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SIGNIFICANCE OF ELECTROLYZED WATER-ICE (EW-ICE) IN FISH INDUSTRY

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ABSTRACT

Slightly Alkaline electrolyzed water (SAlEW) is used as cleaning agent for removal of greasy materials and organic matters from surfaces and also appears to have antimicrobial effect. While, slightly acidic electrolyzed water (SAcEW) act as sanitizing agent for food and food contact surfaces. In the current work, Aerobic plate count (APC) of examined fresh fish samples (mean log10cfu/g) was within the permissible limit (5.7±0.01, 5.94±0.015, 5.99±0.04 and 5.9±0.01) till the 5th, 7th, 9th and 11th days of preservation in ordinary non-treated water ice, SAlEW, SAcEW and SAlEW followed by SAcEW-ice, respectively. The judgment depends on the permissible limits mentioned by ES (No.3494/2019). By matching Psychotrophic count using APC as a guide, the samples recorded 3.94±0.015, 4.96±0.02, 4.87±0.02 and 4.93±0.01 till the aforementioned days as well as types of water used in the experiment. Meanwhile, Coliform count recorded 2±0.07, 1.99±0.09, 1.95±0.02 & 2±0.02 for the same former days and also by using the same water treatments. In addition, *Staph. aureus* count recorded 2.94±0.01, 2.99±0.07, 2.91±0.02 & 2.93±0.02 at the 6th, 7th, 10th and 12th days respectively by using the same aforementioned water types. In conclusion, using of SAlEW followed by SAcEW had a highly decontamination effect as well as prolong the shelf-life of examined fish followed by SAcEW, SAlEW and finally ice made from ordinary water. Chemical analysis (TVB- N & TBA) should not be relied to determine the freshness of the fish, as they were within the permissible limits, despite the bacteriological spoilage of the samples.Overall Sensory parameter scores showed balanced results in their acceptability scores which were parallel to the bacteriological analysis.

KEYWORDS: Slightly alkaline electrolyzed water-ice (SAlEW-ice), slightly acidic electrolyzed water (SAcEW-ice). Detergent, Sanitizers, Fish, Foodborne pathogens.

INTRODUCTION

Food in generally and fish including sea food specifically contains high levels of nutrients such as protein, fats, minerals as calcium, potassium, phosphorus and iron that support the growth of human body and at the same time supports the growth and multiplication of microorganisms which causes corruption, spoilage and shortened shelf-life of the fish. The origins of the microbial contamination in fish originated from the resident microbial flora associated with fish skin, intestinal content, gills, contaminated equipment surfaces and workers during catching and processing or due to the long-elapsed time between catching and processing or when using unhygienic ice produced from non-treated water. Also, the contamination initiates resulting microbiological spoilage of seafood products during unhygienic storage causing off- odors, off-flavors, slime and discoloration (**Hsu, 2005 and U.S. Department of Agriculture (USDA), 2019).** There is a high possibility that contamination also comes from environmental sources in the fish processing plants (**Prendergast *et al.*, 2004**). Besides, spoilage can occur due to chemical reactions such as autolytic reactions and oxidation as well as physical damage **(Mohan *et al.*, 2010)**. Ice storage effectively prevents decomposition and extends the shelf life of fish **(Fan *et al.*, 2007)**. Storage of cached fish and transportation to the processing factory depends mainly on ice storage. However, unhygienic practices applied during the processes or contamination of ice water will resulted in fish deterioration **(Lee, 2006).** Recently, the effects of ice containing antimicrobial agent, like plant extract ice or Electrolyzed water ice, on biochemical and microbiological properties related to fish spoilage have been reported **(Bensid *et al.*, 2014).**

Sea bass, (family Serranidae), any of the numerous fishes of the family Serranidae (order Perciformes), most of which are marine, found in the shallower regions of warm and tropical seas. The family includes about 475 species, many of them well-known food and sport fishes. This fish family according to **ES (3494/2019)** for chilled fish, the Total volatile basic nitrogen (TVB-N) should not exceed than 30 mg/100g and Thiobarbituric acid (TBA) not more than 4.6 mg malonaldehyde/kg fish flesh, APC should be <6 log10 cfu/g, Coliforms <2 log10 cfu/g and *Staph. aureus* (<3 log10cfu/g).

Furthermore, fresh fish should be free from *Salmonella*, *Listeria monocytogenes* and *Vibrio parahemolyticus*. Shelf-life of chilled whole fish at a temperature higher than zero ^{o}C (32°F) and not more than 4°C (39.2°F) shall not exceed 7 days from the date of catching time.

Conventional chemical sanitizers used for food and food contact surface for cleaning and sanitization could result in toxic residues which may have adverse risk effect on human health. Nowadays, slightly alkaline electrolyzed water (SAlEW) and slightly acidic electrolyzed water (SAcEW) are known as a novel detergent and sanitizing agents for cleaning and decontamination of food, utensils, tools, surfaces and equipements due to its high efficiency and no harmful residues. Electrolyzed water is obtained from the electrolysis of a salt solution, generally NaCl (2g/L). When electricity flows through the solution, two types of water are generated: at the cathode, slightly alkaline electrolyzed water (SAlEW) and also known as electrolyzed reduced water (ERW) containing sodium hydroxide (NaCl, pH 8-10), while at the anode, slightly acidic electrolyzed water (SAcEW) also known as electrolyzed oxidized water (EOW) which containing hypochlorous acid (pH 5.4-6.5) (**Fukuzaki *et al.*, 2004).**

Between pH 5.0 and 6.5, HOCl (95%) is the most common active form amongst different chlorine compounds including hypochlorite ions (ClO^{-}), chlorine gas (Cl_2), which discouragement pathogenic microbial activation and vitality. The relative amounts of the HOCl, Cl_2, and ClO^{-} species formed in SAEW solutions depends on the level of pH; therefore, changes in pH have a significant effect on the formation and efficacy of chlorine compounds. Previous studies demonstrated that the SAEW is most effective in eliminating pathogenic microorganisms at a pH of approximately 5.5, when the proportion of HOCl is the highest (**Hricova *et al.*, 2008**). Moreover, a specific oxidation-reduction potential (ORP), which indicates the ability to oxidize or reduce, has been reported to be the main factor influencing the antimicrobial activity of SAEW (**Al-Haq *et al.*, 2005**).

Slightly acidic electrolyzed water (SAcEW) can be used to decontaminate fresh shrimp and pork. Based on these experimental results, the United States Environmental Protection Agency (EPA) has approved the use of EW for disinfection in the food processing field. Moreover, the Japanese Ministry of Health, Labor and Welfare has also authorized EW as a food additive to reduce pathogenic microbial populations in various foods, food contact surfaces, and food processing surfaces **(Rahman *et al.*, 2013 and Wang *et al.*, 2014)**.

