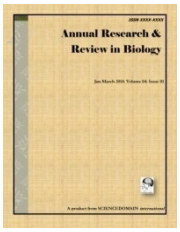

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Study the Chemical, Physical Changes and Microbial Growth as Quality Measurement of Fish

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Authors' contributions

This work was carried out in collaboration between all authors. Author MSAJ the study, wrote the protocol, and performed chemical analysis. Author FMAJ performed microbiological analysis, performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

This study examined the effects of chilling, freezing, packaging, temperature and storage time on the physical, chemical changes and microbiological growth in barred Spanish mackerel (*Scomberomorous commerson*). At time 0 the free fatty acid, trimethylamine, and thiobarbituric acid reactive substances (TBARS) valueswere1.2%, 0.30 mg- N/100g and 0.17mgmalonaldehyde/kg, respectively. Total viable cell count and coli forms fecal were 4.6 and 3.3 $\log_{10}$ CFU/g, respectively. Barred Spanish mackerel was chilled at two different temperatures (4 and 7ºC) for6 days. Also, fresh mackerel was kept frozen up to 6 months at –10 and -18ºC. Sampling was carried out on the initial and at 1, 3, and 6 days for chilling and 0, 1, 3 and 6 months for freezing. Physical and chemical changes of the mackerel were evaluated by increasing TBARS values, trimethylamine and free fatty acid. Drip loss and Torry meter were also measured at various time intervals for six days at 4ºC, for three days at 7ºC and for 180 days at –10 and -18ºC. Shelf life was found to be 6 and 3 days for barred Spanish mackerel stored at 4ºC and 7ºC, respectively. Stored

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barred Spanish mackerel either at -10 and -18ºC showed longer acceptability tolerance (180 days). Generally, increasing temperature from 4 to 7ºC has led to about 50% decrease in the freshness period and shelf life. Freezing storage allowed the retention of freshness qualities of barred Spanish mackerel for extending their shelf life and the improved quality.

Keywords: Chilling; freezing; torry meter; mackerel; drip loss; TBARS; TMA; FFA.

1. INTRODUCTION

Fish quality is influenced by many factors such as raw material quality, cooling methods, processing, packaging and storage conditions. The quality and freshness of fish rapidly decline due to a variety of microbial and biochemical degradation mechanisms [1]. Thus, bacteria naturally found in fish skin, gills, and gut but they are not capable to grow and cause spoilage because of the natural defensive mechanism in fish [2]. Fresh mackerel (*Scomberomorous commerson)* spoil immediately after capture within 12 hours if kept at ambient temperatures because the fish meat chemical composition provides excellent media for growth of spoilage microorganisms [3].

Using good fishing techniques and cooling the fish, with the keep of ice on board will improve both fish quality and safety and increasing shelf life [4]. According to Berkel et al., [5], the rate of fish meat spoilage depends not only on hygienic conditions and storage temperature, but also on the acidity of the meat and the structure of the muscular tissues. The rate of fish spoilage during keeping in ice storage depends on microbial contamination and conditions of storage after catching [6]. Fish quality can be determined by different ways such as sensory tests, microbial measurement or by chemical methods such as measuring volatile compounds, lipid oxidation, determination of TBARS, and catalase activities [7,8,9].

Trimethylamine (TMA) is a volatile amine often gives the fish odor. Measurement of TMA can provide an accurate indication of bacterial spoilage in some species of fish [10]. Bacteria reduce the trimethylamine oxide (TMAO) which is naturally present in the living tissue of many marine fish species. TMA is useful as rapid method means of measuring the eating quality of many species of fish [11]. Estimating TMA has the advantage because the amount of TMA isa directs proportion to the numbers of bacteria in the fish [12]. High ratio of TMA than the permissible limits indicates that the fish load high microbial count [10]. Sensory panels can reject the fish when the level of TMA is around 10-15 mg/ TMA-N/100 g in aerobically stored and at level of 30 mg TMA-N /100 g in packed cod [10].

The Torry meter (TM) is used for various applications such as to estimate the freshness of fish and grading the fish. Also, TM use to determine if fish have been frozen or irradiated, and to estimate shelf-life. TM is used to detect the freshness of caught, processed and sold fish. TM measures the dielectric properties of the fish.

Sensory quality of chilled fish deteriorates as a result of different degradeative pathways such as microbial development and lipid oxidation mechanisms [13]. Lipid per oxidation depends upon the degree of UN saturation of the fatty acids. Increasing the degree of UN saturation of the fatty acids are results in a decrease in color and oxidative shelf-life. Lipid oxidation is a main factor which contributes to flavor deterioration in fish meat. Postmortem

can influence lipid oxidation and can decrease both the shelf life and fish quality due to the initiation of per-oxidation [14].

The aims of this study were to investigate the stability of fish during storage and to measure quality and freshness of fish meat at wholesale and during storage at 4, 7, -10, -18ºC, and 4 and -18ºC under vacuum package. Drip loss, Torry meter, TBARS, TMA, FFA, and microbiological were measured. Also, study the quality of fish at the points of wholesale fish as well as the impact of storage temperature on quality of fish.

