Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.53722

The quality of handling and extended the shelf life and preservation of lagoon mullets fish (*Mugil cephalus*)

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Abstract

The deterioration of fish by microorganisms is very quickly, because it's a highly perishable foods and short shelf life, for this reason the handling and stored condition very important to slow down and extend the shelf life and its quality. There are a number of strategies used as traditional methods to extend the shelf life of fishes included: chilling, freezing, icing, drying, smoking and salting. From the innovative processing technologies using of icing system with organic acids is considered to be a preservative method and delaying the changing in the fish and able to guarantee the quality with extension of their shelf-life. The present study provides the addition of organic acid as lactic acid (0.1%), propylene glycol (7%), potassium sorbate (1000 ppm) and mixture of potassium sorbate (1000 ppm) + sodium benzoate (0.2%) to fresh water and dipping the mullet fish in it then preserved in fresh water crushed ice medium containing the same concentration of organic acids. Different parameters were determined to limit the quality and safety of mullet during storage periods as total volatile nitrogen base, trimethylamine, thiobarbituric acid-value, refractive index of the muscle fluids, electrical resistance of the fish muscles, organoleptic evaluations and in addition to total viable count of bacteria and psychrophilic bacteria. The mixture of potassium sorbate and sodium benzoate using icing system method extended the shelf life of mullet fish up to 17-19 days of storage period in comparison with control and other organic acids.

Keywords: mullet fish; icing organic acids; total volatile base nitrogen; trimethylamine; thiobarbituric acid; electrical resistance.

Practical Application: The strategy of icing organic acids or its salts (as lactic acid, propylene glycol, potassium sorbate, and a mixture potassium sorbate and sodium benzoate) on mullets fish treatment to extend the shelf life and preserved the quality of it. The mixture of potassium sorbate and sodium benzoate and by icing system method could to extend the shelf life of mullet fish up to 17-19 days of storage period in comparison with control and other organic acids.

1 Introduction

Fish and fish products are considered to be the most perishable foods even when preserved under appropriate condition and its quality quickly reduced and deteriorates (Sikorski & Kolakowski, 2000; Ozogul, 2009). The action of enzymes found in the gut and on the flesh of fish leads to autolysis deterioration and caused a loss in the quality of fish. The microorganisms were followed by growth on the surface of fish and manifests as slime layer. These bacteria were then invading the flesh of fish causing breakdown of tissues and appearance of deterioration carried out and in the fatty acid species of fish lipid oxidation and an off-odor and off-flavor can have produced in parallel with autolytic and microbial deterioration, all these caused a loss of the quality and this rates of deterioration depend on the temperature which use for storing of the fish (Ghaly et al., 2010). Chilling is the most employed strategies to keep the original properties of fish species and a high quality of accept by the consumers (Ashie et al., 1996; Medina et al., 2009). The addition of a mixture of organic acids (ascorbic, citric, and lactic) at different concentrations was used in the preservation of different fish species. By new methods and strategies, the organic acids were added to the icing medium and stored up to 15 days of chilling storage (García-Soto et al., 2011). For enhancing the quality of chilled blue whiting (*Micromesistius poutassou*), a natural organic acid-mixture, including ascorbic, citric, and lactic acids was added by using two-step processing strategy: (i) as an aqueous dipping medium prior to chilling storage, and (ii) included in the flake ice employed as the chilling system (Sanjuás-Rey et al., 2011). Organic acids with a low molecular weight as acetic, lactic, citric, malic and scorbic and other many are naturally found in food of plant origin as well as produced through the fermentation process in foods. Our study aimed to using the strategy of using icing organic acids or its salts (as lactic acid, polyglycol, potassium sorbate, and a mixture of potassium sorbate and sodium benzoate) at different concentrations in the treatment of mullet's fish to extend the shelf life and preserved the quality of it. The preservative action of such treatments is determined according to different

Received 01 May, 2022
Accepted 01 June, 2022
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parameters caused deterioration as total volatile base nitrogen, trimethylamine content, thiobarbituric acid-value, changing in pH values, refractive index of the muscle fluids, electrical resistance of fish muscles, aerobic total viable count and total psychrophilic bacteria as well as organoleptic evaluation and sensory acceptability.

2 Materials and methods

2.1 Mullet fish samples

The mullet fishes in this study were caught off the Bardawil lagoon (North Sinai, Egypt). In order to maintain the fish in a fresh condition, immediately after catch where the fishes were still alive, on boat, the fishes divided into 5 groups and then treated with the follows:

Control samples (covered with one-time fresh water crushed ice).

Fish immersed in 7% propylene glycol fresh water solution for 5 min and then covered with propylene glycol fresh water crushed ice.

Fish immersed in 0.1% lactic acid fresh water solution for 5 min and then covered with lactic acid fresh water crushed ice.

Fish immersed in 1000 ppm potassium sorbate fresh water solution for 5 min and then covered with potassium sorbate fresh crushed ice.

Fish immersed in combination of (1000 ppm potassium sorbate + 0.2% sodium benzoate) hash water solution for 5 min and then covered with potassium sorbate + sodium benzoate fresh and crushed ice.

Each layer of fish alternating with a layer of ice. The length of time from mullet capture to receipt at the laboratory varied from 4 to 6 h.

In the Laboratory 3 fishes for each treatment were used to make the first sampling (day 0), and the others were stored in foam boxes with alternating layers of mullet fishes and ice. The liquid from melting ice was drained from an orifice made in the bottom of the storing box and collect out. The ice was replenished when necessary, almost daily.

Samples were taken every day for Chemical, physical and frequently for sensory and bacteriological analyses. The chemical, physical and bacteriological estimation was stopped when the organoleptic evaluation judged the samples as putrid.

2.2 Total Volatile Bases (TVB-N) and trimethylamine content

The method recommended by the Analytical Method Committee (1979) for determination of total volatile bases nitrogen (TVB-N) and trimethylamine (TMA) which based on a semi-microdistillation procedure. Extracts or solutions are made alkaline with sodium hydroxide. The bases are steam distilled into standard acid and back titrated with standard alkali. Formaldehyde is added to the neutralized mixture and the acid released is equivalent to the volatile bases other than trimethylamine

2.3 Thiobarbituric acid-value (TBA)

Increase in the amount of red pigment formed in the reaction between 2- thiobarbituric acid (TBA) and oxidized lipids as oxidative rancidity advances has been applied to a wide variety of fatty foods. The TBA test appears to measure deterioration in both extractable and non-extractable lipids and therefore has been more frequently applied to compound fatty foods, particularly flesh foods, rather than to "pure" oils and fats.

