



Improvement the Shelf Life of Tilapia Fillets Stored at Chilling Condition

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ABSTRACT

The main purpose of this investigation was to study the effect of immersing fillets of Nile Tilapia (*Oreochromis niloticus*) in 1% acetic acid and storage under standard conditions (80% CO₂: 20% N₂) at a temperature of 2°C on their shelf life. Fillets samples were divided into four groups, Group 1 (G1): the fillets immersed in sterilized distilled water for 2 minutes and packaged in Polyamide/ Polyethylene (PA/PE) bags. Group 2 (G2): the fillets immersed in sterilized distilled water and stored under standard conditions. Group 3 (G3): the fillets immersed in 1% acetic acid for 2 minutes and packaged in PA/PE bags. Group 4 (G4): the fillets immersed in 1% acetic acid and stored under standard conditions. All samples were stored at a temperature of 2°C for 21 days and analyzed at the beginning of the experiment and after 7, 14 and 21 days of storage. Results of the study showed that the organoleptic properties of tilapia fillets dropped by the extension of the cold storage time to 21 days. The average values of Thiobarbituric acid-reactive substances (TBARS) in the G1, G2, G3 and G4 are 9.23, 2.56, 8.07 and 0.98 at day 21 of the cold storage at a temperature of 2 °C, respectively. Tilapia fillets kept under CO₂-enriched atmosphere had lower total volatile base nitrogen (TVB-N) than those stored in air where the average values were 19.9, 15.93, 18.97, 15.93 mg/100g in G1, G2, G3 and G4, respectively at the end of the storage period. The results also showed that the total viable count (TVC) remained at permitted in G4 as the average number was $6.14 \pm 0.05 \times 10^3$ after 21 days of storage at a temperature of 2 °C, while in G1, G2 and G3 were $7.53 \pm 0.06 \times 10^6$, $7.63 \pm 0.11 \times 10^5$ and $5.30 \pm 0.09 \times 10^6$ respectively. Thus, the results indicated that the tilapia fillet immersion in 1% acetic acid and storage under standard conditions that have been applied in our study contributed to the extension of the validity period for tilapia fillet for a longer period of cold storage of up to 21 days.

Keywords: Nile tilapia, fillets immersion, validity period, TBARS.

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1. INTRODUCTION

Fish is one of the most highly perishable foods and its shelf life is limited in the presence of normal air due to the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms. Freshness is one of the most important aspects of fish because consumers have a strong tendency to select very fresh fish (Luten and Martinsdóttir, 1997; Ross, 2000). Typical shelf life under chilled storage conditions ranges from 6 to 20 days (Cyprian et al., 2008; Martinsdóttir et al., 2001) depending on species, harvest location and season, and can result in heavy economic loss (Reddy et al., 1995; Sivertsvik et al., 2002). Extending fish shelf life is greatly advantageous to industry, as it reduces losses during product distribution and display, which may result in marketing improvements for fresh products and in a regular supply at reasonable prices (Lioutas, 1988). Shelf life of fishery products is usually

limited by microbial activities that are influenced most importantly by storage temperature (Huss, 1994; Simpson et al., 2003). Bacterial changes are considered the most important cause of fish spoilage (Gram and Dalgaard, 2002). This is because spoilage is often a result of off-odors and off-flavors caused by bacterial metabolism (Gram et al., 1990). Modification of the atmosphere within the package by decreasing the oxygen concentration, while increasing the content of carbon dioxide and nitrogen, has been shown to significantly prolong the shelf life of perishable food products at chill temperatures (Parry, 1993; Reddy et al., 1995; Siah and Mohd Ariff, 2002). The shelf life of fish products in Modified atmosphere packaging (MAP) can be extended, depending on raw materials, temperature, gas mixtures and packaging materials (Farber, 1991). MAP of fishery products has been shown to inhibit the normal spoilage flora and increase shelf life