2. MATERIALS AND METHODS

Mackerel fish were weighed before and after thawing and the drip loss was measured as the loss in mass divided by the initial mass of the mackerel fish. Before weighing, excess drip from the surface of the thawed mackerel fish was wiped off using a paper towel.

2.1 Sample Preparation

Upon arrival of the mackerel fish at the marketplace in Al-Qatif city, they were transported to the laboratory in ice box. Fish were washed with cold water, eviscerated and packaged immediately. The first group was stored in a refrigerator at 4ºC, the second group at 7ºC as abuse temperature, the third at 4ºC with vacuum package, the fourth group at -10ºC, -18ºC, while the last group was stored in a freezer at -18ºC with vacuum package. In order to carry out fish analyses during storage for 6 days and 180 days, samples were taken from each group at various time intervals and used for chemical and microbiological analysis. This experiment was repeated three times (n=3).

2.2 Physical Analyses

2.2.1 Torrymeter score

The Torry meter (Distell, Ind. LTD, Scotland, and U.K) is an electrical device was used to determine fish freshness. To measure the freshness of fish, place the base of the meter firmly on the fish skin. The device was parallel to the surface of the fish shoulder just behind the gill cover. Each time electrodes were cleaned to remove the scales and materials in contact with the measuring surface and the numbers were read from the digital display.

2.2.2 Drip loss

Drip loss is defined as the amount of liquid loss during storage and is expressed as a percentage of weight loss. Thawing loss of thawed samples was determined through the known weights of samples before and after thawing and expressed as % thawing loss (Eq. 1). Samples were thawed in a cold room (4˚C) for 24 hours [15] and weighed to calculated drip loss after thawing. Drip in the package from each treatment was poured off and the meat was blotted with a paper towel and reweighed to determine the drip loss. Drip was expressed as percentage of weight before storage. The drip loss measurement was taken on a 0, 30, 90, 180 days basis. The samples were taken out from each of the freezing media (-10ºC and -18ºC). Fresh fish were weighed on 0 day and recorded. Fish were put in refrigerator at 4 and 7ºC and were taken after 24, 72 and 124 hours for weighting. It was determined by the weight of the fish before and after storage:

Drip loss (%) = A-B/A X100 Eq. 1. [16].

A= weight of the mackerel fish at first day.
B= weight of the mackerel fish after storage period.

2.3 Chemical Analyses

2.3.1 Thiobarbituric acid reactive substances (TBARS)

The TBARS values were measured with the method described by [17]. For extraction, 10 g portion of the fish meat was homogenized (15000rpm, 30s, 20°C) with 30 mL of a 7.5% aqueous solution of trichloroacetic acid in a Bühler homogenizer Type H04 (7400 Tübingen, Germany). After filtration, 5.0 mL of the filtrate were then pipetted into test tubes and 5 mL 0.02 M aqueous solution of TBA in a stoppered test tube, kept at 100°C for 35 min in a water bath, and cooled for 10 min in cold water. The developed color was measured using spectrophotometer at 530 nm by Ultra spec 3000 (Pharmacia Biotech, Cambridge, U.K.) against a control containing 5.0 mL distilled water and 5.0 mL TBA reagent. The results were expressed as mg malonaldehyde/kg fish meat and were measured from the 1, 1, 3, 3-tetraethyoxypropane (TEP) based standard curve.

2.3.2 TMA determination

TMA-N was determined by using the method of AOAC [18]. TMA-N contents were expressed as mg TMA-N/100 g fish muscle.

2.3.3 Free fatty acids (FFA)

The method of Koniecko [19] was used to measure the FFA and was expressed as percentage meq/kg of the fish meat.

2.4 Microbiological Analyses

A 25 g of each sample were 10-fold diluted in 225 ml buffered peptone water and homogenized in a stomacher bag for 1 min. Serial decimal dilutions were prepared and the following analyses were carried out in duplicates: (i) Total viable counts in aerobic count agar plate which was incubated at 30ºC for 48h, (ii) *Pseudomonas* count in aerobic *Pseudomonas* agar media which was incubated at 30ºC for 24 hours, (iii) *Streptococcus* count on Tryptic Soy Agar which was incubated at 35ºC for 24h, (iv) Coli form fecal at Violet Red bile Agar aerobic with incubation at 30ºC for 24 hours, (v) *Staphyloccous* and *Staphylococcus aureus* at *Staphylococcus* Medium 110 aerobic with incubation at 35ºC for 48h. Bacteriological Analytical Manual [20] and American Public Health Association [21] were used to enumerate the total viable counts and identify pathogens in fish meats.

2.5 Statistical Analyses

All analyses were performed using three samples (bags) per each separate replicate. Then all data were statistically analyzed using the ANOVA and standard deviation. Significant differences were defined as $P < 0.05$.The means were compared using the least significant difference (LSD) at the 5% level according to Waller and Duncan [22]. SAS software package was used [23].

3. RESULTS AND DISCUSSION

3.1 Physical Changes

3.1.1 Drip loss

Drip loss was low for all the samples stored at 4^{o}C and 4^{o}C under vacuum. There was no significant deferent between the samples stored in 4 or 4^{o}C under vacuum. However, there was significant deferent between samples store at 7^{o}C and sample stored at 4 or 4^{o}C under vacuum. Table 1 shows the percentage of weight loss measured in a different storage temperature. The losses are expressed as the decrease in fish weight. These results clearly show that there are great differences between the qualities of chilled fish which may be attributed to storage temperature. According to Olsson et al. [24], the decreasing of water holding capacity is related to changing in the muscles post mortem.

