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EFFECT OF PACKAGING METHOD AND USE OF ACETIC ACID ON THE SHELF LIFE OF FISH DURING REFRIGERATION STORAGE.

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Received: 27 August 2023; **Accepted:** 10 September 2023

ABSTRACT

This experiment was conducted to evaluate the efficacy of application of acetic acid 1%, non-vacuum packaging and vacuum packaging on the shelf-life of tilapia fish filets at refrigerated temperature (2±1°C) during 21 days of storage period. In this regard, the current study investigated the quality attributes including sensory, TBA, TVB-N, pH and microbial loads of examined fish. Vacuum packing and acetic acid were found to possess potent antibacterial effects on a variety of microorganisms. The fish were divided into four groups after being gutted and filleted: Group A (GA): fish fillets were vacuum packaged in Polyamide/ Polyethylene (PA/PE) bags. Group B (GB): the fillets were stored in non-vacuum containers. Group C (GC): the fillets underwent chemical treatment by immersion in 1% acetic acid for 2 minutes at room temperature, drained for two minutes then vacuum packaged in Polyamide/ Polyethylene (PA/PE) bags. Group D (GD): the fillets treated as in GC stored in non-vacuum containers. All groups were stored at 2±1°C, 80% CO_2 and 20% N_2 for 21 days. The experiment was repeated in triplicate. The sensory analysis of fish fillets revealed that samples in group C which were treated with acetic acid 1% in combination with vacuum packaging during storage for 21 days at 2±1°C had the best sensory properties and the best shelf life, and a low pH value reduced or even limited microbial levels, and ensured the safety of different microbial counts as well as reduction of TBA and TVB-N in acetic acid-treated groups compared with other groups. Together with the sensory characteristics, the low or limited microbial counts (total viable count, total psychrotrophic count, and total coliform counts) suggested that autolysis or other causes other than microbial activity may be to blame for the deterioration. Meanwhile, the shelf-life of fish treated with vacuum packaging had a longer shelf-life than that treated with non-vacuum packaging. Overall, the research underscores the need for more research and development to produce seafood that is stable and safe against microbes and has a long shelf life in order to meet customer demand.

***Keywords**:* Fish; Shelf life, Packaging; Acetic acid; Aerobic bacterial count.

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INTRODUCTION

Seafoods play a significant role in the human diet, mainly because of their high (nutritive value) and quality, given their abundance in proteins, vitamins, minerals, and omega-3 polyunsaturated fatty acids. Also, a number of marine products are directly linked to nutritional quality and improvement of human health (Tacon and Metian 2018; Jayasekara *et al.*, 2020). Modern dietary trends over the past two decades have driven great attention to the aquaculture industry, which is considered now one of the main columns to global trade, to respond to an incredible rise in demand for fish and fish products on a global scale and in order to meet market needs (FAO 2020). Since the proliferation of microorganisms quickly changes the odor, flavor, color, and texture of fish products, quality losses of fish meat result, making fish products highly perishable food (Tavares *et al.,* 2021; Walayat *et al.,* 2023). A food product's shelf life is determined by how long it maintains the necessary microbiological, physicochemical, and sensory properties to be of high quality and stay safe for consumption (Awulachew 2021).

Several variables, such as storage temperature, time, species, and post-mortem stress, have an impact on the fish's shelf life (Mahmud *et al.,* 2018). Numerous alternatives have been proposed by scientists, including new advancements in conventional fish storage techniques (Nagarajarao 2016). Therefore, it is urgently necessary to create new, useful as well as novel treatments to increase fish's shelf life and to improve its general (characteristics). Therefore, fish can now be kept fresh for a longer time because of the powerful antibacterial (properties) of organic acids as in the case of using acetic acid (Islam *et al.,* 2019).

Therefore, our goal of this study was to assess the efficacy of acetic acid and different packaging methods (vacuum and non-vacuum packaging) to extend the shelf life during refrigeration storage of fish. A variety of techniques, including chemical, microbiological, and sensory examination, were used to evaluate quality features.