significantly at cool temperatures (Sivertsvik et al., 2002). The application of this technology to foods has become increasingly more available in recent years as food manufacturers have attempted to meet consumer demands (Sveinsdottir et al., 2010). The techniques for modified atmosphere packed products involve the use of several gases, such as CO₂, N₂ and O₂. Carbon dioxide, whether alone or associated with other gases, being the most effective and common amongst them. Such an effect is influenced by the CO₂ concentration, initial bacterial population, storage temperature and product type (Reddy et al., 1992). Also, Reddy et al. (1994) evaluated the effect of modified atmospheres (75% CO₂/25% N₂; 50% CO₂/50% N₂; 25% CO₂/75% N₂) on the shelf life of tilapia (*Tilapia spp.*) fillets packed in high barrier film at 4°C. The authors observed that tilapia fillets packed in 75% CO₂/25% N₂ showed an increased shelf life of more than 25 days, presenting acceptable sensory characteristics. Duun and Rustad (2008) reported ice chilled salmon fillets in combination with MAP to have maintained good quality with negligible microbial growth for more than 17 days based on both sensory and microbial analyses. According to Marel et al. (1988), the superficial application of organic acids is used for meat decontamination, aiming mainly at reducing the deteriorating and pathogenic microorganisms naturally found in food. Some studies on the possibility of combining MAP with preservatives to preserve fresh fish have been conducted, in order to develop fish products presenting better quality and longer shelf life. Acetic acid and its salts are very efficient and widely used as acidulating agents and preservatives for food. The presence of 1-2% of non-dissociated acid in meat, fish or vegetable products is generally sufficient for bacterial inhibition, as long as good hygiene practices are observed (Pardi et al., 1994). Acetic acid diluted to 0.5% on catfish fillets would be suitable for prolonging shelf life and appealing to consumers. Acetic acid as a natural antimicrobial product can improve the shelf life and safety of food products providing acceptable sensory quality at an affordable price and reducing economic lost due to spoiled catfish and other food products (Lingham et al., 2012).

The purpose of this experiment is to assess the effect of MAP and the efficacy of acetic acid, when used in conjunction with 80% CO₂: 20% N₂ atmosphere to extend the chilling storage life of fresh tilapia fillets. Quality attributes were assessed by different methods including chemical, microbiological and sensory evaluation.

2. MATERIALS AND METHODS

2.1. Fish preparation

Forty tilapia fish weighing between 450 and 500 g were harvested and transported iced to laboratory within three hours. The fish were gutted, skinned, filleted, and divided into four batches: Group 1 (G1): the fillets immersed in sterilized distilled water for 2 minutes at room temperature, then laid on racks for 2 minutes to drain the solution and were packaged in Polyamide/ Polyethylene (PA/PE) bags and stored at 2°C for 21 days. Group 2 (G2): the fillets were immersed in sterilized distilled water under the same conditions in (G1), and then laid on racks for 2 minutes to drain the solution. The fillets were stored in gas-tight plastic containers. A mixture of liquefied CO₂ and N₂ was accomplished using Oxoid's Atmosphere Generation System (AGS). The storage conditions were as follows: temperature 2 °C, concentration of gaseous CO₂ (80%), and concentration of N₂ (20%) for 21 days. Group 3 (G3): the fillets underwent chemical treatment by immersion in 1% acetic acid at the proportion of 1.2:1, that is, 1.2 kg of fish per 1 liter of solution, for 2 minutes at room temperature. The fillets laid on racks for 2 minutes to drain the solution and were packaged in Polyamide/ Polyethylene (PA/PE) bags and stored at 2°C for 21 days. Group 4 (G4): the fillets underwent chemical treatment by immersion in 1% acetic acid as the same in (G3). The fillets were stored in gas-tight plastic containers. A mixture of liquefied CO₂ and N₂ was accomplished using Oxoid's Atmosphere Generation System (AGS). The storage conditions were as follows: temperature 2 °C, concentration of gaseous CO₂ (80%), and concentration of N₂ (20%) for 21 days.

The experiment was repeated in triplicate. All previous groups of samples were undergone the following analysis at day 1 and every 7 days:

2.2. Chemical analyses

Total volatile base nitrogen (TVB-N) was determined by protein precipitation using trichloroacetic acid (TCA) and evaluation of the total volatile base nitrogen in the TCA extract using the Micro Kjeldhal method, according to Ang (1988). Thiobarbituric acid reactive substances (TBARS) was determined by the precipitation of proteins associated with lipids and phospholipids. The spectrophotometer reading was taken at 535 nm, using a 7.8 conversion factor to transform mg of malondialdehyde to kg of food, according to Schmedes and Holmer (1989). pH was determined by means of a Digimed digital potentiometer, using 5 g of muscle homogenized with 45 ml of CO₂ free distilled water (Lim, 1987).

2.3. Microbiological analysis

Total viable count (TVC): Total viable microbial count on the fillets were determined using the pour plate method according to Maturin and Peeler (2001). Duplicate samples (about 10 g) were taken from each fillet at predetermined intervals. Samples were placed in a sterile stomacher bag and homogenized with 90 ml Ringer's solution in the Seward Stomacher (400 Lab Blender) to give 10^{-1} dilution. Further 10-fold serial dilutions were made as required using the same diluent. One ml of appropriate dilutions was pipetted into two plates and molten standard plate count agar (cooled to 42–45 °C) was then poured in. Plates were incubated at 37 °C for 48 h. Plates were counted and expressed as log CFU/g sample.