Packaging fresh meat is carried out to reduce weight loss at retail or customer level [25]. After 6 days the drip loos in mackerel stored at 4ºC, and 4ºC under vacuum package stored was 3.43 and 3.46%, respectively. However, the fish stored at 7ºC, for 3 days, the drip loss was 3.4 and after 6 days the fish spoilage. Results obtained are consistent with the results obtained by Laleye et al. [26] who reported an increase in drip loss in vacuum packed pastrami during storage at a 0ºC and 3ºC. Randell et al. [27] observed that Baltic herring fillets in over-wrap packages (polystyrene or wood fiber) had lower drip than vacuum packed herring fillets during storage at 2ºC. Ozogul et al. [7] observed that an increase in drip loss lowered the sensory quality of vacuum packed sardines stored at 4ºC. Drip loss was found to be comparatively more in case of vacuum packed samples than those of air packed samples.

Fish stored -18ºC under vacuum package had lower drip loss comparing with fish stored at -10ºC and -18ºC. At the end of storage after 180 days the drip loos in fish stored -18ºC under vacuum package, -18ºC and -10ºC were 2.79, 4.33, and 4.79%, respectively. The results indicated the drip loss of mackerel was highest for samples stored at high temperatures and decreased with decreasing temperature in storage. According to Paine and Paine [28] the benefit of vacuum packaging is to eliminate moisture loss an initial freezing and also during the first 6 month of storage, and drip loss on thawing.

During chilled storage samples at 4ºC had higher water holding capacity compared to samples stored at 7ºC. Also, frozen storage samples stored at -18ºC under vacuum package had higher water holding capacity than sample stored at -10 and -18ºC. After six months there are no significant different between samples stored at -10 and -18ºC. During freezing the drip loss is caused by removing water from its original location in the fish and collected elsewhere in the form of ice crystals. When fish is thawed, the water may or may not be reabsorbed into its original location and form drip loss.

There was no difference in the percentage loss in weight between the samples stored at temperature of -10 and -18ºC after 180 days in the freezer. However, there are significant different between sample stored at -18ºC and samples stored at -18°C under vacuum package. Freezing process will convert most of water into ice. The main factor effect on the quality of frozen fish is the rate of freezing slow or fast. The fast freezing can formulate small ice crystals than slow rate. Small crystal can prevent of losing weight.Table1 shows the drip loss in fish during stored in freezer.

Table 1. The drip loss in fish stored at 4ºC, 4ºC under vacuum and 7°C, -10°C, -18ºC, and -18ºC under vacuum

Fish kept in the refrigerator				Fish kept in the freezer			
Time (Hours)	4°C	4°C under vacuum package	7°C	Time (Days)	-10°C	-18°C	-18°C under vacuum package
	%	%	%		%	%	%
0	0^{d}	0^{d}	0^{d}	0	0^{d}	0^{d}	0^{d}
24	1.90 ± 0.2^{c}	2.0 ± 0.3^{c}	2.4 ± 0.1^{c}	30	2.70 ± 0.2^{c}	2.27 ± 0.1^{c}	1.3 ± 0.4^{c}
72	2.79 ± 0.3^{b}	2.91 ± 0.3^{b}	3.42 ± 0.4^{b}	90	4.03 ± 0.25^{b}	3.42 ± 0.2^{b}	2.42 ± 0.2^{b}
144	3.43 ± 0.3^{a}	3.46 ± 0.5^{a}	4.4 ± 0.3^{a}	180	4.79 ± 0.5^{a}	4.33 ± 0.3^{a}	2.78 ± 0.1^{a}
R^2	0.76	0.76	0.81		0.77	0.82	0.81

- Data is mean of three replicates ± standard error.

- Means with different letters in the same column are significantly different at (p <0.05)

3.1.2 Torrymeter value

The initial Torrymeter reading for the mackerel fresh fishes was 11. Table 2 shows the Torry meter results for fish chilled and frozen during the storage. Similar result was obtained by Ravi Shankar' et al. [29], the Torrymeter score for Mackerel was recorded an initial reading of 11 [30]. The Torrymeter score was decreased consistent during storage period of 1 to 6 days in all the fishes stored at 4°C, 4ºC under vacuum package, and 7ºC. The decline of Torrymeter values of differently stored fish with storage time is shown in Table 2. The Torrymeter score was recorded with storage time show significant changes ($p<0.05$) for samples stored at 4ºC and 7ºC for 6 days. At day 0 the Torrymeter score was 11 out of 12 which indicated that the fish is fresh. Fish that were caught directly from the sea, would score 12, with rejection point is 5.5 [31].

Torrymeter score showed significant decline with time and storage temperature. The Torrymeter score of fish stored at 4ºC, 4ºC under vacuum package and 7ºC for 6 dayswas 6, 6and 4, respectively. The results indicated that packaging of fresh barred Spanish mackerel has an advantage decreased Torrymeter readings in comparison with non-vacuum packaging. A Torrymeter score of 6 is considered the cut off point for sale, as it is the points just before off flavors and odors are detected.

