Enhancement of Quality Characteristics and Shelf–Life of Tilapia (Oreochromis niloticus) Fillets by Propolis

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ABSTRACT

Fish is not only susceptible to rapid spoilage but also to pathogenic infection. Therefore, addition of chemical preservatives is a must in commercial fisheries. Natural preservatives are gaining an increasing attention due to the increased concerns about the possible side effects of chemical preservatives on consumers. Thus, current study aimed to evaluate the positive potential effects of ethanolic extract of propolis on chemical, microbiological, and sensory quality parameters of market fillets of tilapia (*Oreochromis niloticus*). The samples were treated with 0 (control), 0.5, 1, and 1.5% of the ethanolic extract of propolis and stored at 4 $\pm 1^0$C. Then samples were tested for microbiological, chemical, and sensory characteristics after 0, 3, 6, 9 12, and 15 days of storage. Based on the obtained results, the addition of the propolis extract significantly extended the shelf-life of fillets up to 12 days compared to the control group that was completely spoiled after 6 days of treatment. The results suggest that using the ethanolic extract of propolis would be a suitable natural preservative, which might enhance the chemical, microbiological, and sensory characteristics and storability of refrigerated fillets of tilapia fish.

Keywords: Nile tilapia; Fillets; Ethanolic extract of propolis; Quality parameters; Shelf-life

1. INTRODUCTION

In recent years, fish and other aquatic food commodities have gained popularity among international consumers and considered as an important part of Egyptian healthy diet. Fish secure about 16 % of animal proteins consumed by the world's population, and over one billion people depend on fish as their main source of animal proteins **(FAO, 2012)**. This owing to the health benefits resulted from being easily digestibility, one of the best protein sources available to human body in quality and quantity, and richness in vitamin A, B, iodine, and oil that is source of long-chain n-3 polyunsaturated fatty acids **(Adebayo et al., 2012)**. Noticeably, fresh fish is highly perishable due to their biological composition and shelf life, which is restricted by enzymatic and microbiological spoilage under normal refrigerated storage conditions **(Ashie et al., 1996)**. Therefore, preservation and processing are critical in commercial fisheries to conserve freshness and appropriateness of fish for a long time with a minimum loss of flavor, taste, odor, nutritive value, and the digestibility **(Pal et al., 2016)**.

The increase in processed fish products have enlarged the use of chemical preservatives in modern food industry, which limits or prevent nutritional loss caused by microbiologic, enzymatic or chemical changes, and improves the shelf life **(Özdemir et al., 2012)**. There is a large debate about the safety of chemical preservation, as it might cause teratogenic and carcinogenic effects and residual toxicity. Accordingly, consumers tend to be doubtful of the chemical substances addition and have been

demanding foods that are fresh, natural and minimally processed for increased safety and quality (**Skandamis et al., 2001)**. This perspective has put stress on the food industry for the progressive elimination of chemical preservatives and use of alternative natural antimicrobials and antioxidants preservatives **(Cox et al., 2010)**.

Propolis (bee glue) is a solid substance produced by honey bees after mixing their own waxes with resinous materials collected from plants. Afterwards, bees utilize it for sterilizing the hives **(Cuesta et al., 2005)**. Traditionally, propolis has been used as a medicine, due to its antimicrobial, antifungal, antiprotozoal, and antiviral potential **(Wagh et al., 2013)**. It has different bioactive constituents such as terpenoids, flavonoids, phenolic acids, steroids, sugars, and amino acids which act as antioxidants and also consider antagonistic factors against the principal bacteria and fungi **(Tylkowski et al., 2010)**. Propolis prevents the cell division so it inhibits the bacterial growth by disorganized the cytoplasm, cytoplasmic membrane and the cell wall which cause a partial bacteriolysis and inhibited protein synthesis **(Takasi et al., 1994)**.

This remarkable combination makes propolis an excellent candidate for preserving different foods. Successful experiments have been performed for the utilization of propolis in the preservation of various products as meat and fish products **(Ôzcan 1999; Han et al., 2001; Koc et al., 2007; Silici et al., 2013; Ozturk 2014; Spinelli et al., 2014)**. Development of natural preservative with high antioxidant, antibacterial activities that prolong the shelf life of fish and fish products is desirable, so the objective of the present work was to evaluate the benefits of using the ethanolic extract of propolis as a natural preservative for tilapia fish fillets. Through the monitoring of the chemical, microbiological, and sensory characteristics fish fillets and relate it to the quality changes and shelf-life.