Tarladgis et al. (1960) method was applied for the determination of TBA value. The procedure could be summarized as follows:

Macerate 10 g of fish muscle with 50 mL water for 2 min and wash into a distillation flask with 47.5 mL water. Add 2.5 of 4M hydrochloric acid to bring the pH to 1.5, followed by an antifoaming preparation and a few glass beads. Heat the flask by means of an electric mantle so that 50 mL distillate is collected in 10 min dam the time boiling commences. Pipette 5 mL distillate into a glass-stoppered tube, add 5 mL TBA reagent (0.2883 g/100 mL of 90% glacial acetic acid), stopper, shake and heat in boiling water for 35 min. Prepare blank similarly using 5 mL water with 5 mL reagent. Then cool the tubes in water for 10 min and measure the absorbance (D) against the blank at 538 nm using lcm cells.

TBA no. (as mg malonaldehyde per kg sample) = 7.8D

2.4 pH-value

The pH was of minced sample (5 g) used during bacterial enumeration was using an Allied model 810 pH meter (Fisher Scientific, U.S.A), by dipping the glass electrode for 1 min in the homogenate of fish muscle and distilled water (5 mL). The values for three samples in each treatment were averaged for every storage day evaluated.

2.5 Total Titratable Acidity (TTA)

Using the same homogenate prepared for the determination of pH, the TTA was measured by titrating against 0.1N sodium hydroxide to a final pH of 8. The % w/w lactic acid in the sample was calculated by multiplying the volume of alkali (mL) by the factor 0.09 (Association of Official Analytical Chemists, 1995). This assumed that all the acid present in the sample was lactic acid.

2.6 Optical density of gills extract

Ghoneim (1974) method was applied for the determination of optical density of gills extract. The procedure could be summarized as follows:

Twoistance grams of ground gills tissues were located in a glass cylinder with stopper. 50 mL of distilled water were added. The mixture was shaken for 2 min. Cylinder was left for 15 min in complete darkness, after which filtration took place, and rapidly the optical density of the extract was colorimetrically measured at 542 nm.

2.7 Refractive index of the muscle fluids

Refractive index of the muscle fluids as an index of proteolysis was measured according to the method given by Ghoneim (1974).

The same Huid prepared for measuring the optical density of muscle fluids was used for measuring the refractive index, however no dilution was carried out at 20 °C. Refractometer type RL3 Nr 21809/90 was used.

2.8 Electrical resistance of the fish muscles

Electrical resistance of the fish muscles was measured according to the method given by Ghoneim (1974).

Avometer apparatus type (New General model 500) was used. The electrodes were immersed in the dorsal fish muscle, nearly at the middle of the muscle length, taking into consideration that electrodes did not contact with bones. The distance between the two electrodes was constant, being 1cm, and the electrodes were immersed in the tissues for about 2 cm, Electrical resistance was measured in ohmes. The measures were repeated 5 times for each sample and the mean values were recorded.

2.9 Organoleptic evaluation

To measure the consumer acceptability of samples before mullet fishes were subjected to the organoleptic tests according to: Klein & Bardy (1984).

Quality characteristic

Five members of the laboratory were asked to evaluate the acceptability of samples using the above scale of 10 points for general appearance, eyes, gills, scales, rigor-mortis, abdominal coat, peritonium, anus, flesh colour, blood colour and overall quality for treated fresh water crushed ice samples. The chemical, physical, and bacteriological estimation was stopped when the organoleptic evaluation judged the samples as putrid.

2.10 Total bacterial count

The plate count method using tryptone glucose beef extract agar medium as described by Association of Official Analytical Chemists (1995) was applied. The medium was sterilized at 121 °C for 15 min. One mL of each dilution was plated in the above medium in duplicates and incubated at 37 °C for 2 days. The count was calculated per gram muscle on wet weight bases.

2.11 Psychrophilic bacteria

Psychrophilic bacteria were counted per gram using standardplate count agar medium (Oxoid, Code: CM0463, Fisher Scientific UK Ltd, Bishop Meadow Road, Loughborough, LE11 5RG). The plates were incubated at 5 °C for 7 days (Sharf, 1966).

2.12 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Microsoft Office Excel 2013 (Microsoft Inc., Redmond, WA, U.S.A.) and XLSTAT 2014 (Addinsoft Inc., New York, NY, U.S.A.). The program calculates multiple comparisons using Tukey's multiple comparison test. The significance level was set at 95% ($p < 0.05$), if not stated elsewhere.

3 Results and discussion

3.1 Total Volatile Bases Nitrogen (TVB-N)

The data in Figure 1 shows the changes in the total volatile bases content of mullet fish tissues stored in crushed ice made from fresh water and were mixed with propylene glycol, lactic acid, potassium sorbate, and potassium sorbate + sodium benzoate before freezing. It could be observed from this figure that the amount of TVB-N continuously increased during cold storage of mullet fish. Such results agree with those reported by Bennour et al. (1991), Ghoneim (1974).

At the end of storage period the total volatile bases content was 62.27, 61.37, 59.36, 64.32 and 57.50 mg% for control and fresh water crushed ice treated with propylene glycol, lactic acid, potassium sorbate, and potassium sorbate + sodium benzoate, respectively.

TVB-N content was relatively high after 8 days of storage days in untreated sample (control), while in the sample treated with potassium sorbate + sodium benzoate, the ratio was reduced the deterioration to 57.50 mg% to the end of the 17th days of storage period.

Total volatile bases nitrogen (TVB-N) may be also used as freshness test. Sengupta et al. (1972), showed TVB-N value to be about 20 mg% sample when fish was fresh, and to increase to about 30 mg% samples when spoilage commenced. Physical characteristics changed with increase in TVB-N value showing that this could be used as rapid method for determining state of fresh fish. Total volatile bases nitrogen increased from 19.26 mg% (0.5 days) to 28.66 mg% (9 days) in cod muscle and from 19.73 mg% (0.5 days) to 29.13 mg% (6 days) in herring when stored in ice at as 1 °C mentioned by Kołakowski et al. (1977). At spoilage of cod-stored fish at 0-5 ^{0}C, spoilage bacteria predominating in the skin and muscle were non-halophilic and halophilic *Pseudomonas spp.* and *Vibrio spp.*

Billon et al. (1979) mentioned that marine fish may be classified into 3 freshness groups on basis of TVB-N concentration: Class

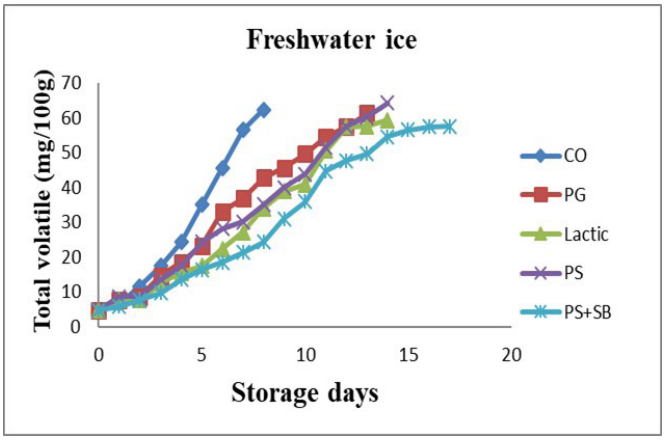

Figure 1. Total volatile bases nitrogen content of mullet fish during cold storage in ice made from freshwater and different additives. CO = Control; PG = Propylene glycol; Lactic = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

1 > 30 mg%; class 2, 30-40 mg%, and class3 (unfit for human consumption) > 40 mg TVB-N%.

