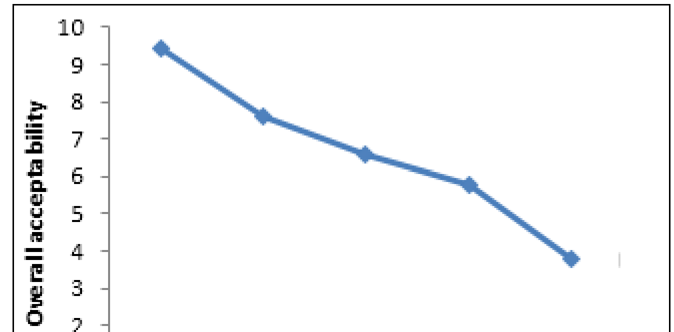

Fig 9: Changes in Overall acceptability

4. Conclusion

The present study was conducted to estimate the shelf life of mackerel in the indigenously developed refrigerated unit, and the results obtained were much better than the conventional chilling methods for short-term fish preservation. The stored Mackerel were acceptable upto the 8 days whereas in conventional chilling methods the shelf life of mackerel is found for 4-5 days only. Hence, the unit was found suitable for fisher folks to market the fish in fresh condition and thus preventing them to overcome from the loss occurring due to spoilage at ambient temperature.

5. Acknowledgement

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6. References

1. Fey MS, Regenstein JM. Extending shelf life of fresh wet red hake and salmon using CO_2-O_2 modified atmosphere and potassium sorbate ice at 1 °C. Journal of Food Science. 1982; 47:1048-1054.
2. Tidwell JH, Allan GL. Fish as food: Aquaculture's contribution. EMBO Reports, 2001; 2(11):958-963.
3. Jeyasekaran G, Ganesan P, Anandaraj R, Shakila RJ, Sukumar D. Quantitative and qualitative studies on the bacteriological quality of Indian white shrimp (*Penaeus indicus*) stored in dry ice. Food Microbiology, 2006; 23(6):526-533.
4. Connor WE. Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition. 2000; 71(1):171-175.
5. HUSS HH. Quality and Quality changes in fresh Fish. FAO. Fish. Technol. Pap. UN, Rome. 1995; 348:48.
6. Simpson R, Almonacid S, Acevedo C, Cortes C. Mathematical model to predict effect of temperature

abuse in MAP systems applied to Pacific hake (*Merluccius australis*). Journal of Food Engineering. 2003; 26:413-434.