2. MATERIALS AND METHODS

2.1. Ethanol extract of propolis (EEP)

The crude propolis was obtained from Faculty of Agriculture, Damanhour University. Alcoholic extraction was completed using 100 g of powdered samples in 400 ml absolute ethanol (96%). The mixture was shaken for 2 hours and kept overnight at 4ºC. Samples were filtered using Whatman filter paper No.2 on a Buchner funnel. The filtrate was dried over a rotatory evaporator to evaporate the ethanol. Crude ethanolic extract was stored in brown-dark bottles in the fridge until used.

2.2. Dipping solutions

About 0.5, 1, and 1.5 g of pure extract were added to 99.5, 99, and 98.5 ml of sterile distilled water to obtain 0.5, 1 and 1.5 % propolis extract, while control groups were of 100 ml sterile distilled water.

2.3. Preparation and dipping of fish fillets

Approximately 36 samples of Nile tilapia (*Oreochromis niloticus*) each weighting 500 ±100 g were purchased from the fish market in Damanhur City, El-Beheira government, Egypt on the same day of harvesting. The samples were rapidly transferred in sterile and labeled plastic bags in a cool ice box to the postgraduate laboratory, Food Control Department, Faculty of Veterinary Medicine, Damanhur University, under complete aseptic conditions without undue delay. Upon arrival, fish samples were

washed thoroughly with tap water then the head removed, gutted and washed again to get their fillets. The samples were filleted by transversal cuts and remove the intramuscular bones to obtain about 72 slices each weighting 80 ± 5 g (5 x 7 x 1 cm). The slices were divided into 4 groups (control, 0.5, 1, and 1.5 % propolis extract), each group has 18 slices. Samples were subjected to organoleptic, chemical, and bacteriological examinations after dipping for 30 minutes in the dipping solution and immediately drained well for 5 min on a sterile stainless wire mesh screen and then examined after 0 (2 hours), 3, 6, 9, 12, and 15 days. The scheme replicates 4 times.

2.4. Packaging and storage

The previously treated fish fillets of *O. niloticus* were labeled, separately-bagged aerobically in polyethylene bags, and stored at 4±1ºC. All groups were subjected to organoleptic, chemical and bacteriological assessment at zero day (within 2 hours after treatment) then periodically every three days until decomposition.

2.5. Sensory evaluation

Twenty panelists (adult males and females) were asked to evaluate the sensory attributes of cooked fish fillets. The fillet samples were blindly–coded. The panelists were not informed about the experimental approach. The samples without salt and spices were fried using high-quality sunflower oil, then served to the panelists to complete the evaluation of the sensory attributes. The panelists were asked to wash their mouths between samples. Nine-point descriptive scale was used. A score of 7–9 indicated ‘‘very good’’, 4.0–6.9 ‘‘good’’, and 1.0–3.9 denoted as spoiled **(Amerina et al., 1965)**.

2.6. Chemical analysis

The pH values were measured using a pH meter (Digital, Jenco 609). Calibration of the pH meter was done using buffer solutions of exactly known pH standards (pH 7.01 and 4.01). Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) (mg/100g) was assayed according to the method recommended by **(EOS63/10, 2006)**. Thiobarbituric acid (TBA) number was expressed as milligrams of malondialdehyde equivalents per Kilogram of samples and was performed according to **(EOS63/9, 2006)**. Peroxide values (PV) were determined according to the methods described in **(AOAC 2000)** and expressed as milliequivalents of oxygen/kilogram of lipid.