Electrolyzed water is an effective disinfectant that facilitates preservation of freshness and safety of fish **(Quan *et al.*, 2010; Zhang *et al.*, 2015 and Xuan *et al.*, 2017)** and is used widely used in medicine and in controlling and reduce the microorganismscontaminating the surface of fruits and vegetables (**Xie *et al.*, 2012 and Mansur and Oh, 2015**). Electrolyzed water is a novel application obtained by electrolysis of water containing sodium chloride (NaCl) or hydrochloric acid (HCl), leading to productionof sodium hypochlorite (NaClO) or hypochlorous acid (HOCl) (**Yoo and Jang, 2011).** Slightly acidic electrolyzed water (SAEW) has been produced by electrolysis of 2–6% HCl or NaCl of 0.1 - 0.2% NaCl (**Athayde *et al.*, 2018)**. in an Electrolysis tank and has high sterilization effects at low effective chlorine concentrations **(Kim *et al.*, 2015**). Previous studies have demonstrated that SAEW has strong bactericidal activities against many foodborne pathogens, including *Vibrio parahaemolyticus*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enteritidis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* **(Diza *et al.*, 2007; Cao *et al.*, 2009 and Issa-Zacharia *et al.*, 2010).**

Because its potent and safe-to-handle sanitizer, the EOW has been easily applied into various industries, as disinfectant for food and food contact materials including processing equipment (stainless steel, glass) (**Park *et al.*, 2002 and Serraino *et al.*, 2010),** or directly on food (vegetables, meat, poultry, eggs, fish) (**Athayde *et al.*, 2018; Fabrizio *et al.*, 2002 and Huang *et al.*, 2008**). Additionally, EW insures safety for workers because it is manufactured in site in a dilute form which lowers the risk of employee injuries from concentrated chemicals (**Dickerson, 2009).**

Electrolyzed oxidizing water (EOW) is novel product obtained by electrolysis of water containing sodium chloride to yield primarily chlorine-based sanitizing products. At acidic pH this results in hypochlorous acid, hypochlorite ions and chlorine gas in the un-protonated form which has the greatest sanitizing and oxidizing potential propertiesand ability to penetrate microbial cell walls to disrupt the cell membranes. EOW has been shown to be an effective method to reduce microbial contamination of food products as well as food contact surfaces **(Al-Haq *et al.*, 2005; Huang *et al.*, 2008and Issa-Zacharia *et al.*, 2010).**

Lipid oxidation is an important factor of oxidative deterioration of food which can be measured by the content of TBA which acts as indicator of the degree of lipid oxidation of food (**Campo *et al.*, 2006).** In addition, lipid oxidation leading to formation of off-flavor and off-odor, thus limiting the shelf life (**Patsias *et al.*, 2006).** Recently, consumer's preferences have moved toward natural antimicrobials rather than chemical antimicrobials (**Yang *et al.*, 2011)**. Although there are several studies that have examined the effect of SAEW-ice on fishery products, there are no sufficient reports on ice made with natural antimicrobials In this study, the possibility of shelf life extension of mullet fish by using different types of EW-ice were identified through determination of acceptable microbiological, chemical, and sensory analyses markers.

The available information about the impact of EW water-ice treatment on the microbial ecology of catches and stored fish and fishery products is not fully documented and need additional researches to maximize the aim of reduction in initial microbial contamination and influencing the types of microbial flora that grow on fish and its products and negatively influences its shelf-life as well as product safety and quality. Therefore, the main purpose of this research is to help the fish producers finding the best way to maintain safety, hygienic quality and to extend the shelf-life of catch fish through using SAcEW-ice & SAlEW-ice to prevent corruption during the elapsed period from fishing until the distribution of the fish to the market, retailing, handling or delivery the processing factory, which may take some days which may result in fish corruption and decomposition specially in unhygienic storage condition.

MATERIALS AND METHODS

Sample preparation

Total 8 kg of freshly cached Mullet type fish were collected from a retail fish market at Cairo Governorate and transferred under strict hygienic measures to laboratory as soonas possible to carry out the experiment. Samples were divided in laboratory into four groups (2kg for each group), **1st group** was covered with crushed ice prepared from ordinary drinking water (**OW-ice)** as control, while the **2nd group** was covered with crushed ice prepared from Slightly alkaline electrolyzed water (**SAlEW-ice**), the **3rd group** was covered with crushed ice prepared from slightly acidic electrolyzed water (**SAcEW-ice**), while the **4th group** was covered with crushed ice prepared from SAlEW for 5 minutes then replaced by crushed ice prepared from SAcEW till the end of the experiment with a 2:1 (ratio of ice/sample). All groups were stored refrigerated at 4°C in polyethylene bags and examined daily bacteriologically, chemically and sensory till the appearance of the deterioration singes, with changing of ice specific to each group if necessary. The experiment was repeated in triplicate.

Preparation of electrolyzed water (EW) according to Al-Haq *et al.* (2005); Hricova *et al.* (2008) and Athayde *et al.* (2018)

Electrolyzed water (EW) of both SAlEW (pH, 8.5) and SAcEW (pH, 6) was prepared through electrolysis of tap water with sodium chloride (NaCl) 0.2% (2 g for each liter of tap water). A current of 9-10 volt and 8-10 amber was passed through electrolysis chamber with two poles, anode (+) and cathode (-) for 10 min. The exchange of ions occurred between two separate sides through a bridge. At the anode side, SAcEW was formed due to the generation of hypochlorous acid (HOCl), hypochlorite ions (OCl-) and chlorine gas (Cl2). While, at the cathode side, SAlEW was formed as a result of generation of sodium hydroxide (NaOH). The pH of EW was estimated using a digital meter (FSSAI, 2015) followed by preparation of crushed ice from each kind of prepared EW to form EW-ice used to preserve the samples along the experimental.

Anode: **2 NaCl→Cl2 (g) + 2 e— + 2 Na+, 2 H2O (l)→4 H + (aq) + O2 (g) + 4 e—, Cl2+ H2O (l) →HCl + HOCl**
Cathode: **2 H2O (l) + 2 e—— →2 OH—— (aq) + H2 (g), 2 NaCl + 2OH—— →2NaOH + Cl**

Preparation of sample homogenate (ISO, 6887-3/ 2017).

Twenty-five grams of the examined samples were aseptically transferred to a sterile stomacher bag and homogenized with 225 ml sterile buffered peptone water (0.1%) for 30-60 seconds to give an initial dilution of 1/10. Transfer by means of pipette 1 ml of the initial suspension into a tube containing 9 ml of sterile diluent. Mix thoroughly by using vortex for 5-10 seconds to obtain 1:100 dilution. Repeat this operation to obtain dilutions 1:1000, 1: 10000 and etc. dilutions.