Psychrotrophic bacteria: Petri dishes containing Standard Agar were used for the PCA count. The dishes were incubated for 10 days at 7°C (Collins and Lyne, 1984).

Total coliforms counts: Most Probable Number (MPN) method is used for the quantitative estimation for coliform were performed according to the techniques recommended by Feng et al. (2002).

Salmonella spp: Fish muscle was homogenized with buffered peptone water (Oxoid, CM 509, Hampshire, England). After 18 h incubation, the samples were inoculated into tubes with tetrathionate broth base (Oxoid, CM0029) with iodine-iodide solution. Samples were incubated for 24 h in 37 °C, and then aliquots were spread onto plates containing with SS agar (Merck, 1.07667) and incubated again in 37

°C for 24 h (Andrews and Hammack, 2007). *Aeromonas spp.* were enumerated and isolated by plating on Dextrin-Ampicilin Agar (ampicilin 10 mg.L⁻¹) and incubated at 30 °C for 48 hours. Colonies were subjected to test of motile, Gram stain and biochemical tests of cytochrome oxidase, D-glucose fermentation, arginine dihydrolase, and ornithine decarboxylase, ONPG, H₂S from cysteine, acetoin from glucose, gas from glucose, L-arabinose utilization and fermentation of salicin (FDA, 2001).

2.4. Sensory analysis

Sensory evaluation was performed by 15 trained panelists. They were required to evaluate the raw fillets based on the color, flavor, texture (from firm to soft) and overall acceptability using a 7-point hedonic scale. Scores below 4 points were considered unacceptable according to Ruiz-Capillas and Moral (2001).

2.5. Statistical analysis:

Triplicate samples ($n = 3$) were analyzed for each property. The results were expressed in terms of mean and Stander Error (SE) of mean. Statistical analysis (ANOVA) was applied to the data followed by Duncan's Multiple Range Test (Duncan, 1955) using SPSS software. Differences between means were determined by the least significant difference test, and significance was defined at $P < 0.05$.

3. RESULTS

Table 1: The mean values of changes in TVB-N (mg/100 g) values of Tilapia fillets during storage at 2 C°.

| Time of storage (days) | TVB-N (mg/100 g) | | | |
|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | G1 | G2 | G3 | G4 |
| 1 | 15.00±0.2 ^{Aa} | 14.07±0.4 ^{Aa} | 14.07±0.6 ^{Aa} | 13.13±0.5 ^{Aa} |
| 7 | 14.30±0.3 ^{Aa} | 12.90±0.5 ^{Aa} | 12.27±0.3 ^{Aa} | 13.16±0.7 ^{Aa} |
| 14 | 15.00±0.5 ^{Aa} | 14.07±0.2 ^{Aa} | 13.37±0.1 ^{Aa} | 14.07±0.3 ^{Aa} |
| 21 | 19.90±0.7 ^{Aa} | 15.93±0.3 ^{Aa} | 18.97±0.2 ^{Aa} | 15.23±0.5 ^{Aa} |

^{AB} Values followed by different capital letter within the same column are significantly different ($P < 0.05$).

^{ab} Values followed by different small letter within the same row are significantly different ($P < 0.05$).

Table 2: The mean values of changes in TBARS values of Tilapia fillets during storage at 2 C°.

| Time of storage (days) | TBA mg Malonaldehyde/Kg | | | |
|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | G1 | G2 | G3 | G4 |
| 1 | 0.79±0.06 ^{Aa} | 0.84±0.10 ^{Aa} | 0.57±0.07 ^{Aa} | 0.04±0.08 ^{Aa} |
| 7 | 3.00±0.04 ^{Bb} | 1.58±0.08 ^{Aa} | 1.36±0.03 ^{Aa} | 0.78±0.05 ^{Aa} |
| 14 | 6.16±0.03 ^{Cc} | 2.38±0.05 ^{Bb} | 2.22±0.08 ^{Bb} | 0.79±0.03 ^{Aa} |
| 21 | 9.23±0.06 ^{Dc} | 2.56±0.03 ^{Bb} | 8.07±0.01 ^{Cc} | 0.98±0.01 ^{Aa} |