MATERIALS AND METHODS

1. Samples collection and processing:

Two kilograms of freshly caught tilapia fish were procured from one market in Beheira Governorate, Egypt, and transferred to the fish processing laboratory at Animal Health Research Institute in Damanhur City in an iced insulated box. They were processed immediately under hygienic and sanitary conditions to prevent any further contamination. The fish were separated into four groups after being gutted and filleted. Group A (GA): For storage at 2±1ºC for 21 days, the fillets were vacuum-sealed in Polyamide/ Polyethylene (PA/PE) bags. The fillets kept in non-vacuum containers are in Group B (GB). Group C (GC): For a chemical treatment, the fillets were submerged in 1% acetic acid for two minutes at room temperature. The fillets were vacuum-wrapped in Polyamide/ Polyethylene (PA/PE) bags and stored after being placed on racks to drain the solution for two minutes. As in Group C (GC), the fillets in Group D (GD) received chemical treatment by being submerged in 1% acetic acid before being kept in non-vacuum containers. The following storage parameters were used: a temperature of 2±1ºC, an 80% gaseous CO_2 content, and a 20% N_2 concentration for 21 days. In triplicate, the test was conducted again. On days 7, 14, and 21, the analysis listed below was performed on all preceding groups (Thabet *et al.*, 2016).

2. Physico-chemical examination:

2.1. Sensory analysis:

Fifteen qualified panelists carried out the sensory analysis. They were instructed to use a 7-point hedonic scale to assess the uncooked fillets' appearance, flavor, aroma, texture (from firm to soft), and overall acceptability. Ruiz-Capillas and Moral (2001) deemed scores of less than 4 to be undesirable.

2.2. Hydrogen ion concentration (pH) measurement:

The pH value was obtained using an electrical pH meter (Bye model 6020, USA) in accordance with (AOAC, 2000). Two buffer solutions with known pH values were used to calibrate pH meters. When the temperature control system was changed, neutralized water was used to clean the pH electrode before it was put to the homogenate.

2.3. Total volatile basic nitrogen measurement (TVBN):

The procedure for calculating total volatile nitrogen (TVBN) in fish meat was validated according to the Food and Agriculture Organization (AOAC, 2000).

2.4. Thiobarbituric acid reactive substances measurement (TBARS) was performed according to (AOAC, 2000):

Homogenize (20g) sample for 2 minutes after adding 100 ml of a 7.5% trichloroacetic acid solution. A filter was applied to the homogenate. After filtration, 5 ml of the filtrate was combined with 5 ml of TBA reagent (0.02M TBA) in a test tube with a screw cover. The test tubes were submerged in water for 40 minutes, and then the absorbance at 538 nm was measured using a spectrophotometer. Malon-aldehyde(MAD) milligrams per kilogram s of fish flesh were used to calculate the value of TBARs.

3. Microbial analysis:

The microbiological analysis of fish fillets was aimed to determine both total viable aerobic bacteria and total psychrotrophic count according to APHA (2001) as well as, total coliform count by most probable number (MPN) (ISO 2007).

4. Statistical analysis:

Using SPSS Version 25 (SPSS Inc. Chicago, IL, USA), analysis of one-way variance (ANOVA) was carried out on the collected data. Duncan's multiple-range tests were used in a statistical model to compare the means of the treatments. Significant differences were detected at $p<0.05$.

RESULTS

Table 1: Mean±SD (log10cfu/g) scores of sensory characteristics of fish fillets during storage at 2±1°C.

