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Investigation on the Freshness, Quality and Shelf-Life of Marine Fish in Ice Storage

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A B S T R A C T

Consumer consumption is increasing for minimally prepared fish that keeps its sensory and nutritional characteristics during processing and storage. The present study aimed to contribute more knowledge about the storage of marine fishes like Indian mackerel, red snapper, Indian whiting Lady fish, Indian goat fish and silver belly fish. During the storage, the lipid inside the fish muscle oxidises and breaks down into simpler molecules such as free fatty acids, peroxides, and thiobarbituric acid and microbial growth due to rich in protein. Spoilage was examined by sensory analyses, chemical and microbiological for the period of 1 to 20 days storage time at 4°C.

***Key words*:** Fish spoilage, Ice storage, Freshness, TBA, TVBN

Tamil Nadu is India's third longest coastal state in the southern portion of the Indian peninsula. It is rich in marine resources in two main regions, Palk Bay and Mannar Gulf [1]. From 9.9 kg per capita in the 1960s to 20 kg in 2016, worldwide fish consumption has risen significantly [2]. Fish is nutrient-dense, containing protein, vitamins, and minerals that our bodies require for optimal health and well-being. [3] Seafood, on the other hand, has a short shelf life due to its high nutritional content, neutral pH, and high moisture content [4]. The early microbiological and biochemical processes that occur in seafood after death cause changes in sensory and nutritional characteristics, reducing shelf life [5]. Seafood, in general, is high in polyunsaturated fatty acids (PUFAs), making it more prone to lipid oxidation. Lipid oxidation in seafood causes unpleasant odours and flavours, as well as nutritional loss, the formation of harmful compounds, and colour changes [6].

The difficulty of seafood spoilage is amplified by microbiological, chemical, and physical changes. Indigenous enzymes and chemical reactions are responsible for the first loss of freshness in fish, whereas microbial metabolic activity is responsible for total spoiling [7]. The methods for determining fish freshness date back to the mid-20th century, with the fastest and creative advancements occurring in the previous 20 years. However, some of these approaches are simple to apply but time demanding, whilst others require less time to produce results but are costly and complicated procedures [8]. In recent years, consumer preference has led to the increasing need for sensitive and quick analytical technologies in terms of food quality and food safety problems. Several conventional physical, textured, sensorial, and chemical analyses like TBA, TVBN, PV, FFA approaches have been employed in the assessment of freshness of the fish and other seafood items and have been authenticated.

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MATERIALS AND METHODS

Collection and preparation of samples

Samples were Collected from the Chidambaram Fish market and Experimental were conducted at Annamalai University, Department of Agricultural Microbiology laboratory. Indian mackerel (*Resatrelliger kanagurta*), Red snapper (*Lutjanus campechanus*), Indian whiting Lady fish (*Elops attinis*), Indian goat fish (*Parupeneus indicus*), Silver belly fish (*Gerres subtasciatus*) The fish were gutted and washed. After that they were surrounded by flake ice at a fish-to-ice ratio of 2:1 (w/w) and stored in a refrigerated box at 4 °C. During the storage, ice was added to the fish samples as required. All analyses were performed on days 1, 5, 10, 15 and 20.

Sensory analyses

Spoilage is defined as any change in the initial state of seafood that results in an unpleasant odour, taste, look, or texture. This transition can be related to enzymatic, chemical, and biological factors or microbiological activity in seafood [9]. Colour and texture measures are relevant to physical characteristics, but form, size, volume, and weight also play a role in identifying freshness [10]. The quality index technique (QIM) was used to evaluate raw fish, as indicated in (Table 1). This sensory rating is based on [11] freshness quality criteria for herring. Each evaluator was given basic descriptors and was

awarded demerit points ranging from 0 to 3, with 0 representing best quality and any higher score indicating lower quality. The panellists were asked if the fish were acceptable or not. That was used to determine the shelf life of Indian mackerel, red snapper, Indian whiting Lady fish, Indian goat fish and silver belly fish.