Table 3: The mean values of changes in pH values of Tilapia fillets during storage at 2 C° :

| Time of storage (days) | pH value | | | |
|------------------------|------------------------|------------------------|------------------------|------------------------|
| | G1 | G2 | G3 | G4 |
| 1 | 6.5±0.02 ^{Aa} | 6.4±0.01 ^{Aa} | 6.2±0.01 ^{Aa} | 5.9±0.04 ^{Ba} |
| 7 | 6.4±0.01 ^{Aa} | 6.4±0.02 ^{Aa} | 6.1±0.04 ^{Ba} | 6.2±0.01 ^{Aa} |
| 14 | 6.6±0.03 ^{Aa} | 6.5±0.02 ^{Aa} | 6.2±0.02 ^{Ba} | 6.1±0.01 ^{Ba} |
| 21 | 6.6±0.01 ^{Aa} | 6.5±0.01 ^{Aa} | 6.3±0.01 ^{Ba} | 6.2±0.02 ^{Ba} |

Table 4: Mean scores of sensory characteristics of Tilapia fillets during storage at 2 C°

| Time of storage (days) | Sensory Scores | | | |
|------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| | G1 | G2 | G3 | G4 |
| Color | | | | |
| 1 | 6.60±0.21 ^{Aa} | 6.40±0.22 ^{Aa} | 6.40±0.1 ^{Aa} | 6.30±0.11 ^{Aa} |
| 7 | 6.20±0.13 ^{Aa} | 6.10±0.12 ^{Aa} | 5.70±0.14 ^{Bb} | 6.00±0.16 ^{Aa} |
| 14 | 4.00±0.19 ^{Bb} | 5.50±0.18 ^{Ba} | 4.50±0.21 ^{Bb} | 5.80±0.12 ^{Ab} |
| 21 | 2.90±0.26 ^{Cc} | 3.80±0.16 ^{Cb} | 3.9±0.15 ^{Cb} | 5.50±0.14 ^{Aa} |
| Odor | | | | |
| 1 | 6.40±0.12 ^{Aa} | 6.60±0.11 ^{Aa} | 6.50±0.15 ^{Aa} | 6.10±0.16 ^{Aa} |
| 7 | 4.80±0.22 ^{Bb} | 6.10±0.13 ^{Aa} | 5.00±0.14 ^{Ab} | 6.00±0.21 ^{Aa} |
| 14 | 3.70±0.24 ^{Bc} | 5.40±0.21 ^{Bb} | 5.10±0.12 ^{Bb} | 5.70±0.14 ^{Bb} |
| 21 | 2.80±0.12 ^{Cd} | 3.90±0.16 ^{Cc} | 3.7±0.11 ^{Cc} | 5.30±0.12 ^{Bb} |
| Texture | | | | |
| 1 | 6.80±0.16 ^{Aa} | 6.70±0.14 ^{Aa} | 6.50±0.11 ^{Aa} | 6.70±0.12 ^{Aa} |
| 7 | 6.20±0.11 ^{Aa} | 6.30±0.12 ^{Aa} | 6.00±0.16 ^{Aa} | 6.60±0.11 ^{Aa} |
| 14 | 5.40±0.13 ^{Bb} | 6.10±0.15 ^{Aa} | 5.50±0.18 ^{Bb} | 6.30±0.21 ^{Aa} |
| 21 | 4.30±0.12 ^{Cc} | 5.30±0.16 ^{Bb} | 5.40±0.21 ^{Bb} | 6.10±0.17 ^{Aa} |
| Overall Acceptability | | | | |
| 1 | 6.70±0.11 ^{Aa} | 6.60±0.14 ^{Aa} | 6.20±0.15 ^{Aa} | 6.60±0.13 ^{Aa} |
| 7 | 5.70±0.14 ^{Bb} | 6.20±0.12 ^{Aa} | 6.10±0.22 ^{Aa} | 6.30±0.12 ^{Aa} |
| 14 | 4.10±0.16 ^{Cc} | 4.90±0.13 ^{Bb} | 5.30±0.17 ^{Bb} | 6.10±0.15 ^{Aa} |
| 21 | 3.80±0.16 ^{Cd} | 4.30±0.12 ^{Cb} | 3.20±0.16 ^{Cc} | 5.90±0.12 ^{Bb} |