Aerobic plate count (APC) as per the procedure of **APHA (2001).**

Aerobic plate count of preserved fish samples in EW-ice and OW-ice was determined using Standard Plate Count Agar (Oxoid). Incubation was run at 35°C ±1°C for 48 h. Bacterial counts were given in log10 cfu/g.

Total Psychrotrophic count (APHA, 2001).

Psychrotrophic count was determined in a similar method to that for APC, except that plates were incubated at 7±1oC for 10 days. The colonies were counted and expressed as log10cfu/g of sample.

Enumeration of *Staphylococus aureus* (FDA, 2001)

About one ml. of food homogenate was transferred and distributed over the surface of 3 plates of Baired-Parker agar (eg. 0.4 ml, 0.3 and 0.3 ml), using sterile bended glass spreader. The plates were retained in upright position until inoculum is absorbed by agar for about 10 mints. The plates were inverted and incubated for 24-48 hours at 35°C and examined for determination of *Staph. aureus* count.

Total Coliform count (Most Probable Number (MPN) according to FDA (2002): I- MPN-Presumptive test for Coliforms.

One ml from each serially diluted food homogenate was inoculated into 3 tubes of LST broth tubes for a 3 tubes MPN analysis, using at least 3 consecutive dilutions. LST broth tubes incubated at 35˚C ± 0.5˚C and examined for gas production after 24h. Gas-negative tubes re-incubated for an additional 24h and examined for the gas production.

II- MPN-Confirmed test for Coliforms

From gas producing LST broth tubes, a loopful of suspension was transferred to a tubes of BGLB, incubated at 35˚ C ± 0.5˚ C and examined for gas

production after 48h ± 3h. MPN of coliforms calculated based on proportion of confirmed gassing LST broth tubes for three consecutive dilutions.

Isolation and identification of Salmonellae according to (ISO, 6579-1/2017)

Incubate the previously prepared food homogenate for 18 h ± 2 h. at 37 °C±1°C. Then, 0.1 ml of Pre-enrichment broth culture added to 10 ml Rappaport-Vassiliadis broth with Soya (RVs broth) incubated at 41.5°C ± 1 ° C for 24 hr. ± 3h. and 1 ml of Pre-enrichment broth added to Muller-Kauffmann Tetrathionate/novobiocin broth (10 ml MKTTn) incubated at 37 ° C ± 1 ° C for 24 hr. ± 3 h. Loopful from both RVS broth and MKTTn was streaked over the surface of Xylose lysine Deoxycholate agar (XLD agar) and Brilliant Green (BG) agar, incubated at 37°C±1°C for 24 hr. ± 3 hrs. Suspected colonies were inoculated in nutrient agar slant for further identification.

Isolation and identification of *V. parahemolyticus* according to ISO/TS 21872- 1:2007/Cor 1:2008

Primary selective enrichment (in a liquid selective medium): by adding 25 g of sample to 225 ml of alkaline saline peptone water (ASPW) in a stomacher for 30 seconds, incubated at 41.5°C±1°C for 6±1hr. (for *Vibrio parahaemolyticus* and *Vibrio cholera* in fresh products. Transfer 1 ml of the culture obtained (taken from the surface) into each of 3 tube each containing 10 ml of ASPW then incubate at 41.5 °C ± 1 °C for 18±1hr. From the obtained culture, inoculate with a sampling loop the surface of a thiosulfate citrate bile sucrose agar plates (TCBS) and incubates set at 37 ˚C for 24hr. Typical colonies of *V. parahaemolyticus* a smooth green and 2 to 3mm diameter.

Determination of Total Volatile Basic Nitrogen (TVB-N) According to **Egyptian Standard "ES" (63-9/2006)**

Ten grams of each examined sample was added to 300 ml of distillated water and two grams of magnesium oxide then thoroughly mixed by a blender for 2 minutes and then was boiled till obtained 100 ml of distillate which received in flask contained 25 ml boric acid 2% and 2 drops of indicator. Flask was boiled tell 100 ml distillate was obtained. Sample was titrated with 0.1 M H2SO4 (R1). Steps were repeated using distilled water instead of sample as blank (R2). TVBN expressed as mg/100 gm = (R1-R2) X 14.

Determination of thiobarbituric acid (TBA) according to **Egyptian Standard "ES"(63-10/2006).**

In a clean blender, about 10 g of the examined sample was blended with 50 ml of D.

W. for 2 minutes, and then washed in distillation flask with 47.5 ml water. 2.5 ml of 4 M hydrochloric acid was added to bring the pH to 1.5, boiled till 50 ml distillate was obtained, and then filtrated. Five ml of TBA reagent (0.29 g/100 ml 90% glacial acid) was added to 5 ml of the filtrate in a screw capped test tube. The tubes then heated in awater bath for 35 minutes and the absorbance of the resulting color was measured by using of a spectrophotometer (Spectronic 21 Germany) at wave length 538 nm. The TBA values were recorded as mg malonaldehyde / Kg of the samples. Concentration of malonaldehyde = 7.8 X S mg/ Kg sample where S = the reading of absorbance.

Sensory analysis

The organoleptic quality of fish was determined by conducting sensory evaluations forvarious attributes such as appearance, odor, texture, consistency and overall acceptability by using a 10- point hedonic scale according to **Codex Alimentarius (CXG 31-1999).** Sensory evaluations were performed by the sensory team- specialist's staff from Reference Laboratory-Animal Health Research Institute that has the competency to perform objective assessments according to the following Evaluation Sheet Key.

Sensory Evaluation Sheet Key									
Very poor (Dislike)	poor		Border line	good		Excellent			
1	2	3	4	5	6	7	8	9	10

Regarding the overall acceptability score, quality score is 7 to 10 means the samplehas excellent quality, if the final score is 5-6, means good, while score 4 indicated border line and score less than 4 meaning the sample is unacceptable (Poor or very poor quality).

Statistical analysis

Statistical analysis of the obtained data was run in triplicate by using of Statistical Packaging for the Social Science (SPSS) Ver. 20 and the results were expressed as mean and standard deviation (Mean±SD). Data were analyzed using analysis of variance (one-way ANOVA). The results with p-value less than 0.05 ($p \leq 0.05$) was considered statistically significant**.**

RESULTS

Effect of various types of water-ice used for preservation of fish on APC and the shelf-life of examined fish.