Chemical methods

TBA method

The levels of thiobarbituric acid (TBA) were monitored throughout time. The TBA values were determined using a spectrophotometer with a timer, in which 10g of minced fish was macerated with 50ml of distilled water at 2-minute intervals, then rinsed and add 47.5ml of water transferred into a distillation flask. 5 glass beads and 2.5ml 4N HCL (antifoam liquid) were added. The mixture was distilled at a rate of 5ml/min, the distillate (5ml) was placed in a glass stoppered tube, 5ml of TBA reagent was added, and the tube was heated for 35 minutes in boiling water. After allowing the sample to cool, the absorbance was measured at 538nm against a blank. The TBA value was calculated using each absorbance [12]. The rate of degradation in these tropical fish species has also been tracked and evaluated on a scale of 1 to 10, with 1 signifying excellent condition, 2 - 3 good, 4 to 5 terrible, and 5 to10 spoiled.

Determination of TVBN

The presence of volatile nitrogenous bases in the original material (fishmeal) is assessed using the TVB-N parameter. According to Ricque-Marie [13], it provides a reliable estimate of the final product's quality. 100 g of fresh fish flesh was weighed and combined with 300 ml of 5% Tricholoroacetic acid in a blender. The mixture was then centrifuged for 1 hour at 3000 g to produce clear extract. 5 ml of the extract and 5 ml of 2 M sodium hydroxide (NaOH) were pipetted into the Markhan apparatus. This was steam distilled into 15 mL of 0.01 M hydrochloric acid (HCl) with 0.1 mL of rosolic indicator. The surplus acid was then titrated in the receiving flask with standard 0.01 M NaOH to a pale pink end point after distillation. A procedural blank was performed with 5 mL Trichloroacetic acid and no sample, and the titration was performed as previously [14]. The following formula was used to calculate TVBN concentration (in mg N/100 g sample):

$$\text{TVBN (mg N/100g Sample)} = (M)(VB - VS)(14)(300 + W) / 5$$

Where;

VB = ml NaOH used for blank titration

W = water content of sample in g/100 g

M = molarity of NaOH standard solution and

VS = ml NaOH used for sample titration

Peroxide value (PV) – Iodometric titration method

The peroxide value (PV) is used to calculate the amount of peroxide/hydroperoxide produced during the initial stage of oil oxidation by measuring the amount of iodine released from potassium iodide during the titration process [15-16]. The iodometric titration technique is a method for estimating the total quantity of the principal oxidation products: hydroperoxides, and it is based on the redox characteristics of the hydroperoxides. As shown in equations 1 and 2, the hydroperoxides in the oil sample react with iodide ions (I-) in a saturated potassium iodide (KI) solution to generate iodine (I_2), which is then titrated against a standardized sodium thiosulfate solution ($Na_2S_2O_2$). The end point can be measured using a starch indicator, but recently it is most often determined potentiometrically by electrochemically detecting freed iodine reduction at a platinum electrode [17]. PV is measured in milliequivalents of peroxides per kilogramme (meq/kg) [18].

Free fatty acid (FFA) titration method

The lipid inside the fish muscle oxidises and breaks down into simpler molecules such free fatty acids, peroxides, and thiobarbituric acid, resulting in muscle deterioration [19]. The quantity and type of the sample were taken into consideration when developing this technique. The fish lipid extract was nitrogen evaporated and redissolved in ethanol that had been neutralized with *m*-cresol purple and heated to 60 °C. A violet end point was reached after titrating three replicates per sample. The use of *m*-Cresol purple to increase the sensitivity of the method has been proposed, and it has been used for the titration of lipid fish extracts [20]. The extract, which included 100 mg of lipids, was titrated with 0.05 N NaOH in this example. FFA levels are often expressed as a percentage of oleic acid:

$$\% \text{ Oleic acid} = (\text{mL of NaOH} \times \text{NaOH normality} \times 28.2)/\text{weight of sample (g)}$$

Microbiological analysis

Seafood is easily vulnerable to opportunistic and harmful bacteria. The microbial load of seafood is mostly determined by its habitat, which is a microbe-rich environment [21]. Samples, from three different fish stored in ice for each group, were taken to estimate total viable counts (TVC). Ten grams of fish muscle were mixed with 90 ml of ringer solution for 3 min. Further decimal dilutions were performed before pipetting 0.1 ml of each dilution over the surface of plate count agar (Oxoid) plates. They were then incubated at 30 °C for two days.