7. Sivertsvik M, Jeksrud WK, Rosnes TJ. A review of modified atmosphere packaging of fish and fishery products- significance of microbial growth, activities and safety. International Journal of Food Science. 2002; 37:107-127.
8. Erkan N, Ozden O. Quality assessment of whole and gutted sardines (Sardina pilchardus) stored in ice. International Journal of Food Science and Technology. 2007; 43(9):1549-1559.
9. Whittle KJ, Hardy R, Hobbs G. Chilled Fish and Fishery Products. In: Gormley T. R. Chilled Foods. Elsevier Science Publishers Ltd, 1990, 87-116.
10. Sharifian S, Alizadeh E, Mortazavi MS, Moghadam MS. Effect of refrigerated storage on the microstructure and quality of Grouper (*Epinephelus coioides*) fillets. Journal of Food Science and Technology. 2014; 51(5):929-935.
11. Losada V, Pineuro C, Barros-velazuez J, Aubourg SP. Inhibition of chemical changes related to freshness loss during storage of horse makerel (*Trachurus trachurus*) in slurry ice. Food Chemistry, 2005; 93:619-625.
12. Althouse AD, Turnquist CH, Branco AF. Modern Refrigeration and Air Conditioning. *Good Heart Willox Co., London*, 1978.
13. Fan Y, Luo L, Souyri B. Review of solar sorption refrigeration technologies: Development and applications, Renewable and Sustainable Energy Reviews. 2007; 11:1758-1775.
14. Balakrishnan V. The Indian mackerel. Bull. CMFRI, 1970; 24:15-21.
15. Suzuki T. Fish and krill protein: Processing technology. Appl. Sci. pub. London, 1981, 62-112.
16. Beaty SA, Gibbons NE. The measurement of spoilage of fish. Journal of Biological Board of Canada. 1937; 3:77-91.
17. Pope CG, Stevens MF. The determination of amino nitrogen using a copper method. J. of Biochem. 1939; 33:1070-1076.
18. AOAC. Official Methods of Analysis; AOAC. Int. Gaithersburg, MD, USA, 2005, 18.
19. Jacobs MB. The chemical analysis of food and food products. Kreiger Publ. Co. Inc. New York, 1958, 393-394.
20. Raghavan S, Hultin HO. Model system for testing the efficacy of antioxidants in muscle foods. J. Agric. Fd. Chem. 2005; 1(53):457
21. Dyer WJ, Morton ML. Storage of frozen filter. Journal of fisheries Research board of canada. 1956; 13:129-134.
22. Sukumar D, Jeyasekaran G, Shakila RJ, Anandraj R, Ganeshan P. Sensory, microbiological and biochemical quality of black pomfrets (Pampus niger) stored in dry ice and ice. Fish Technol, 2007; 44(1):33-42.
23. Massa AE, Palacios DL, Paredi ME, Crupkin M. Postmortem Changes in Quality Indices of Ice-Stored Flounder (*Paralichthys patagonicus*). Journal of Food Biochemistry. 2005; 29:570-590.
24. Dergal NB, Abi-ayad SMEA, Deganad G, Douny C, Breose F, Daube G, *et al*. Microbial, biochemical and sensorial quality assessment of Algerian farmed tilapia *(Oreochromis niloticus*) stored at 4 ºC and 30 ºC. African Journal of Food Science. 2013; 7(12):498-507.
25. Huss HH, Fresh Fish, Quality and quality changes. FAO Fish, Rome, 1988, 29:132.
26. Ozogul Y, Boga EK, Tokur B, Ozogul F. Changes in biochemical, sensory and microbiological quality indices of common sole (*Solea solea*) from the Mediterranean sea, during iced storage. Turkish Journal of Fisheries and Aquatic Science, 2011; 11:243-251.
27. Adebona MB. Changes in total volatile bases during salt preservation of *Sardinella eda* and *Clupea harengus.* IPFC proceedings, monila, Philippines, 1978, 370-374
28. Shakila RJ, Vijayalakshmi K, Jeyasekaran G. Changes in histamine and volatile amines in six commercially important species of fish of the Thoothukkudi coast of Tamil Nadu, India stored at ambient temperature. *Food Chem*istry, 2003; 82:347-352.
29. Marrone R, Vollano L, Chirollo C, Palma G, Mercogliano R. Shelf life of different fish species stored with passive refrigeration (PRS). Veterinary Research Communications, 2009; 33:241-243.
30. Uchoi D, Mandal SC, Barman D, Kumar V, Andrade AJ, Phanna N. Physical, chemical and microbial changes during chilled storage. Aquafind, 2013, 1-4.
31. Joseph J, Perigreen PA, Gopalakrishna-iyer TS. Storage characteristics of cultured *Penaeus indicus* in ice and at ambient temperature. Fish. Technol, 1998; 35(2):84-89.
32. Naik RP, Nayak BB, Chouksey MK, Anupama TK, Moses TLSS, Kumar V. Microbiological and biochemical changes during ice storage of farmed black tiger shrimp (*Peneaus monodon*). Bionano Frontier, 2014; 7(2):249-253.
33. Praneetha SS, Dhanapal K, Reddy GVS, BALASUBRAMANIAN GVS. Development of fish finger from rohu (*Labeo rohita*) and its quality evaluation during refrigerated storage. International Journal of Environmental Science and Technology. 2015; 4(6):1457-1468.
34. Viji P, Tanuja S, George N, Zynudheen AA, Lalitha KV. Quality characteristic and shelf life of sutchi cat fish (*Pangasianodon hypophthalamus*) steaks during refrigerated storage. International Journal of Agriculture Food Science & Technology. 2014; 5(2):105-116.
35. Aubourg SP. Review: Interaction of malondaldehyde with biological molecules-new trends about reactivity and significance. International Journal of Food Science and Technology. 1993; 28:323-335.
36. Vanitha M, Dhanapal K, Reddy VSG. Quality changes in fish burge from Catla (*Catla catla*) during refrigerated storage. Journal of Food Science and Technology. 2013; 52(3):1766-1771.
37. Aubourg S, Lehmann I, Gallardo J. Effect of previous chilled storage on rancidity development in frozen horse mackerel (*Trachurus trachurus*). Journal of the Science of Food and Agriculture. 2002; 82:1764-1771.