3.2 Trimethylamine (TMA) content

Figure 2 show the TMA content in mullet fish tissues stored in crushed ice made from fresh water and was mixed with propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate before colding.

It could be observed from these figure that the amount of TMA continuously increased during cold storage of mullet fish, such results were in agreement with those of Ghoneim (1974), Thanaa (1984), Marrakchi et al. (1990) and Bennour et al. (1991).

By spoilage TMA content increased, being 33.64, 36.46, 28.53, 31.32 and 28.88 mg% for control and fresh water crushed ice treated with propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate, respectively. The results indicated that the TMA content was relatively high in the case of control and reached to 33.64 mg% while it was reduced to 28.88 mg% at the end of storage period (17th days).

Thakur & Patel (1994) reported that sorbates retard chemical changes occurring in fish during storage and inhibit formation of trimethylamine and other compounds responsible for fish spoilage. Sorbates prevent development of off-odours in fish and fish product by preventing oxidation. Acceptability scores of sorbate treated products tend to be higher than for untreated products. It is concluded that use of sorbates in fish and fish products in combination with other compounds can effectively presrve fish and fish products and extend their shelf life.

Tarr & Bailey (1939) found that ice containing 0.1% benzoic acid improved the keeping qualities of dressed halibut and black cod. Tarr & Sunderland (1939) also demonstrated that benzoic acid; benzoates suppressed the formation of trimethylamine in fish muscle undergoing spoilage. It would be erroneous, however, to base treatment with benzoic acid on the amounts of trimethylamine used as an index of quality. Fresh fish contain about 2 or below (mgN/100 g flesh) trimethylamine, while in spoiled fish, up to 80 mg may be found Tarr & Ney (1949), Dyer et al. (1945).

3.3 Thiobarbituric acid (TBA) value

The data presented in Figure 3 show the TBA values during storage of mullet fish in crushed ice made from fresh water and were mixed with propylene glycol, lactic acid, potassium sorbate, and potassium sorbate+ sodium benzoate before freezing.

It could be noticed that the TBA values were low in fresh fish ranging between 0.789 and 0.962 mg/1000 g and that by storage the TBA Values increased markedly. These results agree with the findings of Ghoneim (1974), Thanaa (1984). By complete spoilage the TBA values increased being 3.88, 3.76, 3.78, 3.68 and 3.542 mg malonaldehyde/ 1000 g for control and fresh water crushed ice treated with propylene glycol, lactic acid, potassium sorbate, potassium sorbate + polyphosphate and potassium. sorbate + sodium benzoate, respectively. This means that lipid oxidation accelerated by NaCl, consequently the occurrence of rancidity. These results agree with those reported by Borgstrom (1965).

The increase of the TBA values during cold storage indicates the fat oxidation with the formation of malonaldehyde. Such test has an advantage of being a measure to the fat oxidation, since there is no need for fat extraction to carry out such test, Ghoneim (1974).

The TBA test is selected by many authors for determining oxidative rancidity for two reasons: First it can 'be applied directly to the tissues without the necessity of extracting the fat, second, the fat decomposition product responsible for the test is obtained in much greater amounts from 8 highly unsaturated fatty acids, such as are present in marine fats, Schwartz & Watts (1957). The TBA test, cannot be used as a satisfactory measure of rancidity in fish muscle where the oxidation has been catalyzed by the addition of trace amounts of inorganic iron salts, since the value of TBA will be very high, Castell et al. (1966).

During the secondary lipid oxidation, the most aldehyde generated is malondialdehyde (MDA) and considered as the

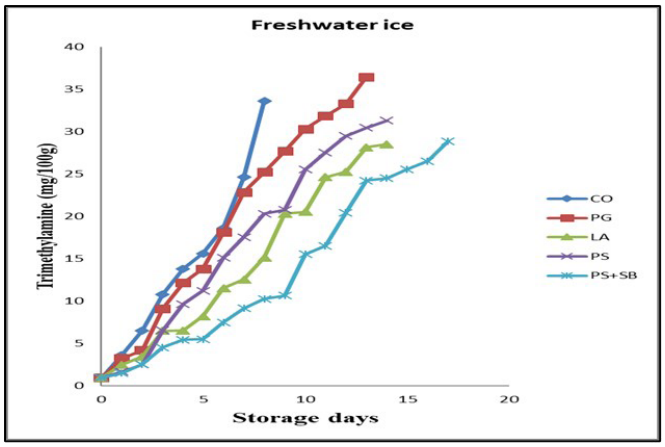

Figure 2. Trimethylamine content of mullet fish during cold storage in ice made from fresh water and different additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

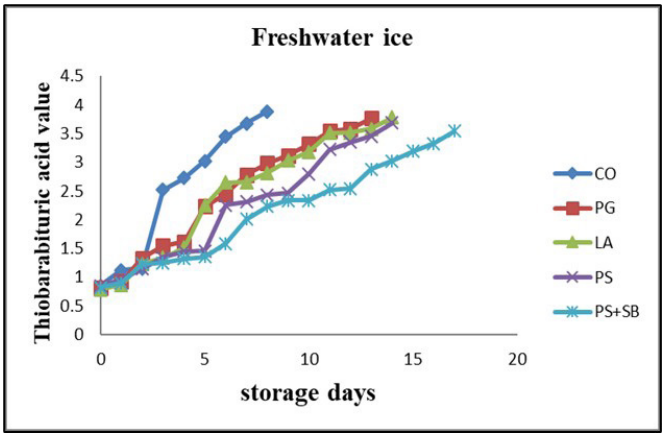

Figure 3. TBA values of mullet fish during cold storage in ice made from freshwater and different additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

most marker of oxidation (Barriuso et al., 2013). Found of oxidized lipids in the diet of humans and animals resulted in enhancement of thiobarbituric acid reactive substances (TBARS) in plasma and tissue (Ruban, 2009). Lipid peroxidation in food resulted in MDA and accumulation of aldehyde. Malondialdehyde concentration found in fish and meat and its products are about 300 μM or more (Kanner, 2007).