storage (days)	Sensory Scores			
	GA vacuum packaging	GB non-vacuum packaging	GC 1% acetic acid vacuum packaging	GD 1% acetic acid non-vacuum packaging
		Color		
0	6.50 ±0.15a	6.48±0.11a	6.49±0.22a	6.45±0.31a
7	6.30±0.12a	6.25±0.11a	6.35±0.22b	5.90±0.14a
14	4.20±0.12b	4.10±0.15a	4.50±0.33b	4.00±.0.11b
21	2.80±0.13c	2.70±0.15b	3.80±0.13b	3.10±.0.11a
		Odor		
0	6.50±0.13a	6.49±0.12a	6.48±0.17a	6.10±0.18a
7	4.80±0.21b	4.30±0.12a	5.10±0.15b	5.00±0.22a
14	3.70±0.25c	3.40±0.23b	5.00±0.13b	4.70±0.15b
21	2.90±0.14d	2.70±0.15c	3.70±0.12c	3.30±0.11b
		Texture		
0	6.90±0.15a	6.80±0.13a	6.70±0.12a	6.60±0.13a
7	6.25±0.12a	6.30±0.15a	6.20±0.15a	6.50±0.11a
14	5.50±0.15b	6.00±0.11a	5.60±0.15b	5.20±0.11a
21	4.20±0.11c	4.30±0.16b	5.40±0.25b	5.10±0.17a
		Overall Acceptability		
0	6.65±0.12a	6.60±0.11a	6.55±0.12a	6.50±0.11a
7	5.70±0.11b	5.20±0.11a	6.30±0.21a	6.10±0.13a
14	4.10±0.14c	4.90±0.12b	5.30±0.15b	5.10±0.14a
21	3.80±0.15d	3.30±0.13b	4.20±0.11c	4.10±0.112b

Values followed by different small letters within the same column are significantly different (P<0.05). The values represent Mean ± SD of three experiments.

Table 2: The mean±SD values of pH of Fish fillets during storage at 2±1°C.

storage (days)	pH values			
	GA vacuum packaging	GB non-vacuum packaging	GC 1% acetic acid vacuum packaging	GD 1% acetic acid non-vacuum packaging
0	6.4±0.03a	6.4±0.01a	5.8±0.05a	6.1±0.02a
7	6.5±0.02a	6.4±0.05a	6.1±0.02a	6.2±0.01c
14	6.6±0.01b	6.5±0.01a	6.2±0.01a	6.2±0.06a
21	6.7±0.03a	6.5±0.04a	6.2±0.07a	6.3±0.01a

Values followed by different small letter within the same column are significantly different (P<0.05).

Table 3: The mean±SD values of TBARS of Fish fillets during storage at 2±1°C.

Storage (days)	TBA mg /kg MAD)			
	GA vacuum packaging	GB non-vacuum packaging	GC 1% acetic acid vacuum packaging	GD 1% acetic acid non-vacuum packaging
0	0.39±0.05a	0.40±0.04a	0.37±0.07a	0.38±0.05a
7	0.75±0.02b	0.97±0.05a	0.41±0.03a	0.49±0.02a
14	1.02±0.05a	1.45±0.02b	0.46±0.08b	0.57±0.04a
21	1.35±0.07a	2.01±0.05b	0.52±0.02c	0.76±0.03a

Values followed by different small letter within the same column are significantly different (P<0.05).

Table 4: The mean±SD values of TVB-N (mg/100 g) of Fish fillets during storage at 2±1°C.

Sstorage (days)	TVB-N (mg/100 g)			
	GA vacuum packaging	GB non-vacuum packaging	GC 1% acetic acid vacuum packaging	GD 1% acetic acid Non-vacuum packaging
0	14.03±0.1a	15.05±0.3a	12.07±0.6a	13.13±0.5a
7	15.80±0.2a	16.40±0.1a	12.87±0.2a	13.96±0.5a
14	16.50±0.4a	18.57±0.4a	13.05±0.5b	14.85±0.2a
21	18.70±0.6a	19.62±0.1a	14.52±0.2a	15.83±0.5a

Values followed by different small letter within the same column are significantly different (P<0.05).

Table 5: Total viable microbial count (Mean±SD) (TVC) in Fish fillets stored at 2±1°C.