Examined fish samples maintained in OW-ice water, remained acceptable in terms of amean±SD (log10cfu/g) of Total aerobic bacterial count (APC) until the 5th day of storage (5.7±0.01) but exceeded the permissible limit on the 6th day of storage (6.48± 0.01) as per the criteria established by Egyptian standard [ES No.3494/2019 (APC should be <6 log10cfu/g)]. Meanwhile, samples kept in SAlEW-ice remained intact until the 7th day as they recording APC (5.94±0.01) and were considered rejected on the 8th day of storage (6.48 ± 0.01). While, SAcEW-ice kept the samples safe till the9th day (5.99± 0.04) and exceeded the limit at the 10th day which

recorded APC (6.93±0.01). Treatment using SAlEW-ice for 30 minutes followed by continuous preservation in SAcEW-ice resulted in keeping the samples sound till the 11th day (5.9±0.01) while at the 12th day, APC elevated to unacceptable level (6.6±0.01). Highly significance difference (P<0.001) between preserved fish with SAlEW-ice followed by continuous preservation using SAcEW-ice and that fish preserved by using ordinary ice water and SAlEW. Moreover, there were significance difference (P<0.05) between preserved group using SAlEW + SAcEW-ice and the group preserved using SAcEW-ice alone. It could be concluded that SAcEW-ice and mix treatment with SAlEW followed by SAcEW-ice were able to keep the fish safety and quality till the 9th and 11th day of storage respectively, as compared with Ordinary water-ice (till the 5th day of storage only), which resulted in addition of 4-6 days for the shelf-life of examined fish **(Fig. 1).**

Effect of various types of water-ice used in fish preservation on Psychotropic count and the shelf-life of examined fish

Mean log10cfu/g±SD of Psychotrophic count of examined fish samples was 3.94±0.15 at the 5th day of storage using OW-ice, at the 7th day, it was (4.96±0.2) using SAlEW- ice, while by using SAcEW-ice the mean Psychotrophic count recorded 4.87±0.02 at the 9th day. Moreover, keeping the fish in SAlEW-ice for 30 minutes followed by permanent storage in SAcEW-ice resulted in psychotrophic count of 4.93±0.01 at the 11th days, respectively **(Fig. 2).** Highly significance difference (P<0.001) between treated group with (SAlEW-ice for 30 min followed by SAcEW-ice for the end of the trial) and both groups of fish preserved either by using OW-ice or SAlEW-ice. Such significance difference was (P<0.05) between preserved group using SAlEW + SAcEW-ice in one side and that group preserved using SAcEW-ice only in the other side. Bearing in mind that the time table for carrying out the Psychotrophic count was determined based on the acceptance limit in terms of APC results, since the latter has a permissible limit mentioned in the aforementioned Egyptian standard. So based on APC results, the examined fish samples were considered rejected when the Psychotrophic count recorded 5.3±0.07 at the 6th day, 5.78±0.04 at the 8th day, 5.62±0.33 at the 10th day and 4.98±0.07 at the 12th day using OW-ice, SAlEW-ice, SAcEW-ice and mix of SAlEW followed by permanent preservation using SAcEW- ice, respectively.

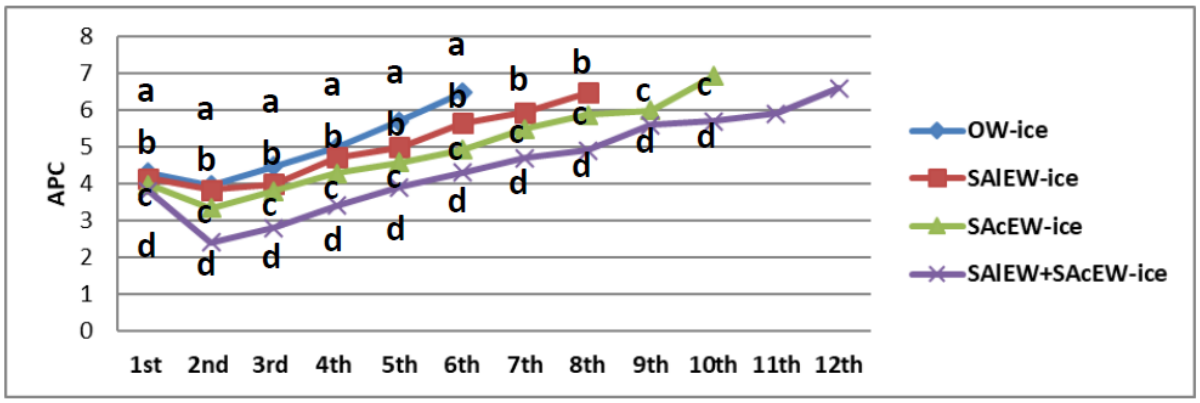

Fig. 1: APC (log10 cfu/g) of different water-ice treatments during storage days of fish.

NB: Same letters of the same day means no significance differences and this applied for allfigures.

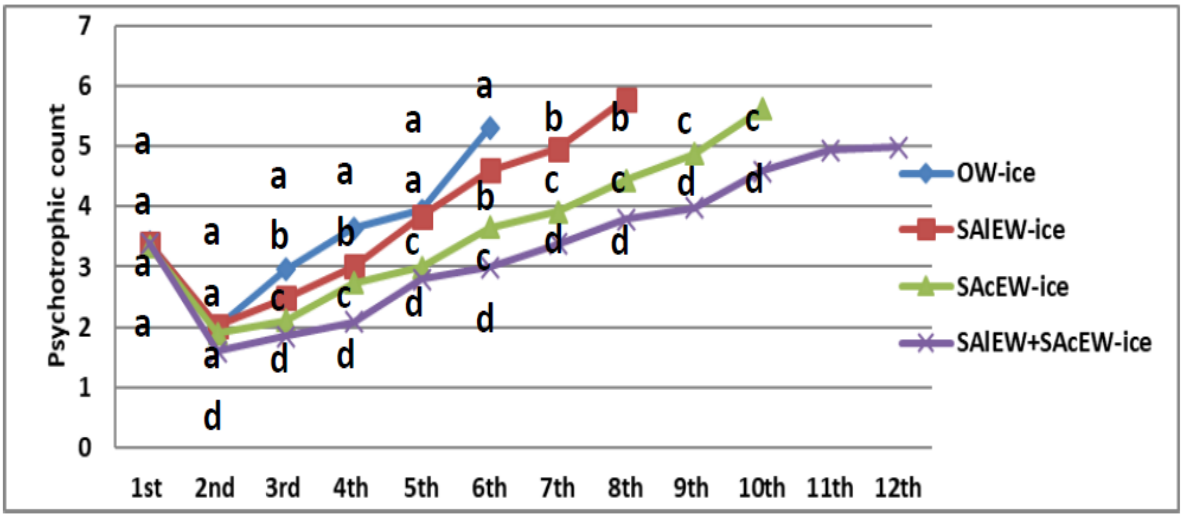

Fig. (2): Psychotropic count (log10 cfu/g) of different water-ice treatments during storage days offish.