Further oxidation of lipids products when exposed to further oxidation resulted in secondary oxidation products such as aldehyde, ketones, epoxides, hydroxyl compounds polymer and, oligopolymer. These compounds characterized by its physicochemical properties, different in volatility, polarity, and molecular weight. The most groups of compounds detected are aldehydes, volatiles, and polymers as well as a particular molecule very frequently used as an oxidation marker (malondialdehyde) (Reitznerová et al., 2017).

Ghoneim (1974), concluded that spoilage caused the increase of TBA values in tilapia fish reaching 3.19-4.29, 3.17-4.36 mg malonaldeyde/1000 g by weight, during storage in the refrigerator at 40 °F and in crushed ice, respectively. by spoilage, TBA values increased by about 1.5-4 folds of the values shown in all fish samples.

The increase in the TBA value is a measure of the extent of 'oxidative deterioration in oily fish, but, as in the case of peroxide value, The TBA value can fall again at a later stage of Spoilage (Torry Research Station, 1992).

3.4 Analysis of pH

The data presented in Figure 4 show the pH values of mullet fish muscles during cold storage under conditions explained in the present study. It could be observed from the figure that the pH value of mullet fish increased continuously during cold storage. The pH value decreased at the first few days of cold storage may be due to lactic acid accumulation, but increase of pH value especially at late periods of storage may indicate spoilage.

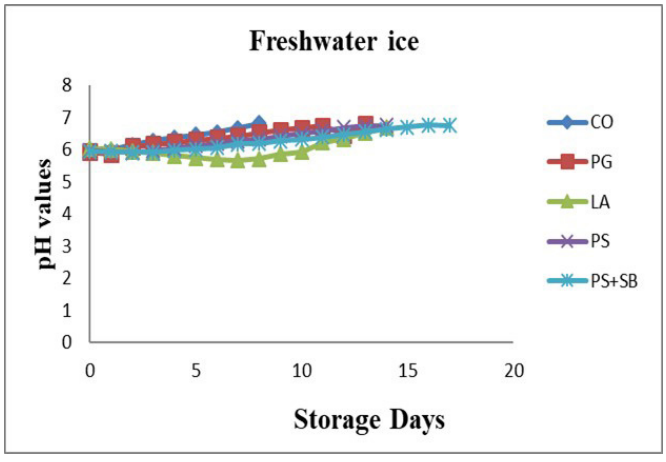

Figure 4. pH values of mullet fish during cold storage in ice made from freshwater at different additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

At the end of storage period the pH value increased being 6.81, 6.77, 6.69, 6.75 and 6.74 for control and fish water crushed ice treated with propolyn glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate. The increase pH value resulted from proteolysis leading to the increase of free basic amino acids as well as the accumulation of ammonia, amines and other basic products of bacterial breakdown as pointed out by Khouraiba (1981). In the present study the increase in pH for all the treatments between day 0 and spoilage was only on 0.78 unit for fresh water crushed ice (Bennour et al., 1991).

As show in Figure 4, the pH of the mullet fish at days 0 time was ~ 6.0 and increased with storage time to 6.81 unit in the control treatment while is about 6.7 for the other treatments at the end of the storage period. Normally using the pH as an indicator to detect the deterioration in fish (Howgate, 2009). The high pH values lead to the short shelf life of fresh fish as compared with the chill stored. However, at pH ~ 6-7 have been found in a tropical fish species (Gram et al., 1989). Skin properties considered as the factor contribute in the extended of shelf life of flatfish.

Similar trends in the increasing of pH values were reported in saithe fillets by different researchers (Susanto et al., 2009; Abelti, 2013; Magnusson et al., 2009b). This elevated of pH due to the accumulation of alkaline compounds as amines (trimethylamine) and ammonia which produced from the microorganisms which contaminated the fish muscles (Ruiz-Capillas & Moral, 2005). The pH of spoiled fish may be more than 7.2. The accumulation of volatile aromatic alcohols, such as cresol and phenol, the volatile bases compounds and heterocyclic compounds such as indole and skatole are the main causes of the appearance of offensive odors of the spoiled fish. Furthermore, the cyclic monoarnines, the diamines, the oxyammonium base (neurin), and phenol, cresol, indole, and skatole are all toxins and can cause food poisoning (Zaitsev et al., 1969).

Poulter & Lawrie (1977) mentioned that the increase in the pH value of fish muscle during frozen storage could be probably due to the breakdown of urea to ammonia.

Marrakchi et al. (1990) reported that the average pH at day 0 (4 to 6 h after catch) was 5.83. The pH increased to 6.36 and 6.57 at day 9 and day 18, respectively. The muscle pH rapidly reaches, in a few hours after capture, values appropriate for bacterial histidine decarboxylase synthesis (Eitenmiller et al., 1982) and optimal for the activity of this enzyme. The increase in pH between day 0 and day 18 was only a 0.8 unit. This slow rise in pH was also observed by other authors Reppond & Collins (1983) and Ryder et al. (1984), who explains the inability of this parameter to evaluate the quality of iced sardine. Bennour et al. (1991), reported that a few' hours after catch, the pH of mackerel was 5.69. It varied from 5.95 to 6.02 at different rejection times. At the end of storage, the pH values were 6.24 to 6.52. The increase in pH between day 0 and day 12 was less than one unit.

3.5 Total acidity content

The data presented in Figure 5 showed the total acidity content as percentage during cold storage of mullet fish muscles. It could be observed that the total acidity content decrease continuously

during cold storage of mullet fish. The total acidity content increased in the first few days of cold storage which may be due to lactic acid accumulation but decrease of total acidity content especially at late periods of storage may indicate spoilage and proteolysis leading to the increase of free basic amino acids as well as the accumulation of ammonia amines and other products of bacteria breakdown as pointed by Khouraiba (1981).

At the end of storage period the total acidity content decreased being 0.23, 0.21, 0.31, 0.22 and 0.31% as lactic acid for control and fresh water crushed ice treated with propylene glycol, lactic acid, potassium sorbate and potassium sorbate+ sodium benzoate. The total acidity content at spoilage, calculated as % original value was 36.35% in the case of storage in fresh water crushed ice. The results indicated that the total acidity content was relatively low in the fresh water crushed ice sample.

3.6 Optical density of muscles extract

The data presented in Figure 6 showed the optical density of muscle extract during storage of mullet fish in crushed ice made from fresh water and mixed with propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate before freezing. From the Figure 6, it could be observed that the optical density of fresh fish was decreased during storage of fish until being spoiled, such decrease may be due to that by spoilage more extracted proteins were broken down to small particles of lower molecular weight, Zaitsev et al. (1969) and Ghoneim (1974).