The Torrymeter score in fish stored at -10, -18 and -18ºC under vacuum package for 180 days dropped from 11 to 8, 9 and 9, respectively. Fish is considered fresh and high quality when Torrymeter score is 10 or above, and are spoiled when Torrymeter scores are less than 4. The Torrymeter score in fish stored at -10, -18 and -18ºC under vacuum package after 180 day was 8, 9 and 9, respectively. The results indicate that there were notsignificant different between temperature -10, and -18ºC. The results indicated that the Torrymeter readings showed good correlation with the biochemical parameters such as drip loss, TMA, TBARS, FFA and microbial count. Therefore, Torrymeter can be used to monitor the fish quality. Cheyne, [32] and Bull, [30] mentioned that Torrymeter had been studied by many investigators and they found that the Torrymeter can be used to measuring the freshness for cold-water fishes and freshness of ice-stored fish.

Table 2. The Torrymeter number in fish stored at 4ºC, 4ºC under vacuum and 7ºC, -10ºC, -18ºC, and -18ºC under vacuum

Fish kept in the refrigerator				Fish kept in the freezer			
Time (Hours)	4ºC	4ºC under vacuum package	7ºC	Time (Days)	-10ºC	-18ºC	-18ºC under vacuum package
0	11^{a}	11^{a}	11^{a}	0	11^{a}	11^{a}	11^{a}
24	10^{a}	11^{a}	9^{b}	30	10^{a}	11^{a}	11^{a}
72	9^{ab}	10^{a}	5^{c}	90	9^{ab}	10^{a}	10^{a}
144	6^{c}	6^{b}	4^{d}	180	8^{abc}	9^{ab}	9^{ab}
R^2	0.98	0.90	0.87		0.95	0.97	0.76

- Data is mean of three replicates ± standard error.
- Means with different letters in the same column are significantly different at (p <0.05)

3.2 Chemical Composition Change during Chilling and Freezing

3.2.1 Thiobarbituric acid reactive substances – TBARS

All samples were significant increase of TBARS values after 1, 3, 6, 30, 90, and 180 days at 4ºC, 4ºC under vacuum package, 7°C, -10ºC, -18ºC, and -18ºC under vacuum package, respectively. Mackerel samples stored at 7ºC generally had higher TBARS content than samples stored at 4ºC, but there were no differences between samples stored at 4 and 4ºC under vacuum package. There was a small but significant difference in TBARS content between frozen mackerel stored at -10 and -18°C up to 6 months. According to Dulavik et al. [33] The TBARS content in fish increased with time stored at -10 and -20ºC. Also, they found that the TBARA was significantly higher than samples which stored at or below -30ºC. Aubourg et al. [34] Found that the peroxide value (PV) and TBARS content in horse mackerel fillets stored at -20°C higher than samples stored at -80ºC. During chilled and frozen fish the lipids changes in fish muscles and lead to reduce the fish quality. Fish often contain a high ratio of poly unsaturated fatty acids and oxidized rapidly during cooling or freezing and produced TBARS.

Oxidative rancidity increased in fish meat during storage time at 4ºC, 7ºC and 4ºC with vacuum package. Fish samples stored at 7ºC generally had higher TBARS content than samples stored at lower temperatures (4ºC), but there were no significant differences between samples stored at 4ºC and at 4ºC under vacuum package. The change in TBARS for fish samples at different temperatures over time is presented in Table 3. The TBARS content increased with time for samples stored at 7ºC and was significantly higher compared to samples stored at 4ºC ($p<0.05$) during the time of the experiment. The highest values of the reactants with the TBARS were reached on the sixth day at 4ºC but the unwanted odors began to appear on the third day of the chilling at 7ºC. According to Chang et al. [35], the trained judges were able to detect the undesired odors and flavors when the values of the TBARS in meat were between 0.5-1.0 ppm. The values of TBARS in fish meat samples collected were 0.17 mg malonaldehyde/kg, indicating a low degree of lipid oxidation. The low level of TBARS in fish indicates that the fish has a good quality. After 3 days of storage at 4ºC, the collected fish meat had significantly ($P<0.05$) higher TBARS than in day 1 and the levels of TBARS were positively correlated with the storage time. At day 6, the levels of TBARS were increased and reached 0.52 mg malonaldehyde/kg in the

fish stored at 4ºC and 1.05mg malonaldehyde/kg in fish stored at 7ºC and 0.42 mg malonaldehyde/kg in fish packaged under vacuum and kept at 4ºC.The results indicated that the fish stored at 7ºC had a higher level of rancidity than fish stored at 4ºC and fish packaged under vacuum and kept at 4ºC. The lower value of TBARS found in fish packaged under vacuum and kept at 4ºC is due to the low storage temperature. However, after 6 days at 7ºC, there was a significant rise in the value of TBARS. This result of this study is consistent with many studies that have investigated the effect of temperature at various periods of storage on the value of TBARS.

TBARS values in frozen fish at -10, -18 and -18ºC under vacuum package increased form 0.17±0.07 at day 0 for the same storage temperature to 0.30±0.07, 0.26±0.04 and 0.27±0.05 mg malonaldehyde/kg after 30 days in freezer, respectively. After 90 days in freezer the TBARS increased to 0.38±0.07, 0.32±0.06 and 0.34±0.06 respectively. After 180 days the values of TBARS in fish increased and reach to 0.64±0.09, 0.49±0.13 and 0.51±0.08 mg malonaldehyde/kg respectively.