Effect of various ice water types used in fish preservation on coliform count and the shelf-life of examined fish

As listed in the aforementioned Egyptian standard, that coliform count should not exceed 2 log10cfu/g, where the obtained results showed that the fish that were kept in OW-ice were within the permissible limits in term of mean coliform count expressed as (mean log10cfu/g±SD) till the 5th day (2±0.07) while the count exceeded the permissible limits at the 6th day (2.62±0.01). As for the use of SAlEW-ice, the count was (1.99±0.09) at the 7th day, while on the 8th day, it exceeded the permissible limit, recording (2.83±0.02). The results of SAcEW-ice were within the permissible limit on the 9th day (1.95±0.01), but at the 10th day, it exceeded the limit (2.07±0.08). At the same time, the results of coliform count as a result of preserving fish in SAlEW-ice for 30 minutes, followed by continuous preservation in SAcEW-ice were within the permissible limits until the 11th day (2±0.02), while they were higher than the limit (2.48±0.01) on the 12th day of preservation **(Fig. 3**). The obtained results summarized that using of SAlEW-ice followed by SAcEW-ice was the best way for fish preservation and keeping its shelf-life for as long as possible followed by using SAcEW-ice and finally, SAlEW-ice.

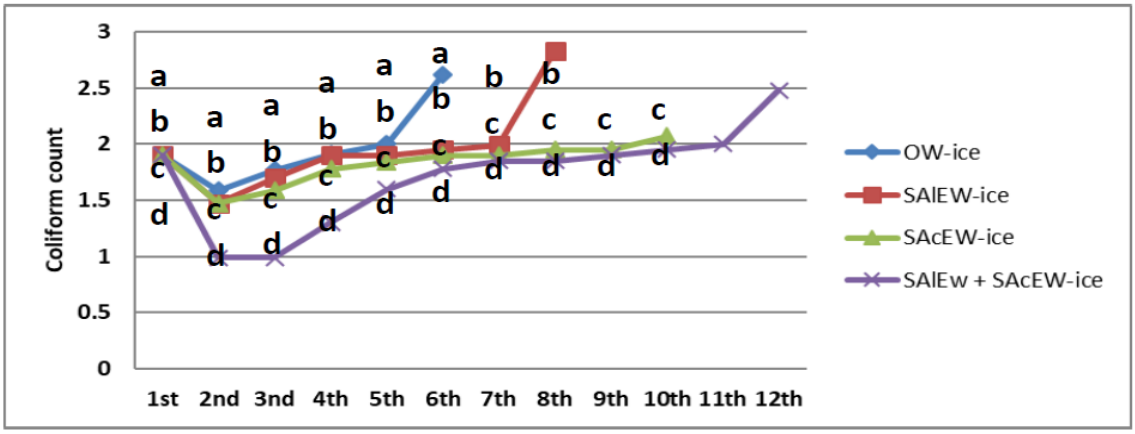

Fig. (3): Coliform count (log10 cfu/g) of different water-ice treatments during storage days offish.

Effect of various ice water types used in fish preservation on *Staph. aureus* count and the shelf-life of examined fish

Staph. aureus count should not exceed 3 log10cfu/g of fish as mentioned in **ES No. 3494/2019**. According to this criterion, the fish samples preserved using OW-ice water were fit until the 5th day (2.94±0.01), while it exceeded the permissible count at the 6th day of storage (3.3±0.04). As for SAlEW-ice, it exceeded the permissible count at the 8th day of storage, recording (3.84±0.03) while, the count was within the permissible limits till the 7th day of storage (2.99±0.07). Furthermore, fish samples which kept in SAcEW-ice, *Staph. aureus* count was within the permissible limit till the 10th day (2.91±0.02). In addition, storage of fish samples in SAlEW-ice for 30 minutes followed by continuous preservation in SAcEW-ice till the end of the experiment resulted in prolongs the shelf-life of examined fish till the 12th day of storage (2.93±0.01**) (Fig. 4).** This means that both SAcEW alone or in combined with SAlEW have a positive effect on the shelf-life of fish when it is limited only to *Staph. aureus* count, but when compared to the APC and *E. coli* counts, the shelf-life are declined which means that both types of EW had a significant effect on Gram-positive bacteria more than Gram-negative bacteria.

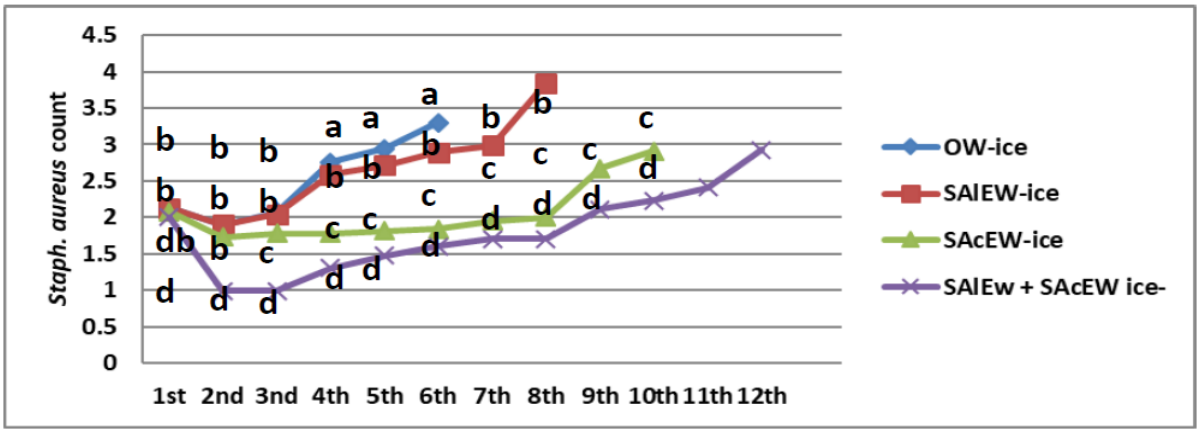

Fig. 4: *Staph. aureus* count (log10 cfu/g) of different water-ice treatments during storagedays of fish.

Effect of various ice water types used in fish preservation on the formation ofTVB-N

Total volatile base nitrogen (TVB-N) resulted from the degradation of proteins and non-protein nitrogenous compounds, mainly due to the microbial activity. It is believed that TVB-N considered as an important and sensitive indicator of meat freshness during storage. The TVB-N values of the samples during storage are showed in **Figure (5).** The Mean ± SD values of TVB-N of fish samples preserved in OW-ice water at the 1st day of the experiment was 10.04±0.1 mg/100g and increased gradually to record 24.05±0.18 at the 6th day of storage. While, treated samples with SAlEW-ice, SAcEW-ice and combination of both recorded 22.96±0.2, 20.23± 0.4 and 21.2± 0.3 mg/100g at the 8th, 10th and 12th day of storage, respectively. The TVB-N values showed to be increased with storage time in all the treated groups. The TVB-N was substantially slower and suppressed through preservation of samples in combination of SAlEW-ice followed by SAcEW-ice ($p < 0.001$) orthrough using of SAcEW alone ($p < 0.05$) as compared with samples preserved in OW-ice. The obtained results showed that TVB-N values were within the permissible limit (4.6 mg/kg MAD) at the time which the samples were exceeded the microbiological limits. This indicated that TBA shall not be considered as an accurate measurement fordetermination of fish freshness.