As the storage period was progressed the optical density of muscles water extract was decreased reaching 0.20, 0.22, 0.21, 0.23 and 0.31 for control and fresh water ice treated with propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate, respectively at the end of storage period. It was also noticed that when the optical density of muscles reached to about 34.22-38.78% of the original optical density the fish was fairly considered unfit for human consumption. The results indicated that the optical density of muscles extract during storage of mullet fish was relatively low in the case of fresh after crushed sample.

Proctor et al. (1959) successfully measured the optical density of eye fluids, and used it for the determination of freshness during cold storage of haddock.

Ghoneim (1974) mentioned that the optical density of gills extract was increased continuously by cold storage reaching 0.85-0.97; and 0.82- 0.97 at complete spoilage in the refrigerator at 40 °F and crushed ice, respectively. Spoilage of tilapia fish took place, as the optical density of gills extract increased by 3 folds as compared with the fresh samples. The same author found that the optical density of muscle fluids decreased by spoilage reaching 0.64- 0.86, and 0.61- 0.98 during storage in the refrigerator at 40 ^{O}F and in crushed ice, respectively. The decrease of the optical density of muscle extract to 50-75% of the original value indicate complete spoilage. The values obtained, using such method was found to be greatly affected by leaching during storage in crushed ice.

3.7 Optical density of gills extract

The data in Figure 7, show the optical density of gills extract for the stored mullet fish. From these figure it could be observed that

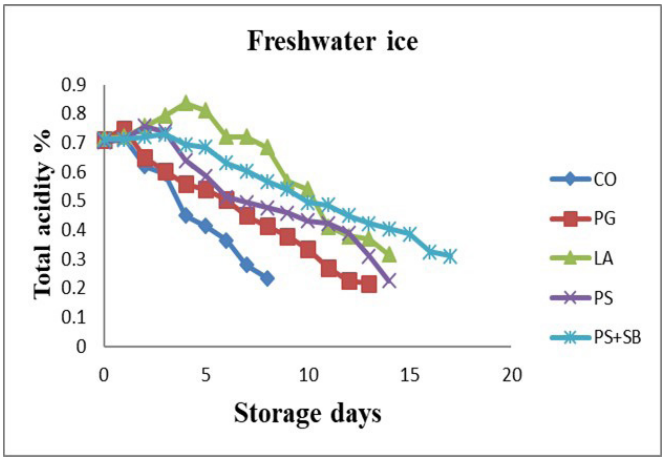

Figure 5. Total acidity (%) of mullet fish during cold storage in ice made from freshwater at different additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

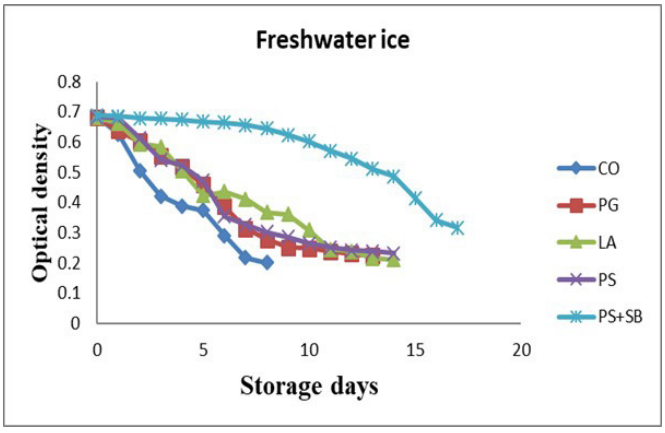

Figure 6. Optical density (%) of mullet fish muscle extract during cold storage in ice made from fresh water and different additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

the optical density of gills extract increased continuously during cold storage and spoilage which may be due to changes in color and breakdown of tissue structure beside the changes in protein Ghoneim (1974). The value of the fresh fish was ranging from 0.518, 0.531. they increased as the storage period was progressed reaching 1.203, 0.903, 0.743, 0.835 and 0.813 at the end of storage period for control and fresh water crushed ice treated with propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate respectively. The optical density of gills extract was relatively high in the fresh water crushed ice samples. The separation of more broken down tissues, more proteins (native and denaturation) and the formation of metamyoglobin may be explain the obtained results of Ghoneim (1974).

3.8 Refractive index of muscle fluids

The data presented in Figure 8, showed the changes in the refractive index of the conditions explained in the present study.

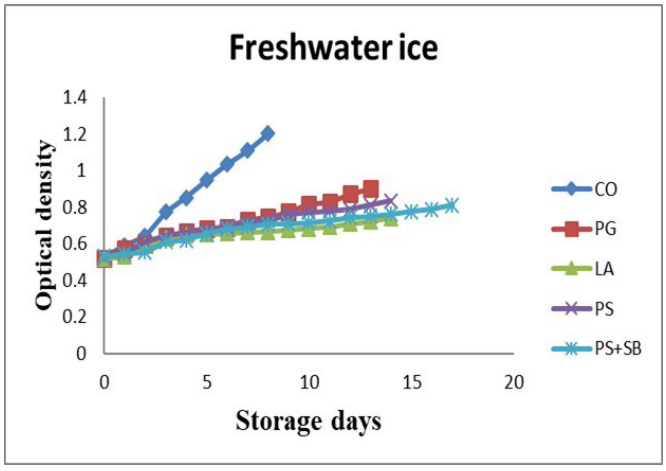

Figure 7. Optical density (%) of mullet fish gills extracts during cold storage in ice made from freshwater and different additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

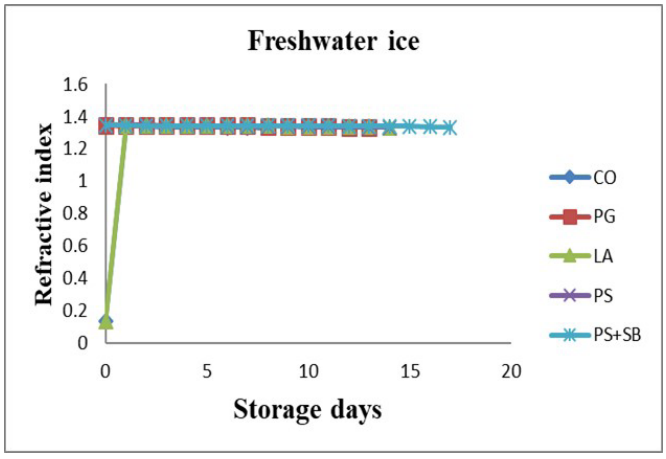

Figure 8. Refractive index of mullet fish muscles extract during cold storage in ice made from freshwater and different additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

It could be seen that the refractive index of mullet fish muscle fluids was decreased during cold storage. Such results were in agreement with Ghoneim (1974), who found a steady decline of the refractive index of fish muscle juice during cold storage.