3.2.2 Trimethylamine (TMA)

Table 4 shows the development of TMA in fish stored in refrigerator and freezer temperature. All samples were significant increased of TMA values after 1, 3, 6, 30, 90, and 180 days at 4^{o}C, 4^{o}C under vacuum package, 7ºC, -10^{o}C, -18ºC, and -18ºC under vacuum package, respectively. The amount of TMA in mackerel refrigerated was 12.9, 11.8, 14.5 mg /100g for 6 days at a temperature of 4ºC, 4ºC under vacuum package, and 7ºC, respectively. Also, the amount of TMA in mackerel frozen was 2.4, 1.5, 1.4 mg /100g for 180 days at a temperature of -18ºC, -18ºC under vacuum package, and -10ºC, respectively. The frozen mackerel, examined was fresh based on the observed values of TMA based on the acceptance limit (10-15mg-N/100g) for TMA proposed by Connell [36] for fresh fish. The maximum allowable levels of TMA in fish are between 5 and 10 mg/100 g.

Trimethylamine is a product of decomposition of fish and it is responsible for the off odor. At day 0, the TMA in fresh fish was 0.3 mg/TMA-N/100g. TMA increased in fish stored at 4ºC under vacuum package 7ºC and for 3 days from 0.3 to 2.4, 2.2 and 8.3 mg/TMA-N/100g, respectively. However, fish samples stored at 7ºC had been rotten on the sixth day in the refrigerator. Fish samples stored at 7ºC generally had higher TMA content than samples stored at lower temperatures (4°C) but there were no differences between samples stored at 4°C and 4ºC under vacuum package.

TMA value of frozen mackerel low changed at –10°C and little at –18°C and –18°C under vacuum package. Storage temperature was one of the factors affecting on developments of TMA in fish. It has been proposed that TMA levels between 5 and 10 mg/100 g tissue should be considered the maximum allowable levels in international trading.

3.2.3 Free fatty acid

This study has shown that the rapid rise in the free fatty acids (FFA) content of fish during storage. Table 5 shows the percentage of free fatty acids in fish during storage time at refrigerator and freezer temperature. Accumulation of FFA does not affect fish quality but has been shown to interrelate with lipid oxidation and has been proposed to have a pro-oxidant effect on lipids [37]. The mean values of the free fatty acids increased significantly ($p<0.05$) throughout the storage period. The mean values of FFA in fish stored at 4ºC

observed at 0, 1, 3 and 6 day were 0.96, 1, 1.3 and 1.9 (% oleic acid), respectively. Also, the mean value of FFA in fish stored at 7ºC was observed 0.96, 1.3, and 1.7 % at day 0, 1, and 3 and 0.96, 1.2and 1.6% in fish stored at 4°C, 7ºC under vacuum package, respectively. There are significant different between the fish stored at 4ºC, 4ºC under vacuum package and 7ºC.

The mean values of FFA in fish stored at -10ºC observed at 0, 30, 90 and 180 day were 0.96, 0.95, 1.2 and 1.5 (% oleic acid), respectively. Also, the mean values of FFA of fish stored at -18ºC were observed to be 0.96, 0.98, 1.1 and 1.4% at day 0, 30, 90 and 180, respectively. The mean values of FFA in fish stored at -18ºC under vacuum observed at 0, 30, 90 and 180 day were 0.96, 0.92, 1.0 and 1.2 (% oleic acid), respectively. The FFA in frozen fish was increased slightly due to low temperature. However, there are not significant different between the fish stored at -10ºC and-18ºC and -18ºC under vacuum package.

3.3 Microbiological Assessment

Total viable bacterial, *Pseudomonas*, *Streptococcus* fecal, coliform fecal *Staphylococcus*, and *Staphylococcus aureus* counts were increased as time increasing in chilled temperature. The initial count of total viable bacterial counts, *Pseudomonas* counts, *Streptococcus* fecal, coliform fecal *Staphylococcus*, and *Staphylococcus aureus* 4.6, 3.9, 3.3, 3.3, 4.1 and 3.7 $\log_{10}$ CFU/g, respectively. *Salmonella* was not detected at 0 days until the end day of storage. The results indicated that the mackerel under this study was less contaminated but the mackerel loaded moderate counts which mean the hygienic of market need more improvement. The presence of pathogenic bacteria in mackerel leads to a lack of quality and high risk, therefore; it be conducted in a few precautions during manufacturing, cleaning, processing and handling of products. According to Liston [38] coliforms, *Escherichia coli, Staphylococcus* and Enterococci should not be found on fresh fish. International Commission on Microbiological Specifications for Foods (ICMSF) [39] recommended that total coliforms in frozen fish should be between 11-500 CFU/g. In the study, all the frozen fishes from observed were out of the acceptance range. The presence of pathogens indicated that contamination have occurred during catching, material, chilling, and handling.