2.7. Microbiological analysis

Preparation of samples was done according to the **(ISO 6887-6, 2013)**. Approximately, 10 g of each sample were removed by sterile scissors and forceps after surface sterilization under complete aseptic conditions. The weighted samples were transferred into homogenizer flask containing 90 ml sterile peptone water 0.1% (Oxide CM0009). The content was homogenized at 4000 rpm for 2.5 min. To provide a dilution of 10^{-1}, the original homogenate was allowed to stand for 5 min at room temperature then mixed thoroughly by shaking. One milliliter from the original homogenate was transferred to sterile test tubes containing 9 ml of sterile peptone water 0.1%. Then tenth-fold serial dilution were prepared up to 10^{-6}. Total aerobic bacterial count (TBC) were done according to the method described by **Swanson et al. (1992)** and **ISO2293 (1976**). The pour plate technique was applied on plate count agar medium. The inoculated plates were incubated in a thermostable and controlled incubator in an inverted position at 37º

C for 24 hours. Psychrophilic bacterial count (PBC) was completed according to **Swanson et al. (1992)** by pour plate technique after incubation at 4°C for 5-7 days. Staphylococcal were enumerated on Baird Parker Agar medium and incubated at 37ºC for 48 hours. Enterobacteriaceae **(ISO21528-2, 2004)** were enumerated on violet red bile glucose agar (Oxide CM 485B) medium and incubated at 37º C for 24 hours.

2.8. Experimental design and analysis of the data

Results of the preservative effects of propolis extract (EEP) on the quality attributes of the fish fillets were statistically analyzed as a factorial design with two factors was used (time and concentration). Four concentrations of EEP: 0, 0.5, 1, and 1.5% and six levels of storage time (0, 3, 6, 9, 12, and 15 days) were tested. The data were statistically analyzed using the MIXED PROC of the Statistical Analysis Software **(SAS, 2016)**. Significant means were compared using the Tukey post-hoc test at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Sensory analyses

The organoleptic evaluation considers a good tool for evaluating immediate quality and freshness of fish in addition to being simple, fast and costless method. Analysis of appearance, odour, flavour and texture are evaluated using the human senses **(Huss, 1995)**. In current study, fish samples were considered to be acceptable for human consumption when the sensory score reached at least 4 out of 9 **(Amerina et al., 1965)**. Results in Figure 1 showed that at zero time, all examined groups of treatments were acceptable with a mean score value of 9, this indicated that all samples at the start of the experiment were in good quality. There were no significant differences between control and treated samples before storage. The organoleptic acceptability for all groups of treatments was decreased gradually with storage time. By the 6th day, a significant loss in the fish quality for control samples ($P < 0.05$), where there were significant changes in organoleptic parameters between control and treated samples. Control and 0.5% propolis treated groups were rejected by the panelists after 6, and 12 days of chilling storage, respectively. On the other hand, the 1 and 1.5% propolis treated groups were acceptable after 12 days of storage with scores 4.16±0.17 and 5.08±0.10 mean values, respectively. Texture, odor, and appearance scores of both control samples and extract of propolis samples were decreased at a slower rate compared to odor and taste (Figure 1). The results showed that samples treated with EEP had high acceptable overall scores compared to the control group. The 1.5% group of EEP-treated samples were the most acceptable products by the panelists.

Our results were in coincide with **(Hassanin and El-Daly 2013)** who found that all the initial samples at zero time had high scores for odor, texture and taste, while throughout the frozen storage period there were significant decreases ($P < 0.05$) in sensory scores for control groups as compared with treated samples and the maximum odor, texture and taste scores were obtained in fillets treated by mixture of 3% garlic mixed with 0.6% propolis. Also, similar results have reported that all the initial samples at zero time had high scores for odor, texture, and taste and throughout the chilling storage period there were significant decreases ($P < 0.05$) in sensory scores for control and all treated samples. The maximum odor, texture and taste scores were obtained in fillets treated by, 0.5, 0.3, and 0.1 % (v/w) water

extract of propolis, respectively compared to control samples which show significantly loss ($P < 0.05$) in the sensory score at the end of the storage period as compared with treated groups **(Doman and Ozpolat, 2014).**