At the stage which the fish were considered spoiled organoleptic ally the refractive index of the muscle fluids decreased reaching 1.33, 1.33, 1.33, 1.33 and 1.33 for control and freshwater crushed ice treated with propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate. The results indicated that the refractive index of muscle fluid of mullet fish was relatively low in the freshwater crushed ice sample.

Such method may be considered as a simple and routine freshness test that did not require long time or complicated operations as in the case of the chemical methods (Ghoneim, 1974).

Proctor et al. (1959), studied the changes in the refractive index of Fish eyes during storage of whole haddock under different conditions. They

reported a good correlation between the value of the refractive index and The organoleptic judgement.

In a similar study with anchovy RI values were found between 1.3352 and 1.3508 at +4 °C for 5 days (Yapar & Yetim, 1998). In the another study, the initial RI value was found to be 1.3355, while at the end of the 6-day storage this value reached 1.3381 at 0 °C and 1.3406 at +4 °C (Gokoglu & Yerlikaya, 2004).

Washing the fish in chlorotetracycline solution before storage did not reduce the refractive index, indicating that such method reflects autolytic changes, and was not affected by the microbial load.

Elerian (1965) reported a method for detecting spoilage and deterioration of fish, in which the refractive index of muscle juice and whole muscle tissue are measured during progress of various treatments. The same author found that the refractive index of whole gutted cod kept in crushed ice at ambient temperature of about 20 ^{0}C showed a steady decline. The values for the tissue fluid taken from the posterior end of the fish were lower than those from the anterior end. The decline of the refractive index was independent of the dilution effect from the melt-Water of the ice, used in preservation. Frozen storage, caused the increase in the refractive index of muscle fluids.

Ghoneim (1974) stated that the refractive index of muscle fluids decreased by spoilage reaching 13329-13430; and 13332-13395 during air storage at 400 F and in crushed ice, respectively. Refractive index of muscle extract could be considered as a routine method. Such a method was found to be good enough in case of cold storage at 40 °F without crushed ice. The same author found that by spoilage, the refractive index of eye fluids increased, reaching 13395-13425; and 13398-13430 during air storage at 40 °F and in crushed ice, respectively. Such a method was autolytic changes, leading to the breakdown of proteins. Such changes enhance the microbial decomposition of fish tissues, which is the main factor causing the spoilage of fish during 'cold storage, while autolysis is of less importance as compared. With microbial decomposition.

3.9 Electrical resistance of fish muscles

The data presented in Figure 9, showed the electrical resistance of mullet fish muscles during cold storage.

The data presented in the Figure 9, showed that the electrical resistance of mullet fish decreased continuously during cold storage. The electrical resistance increased after 1 day of cold storage of mullet fish which may be due to the development of rigor mortis. Such results are in agreement with finding of Thanaa (1984), who shoed the resolution of rigor mortis and spoilage.

At the stage of spoilage electrical resistance decreased reaching 27.19, 28.71, 27.61, 36.24 and 41.68 ohms for control and freshwater crushed ice treated with propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate, respectively.

The electrical resistance of spoilage at spoilage calculated as % of original value showed wide range being 36.38-5756% in the case of storage in freshwater crushed ice, such results are in agreement with the finding of Ghoneim (1974). Early studies showed, that the electrical conductivity was of no practical value as an index of fish spoilage since it varied with storage time and temperature, passing through a minimum during the early stages and rising again as the definite spoilage was developed. Asakawa (1957), reported that electrical resistance of carp muscles changed stepwise during storage as the fish passed through the stage of pre-rigor, and post-rigor to definite spoilage. Ghoneim (1974), reported that the electrical resistance increased by rigor mortis, while decreased by the resolution of rigor mortis and deep autolysis, i.e. spoilage. By spoilage the electrical resistance decreased to about 18-23 ohms, during storage of tilapia fish either in the refrigerator or in Crashed ice.

The electrical properties of fish skin and muscle change systematically after death and can be used as the basis of an instrument, two models are commercially available. The change in electrical properties is not caused directly by bacterial action or other spoilage mechanism, but i the instrumental reading on iced fish can be correlated with the stage of spoilage, as measured by sensory methods or by one of non-sensory methods (Torry Research Station, 1992).

The electrical properties of fish tissue closely related to freshness due to rapid changes in proteins and cell structure postmortem (Sun et al., 2018). It has concluded that electrochemical impedance spectroscopy (EIS) could be used to judge total volatile base nitrogen (TVB-N) of fish including carp, sea bream, and squid (Pérez-Esteve et al., 2014; Sun et al., 2018; Zavadlav et al., 2016). Furthermore, Yuan et al. (2018), proposed that bio-impedance analysis had a good relationship with the K-value after 24 hr ice storage. For the ready to eat raw materials, the textural properties and taste are as important as food safety. However, previous researches mainly focused on the fish shelf-life rather than fish-eating quality.

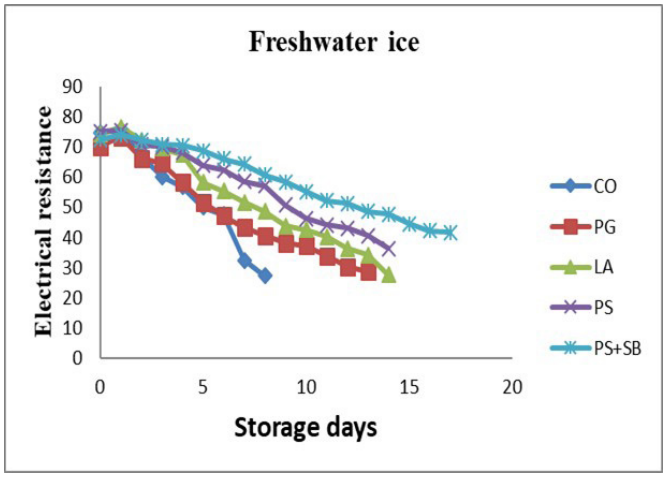

Figure 9. Electrical resistance of mullet fish during cold storage in ice made from freshwater and different additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

3.10 Total bacteria count

Data of the bacterial counts of iced mullet fish are shown in Figure 10 it could be observed that the total bacterial count was increased continuously during cold storage of mullet fish, such results agree with those reported by Thanaa (1984) and Bennour et al. (1991).

It could be noticed that the bacterial counts were low in fresh fish ranging from 0.0015 and 0.0051 x 10^6 cells/g and that by storage the bacterial counts increased markedly.

By complete spoilage the bacterial counts increased being 462, 720, 370, 280, 260 and 170 x 106 cells/g for control and freshwater crushed ice treated with propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate, respectively. The total bacterial counts were relatively high in the freshwater crushed ice samples and similar results has been reported by Zaitsev et al. (1969) who mentioned that common salt has a bacteriostatic and bactericidal action that delay the growth with subsequent killing of the bacterial cells. Thus this may indicate the presence of good correlation between chemical, physical, sensory analysis and the bacterial counts.