Storage (days)	Total viable microbial count (TVC) log cfu/g			
	GA vacuum packaging	GB non vacuum packaging	GC 1% acetic acid vacuum packaging	GD 1% acetic acid non vacuum packaging
0	3.51±0.02a	4.85±0.07a	2.29±0.02a	3.2±0.01a
7	4.35±0.05a	5.32±0.02c	3.12±0.04a	4.62±0.07a
14	5.27±0.07b	7.42±0.03b	4.82±0.05a	5.42±0.01a
21	6.53 ± 0.06a	8.63± 0.11a	5.10 ± 0.09d	6.14 ± 0.05a

Values followed by different small letters within the same column are significantly different (P<0.05).

Table 6: Total psychrotrophic count (log_{10} CFU/g mean±SD) in Fish fillets stored at 2±1°C.

Storage (days)	Total psychrotrophic bacterial count (log CFU/g)			
	GA vacuum packaging	GB non-vacuum packaging	GC 1% acetic acid vacuum packaging	GD 1% acetic acid non-vacuum packaging
0	1.30±0.01a	1.41±0.02b	1.01±0.01c	1.23±0.03a
7	2.95±0.05a	3.51±0.03a	1.03±0.03a	2.07±0.02a
14	3.25±0.02a	5.34±0.02a	2.31±0.09d	3.32±0.02a
21	3.42 ± 0.04a	4.25± 0.01a	1.05 ± 0.01a	3.38 ± 0.06a

Values followed by different small letter within the same column are significantly different (P<0.05).

Table 7: Total coliform count (MPN log_{10} CFU/g) in Fish fillets stored at 2±1ºC.

Storage (days)	Total coliform count (log CFU/g)			
	GA vacuum packaging	GB non-vacuum packaging	GC 1% acetic acid vacuum packaging	GD 1% acetic acid non-vacuum packaging
0	1.24±0.02a	1.35±0.04c	1.00±0.01d	1.12±0.02a
7	1.30±0.01a	2.41±0.01a	1.24±0.04a	1.78±0.01a
14	1.45±0.03b	2.57±0.03a	1.33±0.06b	2.32±0.02a
21	2.75 ± 0.05a	5.51± 0.02a	1.75±0.09a	1.85 ± 0.03a

Values followed by different small letters within the same column are significantly different (P<0.05).

DISCUSSION

Fish is a popular and healthy food item, but keeping it fresh is still difficult due to its perishability (Prabhakar *et al.,* 2020). This food must be kept chilled or frozen, but even then it has a very short shelf life (Tavares *et al.,* 2021; Xiaobao Nie *et al.,* 2022). Typically, the bacteria content of fresh or frozen fillets is determined using the Total Viable Count (TVC) as well as chemical components like Total Volatile Base Nitrogen (TVB-N) and Thiobarbituric Acid (TBA).

1. Sensory evaluation:

One of the most important statistical methods for determining the precise quality and customer acceptance of a particular food or food product is the sensory evaluation of food. The sensory features of the treated tilapia fish fillets are significantly different from those of the untreated fish samples, and they exhibit better sensory qualities. Since the sensory parameters are considered the consumer's primary judging factors for products and the obvious aspects of their visual sense, texture, color, and odor are crucially important (Lazo *et al.,* 2017). According to the Egyptian standard of the Egyptian Organization for Standardization (EOS No. 3494 / 2020) the sensory evaluation of chilled fish fillets should retain the natural sensory characteristics of the species as a result of no change in its chemical or microbiological properties according to the permissible limits.

1.1. Color

According to a study on the color variations in food product quality, the alterations are caused by the properties of protein, fat, and other significant biomolecules being downcast due to water activity and microbial invasion (Masniyom 2011). However, it was discovered that the colour qualities were deteriorating as the storage days went by, regardless of the formulation of various treatments. During the storage trials, there were significant differences ($p < 0.05$) among days of storage in color parameters a* (redness–greenness), and b* (blueness–yellowness) (Table 1). Throughout a 21-day period of storage at a chilled temperature of 2°C, the color of the treated and untreated fish was assessed every seven days. Compared with GA, GB and GD fish fillet samples results, it was found that GC samples (1% acetic acid and vacuum packaging) largely had preserved the color properties.