In the study, the total viable count for all the fresh fishes was 4.6 $\log_{10}$ CFU/g. After 6 days at temperature 4ºC, at 4ºC under vacuum package, and 7°C the total count increased to reach 7.5. 7.1 and 8.9 $\log_{10}$ CFU/g, respectively. *Salmonella* was not detected in all the samples. The *Staphylococcus aureus Pseudomonas*, *Streptococcus* fecal, *Staphylococcus*, and *Staphylococcus aureus* were increased as storage time increased in refrigerator. However, total viable bacterial, coliform fecal decreased as storage time increased in refrigerator. The data were presented in Table 6. According to Oramadike et al. [40] The *Pseudomonas spp. and Shewanela putrefaciens*are dominated in the fish spoilage during icing storage. The total count in fish meat at day 0 was 4.6 log CFU/g which indicated that fish meat load low counts. High count in fish may be related to some factors such as, handling, delay of chilling and high temperature during transportation. High microbial load can reduce the shelf life and quality of fish. Also, it can cause economic losses and health problem. After 2 days of storage at refrigerator temperature 7±1ºC, the total count increased to 5.8 log 10 CFU / g. This study confirmed that refrigeration alone did not interact with microbial populations on fish. The low temperature effectively suppressed the growth of aerobic spoilage bacteria on fish meat and prolonged the shelf life by 3 days. The storage temperature 7ºC is not prolong shelf life of fish and should be lower than 7ºC.

Table 3. Shows the TBARS in fish stored at 4ºC, 4ºC under vacuum and 7ºC, -10ºC, -18ºC and -18ºC under vacuum

Fish kept in the refrigerator				Fish kept in the freezer			
Time (Hours)	4°C	4°C under vacuum package	7°C	Time (Days)	-10°C	-18°C	-18°C under vacuum package
0	0.17±0.07^{a}	0.1±0.077^{a}	0.17±0.07^{a}	0	0.17±0.07^{a}	0.17±0.07^{a}	0.17±0.07^{a}
24	0.25±0.13^{a}	0.2±0.03 ^{a}	0.32±0.10^{b}	30	0.30±0.07^{b}	0.26±0.04^{b}	0.27±0.05^{b}
72	0.37±0.010^{b}	0.33±08^{b}	0.56±0.09^{c}	90	0.38±0.07^{bc}	0.32±0.06^{b}	0.34±0.06^{c}
144	0.52±0.08^{c}	0.4±0.04^{c}	1.05±0.11^{d}	180	0.64±0.09^{d}	0.49±0.13^{c}	0.51±0.08^{d}
R^2	0.99	0.97	0.99		0.97	0.97	0.98

- Data is mean of three replicates ± standard error

- Means with different letters in the same column are significantly different at (p<0.05)

Table 4. Shows the TMA in fish stored at 4ºC, 4ºC under vacuum and 7ºC, -10ºC, -18ºC and -18ºC under vacuum

Fish kept in the refrigerator				Fish kept in the freezer			
Time (Hours)	4°C	4°C under vacuum package	7°C	Time (Days)	-10°C	-18°C	-18°C under vacuum package
0	0.3±0.05^{a}	0.3±0.05^{a}	0.3±0.05^{a}	0	0.3^{a}±0.05^{a}	0.3±0.05^{a}	0.3±0.05^{a}
24	1.5±0.07^{b}	1.2±0.15^{b}	4.7±0.8^{b}	30	1.1±0.2^{b}	0.7±0.1^{b}	0.6±0.08^{b}
72	2.4±0.3^{c}	2.2±0.25^{c}	8.3±1.0^{c}	90	1.8^{c}±0.2	1.3±0.3^{c}	1.1±0.15^{c}
144	12.9±1.3^{d}	11.8±1.3^{d}	14. 5±1.3^{d}	180	2.4±0.3^{d}	1.5±0.18^{d}	1. 4±0.20^{d}
R^2	0.97	0.88	0.88		0.92	0.87	0.94

- Data is mean of three replicates ± standard error

- Means with different letters in the same column are significantly different at (p <0.05)

Table 5. The percentage of free fatty acid in fish stored at 4ºC, 4ºC under vacuum and 7ºC, -10ºC, -18ºC, and -18ºC under vacuum

Fish kept in the refrigerator				Fish kept in the freezer			
Time (Hours)	4°C	4°C under vacuum package	7°C	Time (Days)	-10°C	-18°C	-18°C under vacuum package
0	0.96±0.1^{b}	0.96±0.1^{b}	0.96±0.1^{c}	0	0.96±0.1^{a}	0.96±0.1^{a}	0.96±0.1^{a}
24	1.0±0.12^{b}	1.05±0.2^{b}	1.3±0.1^{c}	30	0.95±0.1^{a}	0.98±0.1^{a}	0.92±0.07^{a}
72	1.3±0.20^{b}	1.2±0.34^{b}	1.7±0.25^{b}	90	1.2±0.15^{a}	1.1±0.2^{a}	1.0±0.14^{a}
144	1.9±0.25^{a}	1.6±0.25^{a}	2. 2±0.15^{a}	180	1.5±0.18^{a}	1.4±0.12^{a}	1. 2±0.16^{a}
R^2	0.96	0.99	0.98		0.97	0.96	0.86

- Data is mean of three replicates ± standard error

- Means with different letters in the same column are significantly different at (p <0.05)