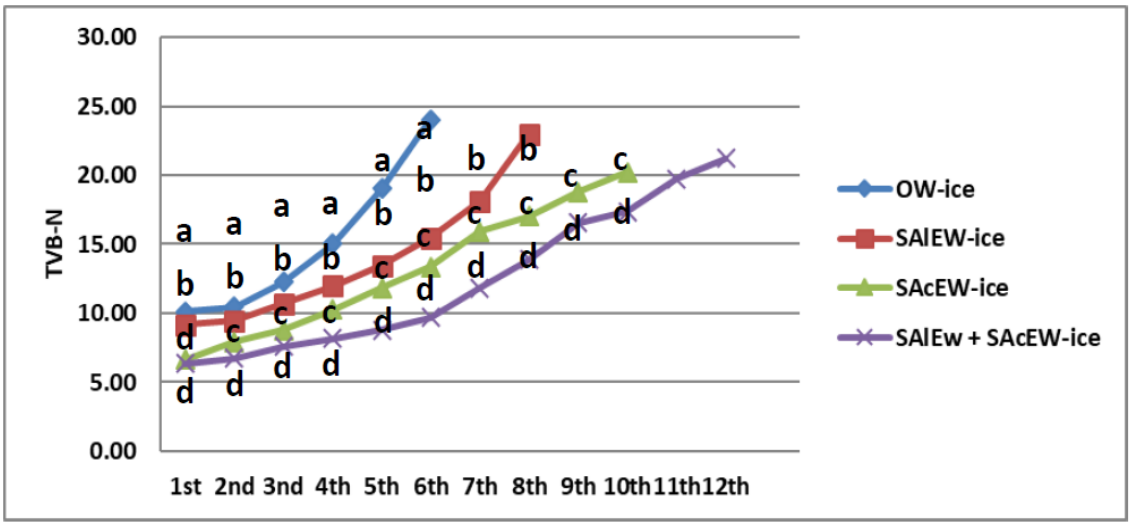

Fig. (5): Mean levels of TVB-N in all treatments through the storage period of each fish group.

Effect of various ice water types used in fish preservation on the formation of TBA

The changes in the values of TBA of treated and untreated fish during storage are illustrated in **Figure (6).** The content of TBA (mg malonaldehyde/kg) was variable significantly at the 1st day between control (0.46±0.05) and both of treated samples with SAcEW (0.36±0.06) ($P<0.05$) or when SAlEW used in combinations with SAcEW (0.3±0.03) ($P<0.01$). An increasing trend in TBA content was observed with increase in storage time for all the samples, although at different rates. The results showed that TBA values of the four groups increased gradually to record 1.85±0.11 at the 6th day, 1.69±0.13 at the 8th day, 1.45±0.07 at the 10th day and 1.53±0.04 at the 12th day for OW-ice, SAlEW-ice, SAcEW-ice and combinations of SAlEW+SAcEW, respectively. The obtained results cleared that the TAB values were within the permissible limit (4.6 mg MAD/kg) at the time which the samples were exceeded the microbiological limits. This indicated that TBA shall not be relied upon to determine the shelf-life and the expiration date of fish.

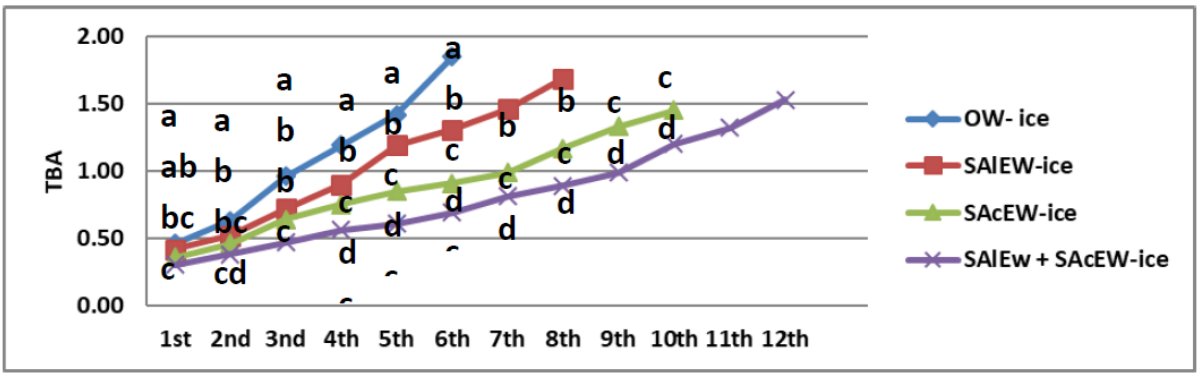

Fig. (6): Mean levels of TBA in all treatments through the storage period of each fish group.

Effect of various ice water types used in fish preservation on the overall sensory parameters

Significance differences of overall Sensory scores were more pronounced at the 5th day of storage between SAlEW and OW-ice ($P<0.05$) and between OW-ice and both of SAcEW and a combination of SAlEW & SAcEW ($P<0.001$). Storage scores of OW- ice, SAlEW-ice, SAcEW-ice and combination (SAlEW+SAcEW-ice) were considered overall sensory unaccepted at the 6th, 8th & 10th days of storage recording 3.83±0.29, 3.87±0.15 & 3.9±0.17, respectively. Only SAlEW+SAcEW samples were marginally accepted (4±0.1 score) at 12th day despite of the samples were contained unacceptable level of APC at the 12th day of storage.

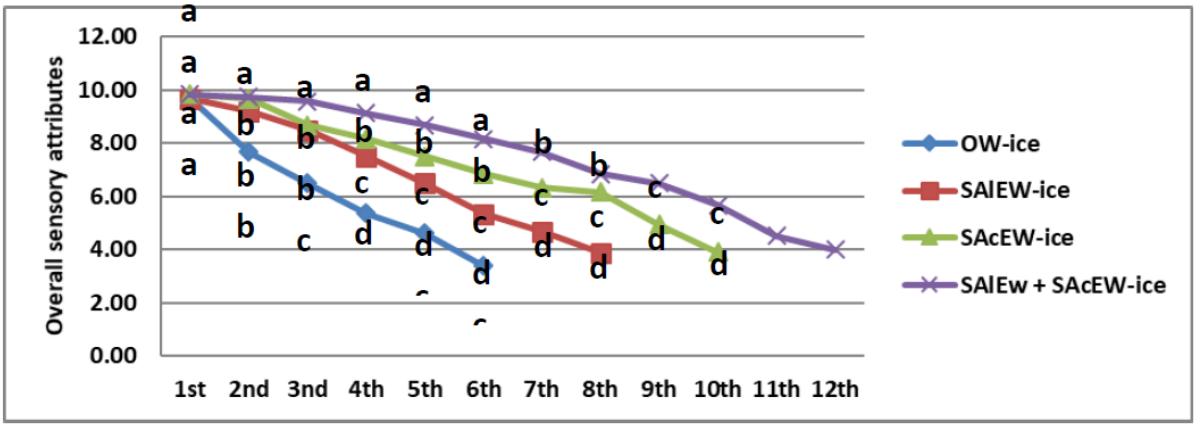

Fig. (7): Overall sensory scores of each fish group.