1.2. Odor

Notably, up to 21 days of research, the sample handled with vacuum packaging, with or without acetic acid, usually preserved the natural odor. When compared with other samples that were kept untreated or treated with vacuum packaging, the GC (1% acetic acid and vacuum packaging) largely preserved the odor properties. However, prior to the day 21 period of refrigerated storage, unfavorable odor characteristics were noticed in the GA and GB samples (Table 1). Rancidity of the fat or putrefaction of the protein are the causes of the unpleasant odour (Emborg *et al.*, 2005). The natural odour of fish quickly degrades due to microbial invasion, which begins shortly after the postmortem.

1.3. Texture

Remarkably, involving regard to 21 days of (storage), the samples handled with vacuum packaging, with or without acetic acid, usually preserved the natural texture. When compared with other samples that were kept untreated or treated with vacuum packaging. The GC (1% acetic acid and vacuum packaging) largely preserved the texture properties. However, prior to the day 21 period of refrigerated storage, unfavorable texture characteristics were noticed in the GA and GB samples. When the texture sensory score was below 4, it was deemed to be outside of the permissible range (Arfat *et al.,* 2015); when it was below 4, it was asserted that the texture was of bad quality. According to Sankar *et al.* (2008), a variety of microorganisms, predominantly from bacterial species, which disrupt the structure of fish protein, are to blame for the soft texture that resulted from the texture quality degrading.

1.4. Overall acceptability

As for the overall acceptability, GC group samples (1% acetic acid with vacuum packaging) showed the highest acceptability up to 21 days, when compared with untreated (GA) as well as treated group samples (GB & GD)

2. Physicochemical quality criteria:

To ensure microbiological stability/safety and increase shelf life, minimally processed foods must have certain physicochemical properties while stored at cooling temperatures, especially when there is less oxygen present. (Prabhakar *et al.,* 2020).

2.1. pH:

The pH value of food is regarded as an essential factor since it controls numerous functions and reactions, which has an impact on the fish quality and shelf durability. According to the Egyptian Organization for Standardization (EOS 3494/2020) the pH of a chilled fish should not exceed 6.5. The achieved data in Table (2) showed the pH values were significantly affected ($p< 0.05$). In the present study, a gradual increase in pH values with storage time from 6.4±0.03Aa, 6.4±0.01Aa, 5.8±0.05Ba and 6.1±0.02Aa at day zero to reach 6.7±0.03Aa, 6.5±0.04Aa, 6.2±0.07Ba and 6.3±0.01Ba at day 21 of refrigeration storage in GA, GB, GC, and GD, respectively. The data also showed that samples treated with 1% acetic acid and vacuum packed (GC) scored the lowest pH value, which was advantageous and efficacious in inhibiting microbial growth and keeping the product fresh for a longer time. In a related study, Fan *et al.* (2009) found that a rise in the pH of silver carp fish fillets was related to the generation of volatile bases as a result of the breakdown of protein by microbial or endogenous enzymes into ammonia and trimethylamine.

2.2. Thiobarbituric acid:

The TBARS value is used widely used for measuring lipid oxidation in fish and fish products (Yanar *et al.,* 2006). After varying times of cold storage, the TBARS value was within the normal levels. All the examined samples were accepted at day zero, TBARS value of all samples was found between 0.37 and 0.40 mg malonaldehyde/kg muscle. TBARS value of all examined samples increased when the storage time increased ($P < 0.05$) (Table 3). The average TBARS value in (GA), (GB), (GC) and (GD) fillet samples were elevated to record 1.35±0.7a, 2.01±0.05b, 0.52±0.2c and0.76±0.03a mg malonaldehyde/kg muscle on day 21 of storage at 2±1^{o}C in the aforementioned groups, respectively. Regarding to present study results, the mean values of TBARS in the examined samples were lower than the maximum permissible limit recommended according to the Egyptian standard of the Egyptian Organization for Standardization (EOS, No. 3494 / 2020), that reported the TBARS value of a cold fish fillets should not exceed 4.6. mg malonaldehyde/kg in fish muscle. Fish that have partially dehydrated and interacting lipids with oxygen in the air may be the cause of the increase in TBARS during storage (Rezaei *et al.,* 2008). There was a significant difference between the studied groups. This result suggested that oxidation of lipids in fish samples could be minimized by using acetic acid with vacuum packaging (GC) which was considered the best group followed by the use of acetic acid with non-vacuum packaging (GD) probably due to the antioxidant activity as well as its low oxygen permeability characteristic of acetic acid and the vacuum packaging effect.