RESULTS AND DISCUSSION

Sensory analyses

The (Table 1) shows the total demerit points of stored fish in ice. The Indian mackerel, red snapper, Indian whiting Lady fish, Indian goat fish and silver belly fish were a very bright look, a firm texture, bright and convex eyes, and fresh smells when they were just harvested. With storage time, demerit points increased in all species. Although the whole species had similar initial sensory ratings on day 0, red snapper had better scores on day 20 Indian mackerel followed Indian goat fish, silver belly fish and Indian whiting Lady fish. The permissible shelf life for red snapper (demerit score: 13.27) followed Indian mackerel (demerit score: 13.56), Indian goat fish (demerit score: 14.20), silver belly fish (demerit score: 14.68) and the last Indian whiting Lady fish (demerit score: 15.39). The results of the sensory analysis indicated the shelf life of these fishes was different from the lives of that the storage.

Chemical methods

TBA method

The aldehyde (malondialdehyde) generated during the rancidity process of the lipid had reduced to the point where the change in TBA was no longer significant after days of storage. This might indicate that the lipids have totally rancidity and that continued storage will not produce more aldehydes. This is in line with the findings of previous researchers [22]. The five fish samples analyzed were found to have TBA values of 0.89, 0.73, 0.75, 0.90 and 0.95mgMA/kg for Indian mackerel, red snapper, Indian whiting Lady fish, Indian goat fish and silver belly fish respectively at first day of keeping. Fresh fish had a TBA value of less than 5.0 mgMA/kg, confirming that the fishes were still fresh. (Table 2) showed during the 20th day of the incubate in

ice the five fish species of Indian mackerel, red snapper, Indian whiting Lady fish, Indian goat fish and silver belly fish. The TBA values were rapid increased viz., 6.80, 5.61, 6.55, 7.20, and 6.75mgMA/kg respectively.

Table 1 Quality index method (QIM) for Sensory evaluation of fish samples (Nielsen and Hyldig 2004)

Quality parameter	Description	Score
Whole fish appearance of skin	Very bright	0
	Bright	1
	Mat	2
Blood on gill cover	None	0
	Some	1
	Much	2
Texture	Hard	0
	Firm	1
	Soft	2
Texture on belly	Firm	0
	Soft	1
	Burst	2
Odour	Fresh sea odour, sea weedy, metallic	0
	Neutral	1
	Slight off odour	2
	Strong off odour	3
Eyes appearance	Bright	0
	Somewhat lustreless	1
Shape	Convex	0
	Flat	1
	Sunken	2
Gill colour	Characteristic red	0
	Somewhat pale, mat, brown	1

Total demerit points (0-18)

Table 2 Investigation of chemical changes in fish samples at different days of storage