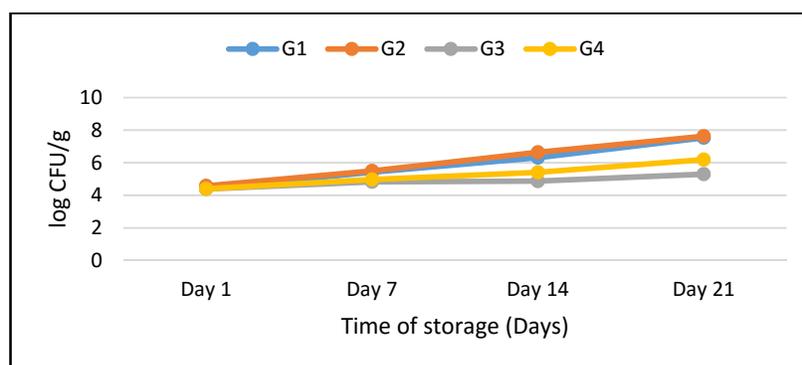


Figure (1): Total viable microbial count in tilapia fillets stored at 2 C°

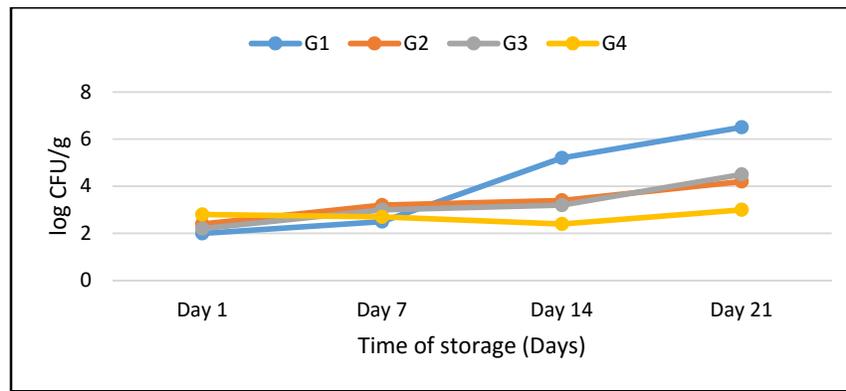


Figure (2): Psychrotrophic bacterial count (log CFU/g) in tilapia fillets stored at 2 °C

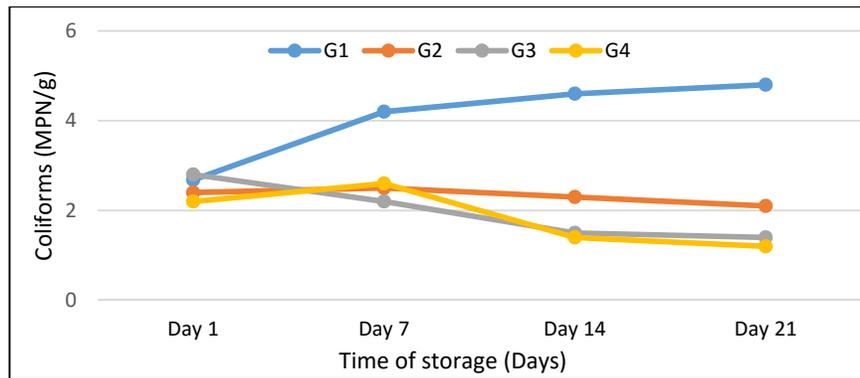


Figure (3): Total coliform counts (MPN/g) in tilapia fillets stored at 2 °C

4. DISCUSSION

TVB-N value is a quality index for unprocessed fishery products indicative of fish spoilage because of metabolic activity of fish spoilage bacteria and endogenous enzymes action (Connell, 1990). A level of 35 mg/100 g has been considered as an upper limit above which fishery products are considered unfit for human consumption (Ludorff and Meyer, 1973; Schormüller, 1968).

In our study, the TVB-N values for all the treatments were below the unaccepted limit and they were not significantly ($p>0.05$) affected by the treatments and storage periods (Table 1). High initial values followed by decreases were observed for some treatments. The average TVB-N value in (G1), (G2), (G3) and (G4) fillet samples were 15.00 ± 0.2 , 14.07 ± 0.4 , 14.07 ± 0.6 and 13.13 ± 0.5 mg N/100 g on day 1, respectively. The average TVB-N level in (G1), (G2), (G3) and (G4) fillet samples were 19.90 ± 0.7 , 15.93 ± 0.3 , 18.97 ± 0.2 and 15.23 ± 0.5 mg N/100 g on day 21 of storage at 2°C, respectively. These findings were in agreement with those reported by Dokuzlu (1997), Aksu et al. (1997), Cascado et al. (2005) and Olgunoğlu (2007).