2.3. Total volatile basic nitrogen:

TVB-N value is an indicator of fish spoilage in unprocessed fisheries products due to the metabolic activity of fish spoilage bacteria and the action of endogenous enzymes (Prabhakar *et al.*, 2021). In the present study, the TVB-N values for all the treatments were below the unaccepted limit and they were significantly ($P<0.05$) affected by the treatments and storage periods (Table 4). The average TVB-N values in (GA), (GB), (GC) and (GD) fillet samples were 14.03±0.1a, 15.05±0.3a, 12.07±0.6a and 13.13±0.5a mg N/100 g on day zero, respectively. Such levels are elevated to record 18.70±0.6a, 19.62±0.1a, 14.52±0.2a and15.83±0.5a mg /100 g on day 21 of storage at 2±1ºC in the aforementioned groups, respectively. Referring to the present study results, the mean values of TVB-N in the examined samples were lower than the maximum permissible limit recommended according to the Egyptian standard of the Egyptian Organization for Standardization (EOS, No. 3494 / 2020) that reported the TVB-N value of a cold fish fillets should not exceed 30 mg N/100 g in fish muscle. (Must not exceed 25mg / 100 mg of fish meat for Sebastes spp.,35 mg for Salmo salar and 30 mg for other types of fish). The obtained data revealed that GC (1% acetic acid and vacuum packaging) is considered the best group that minimizes TVB-N levels than other groups. These results concurred with those made public by Cascado *et al.* (2005) and Olgunolu (2007). The development of bacteria as well as the multiplication of the microflora that contribute to spoiling alterations as shown by an elevated TVB-N level might be attributed to this dynamic shift in TVB-N level. This correlation is consistent with the research of Balamatsia *et al.* (2007), who first reported that trimethylamine (TMA-N) and total volatile nitrogen (TVN) could be used as potential chemical indicators in monitoring the microbial quality of fresh fish meat during chill storage under aerobic and modified atmosphere packaging (MAP) conditions. According to Banks *et al.* (1980), the variations in TVB-N levels must have been brought about by fewer bacteria because of their capacity to affect the oxidative deamination of non-protein nitrogen molecules. The absence of ambient oxygen in the CO_2 MAP may prevent this process from occurring, which is referred to in a second explanation as anaerobic circumstances. The total quantity of volatile nitrogen (TVN) and biogenic amines were shown to have a direct correlation with the microbiological quality of beef (protein-based) by Rokka *et al.* (2004).

2.4. Microbiological analysis:

2.4.1. Total viable microbial count (TVC)

TVC is an indicator of product degradation, which in fish is primarily due to the growth of specific spoilage organisms (Santos *et al.*, 2013). According to the Egyptian standard of the Egyptian Organization for Standardization (EOS No. 3494 / 2020) the TVC of cold fish fillets should not exceed 1×10^6 cfu/g (6 $\log_{10}$ cfu/g) in fish muscle. The results of the total viable counts are shown in Table (5) revealing that the initial TVC value of GA was 3.51±0.02 which indicated that the fish fillets were of good quality at the beginning of the experiment having a low population of microbes and increased to 6.53 ± 0.06 at the end of storage time. While the average TVC value in (GB), (GC) and (GD) fish fillet samples were 4.85±0.07, 2.29±0.02, and 3.2±0.01, on at day zero respectively, which the count increased to 8.63±0.11, 5.10 ± 0.09, and 6.14 ± 0.05, on day 21 of storage at 2±1ºC, respectively. These results revealed that the microbial growth was delayed after the treatment with 1% acetic acid with vacuum packaging (GC) which was considered the best group that reduced microbial growth than other groups followed by that treated with 1% acetic acid with non-vacuum packaging (GD). Acetic acid is regarded as an antimicrobial agent for its decontaminating activity (Tajkarimi and Ibrahim 2011; In *et al.*, 2013). The solution designed to clean the fish's surface decreased the amount of germs present on the fish while also preventing bacterial development. According to Tajkarimi and Ibrahim (2011) and In *et al.* (2013), acetic acid decreased the pH of fish and raised their surface temperatures, both of which are considered unfavorable conditions for bacterial development. Additionally, this study's vacuum packing demonstrated a delay in microbial development. The study's findings also supported prior research by Ozogul *et al.* (2004) and Ogongo *et al.* (2015) showing fish stored in air exhibit bacterial growth rates that are higher than those of fish vacuum-packed at 0°C.