Samples	Storage days	TVB-N mg/100 g	TBA (mg MA kg^{-1})	PV meq/kg	FFA % of oleic acid
Indian mackerel	1	20.36 ± 0.01	0.89 ± 0.03	0.75 ± 0.02	0.95 ± 0.11
	5	22.45 ± 0.03	1.46 ± 0.04	1.73 ± 0.03	1.42 ± 0.13
	10	23.76 ± 0.04	3.25 ± 0.06	3.46 ± 0.04	2.11 ± 0.15
	15	31.25 ± 0.06	5.57 ± 0.07	5.91 ± 0.05	3.20 ± 0.17
	20	34.57 ± 0.08	6.80 ± 0.09	6.55 ± 0.06	3.63 ± 0.19
Red snapper	1	20.26 ± 0.02	0.73 ± 0.01	0.83 ± 0.01	0.81 ± 0.13
	5	23.40 ± 0.04	1.16 ± 0.02	1.25 ± 0.03	1.53 ± 0.14
	10	27.51 ± 0.05	3.25 ± 0.03	3.31 ± 0.04	2.69 ± 0.15
	15	30.23 ± 0.07	4.38 ± 0.05	5.60 ± 0.06	3.25 ± 0.17
	20	33.81 ± 0.09	5.61 ± 0.08	7.13 ± 0.08	4.64 ± 0.19
Indian whiting	1	20.15 ± 0.01	0.75 ± 0.02	0.91 ± 0.01	0.91 ± 0.08
Lady fish	5	22.71 ± 0.03	2.26 ± 0.03	1.26 ± 0.03	1.50 ± 0.10
	10	27.68 ± 0.05	3.18 ± 0.04	3.65 ± 0.05	2.67 ± 0.12
	15	31.24 ± 0.06	5.43 ± 0.06	5.40 ± 0.07	3.25 ±0.15
	20	34.82 ± 0.09	6.55 ± 0.08	6.12 ± 0.09	3.89 ± 0.17
Indian goat fish	1	20.42 ± 0.03	0.89 ± 0.02	0.86 ± 0.03	0.88 ± 0.14
	5	21.25 ± 0.06	1.40 ± 0.04	1.23 ± 0.05	1.60 ± 0.16
	10	25.38 ± 0.08	3.85 ± 0.06	3.51 ± 0.07	2.25 ± 0.19
	15	29.63 ± 0.09	5.73 ± 0.09	4.68 ± 0.08	3.16 ± 0.21
	20	31.79 ± 0.11	7.20 ± 0.10	6.33 ± 0.11	4.37 ± 0.23
Silver belly fish	1	20.75 ± 0.01	0.95 ± 0.03	0.73 ± 0.02	0.76 ± 0.11
	5	22.36 ± 0.03	1.35 ± 0.05	1.18 ± 0.03	1.61 ± 0.14
	10	26.14 ± 0.03	3.43 ± 0.06	2.65 ± 0.04	2.58 ± 0.16
	15	28.31 ± 0.04	4.68 ± 0.07	4.07 ± 0.06	3.40 ± 0.18
	20	30.28 ± 0.06	6.75 ± 0.09	6.35 ± 0.08	3.93 ± 0.20

Mean ± *SD; n* = 3

Determination of TVBN

In this study, the highest value of TVBN is Indian whiting lady fish (34.82) followed Indian mackerel (34.57), red snapper (33.81), Indian goat fish (31.79) and silver belly fish (30.28) at the 20th day of ice storage. Pearson [23] suggested a TVBN limit of 20 to 30 mg N/100 g for fresh fish, whereas Kirk and Sawyer [24] advised the upper limit was proposed to be 30 to 40 mg N/100 g. In addition, Connell's [25] recommendation is the maximum acceptability of fish has been recorded 30 mg N/100 g.

Peroxide value (PV) – Iodometric titration method

The PV value indicates the early stages of oxidative rancidity. Red snapper had the highest PV (7.13 meq/kg)

followed by Indian mackerel (6.55 meq/kg), silver belly fish (6.35 meq/kg), Indian goat fish (6.33 meq/kg) and finally Indian whiting lady fish (6.12 meq/kg). PV for fish oil is acceptable ≤5 meq/kg oil [26]. On the other hand, [27] also reported that PV less than 5 meq/kg oil shows that fat is not oxidized, but PV between 5 and 10 meq/kg oil suggests rancidity.

Table 3 Estimation of total viable count (log_{10} cfu g^{-1}) in fish samples at different days of storage

Days of storage	Population of microorganism in fish samples									
	Indian mackerel		Red snapper		Indian whiting Lady fish		Indian goat fish		Silver belly fish	
	Bacteria	Yeast and molds	Bacteria	Yeast and molds	Bacteria	Yeast and molds	Bacteria	Yeast and molds	Bacteria	Yeast and molds
1 day	2.64±0.14	-	1.01±0.13	-	2.13±0.14	-	2.25±0.15	-	2.85±0.13	-
5 days	3.32±0.15	-	2.65±0.15	-	3.62±0.16	-	3.36±0.17	-	3.14±0.14	-
10 days	4.14±0.17	1.18±0.01	3.12±0.17	1.20±0.01	4.80±0.19	-	4.53±0.20	1.21±0.02	3.75±0.17	1.07±0.02
15 days	5.29±0.22	1.44±0.02	4.53±0.19	1.51±0.03	5.35±0.22	1.08±0.01	5.18±0.22	1.44±0.04	4.28±0.19	1.26±0.03
20 days	6.86±0.25	1.68±0.04	6.20±0.21	1.78±0.05	7.63±0.24	1.22±0.03	7.70±0.25	1.68±0.07	5.41±0.21	1.85±.005