This dynamic change in TVN level could be related to the growth of microorganisms as proliferation of the microflora contributing to spoilage changes as seen by increased TVN level. This correlation is in agreement with the findings of Balamatsia et al. (2007), which firstly reported that trimethylamine (TMA-N) and total volatile nitrogen (TVN) could be employed as potential chemical indicators in monitoring the microbial quality of fresh meat during chill storage under aerobic and modified atmosphere packaging (MAP) conditions. Banks et al. (1980) indicated that the differences in TVB-N amounts must have been caused by a smaller number of bacteria their ability to act on the oxidative desamination of non-protein nitrogen compounds. A second explanation refers to the anaerobic conditions found in the CO₂ MAP, as they may inhibit this reaction due to the lack of atmospheric oxygen. Rokka et al. (2004) showed a clear relationship between the microbiological quality of meat (protein-based) and the total amount of Total Volatile nitrogen (TVN) and biogenic amines.

The TBARS value was widely used for measuring lipid oxidation in fish and fish products (Yanar et al., 2006). Fish lipid typically contained high percentage

of polyunsaturated fatty acids and was consequently prone to oxidation (Huss, 1995).

In perfect quality material, TBA value should be less than 3 mg malonaldehyde/kg and, in good quality material; TBA value should not be more than 5 mg malonaldehyde/kg. Consumption limits are from 7-8 mg malonaldehyde/kg (Schormüller, 1968). Nonetheless, Tarladgis et al. (1960) reported that rancidity was occurred when TBA value exceeded to 4 mg malonaldehyde/kg. The TBARS value was increased to critical values indicating incipient spoilage of these fillet samples after different periods of chilling storage. Storage time was a significant factor for TBA value increase regardless of the packaging effect.

The achieved data in Table (2) showed the TBARS amounts were significantly ($p < 0.05$) affected by the treatments and storage periods, with interactions being observed between these factors. TBARS values of the examined (G1), (G2), (G3), and (G4) fillet samples were 0.79 ± 0.06 , 0.84 ± 0.10 , 0.57 ± 0.07 and 0.04 ± 0.08 on day 1 of storage at 2°C , respectively. TBARS values of the examined (G1), (G2), (G3), and (G4) fillet samples were 9.23 ± 0.06 , 2.56 ± 0.03 , 8.07 ± 0.02 and 0.98 ± 0.01 on day 21 of storage at 2°C , respectively. Rancidity started being sensorially detected by tasters on the 7th day of storage in (G1). The TBARS values observed for (G4) were lower at the end of the storage period, when compared to the values obtained for the other treatments. This was probably caused by the absence of O_2 , which retarded the oxidative process of the polyunsaturated fatty acids in this treatment. O_2 reacts with the fatty acids to produce hydroperoxide without degrading the odoriferous components (Church, 1998).

Generally, the natural pH of live fish is just above 7.0, typically about 7.3, but this value falls markedly after death as the fish goes through rigor mortis and glycogen is converted to lactic acid. In most species, the post mortem pH is between 6.0 and 6.8. Differences among the initial pH values may be due to the species, diet, and season, level of stress during the catch as well as type of muscle (Hernandez et al., 2009).

The achieved data in Table (3) showed the pH values were significantly affected ($p < 0.05$) by the treatments, but remaining stable and varying very little during the storage period. Similar results of pH values at day zero of chilled storage were recorded by Sallam et al. (2007) and Zambuchini et al. (2008). pH values reported in the current study were significantly higher ($p < 0.05$) in (G1) and (G3) than (G2) samples. Higher pH values of the (G1) and (G3) samples could be attributed to the increase in

volatile bases such as ammonia produced by either microbial or muscular enzymes (Li et al., 2012). There was no significant difference between pH values of (G4) samples and (G2) samples up to 11 days of storage. However, from 14 days onward, the pH of (G4) samples reduced significantly ($p < 0.05$). pH values of (G4) fillets samples were significantly lower ($p < 0.05$) than (G1), (G2) and (G3) throughout the whole storage period. Results showed that the treatment of fish fillet in (G4) prior to refrigerated storage acts synergistically with MAP on pH of fish muscle. This is thought to be a result of the effect of both factors on microbial growth. The pH decrease observed for (G3) (6.2-6.1) and (G4) (6.2-6.1) on the 7th and 13th days of storage, respectively, was related to the decrease in psychrotrophic bacteria count, probably due to the antimicrobial action of the acetic acid and the CO_2 in the fish muscle. Debevere and Boskou (1996) reported that CO_2 diffusion in fish muscle showed a somewhat contrary effect to that of the increase in pH due to the production of TVB-N, resulting in pH stabilization.