2.4.2. Total psychrotrophic bacterial count

Total psychrotrophic bacterial count is one of the important indicators of the shelf life of meat (Rubio *et al.*, 2016). The results of the total viable counts are shown in Table (6) showed the initial value of the total psychrotrophic bacterial count of GA (sterilized distilled water vacuum packaging) was 1.30±0.01, which indicates that the fish fillets were of good quality at the beginning of the experiment having a low population of microbes and increased to 3.42 ± 0.04) at the end of storage period (21 days). While the average TVC value in (GB), (GC) and (GD) fish fillet samples were 1.41±0.02, 1.01±0.01, and 1.23±0.03, on day zero, respectively and increased to 4.25± 0.01, 1.05 ± 0.01, and 3.38 ± 0.06, on day 21 of storage at 2±1ºC, respectively. These results revealed that the psychotropic bacterial growth was delayed after the treatment with 1% acetic acid vacuum packaging (GC) which is considered the best group that reduced microbial growth than other groups followed by those treated with 1% acetic acid non-vacuum packaging (GD). When fish were held at low temperatures, Silliker and Wolfe (1980) noticed that high CO2 concentrations impeded the development of psychrotrophic microbes, proving that psychrotrophic bacteria are sensitive to CO2. Reddy *et al.* (1992) and Silva *et al.* (1993) confirmed that CO2 postponed the lag phase and decreased the growth of these degrading bacteria. According to Etemadian *et al.* (2012), tilapia fillets vacuum-packed as opposed to those packaged in air had lower psychrotrophic bacterial counts.

2.4.3. Total Coliform count

Total coliform is an indication of sewage contamination, but it can also happen during other processing phases including transport and handling. Additionally, according to Sanjee and Karim (2016), the contamination might potentially be brought on by the water used for cleaning or icing. According to Egyptian standard (EOS, No. 3494 / 2020), the total coliform count of cold fish fillets should not exceed 1×10^2 cfu/g (2 $\log_{10}$ cfu/g) in fish muscle. The changes in total coliform counts (MPN/g) with storage time in all groups were concluded in Table (7). Total coliform counts in the fish fillets in the first group treated with sterilized distilled water with vacuum packaging (GA) was 1.24±0.02 on day zero and reached 2.75 ± 0.05 at the end of the storage period at day 21. While the group treated with 1% acetic acid vacuum packaging (GC) showed the best inhibitory effect of coliform from day zero (1.00±0.01) to day 21 (1.75±0.09), followed by the group treated with 1% acetic acid non-vacuum packaging (GD) from day 0 (1.12±0.02) to the end of the storage period (1.85 ± 0.03). While the non-vacuum packed (GB) group showed less inhibitory effect from the start of the study to the end of the storage period (1.35±0.04 to 5.51±0.02), respectively. Low temperatures, the application of acetic acid, and low levels of water pollution all contributed to the decrease in coliform growth. These results corroborated those of earlier research by Mahmoud *et al.* (2004) and Hernandez *et al.* (2009) that revealed acetic acid's inhibitory impact against the coliform group of bacteria. The coliform group of bacteria has been identified in fish and fisheries products, and they are thought to be significant in the microbiological conditions that exist throughout the capture, handling, processing, and distribution of these goods, according to the National Academy of Science (1985).