Cutting & Spencer (1968) reported that during spoilage under normal lectrolyz conditions, the microbial count increase to the order of 5 x 10^6/g in fish flesh and to 5 x l0^7/cm2 on the skin.

Lartigue et al. (1960) found that bacterial counts were closely parallel to organoleptic ratings during cold storage. Moreover, Hoff et al. (1967b) showed that total counts were superiorthan the number of coliforms in determining the spoilage during cold storage under crushed ice. The bacterial counts, were 38 x 10^6, 142 x 10^6 after 4 days of storage at 34 °F and in ice respectively. Ghoneim (1974) added, spoilage took place of mullet fish after 8-11 days and 4-6 days when the storage was conducted in the refrigerator at 40 °F. and in crushed ice respectively.

Shetty & Setty (1990) found that the initial total plate count (TPC) of fresh fish was 3.6 x l0^3/g; TPC increased to 8.1 x 10^7/g during storage in chilled (2 ± 1 °C) sea water (CSW). TPC of the medium surrounding the fish also increased. Gram negative

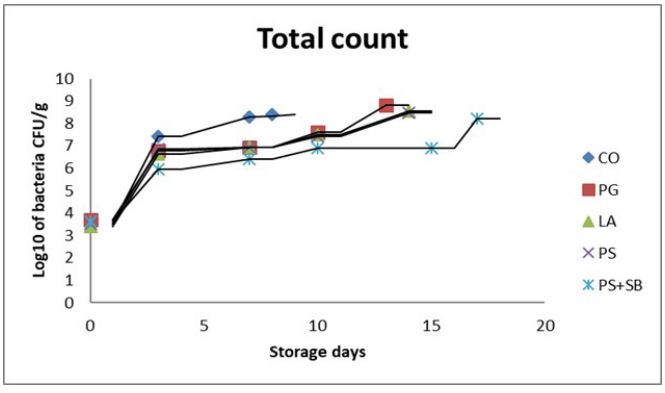

Figure 10. Total viable count of bacteria in mullet fish samples during cold storage in ice made from freshwater and different organic additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

bacteria (60%) predominated over Grain positives (40%) in freshly caught sardine. Among the Gram positives, *Micrococcus spp.* and *Bacillus spp.*, were the predominant organisms and in Gram negatives, *Flavobacterium spp.* Followed. By *Pseudomonas spp.*, *Acinetobacter spp.*, *Vibrio spp.* and *Aeromonas spp.* were predominant. As spoilage advanced, flora changed but _with, Gram negative organisms still predominant, in the order *Psudomonas spp.*, *Vibrio Spp.*, *Flavobacterium spp.*, *Acinetobacter spp.*, *Aeromonas App*, and. *Moraxella spp.* Gram negatives were also predominant in freshly collected sea water with higher incidence of *Vibrio spp.* and *Arthrobacter spp*. During storage the distribution pattern of different bacterial genera in the CSW media surrounding the fish was almost similar to that in the fish.

Gram (1991) reported that mesophilic motile *Aeromonas spp.* Which were classified as specific spoilage bacteria of Nile Prech from lake Victbria stored at ambient temperature were inhibited in laboratory model systems using NaCl, potassium sorbate, and liquid smoke. Growth was not detected when the salt concentration exceeded 5% or the temperature was below 5 °C. At 25-37 °C growth occurred within 24 h when no preservation was applied, but a combination of 5% salt and 1000 ppm sorbate inhibit growth at 25 37 °C. The addition of 1.5% salt (w/w) and 1500 ppm sorbate (w/w) followed by 3. Dayes of sun-dung resulted in a lightly brown, well preserved fish product which could be produced at artisanal level and was palatable to local consumers. Good agreement was seen between results from model experiments and trials with fish.

3.11 Psychrophilic bacteria

Data in Figure 11 show the psychrophilic bacteria counts during cold storage of mullet fish. The results showed that there was a considerable increase for these bacteria during cold storage, these results agree with Frazier (1997) and Franz (1970), who reported that the primary flora of raw materials of fish products comprises psychrophilic bacteria with an optimum at 0 °C.

At the end of storage period the psychrophilic bacterial counts reached 149, 32.4, 26.3, 34.3 and 8.8 x 10^6 cells/g for control and freshwater crushed ice treated with propylene glycol, lactic acid, potassium sorbate and potassium sorbate+ sodium benzoate, respectively.

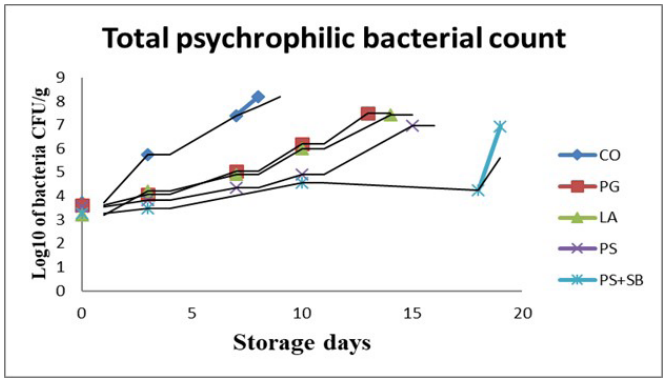

Figure 11. Total psychrophilic of bacteria in mullet fish samples during cold storage in ice made from freshwater and different organic additives. CO = Control; PG = Propylene glycol; LA = Lactic acid; PS = Potassium sorbate; PS + SB = Potassium sorbate + Sodium benzoate.

The results showed that the increasing rate for psychrophilic bacteria bacteria freshwater crushed ice in comparison with sea water which contained NacL. Nacl reducing the counts of psychrophilic bacteria.

The microbial limit of acceptability at 10^6–10^7 CFU g^{-1} for mesophilic aerobic bacteria (Gobantes et al., 1998) and 10^5 CFU g^{-1} for psychrotrophic bacteria (Lapa-Guimarães et al., 2002) were exceeded 7 days of storage. Similar results for mesophilic aerobic counts have been reported by Rehbein et al. (1994), for redfish as 12 days of storage in ice. Eifert et al. (1992) reported hybrid striped bass fillets stored at 4 °C did not reach 10^7 CFU g^{-1} until 12 days. Previous studies on the initial flora of sardines showed a predominance of gram-negative bacteria that were both psychotropic and moderately halophilic (Marrakchi et al., 1992).

3.12 Organoleptic

Organoleptic evaluation

Data presented in Table 1 show the average organoleptic scores of mullet fish during cold storage in cruched ice. It was evident that chilling of mullet fish in freshwater crushed ice treated have a shelflife of 8, 13, 14, 14 and 17 days for control and propylene glycol, lactic acid, potassium sorbate and potassium sorbate + sodium benzoate, respectively. The results may indicate the presence of good correlation between chemical, physical, bacteriological analysis and sensory assessment.