Microbial activity is responsible for spoilage of most fresh and of several lightly preserved seafood. Possibly, for this reason, the total number of microorganisms have been used in mandatory seafood standards (Lund et al., 2000). In this respect, ICMS (1986) stated that the upper acceptability limit of total viable bacterial count in fresh fish is $7 \log_{10}$ CFU/g flesh, and $6 \log_{10}$ CFU/g is the maximum permissible limit of TVC recommended by Egyptian organization for standardization (EOS) (2005) in chilled fish. Özogul et al. (2004) mentioned that when the aerobic plate count reaches 10^6 CFU/g or mL in a food product, it is assumed to be at, or near, spoilage. Furthermore, Parallel Food Testing in the European Union (1995) stated that in a recent European study by consumers, fish was assumed "not to be in a good enough condition to be stored for long" when total plate count were 10^6 CFU/g. The changes in TVC with storage time in all groups were concluded in Figure (1).

The initial TVC ($4.42 \pm 0.03 \times 10^3$, $4.85 \pm 0.07 \times 10^2$, $4.39 \pm 0.02 \times 10^2$, $4.1 \pm 0.01 \times 10^2$ CFU/g) increased progressively ($p < 0.05$) with storage time to final values of ($7.53 \pm 0.06 \times 10^6$, $7.63 \pm 0.11 \times 10^5$, $5.30 \pm 0.09 \times 10^6$, $6.19 \pm 0.05 \times 10^3$ CFU/g) in G1, G2, G3 and G4 samples, respectively. Our result was in agreement with Fu and Labuza (1993) and Zambuchini et al. (2008). In this regard, Fu and Labuza (1993) found that the duration of refrigerated storage of fish had a significant ($P < 0.05$) effect on bacterial count,

which tended to increase as the storage duration increased.

TVC in (G4) was significantly ($p < 0.05$) lower than other groups in each occasion of examination, which indicates the high antimicrobial activities of MAP, associated with 1% acetic acid. Regarding the upper acceptability limit recommended by Egyptian organization for standardization (EOS) (2005) for total viable count in fresh fish (6 log₁₀ CFU/g flesh), it could be observed that (G1) exceeded such limit at 9th day of storage, while such limit has not exceeded by treated groups until time of spoilage. Lower TVC of sample kept under MAP indicated that CO₂ at a concentration 60% effectively inhibited the microbial growth. CO₂ commonly become more effective as antibacterial agent when its concentration is increased (Farber, 1991). It retards the microbial growth of spoilage bacteria such as *Pseudomonas* spp. and *Shewanella* spp. Thus, CO₂-enriched atmosphere has been used in the preservation for fresh fishery products. This was probably because CO₂ entered into mass action equilibrium for enzymatic decarboxylation, leading to inhibition of the metabolic activity of microbial flora as result of an extension in lag phase and a reduction in logarithmic phase of spoilage bacteria (Lalitha et al., 2005; Masniyom et al., 2011). Our result was in agreement with Özogul et al. (2004) who reported that TVC was retarded when sardine (*Sardina pilchardus*) were kept in 60% CO₂-enriched atmospheres. It has been reported that 50% CO₂ inhibited the microbial growth in chub mackerel (*Scomber colias japonicus*) during storage (Stamatis and Arkoudelos, 2007).

Psychrotrophic bacteria are very important among different bacteria causing spoilage, because they are mostly related to the changes in sensory attributes such as odor, texture and flavor and could produce different metabolic compounds such as ketones, aldehydes, volatile sulfides and biogenic amines (Safari and Yosefian, 2006). A proposed limit of psychrotrophic bacteria is 10³ to 10⁴cfu/g, which is consistent with other studies (Pons-Sanchez-Cascado et al., 2006).

Results that obtained in Figure (2) presents the psychrotrophic bacterial count (log CFU/g) during the storage period. The development of psychrotrophic bacteria increased during the storage period, with values above 10⁶ CFU/g being verified for the control (G1). Although the limits for psychrotrophic bacteria are not within the legislative scope, such high counts for this group of bacteria must contribute to the reduction in product shelf life. The increase in the psychrotrophic bacterial count for

the control is also related to the increase in pH, despite the product being sensorially accepted by the tasters, who considered the appearance to be the most important attribute. (G2) and (G3) fillet samples presented a decrease in the bacterial count on the 7th and 13th days of storage, respectively, (G4) fillet samples presenting the lowest count at the end of the storage period. Probably, this fact was a consequence of the high CO₂ concentration associated with the use of acetic acid, as both show antimicrobial action.