DISCUSSION

The obtained results matched with **Jung *et al.* (2018)** who found that slightly acidic electrolyzed water ice (SAEW-ice) affect positively the microbiological, chemical parameters and sensory characteristics of brown sole as compared with traditional water ice (TW-ice). Overall, the quality of fish was maintained for 9–10 days in TW- ice, and 11–12 days in SAcEW-ice due to its lower content of APC than those stored in TW-ice**.** Therefore, storage using SAEW-ice effectively extended the shelf life of brown sole. APC was correlated with storage times which are increased significantly under all ice storage conditions ($p < 0.05$). In this context, **Fabrizio & Cutter (2004)** and **Mahmoud *et al.* (2006)** stated that SAEW treatment exhibited higher disinfectant efficacy of total viable count (TVC). The TVC increased with storage time in all the samples, but at different rates. As expected, the TVC values of the untreated samples increased at a faster rate than those of all treated samples, indicating the strength of the antimicrobial effect of SAEW as compared with that of the distilled water treatment and control fresh samples ($p < 0.05$). This agreed with the results in the present study. Furthermore, fish traders in many tropical countries often use diluted formalin dip or spray in order to encounter quality loss and delay bacterial spoilage (**Wooster *et al.*, 2005** and **Yeasmin *et al.*, 2010a&b**) which is considered carcinogenic to human health (**Marsh *et al.*, 2007).** Chilling of cached fish on ice is good enough if adequate EW-ice is maintained. It has been shown that EW-ice was effective not only in eliminating or reducing APC from fish body but also in improving the keeping quality and shelf life of three wet fish in both ambient (25°C) and chilling (4°C) temperature. Therefore, the use of EW-ice in preserving of cached fish and subsequently fish trading can reduce the chance of using harmful chemicals like formalin in fish. This comes in compliant with the results in our present study.

Moreover, **Lin *et al.* (2013)** added that plate count enumeration demonstrated that (Acidic electrolyzed water) AEW ice had a capability to inhibit growth of bacteria on raw shrimp, and the maximum reductions of population reached at >1.0 log CFU/g (>90%) at the 6th day. Based on above analysis, AEW ice can be a new alternative of traditional sanitizer to better preserve the quality of seafood in the future. Lower results were stated by **Alam *et al.* (2020)** who reported that the viable bacterial count (APC) of the surface of examined fish (pacific mackerel, oil sardine and horse mackerel) has reduced significantly to be non-detectable after treatment with using EW ($P<0.05$). He added that EW found to have strong effect in killing surface bacteria through one minute dipping only.

The obtained results in the current research comply with **Fera *et al.* (2017)** which stated that in fish and seafood processing, maintaining the cold-chain is vital. Fish producers use ice to retain the quality and suppress microbial growth in the products during processing. EO water efficacy increased specially it contains hypochlorous acid in ice (EO-ice). Active EO and the water will be released slowly during melting allowing it to function as sanitizers longer than simple non-ice EO water. EO-ice could be thus be used to replace OW-ice that ice give a greater strength in inhibiting microbial growth during processing. Also, the results complied also with **Feliciano *et al.* (2010)** who observed the inhibitory effect of EO-ice at pH 6.8 on the microbial growth including *E. coli* and psychotropic bacteria of whole tilapia, tilapia fillet and water during storage. Furthermore, the present data agreed with Kim *et al.*

(2006) and Tolba *et al.* (2020) who mentioned that SAcEW had an strong effect on reducing of both APC and total psychotropic count, Many previous research followed the same scientific approach as in the current research because of what is rumored about the sanitization effect of EW and its sanitizing effect against bacteria in many foods, as seafood, fish, poultry and meat as well as its ability to improve the food freshness through keeping the accepted limits mentioned in international Food standards including physicochemical and sensory characteristics (**Zhang *et al.*, 2015; Abo-Zeid, Howida, 2020; Tolba *et al.*, 2020 and Ahmed-Aliaa, 2022**). Spoilage of fish starts with autolytic degradation followed by microbial activity which accelerates the degradation of fish tissues producing off odors and flavors. Even though many microorganisms occur in seafood post-harvest, mainly specific spoilage organisms (SSO) as Psychotropic bacteria, *Vibrio* specially *parahemolyticus* type and coliforms which considered the main spoilage bacteria reported in fish, that are responsible for the degradation and limitation of fish shelf life. To maintain the quality and freshness of products, and to improve shelf life, growth of the SSO must be controlled (**Ray and Bhunia, 2008**).

Furthermore, **Nan *et al.* (2010)** found that treatment with slightly acidic electrolyzed water SAEW (pH 6.0 to 6.5) resulted in 100% inactivation of *Staph. aureus* and *E. coli*. (7.92- to 8.75-log reduction). The bactericidal activity of SAEW was more pronounced on *E. coli* O157:H7 which was much more sensitive than *Staph. aureus* to SAEW as the morphological damage to *E. coli* O157:H7 cells by SAEW were significantly greater than that to *Staph. aureus* cells. SAEW with a near neutral pH may be a promising disinfectant for inactivation of foodborne pathogens. EW has been shown effective control of pathogenic and spoilage microorganisms on fresh fish and seafood (**Rahman *et al.*, 2016**). Uses of EW for inactivating bacteria in raw salmon, tilapia and tuna fillets have been reported (**Huang *et al.*, 2006a & 2008** and **Ozer and Demirci, 2006). Wang *et al.* (2014)** used strong acidic EW to completely suppress the proliferation of *Vibrio parahaemolyticus* in raw and cooked shrimp. **Ozer and Demirci (2006)** found that treating raw salmon with EW at 35 °C for 64 min resulted in 91.1 and 92% reduction in *E. coli* and *L. monocytogenes*, respectively. Furthermore, previous studies reported that EW had strong sanitizing effect against many types of microorganisms including *Salmonella* (**Venkitanarayanan *et al.*, 1999 and Kim *et al.*, 2000b**) and thought be safety to human health and effective in suppressing spoilage bacteria in fresh fish through the distribution chains (**Park *et al.*, 2002 a&b**). In the present work, the examined fresh fish samples were free from either *Salmonella* spp. or *V. parahemolyticus*. In this regard, EW was found to be effective in reducing population of *E. coli* and *V. parahaemolyticus* on tilapia (**Huang *et al.*, 2006a)** based on its bactericidal and preservative effects.