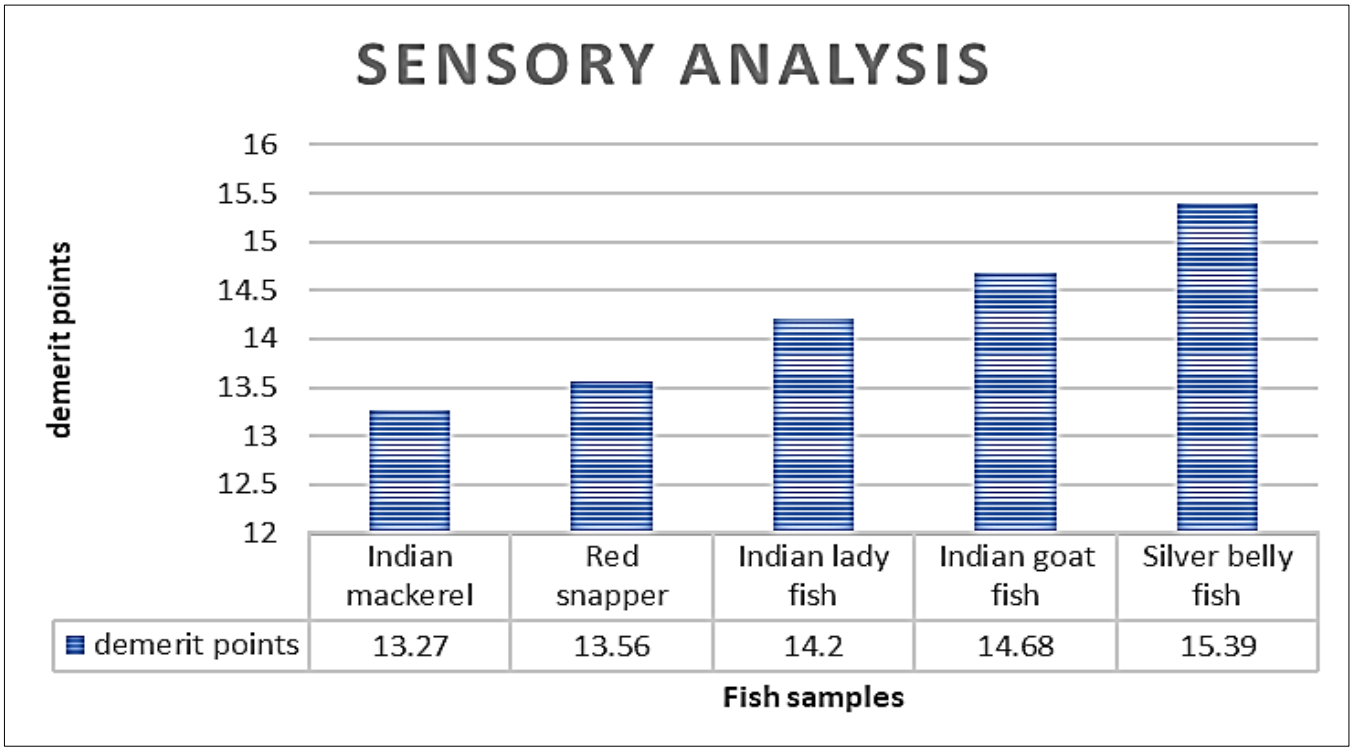

Fig 1 Sensory evaluation of fish samples at 20th day

Free fatty acid (FFA) titration method

The data in (Table 2) shows the Free fatty acid (FFA) content of fish. The free fatty acid level in Indian whiting lady 4.90% of oleic acid followed red snapper 4.64% of oleic acid, Indian goat fish 4.37% of oleic acid, silver belly fish 3.93% of oleic acid and Indian mackerel 3.63% of oleic acid was typically low in the current investigation. FFAs are significant not just in terms of oxidation products, but they have also been found to have a direct sensory influence [28]. It is preferable that the FFA level of edible oil between 0.0 and 3.0 percent oleic acid [29].