In the study, the total viable count, *Pseudomonas*, *Streptococcus* fecal, *Staphylococcus* and *Staphylococcus aureus* in fish stored at 4ºC for 6 days increased to 7.5, 7.2, 5.1, 5.7 and 5.8 $\log_{10}$ CFU/g, respectively. The total viable count, *Pseudomonas*, *Streptococcus* fecal, *Staphylococcus*, and *Staphylococcus aureus* in fish stored at 7ºC for 6 days increased to 8.9, 8.2, 4.8, 5.2 and 5.5 $\log_{10}$ CFU/g, respectively. Also, in the study, the total viable count, *Pseudomonas*, *Streptococcus* fecal, *Staphylococcus*, and *Staphylococcus aureus* in fish stored at 4ºC under vacuum packaged for 6 days increased to 7.1, 7.0, 4.4, 5.5 and 5.5.9 $\log_{10}$ CFU/g respectively.

At day 0, the total viable cell counts, *Pseudomonas*, *Streptococcus* fecal, coliform fecal, *Staphylococcus*, and *Staphylococcus aureus* in mackerel stored at -10ºC were 4.6, 3.9, 3.3, 3.3, 4.1 and 3.7 respectively. The *Salmonella* was not detected at day 0. During frozen storage there is a slow growth of total viable count, however; there was a reduction in total counts after storage for 6 months at –10 and -18ºC. A study on mackerel and ocean perch fillets that were frozen and thawed after no more than 5 weeks in storage showed very little change in total bacterial counts. However, there was a reduction in total counts after storage for 14 weeks at –13ºF [41]. Table 7 shows the influence of freezing temperature on microbiological of aerobically packaged.

The microorganisms of meat fish is generally decrease slightly during freezing, a storage of frozen and the growth of some microorganisms can occur at temperatures below 0^{o}C, with frequent reports of growth of -5ºC and rarely up to -10ºC [42]. According to the results the temperature -10ºC are considered to be effectively inhibits the growth of microorganisms in fish. Generally, the freezing inhibits the metabolism of microorganisms in the process of frozen storage, enzymatic activity still continues. Freezing is effects on sub-lethal damage to vegetative bacteria such as *Salmonella*, *Campylobacter, L. monocytogenes*and *Vibrio* [43]. Some organisms such as *E. coli, B. subtilis, Vibrio cholerae* and some lactic acid bacteria responds to a low temperature, voltage for the production of cold shock protein or cold-induced proteins.

In the study, the total viable count (TVC) for all the frozen fishes ranged from 2.9 to 3.2 log10 CFU/g as shown in Table 6. *Salmonella* was not detected in all the samples. The *Staphylococcus aureus* detected ranged from 2.9 to 3.8 log10 CFU/g. However, total viable bacterial, *Pseudomonas*, *Streptococcus* fecal, coliform fecal *Staphylococcus*, and *Staphylococcus aureus* were decreased as storage time increased in freezer.

In the study, the total viable count, *Pseudomonas*, *Streptococcus* fecal, coliform fecal, *Staphylococcus*, and *Staphylococcus aureus* in fish stored at -10ºC for 180 days decreased to 3.2, 2.8, 3.1, 2.2, 3.5 and 3.4 $\log_{10}$ CFU/g, respectively. The total viable count, *Pseudomonas*, *Streptococcus* fecal, coliform fecal, *Staphylococcus* and *Staphylococcus aureus* in fish stored at -18ºC for 180 days decreased to 2.9, 2.6, 2.4, 2.0, 3.1 and 2.9 $\log_{10}$ CFU/g, respectively. Also, in the study, the total viable count, *Pseudomonas*, *Streptococcus* fecal, coliform fecal, *Staphylococcus* and *Staphylococcus aureus* in fish stored at -18^{o}C under vacuum packaged for 180 days decreased to 2.6, 2.5, 2, 1.8, 2.8 and 2.9 $\log_{10}$ CFU/g, respectively.

Table 6. Influence of refrigerated storage (4±1°C, 7±1°C, and 4±1°C vacuum package) on microbiological of aerobically packaged.