Lartigue et al. (1960) found that bacterial counts were closely parallel to organoleptic ratings during cold storage. Moreover, Hoff et al. (1967b), showed that total counts were superior to the number of coliforms in determining the spoilage during cold storage.

under crushed ice. The bacterial counts, were 38 x 106, 142 x 10^6 after 4 days of storage at 34 °F and in ice respectively. Ghoneim (1974) added, spoilage took place of bolti fish after 8-11 days and 4-6 days when the storage was conducted in the refrigerator at 40 °F. and in crushed ice respectively.

Fey (1980) found that red hake and salmon stored in a MA of 60% $C0_2$, 20% 0_2, 20% N2 with 1% KS ice were organoleptically acceptable after one month of storage at 1 °C. He also reported that KS solutions as dips and ices apparently inhibit the activity of TMA-producing bacteria in red hake, Chinook, and Sockeye salmon stored at 0-1 °C.

Maha et al. (1980) found that 0.1% of KS residue in cured mackerel and smoked milkfish followed by irradiation could retard mold growth and extend the shelf life considerably. Several reports have shown that Sorbates may be useful in extending the shelf-life of fresh poultry meat (Robach & Sofos, 1982). Tompkin et al. (1974) showed that Sorbate retarded growth of *Salmonela spp*. and *Staph. aureus* and probably delayed the growth and toxin production of *Cl. botulinum*

The light preservation techniques often use chemical preservatives like sorbic acid which has been approved in the

Table 1. Average organoleptic scores of mullet fish during cold storage in ice made from freshwater and different additives (Scale of 10 points).

Quality characteristics	Control				Propylene glycol					Lactic acid					Potassium sorbate					Potassium Sorbate + Sodium benzoate					
	days				days					days					days					days					
	0	3	7	8	0	3	7	10	13	0	3	7	8	14	0	3	7	10	14	0	3	7	10	15	17
General appearence	10	5	3	.	10	8	5	3	.	10	5	4	3	.	10	8	5	4	.	10	9	9	5	3	.
Eyes	10	7	3	.	10	8	7	3	.	10	8	3	2	.	10	8	7	3	.	10	9	7	6	3	.
Gills	10	7	3	.	10	9	5	4	.	10	4	4	3	.	10	9	5	4	.	10	9	5	4	3	.
Scales	10	8	4	.	10	8	6	5	.	10	8	5	4	.	10	9	6	5	.	10	9	8	6	4	.
Ringer- morits	10	7	4	.	10	9	9	7	.	10	9	7	4	.	10	9	9	7	.	10	9	9	7	4	.
Abdominal Coat	10	8	4	.	10	8	4	4	.	10	8	3	2	.	10	8	5	4	.	10	9	5	4	3	.
Peritoneum	10	6	2	.	10	5	2	1	.	10	6	2	1	.	10	9	3	2	.	10	9	6	6	2	.
Anus	10	7	3	.	10	7	5	3	.	10	7	5	3	.	10	7	7	3	.	10	9	7	7	3	.
Flesh colour	10	8	3	.	10	8	8	2	.	10	3	2	1	.	10	8	8	2	.	10	8	8	8	2	.
Blood colour	10	9	4	.	10	9	6	2	.	10	3	2	1	.	10	8	8	2	.	10	8	8	8	2	.
Overall quality	10	9	4	.	10	9	6	2	.	10	6	6	3	.	10	6	6	3	.	10	9	6	4	3	.
Overall quality	10	7.2	3.3	.	10	7.9	5.7	3.4	.	10	6.4	4.1	2.6	.	10	8	6.1	3.8	.	10	8.9	7	5.7	3	.

10 = excellent; 7 = medium; 9 = very good; 6 = fair; 4 = very poor; 8 = good; 5 = poor; 3 = extremely poor.

United States as a GRAS. (generally regarded as safe). Substance (Liewen & Marth, 1985). Sorbic acid has proved as effective preservative of chilled fish (Statham & Bremner, 1983) by inhibition the spoilage bacteria (Robach, 1979). Although the effects of Sorbic acid on fish mesophilic spoilage bacteria are not known, it is may also be valuable in preserving fish in the tropical region

It has been shown that sorbate added to fish as dry powder was degraded, whereas proper absorption and antimicrobial effect were seen if the sorbate was added to the brine solution (Doesburg et al., 1969).

The addition of Sorbate was useful in preventing attack of fish product by molds. This observation is similar to results and on smoked fish (Ikeme, 1986).

Raczek (2004) mentioned that sodium benzoate is usually employed in combination with potassium sorbate, because a better effect against acid producing bacteria can be achieved. Moreover, the potassium sorbate content renders the mixture less obtrusive organoleptically than sodium benzoate on its own.

4 Conclusion

It could be concluded that the electrical resistance increased by rigor mortis, while decreased by the resolution of rigor mortis and deep analysis. The optical density of gills extract was increased continuously by cold storage while the optical density of muscles fluids decreased by spoilage. The refractive index of muscle fluids decreased in freshwater crushed ice sample. The total volatile bases content was increased during storage in freshwater crushed sample. Trimethylamine content low in freshwater crushed ice sample. The total acidity content increased at the first days of cold storage and then decreased continuously up to spoilage. In freshwater crushed ice sample the total acidity was relatively low. The total bacterial count was low in fresh fish but during storage the bacterial count increased markedly. The psychrophilic bacteria count increased continuously during spoilage. During storage in freshwater crushed ice sample was reduced in comparison with the control samples. Organoleptically iced mullet fish have a shelf life of 8, 13, 14, 14 and 17 days for control and freshwater crushed ice treated with propylene glycol, lactic acid, potassium sorbate, potassium sorbate + sodium benzoate. in general, the treatment with potassium sorbate + sodium benzoate was the best treatment and recommended to increase the shelflife of mullet fish during transportation and storage.

Ethical approval

All experiments were performed in accordance with the European Community Directive (95/701/EEC). The animal care procedures agreed with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, eighth edition, and were approved by the Institutional Animal Ethics Committee for Laboratory Animal Care at the Zoology Department, Faculty of Science, Helwan University (Approval number: HU2021/Z/MFE0521-02).

Conflict of interest

The authors declare no conflict of interest.

Availability of data and material

All the data regarding this work were represented inside this manuscript.

Funding

Funding Researchers Supporting Project number (RSP-2021/97), King Saud University, Riyadh, Saudi Arabia.

Author contributions

S.H.M., H.M.Y, A.H.A.-M. and M.F.E. conceived and designed the experiments. H.M.Y., E.A.I. and S.H.M. performed

the experiments. S.H.M. analyzed the data. H.M.Y. and S.H.M. wrote the paper.

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