Microbiological analysis

The results depicted in (Table 3) shows microbial counts on Indian mackerel, red snapper, Indian whiting Lady fish, Indian goat fish and silver belly fish maintained on ice. Total viable counts (TVC) increased over the storage period. The Total viable counts (TVC) for fish was exceeded. After 20 days, log cfu g^{-1} was regarded the highest level for acceptability for Indian whiting Lady fish (7.63cfu g^{-1}) followed by, Indian mackerel (6.86 cfu g^{-1}), Indian goat fish (7.70 cfu g^{-1}), silver belly fish (5.41 cfu g^{-1}), and red snapper (6.20 cfu g^{-1}). Bacteria proliferated faster in all species during the storage periods. The results indicating that sensory analysis was strongly linked with microbiological analysis investigation of the fish. The maximum microbiological limit for TVC which separates the good quality fish from bad quality is 5×10^5 cfu g^{-1} [30].

CONCLUSION

The shelf life of the five species of fishes were analyzed for shelf-life study and based on the results it was concluded that (Indian mackerel, red snapper, Indian whiting Lady fish, Indian goat fish and silver belly fish) limits for sensory acceptability for the period of 15 days. And in chemical analysis method of TBA, TVBN, PV and FFA the acceptability of fish was up to 15th day of storage, thereafter, the spoilage begins when kept in ice. The results of microbiological and chemical tests confirmed these conclusions. The range of the microbial population of bacteria, yeast and mold increased during the increase of the storage period. Among the five fishes the Indian whiting lady fish got spoiled during the 15th days of storage followed by Indian mackerel, Indian goat fish, silver belly fish and red snapper.