The obtained results by **Kim *et al.* (2006)** and **Tolba *et al.* (2020)** revealed that SAcEW (EO-ice) of pH (5) or little bit more had inhibitory effect inhibits the microbial growth and delayed formation of total volatile- basic nitrogen (TVBN) and thiobarbituric acid (TBARS) and help in prolong the shelf-life of shrimps and Pacific fish by 4–5 days longer as compared with non-electrolyzed TW-ice. Inhibiting the formation of TVBN and TMAO with EO-ice helped in improve the sensory quality of fish and seafoods. Such results are similar to that obtained in our research study**. Rahman *et al.* (2012)** found that SAEW treated groups had lower total volatile basic nitrogen (TVB-N) and Thiobarbituric (TBA) as compared with control group due to the presence of −OH and HOCl, which has antioxidant effect, and can maintain the oxidation stability of meat. Such results comply with the results in the present study. On the contrary**, Sheng *et al.* (2018)** observed that there were no significant differences ($p>0.05$) between the untreated and SAEW-treated samples, suggesting that SAEW has no antioxidant activity. Also,**Chen *et al.* (2016)** reported that SAEW has no immediate antioxidant activity. This difference in results might be due to the nature, type of meat, stability against oxidation process, the level of unsaturated fatty acids and the levels of natural antioxidants. Furthermore, the obtained data were more or less in accordance with **Alam *et al.* (2020)** who mentioned that mean TVBN (mg/100g) and TBA (mg MAD/kg) of five days storage of pacific mackerel previously dipped in EW were 16.07 ± 1.1 and 1.27 ± 0.06; 16.06 ± 0.07 and 1.6 ± 0.07 for oil sardine and 24.67 ±1.1 and 1.65 ± 0.16 for horse mackerel, respectively**.** In this regard**, Xuan *et al.* (2017)** have reported that SAEW in the form of ice can maintain comparatively low TBA contents during the storage of squid. This indicates that SAEW ice might be a novel technology to ensure the oxidant antagonistic activity and control the deterioration of quality of fresh fish during storage. Moreover**, Lin *et al.* (2013)** Outlined that AEW ice displayed an noticeable significantly ($p<0.05$) improvement of the overall sensory acceptability of examined shrimp when compared with tap water (TW) ice which lead to retardation of changes of color difference and the formation of total volatile basic nitrogen (TVBN). And AEW ice treatment had no adverse effects on the firmness of shrimp.. Further studies are required to increase the antioxidant efficiency of SAEW on fish. **Sheng *et al.* (2018)** concluded that TVB-N values reached 30 mg% after 23 day of SAcEW-ice storage, while our results revealed that TVB-N recorded 20.23±0.04mg after 10 days of storage using SAcEW-ice and 21.2± 0.3 at the 12th day using SAlEW followed by SAcEW-ice.

TVB-N values of brown sole did not reach 30 mg%, in all experiment days till the end using the different ice water preservation treatments whereas the samples were unfitfor consumption **(Kyrana *et al.*, 1997; Tejada and Huidobro, 2002 and Jung *et al.*,2018)** Such results complied with that of the present study as the TVB-N of

examined fish samples did not reach 30 mg/100 g at the 6th, 8th, 10th and 12th day of storage using OW-ice, SAlEW-ice, SAcEW-ice & SAlEW+SAcEW-ice respectively, whereas, APC exceeded the permissible limit (>106 log10cfu/g). These results indicating that TVB-N values alone are insufficient to determine the freshness of aquatic products. Therefore, we suggest the need for multiple indices of freshness, including microbiological changes and sensory analyses.

Previously, **Kamalakanth *et al.* (2011)** found that the shelf-life declined at the 15th and 20th days to 4 points (margin of acceptance) for TW-ice and SAcEW-ice, respectively. Such shelf-life period considered longer than the results of the current search. In this context, **Alam *et al.* (2020)** stated that EW was found to be effective in reducing APC. Fish treated with EW showed better keeping quality in terms of bacterial load and physical properties of fish compared to other treatments [chlorinated water (CW) and some natural herbal extracts]. Therefore, EW can be effectively used to reduce bacterial spoilage and extend shelf life of fish during distribution and marketing through improving its sensory parameters and chemical properties. This agreed with the results in the present study. The obtained results regarding the effect of electrolyzed water (EW-ice) in comparison with OW-ice in prolonging the shelf-life offish were agreed with **Kim *et al.* (2006)** who stated that Sensory analysis was improved and the freshness of fish was better during its preservation in electrolyzed water (EW-ice) than in tap water (TW-ice) and showed more prolonged shelf-life of fish by 4 to 5 days. The obtained data in this study were also incompliant with that previously reported by **Shung *et al.* (2018)** who found that, on day 10 of storage, sensory scores of brown sole stored in TW-ice, SAEW-ice were 4.44 and 5.38, respectively. On day 20, sensory scores of samples stored in TW-ice and SAEW-ice were 2.33 and 4.00, respectively, and there were significant differences among all treatments ($p<0.05$). Fish decomposition is very complex (**Hungerford, 2010**) therefore, the sensory evaluation of most of the fish is not sufficient to detect its quality, therefore chemical testing is required including Volatile amines and lipid oxidation which are commonly used as criteria for assessing the fish quality. (**Gulsun *et al.*, 2009 and Prester, 2011).** Also our results agreed with **Zhang *et al.* (2015)** who concluded that EO-ice could affect positively to extend the shelf-life of peeled frozen shrimp. The treatment successfully inhibited the deterioration including microbial growth, values of TVBN, TMA, TBARS, texture and color. The researcher findings have also shown that EO-ice is a promising technology to improve the quality of fish and sea food. A little published study discusses the effects of EO-ice on fish, sea foodsand fishery products. Therefore, further studies in this field are needed to establish a stronger evidence-base.

In conclusion, the microbial, chemical, and sensory characteristics were highly correlated with freshness of Sea bass fish. According to the different analytical treatments of all the present results, the required safety and quality standard of the Sea bass stored in OW-ice, SAlEW-ice, SAcEW-ice and SAlEW+SAcEW-ice was maintained up to 6, 8, 10 and 12 days, respectively. Also, the obtained combined results of this study indicated that using of SAlEW-ice for 30 min followed by storage in SAcEW-ice is the most coefficients extend the shelf life of Sea bass and can be used as preservation method to improve the safety and quality of fish and fisher products.

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