	Storage at 4±1°C				Storage at 7±1°C				Storage at 4±1°C vacuum package			
	0	**1**	**3**	**6**	**0**	**1**	**3**	**6**	**0**	**1**	**3**	**6**
Total viable cell count	4.6±0.5^{a}	5.8±0.4^{b}	6.5±0.7^{c}	7.5±0.4^{d}	4.6±0.5^{a}	6.2±0.7^{b}	8.3±0.6^{c}	8.9±05^{d}	4.6±0.5^{a}	5.3±0.5^{b}	6.2±0.4^{c}	7.1±0.7^{d}
Pseudomonas	3.9±0.4^{a}	5.7±0.5^{b}	6.9±0.3^{c}	7.2±0.4^{c}	3.9±0.4^{a}	5.5±0.3^{b}	7.5±0.7^{c}	8.2±0.7^{d}	3.9±0.4^{a}	4.9±0.3^{b}	5.9±0.5^{c}	7.0±0.2^{d}
Streptococcus fecal	3.3±0.4^{a}	3.7±0.3	4.6±0.4^{c}	5.1±0.5^{c}	3.3±0.4^{a}	2.9±0.2^{b}	4.1±0.3^{c}	4.8±0.4^{d}	3.3±0.4^{a}	3.0±0.2^{a}	4.0±0.2^{c}	4.4±0.3^{d}
Coliform fecal	3.3±0.3^{a}	1.9±0.3	1.4±0.2^{c}	2.2±0.3^{d}	3.3±0.3^{a}	1.9±0.2^{b}	3.1±0.3^{c}	4.0±0.4^{d}	3.3±0.3^{a}	1.5±0.2^{b}	1.0±0.3^{c}	1.2±0.4^{c}
Staphylococcus	4.1±0.6^{a}	5.3±0.2^{b}	5.5±0.3^{b}	5.7±0.4^{b}	4.1±0.6^{a}	3.6±0.4^{b}	4.9±0.4^{c}	5.2±0.3^{d}	4.7±0.6^{a}	4.3±0.3^{a}	5.2±0.4^{b}	5.5±0.5^{b}
Staphylococcus *aureus*	3.7±0.2^{a}	4.6±0.3^{b}	5.5±0.4^{c}	5.8±0.3	3.7±0.2^{a}	3.6±0.3^{b}	4.9±0.5^{c}	5.5±0.2^{d}	3.7±0.2^{a}	4.2±0.4^{b}	5.1±0.5^{c}	5.5±0.4^{c}
Salmonella	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

- Data is mean of three replicates ± standard error

- Means with different letters in the same column are significantly different at (p <0.05)

Table 7: Influence of freezing storage (-10±1°C, -18±1°C, and -18±1°C vacuum package) on microbiological of aerobically packaged.

	Storage at -10±1°C				Storage at -18±1°C				Storage at -18±1°C vacuum package			
Storage time (Days)	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**	**0**	**30**	**90**	**180**
Total viable cell count	4.6±0.2^{a}	4.8±0.5^{a}	3.5±0.2^{b}	3.2±0.4^{b}	4.6±0.2^{a}	4.4±0.4^{a}	3.1±0.2^{b}	2.9±0.2^{b}	4.6±0.2^{a}	4.0±0.2^{b}	3.0±0.2^{a}	2.6±0.2^{a}
Pseudomonas	3.9±0.3^{a}	4.2±0.3^{a}	3.2±0.5^{b}	2.8±0.3^{b}	3.9±0.3^{a}	4.0±0.2^{a}	3.2±0.5^{b}	2.6±0.3^{c}	3.9±0.3^{a}	3.0±0.2^{b}	2.8±0.3^{b}	2.5±0.3^{b}
Streptococcus fecal	3.3±0.5^{a}	3.6±0.2^{a}	3.4±0.6^{b}	3.1±0.5^{b}	3.3±0.5^{a}	3.2±0.4^{a}	3.0±0.3^{a}	2.4±0.5^{c}	3.3±0.5^{a}	2.9±0.3^{b}	2.3±0.4^{c}	2.0±0.2
Coliform fecal	3.3±0.4^{a}	3.7±0.3^{a}	3.1±0.2^{b}	2.2±0.2^{d}	3.3±0.4^{a}	3.1±0.3^{a}	2.9±0.5^{a}	2.0±0.2^{b}	3.3±0.4^{a}	2.8±0.2^{b}	2.3±0.2^{c}	1.8±0.1
Staphylococcus	4.1±0.2^{a}	4.5±0.5^{a}	4.0±0.4^{b}	3.5±0.3^{b}	4.1±0.2^{a}	3.8±0.3^{a}	3.4±0.6^{ab}	3.1±0.3^{b}	4.1±0.2^{a}	4.2±0.3^{a}	3.1±0.3^{b}	2.8±0.5^{b}
Staphylococcus *aureus*	3.7±0.3^{a}	4.1±0.2^{a}	3.8±0.3^{a}	3.4±0.4^{a}	3.7±0.3^{a}	3.3±0.5^{a}	3.1±0.4^{a}	2.9±0.4^{a}	3.7±0.3^{a}	3.8±0.4^{a}	3.2±0.4^{b}	2.9±0.2^{b}
Salmonella	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

- Data is mean of three replicates ± standard error

- Means with different letters in the same column are significantly different at (p <0.05)

4. CONCLUSIONS

A useful tool for describing fish quality in chilled and frozen temperature is to measure drip loss, Torrymeter values, TMA, FFA, TABRS and microorganisms. Drip loss, Torrymeter values, TBARS, TMA, FFA, total viable count, *Pseudomonas*, *Streptococcus* fecal, *Staphylococcus* and *Staphylococcus aureus* increased ($P<0.05$) with storage period. Different storage conditions influence the changes in drip loss, Torrymeter values TM, TMA, TBARS, FFA and microbial growth. Apparently storage at 7ºC as abuse temperature had less preservation effect than at 4ºC. Whole fish storage at 4ºC temperatures may extend shelf life for 6 days while freezing storage of mackerel generally contributes to longer shelf life. Freshness loss in refrigerator of whole fish depends on the temperature, being shortest for 3 days at 7ºC in comparison to 6 days at 4ºC and 180 days at -10 and -18ºC. Frozen temperature was more effective to reduce or inhibit the pathogenic and spoilage microorganism.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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