LITERATURE CITED

1. Ajmal Khan S, Kadharsha K, Lyla PS. 2017. Fishing ban in Tamil Nadu - reprieve for fishes to breed or enforced summer vacation for fisherman. *Indian Ind. Jr. Geo. Mar. Sci*. 46(11): 2386-2392.
2. Anonymous. 2016. Food and Agriculture Organization (FAO). Global per capita fish consumption rises above 20kilograms a year. Italy.
3. Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MC, Clay J, Naz S, Saeed R. 2018. Oxidative stability of canola oil by physico-chemical analysis and FT-IR spectroscopy. *Asian Journal of Pharmaceutical Research and Development* 6(1): 9-15. https://doi.org/10.22270/ajprd.v6i1.353
4. Viji P, Venkateshwarlu G, Ravishankar C, Gopal TS. 2017. Role of plant extracts as natural additives in fish and fish products-A review. *Fishery Technology* 54: 145-154. https://doi.org/10.1080/10408398.2010.518775.
5. Olatunde OO, Benjakul S. 2018. Nonthermal processes for shelf-life extension of seafoods: A revisit. *Comprehensive Reviews in Food Science and Food Safety* 17: 892-904. https://doi.org/10.1111/1541-4337.12354
6. Secci G, Parisi G. 2016. From farm to fork: Lipid oxidation in fish products. A review. *Italian Journal of Animal Science* 15: 124-136. https://doi.org/10.1080/1828051X.2015.1128687
7. Sriket C. 2014. Proteases in fish and shellfish: Role on muscle softening and prevention. *International Food Research Journal* 21: 433-445.
8. Prabhakar PK, Srivastav PP, Pathak SS. 2019. Kinetics of total volatile basic nitrogen and trimethylamine formation in stored rohu (*Labeo rohita*) fish. *Journal of Aquatic Food Product Technology* 28(5): 452-464.
9. Ghaly AE, Dave D, Budge S, Brooks M. 2010. Fish spoilage mechanisms and preservation techniques. *American Journal of Applied Sciences* 7: 859. https://doi.org/10.3844/ajassp.2010.859.877
10. Olafsdottir G, Nesvadba P, Di Natale C, Careche M, Oehlenschlager J, Tryggvadottir SV, Macagnano A. 2004. Multisensor for fish quality determination. *Trends in Food Science and Technology* 15(2): 86-93.
11. Nielsen D, Hyldig G. 2004. Influence of handling procedures and biological factors on the QIM evaluation of whole herring (*Clupea harengus* L.). *Food Research International* 37: 975-983.
12. Dandago MA, Garba R, Bahago EJ, Komolafe GO. 2004. Analysis of specific food commodities. *In*: (Eds) Balami T. A. Practical manual on food technology, nutrition and dietetics for schools and industries. National Science and Technology Forum, Kaduna. pp 225-226.
13. Ricque-Marie D, Abdo-de La Parra MI, Cruz-Suarez LE, Cuzon G, Cousin M, Pike IH. 1998. Raw material freshness, a quality criterion for fish meal fed to shrimp. *Aquaculture* 165(1/2): 95-109.
14. Anonymous. 1986. Food and Agriculture Organization of the United Nations 1986.
15. Nunes CA. 2014. Vibrational spectroscopy and chemometrics to assess authenticity, adulteration and intrinsic quality parameters of edible oils and fats. *Food Research International* 60: 255-261. https://doi.org/10.1016/j.foodres.2013.08.041
16. Naz S, Saeed R. 2018. Oxidative stability of canola oil by physico-chemical analysis and FT-IR spectroscopy. *Asian Journal of Pharmaceutical Research and Development* 6(1): 9-15. https://doi.org/10.22270/ajprd.v6i1.353
17. Frankel EN. 2005. *Lipid Oxidation*. Oily Press.
18. Shahidi F, Zhong Y. 2005. Lipid oxidation: Measurement methods. *Bailey's Industrial Oil and Fat Products.* John Wiley & Sons, Inc.
19. De Abreu DP, Losada PP, Maroto J, Cruz JM. 2010. Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (*Salmo salar* L.). *Food Research International* 43(5): 1277-1282.
20. Brake NC, Fennema OR. 1999. Lipolysis and lipid oxidation in frozen minced mackerel as related to Tg' molecular diffusion, and presence of gelatin. *Chem/Biochemistry* 61: 125-132.
21. Kuley E, Durmus M, Balikci E, Ucar Y, Regenstein JM, O¨ zog˘ul F. 2017. Fish spoilage bacterial growth and their biogenic amine accumulation: Inhibitory effects of olive by-products. *International Journal of Food Properties* 20: 1029-1043. https://doi.org/10.1080/10942912.2016.1193516
22. Saeed S, Howell N. 2002. Effect of lipid oxidation and frozen storage on muscle protein fish. *Journal of Agriculture and Food Chemistry* 82: 579-586.
23. Pearson D. 1976. *The Chemical Analysis of Foods*. 7th Edition Churchill Livingstone, Edinburg, London. pp 289-497.
24. Kirk RS, Sawyer R. 1991. *Pearson's Composition and Analysis of Foods*. 9th Edition (Student edition). England Addision Wesley Longman Ltd. pp 33-36.
25. Connell JJ. 1995. *Control of Fish Quality.* Fourth Edition. Church Hill Livingstone Edinburg, Scotland. pp 245.
26. FAO. 2017. *Standards for Fish Oils*. Italy: Codex Alimentarius Commission.
27. Gracey JR, Collins DS, Huey R. 1999. Meat Hygiene. Philadelphia, PA: W. B. Saunders Company. Health Canada. 2009. Evidence for Quality of Finished Natural Health Products. Ottawa, ON, Canada: Natural Health Products Directorate, Health Canada.
28. Ashton IP, Unilever R, harnbrook D. 2002. Understanding lipid oxidation in fish. *In*: Safety and Quality Issues in Fish Processing. (Eds) Bremner H. A. Cambridge, United Kingdom: Woodhead Publishing Limited. pp 254-285.
29. Bassir O. 1971. *Handbook of Practical Biochemistry*. 2nd Edition. Ibadan, Nigeria: Ibadan University Press. pp 1017.
30. ICMSF. 1986. International Commission of Microbiological Specification for Food. Microorganisms in Food 2. Sampling for Microbiological Analysis: Principles and Specific Applications. 2nd Edition. University of Toronto Press, Toronto, Canada.