CONCLUSION

In conclusion, when vacuum-packaged fillet samples were initially submerged in 1% acetic acid, chemical spoilage and microbial contamination levels were noticeably reduced as well as physical and sensory properties were of good quality. The current findings suggest that treating with acetic acid in combination with vacuum packaging is an effective method for extending the shelf life of fish.

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تأثير طريقة التعبئة و حمض الخليك علي فترة صلاحية الأسماك أثناء التخزين بالتبريد

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أجريت هذه التجربة لتقييم فاعلية إضافة حمض الخليك ١٪ والتعبئة غير المفرغة والتعبئة بالتفريغ على العمر التخزيني لشرائح سمك البلطي عند درجة حرارة مبردة (٢ درجة مئوية) خلال ٢١ يوم من فترة التخزين. في هذا الصدد ، استقصت الدراسة الحالية سمات الجودة بما في ذلك الحسية، حمض الثيوباربتيورك ، المركبات النيتروجينية الطيارة، الأس الهيدروجيني والأحمال الميكروبية للأسماك المفحوصة. تم العثور على أن لحمض الخليك والتعبئة الفراغية أنشطة قوية مضادة للجراثيم ضد الكائنات الحية الدقيقة المختلفة. تم نزع أحشاء الأسماك وجلدها وشرائحها وتقسيمها إلى أربع مجموعات: المجموعة أ :(GA) تم تعبئة شرائح السمك في أكياس مفرغة من مادة البولي أميد / البولي إيثيلين .(PA / PE) المجموعة B (GB): تم تخزين الشرائح في حاويات غير مفرغة. المجموعة ج :(GC) خضعت الشرائح لمعالجة كيميائية عن طريق الغمر في حمض أسيتيك بنسبة ١٪ لمدة دقيقتين عند درجة حرارة الغرفة ، ثم تم تصريفها لمدة دقيقتين ثم تعبئتها في أكياس بولي أميد / بولي إيثيلين .(PA / PE) المجموعة D (GD): الشرائح المعالجة كما في GC المخزنة في حاويات غير مفرغة. تم تخزين جميع المجموعات عند ٢ درجة مئوية و ٨٠٪ CO2 و ٢٠٪ N2 لمدة ٢١ يومًا. التجربة أعيدت في ثلاث نسخ. أظهر التحليل الحسي لشرائح السمك أن عينات المجموعة C التي عولجت بحمض الأسيتيك بنسبة ١٪ مع عبوات مفرغة الهواء أثناء التخزين لمدة ٢١ يومًا عند ٢ درجة مئوية كانت تتمتع بأفضل الخصائص الحسية وأفضل عمر تخزين، وقيمة منخفضة للأس الهيدروجيني. خفض أو حتى الحد من المستويات الميكروبية ، وضمان سلامة تعداد الميكروبات المختلفة وكذلك تقليل حمض الثيوباربتيورك و المركبات النيتروجينية الطيارة في المجموعات المعالجة بحمض الأسيتيك مقارنة بالمجموعات الأخرى. تشير المستويات الميكروبية المنخفضة أو المحدودة (العد الكلي البكتيري، العد الكلي للميكروبات المحبة للبرودة ، والعد الكلي للميكروبات القولونيات المقترنة بالسمات الحسية إلى أن التلف قد يكون ناتجًا عن آليات أخرى مثل التحلل الذاتي بدلاً من النشاط الميكروبي. وفي الوقت نفسه ، العمر الافتراضي للأسماك المعالجة بالعبوات المفرغة من الهواء تتميز بعمر تخزين أطول من تلك المعالجة بتغليف غير مفرغ. بشكل عام ، يسلط العمل الحالي الضوء على إمكانية إجراء مزيد من البحث والتطوير لإنتاج المأكولات البحرية الآمنة والمستقرة من الناحية الميكروبيولوجية مع الأطعمة البحرية ذات العمر الافتراضي الطويل لتلبية طلبات المستهلكين المقابلة.