The values obtained for (G2) and (G4) fillet samples were similar to those found by Randell et al. (1999), when studying salmon fillets stored at 2°C under 60% CO₂/40% N₂ MAP and vacuum packs. Siliker and Wolfe (1980) observed that high CO₂ concentrations inhibited the growth of psychrotrophic microorganisms when the fish was stored under low temperature conditions, evidencing that psychrotrophic bacteria are sensitive to CO₂. Reddy et al. (1992) and Silva et al. (1993) verified that the lag phase was retarded and growth of such deteriorating bacteria reduced by CO₂. Etemadian et al. (2012) reported that tilapia fillets packaged under vacuum packaging reduced the psychrotrophic bacterial count, compared with the samples packed in air.

The changes in total coliform counts (MPN/g) with storage time in all groups were concluded in Figure (3). The National Academy of Science (1985) reported that coliform group of bacteria in fish and fishery products has been considered important in microbiological analysis on account of their significance as indicator organisms for pin pointing the unhygienic conditions during catching, handling, processing and distribution. Total coliform counts in the tilapia fillets during the storage period were not significantly affected ($p > 0.05$) by the treatments and storage period ($< 2 - 3.3 \times 10^1$). The only treatment promoting the growth of total coliforms was showed in (G1) samples ($0.3 - 1.0 \times 10^2$), while the others presented a decrease in growth, probably due to the combination of the low temperatures and use of acetic acid, as well as a low contamination level in the water.

At day 1 and 3rd day of storage, there was no significant difference in coliforms MPN between groups, while at 6th day of storage, (G1) was significantly higher ($p < 0.05$) than treated groups, as well as at 9th day, (G1) and (G2) samples were significantly higher ($p < 0.05$) than (G3) and (G4) samples in coliforms MPN. The inhibitory effect of acetic acid against coliform group of bacteria was previously reported by Harpaz et al. (2003); Hernandez et al. (2009) and Mahmoud et al. (2004).

The presence of *Salmonella* and *Aeromonas* spp. was not detected in any of the samples analyzed in the experiment. In the same way, Passy et al. (1983) and Randell et al. (1999) did not detect the presence of such bacteria in fresh water prawns (*Macrobrachium rosenbergii*), catfish (*Ictalurus punctatus*) and salmon (*Salmo salar*) during MAP storage. According to Lioutas (1988), fish from non-polluted waters are free from *Salmonella* because this bacteria is not naturally found in the fish flora and its presence in fish is mainly due to handling or contact with poorly disinfected surfaces.

There was a significant effect of storage time on the sensory qualities of fish fillets. High scores (>6.60) were given to the color of fillets on day 1 (Table 5). There were no significant differences ($p < 0.05$) for the first 3 days for (G1) samples. From the 7th day, colour score decreased significantly and to the unacceptable levels at the 11th day (<4.0 point). As for (G2) and (G4) samples, no significant difference was noticed up to 7 days of storage. Panelists rejected the fillets in terms of color at the 14th day for (G2) and at the 21st day for (G4) samples.

There were no significant changes in terms of odor for (G1) samples for the first 3 days of storage, and for (G2) and (G4) samples up to 7 days. However, when they were stored longer, an odor developed and the scores decreased significantly ($p < 0.05$). The products continued to deteriorate ultimately having what is often described as an intense and putrid odor and this could be noticed on the 11th day for (G1) samples, 14th day for (G2) samples, and 21st day for (G4) samples. Very high microbial counts were noticed at the later stage of storage days and these could be due to the production of ammonia compounds from spoilage bacteria, resulting in the unacceptable odor.

Similar trends were also observed in texture and overall acceptability of fish fillets. Higher scores were given to all treatments in the first few days of storage and when stored longer, scores given were subsequently lower. Samples of (G1) showed the most marked changes. Fillets became tenderer, less succulent, less firm, less springy, less fibrous, stale, and dull in appearance and produced unpleasant odor. These changes may have resulted from the effect of increasing pH on protein structure (Love et al., 1979) or from bacterial proteolysis (Shewan, 1974).

Generally, (G1) fish samples were rejected by sensory evaluation at 7 days of storage, 14 days for (G2) samples, and up to 21 days for (G4) samples.

According to the sensory evaluation results, (G3) fillets samples that underwent pretreatment with acetic acid were considered unsuitable for consumption from the 7th day of storage onwards, receiving scores below five for the attributes color, odor and appearance.

5. CONCLUSIONS

As conclusion, a delay in chemical, microbiological and sensorial alterations was observed when acetic acid was combined with MAP. Chemical and microbiological levels were significantly lower when MAP fillets samples had been previously immersed in acetic acid. chemical treatment by immersion in 1% acetic acid had significantly increased the time of storage as MAP stored samples were rejected due to the development of off-odors. Control samples had a shelf life of 7 days, 18 days for MAP samples, and 21 days for MAP associated with immersion in 1% acetic acid samples.

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