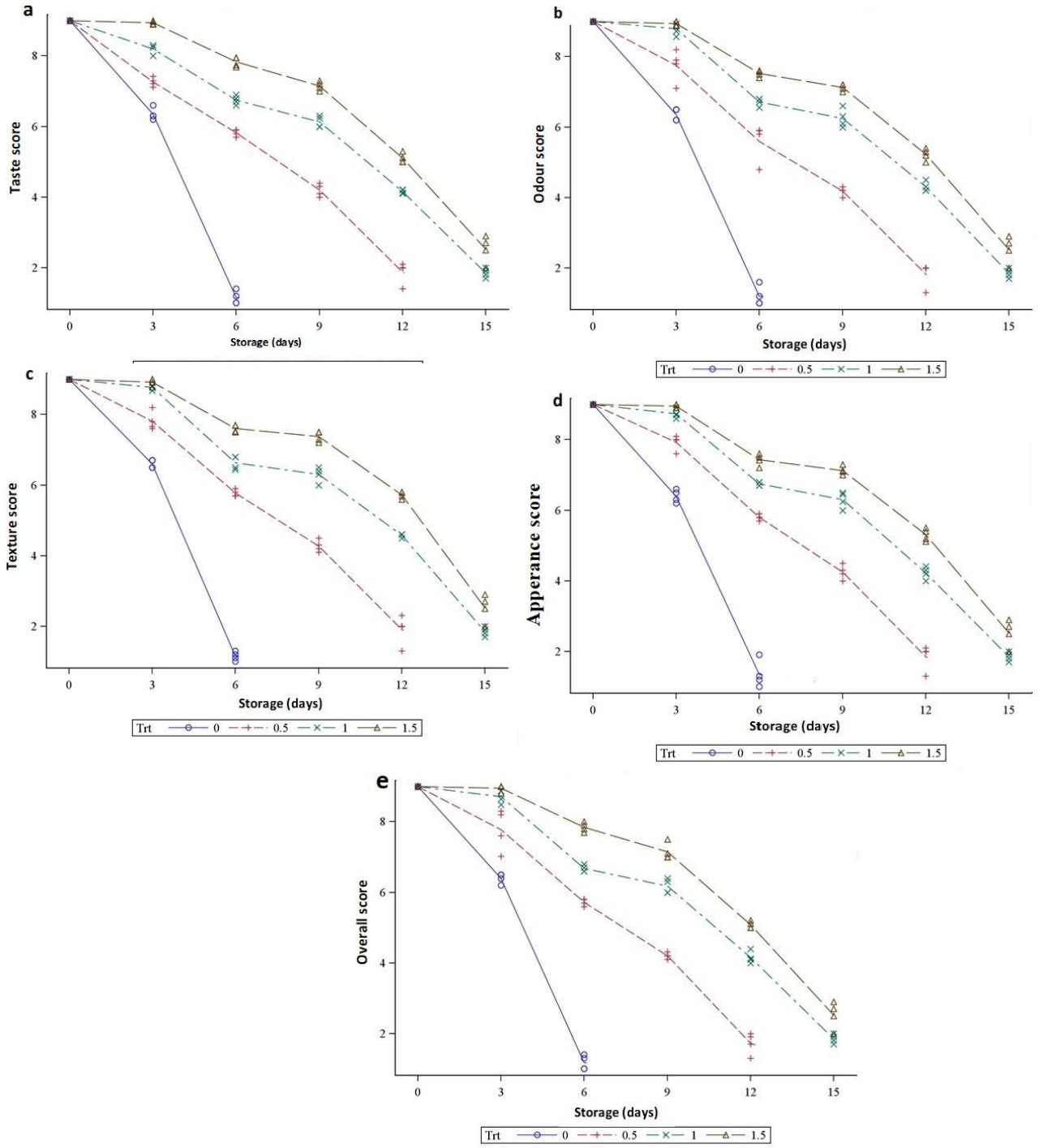

Fig 1. Changes in taste (a), texture (b), dour (c), appearance (d), and overall acceptance (e) of tilapia fillets samples that were treated with 0, 0.5, 1, and 1.5% (v/w) of EEP after 0, 3, 6, 9, and 12 days of storage at 4 ±1^{0}C.

3.2. Chemical analysis

3.2.1. Hydrogen ion concentration (pH)

The initial pH values were 6.07±0.05, 6.05 ±0.02, 6.04± 0.02, and 6.02 ±0.02 for control, 0.5, 1, and 1.5% EEP-treated groups, respectively (Figure 2a). There were no significant differences between control and treated groups at zero time. Meanwhile, after 3, 6, 9, and 12 days of storage, significant effects were observed between different EEP treatments as compared with control samples due to increased pH value (P<0.05). The pH values of control samples were increased to 6.96±0.04 after 6 days of storage. This increase in the pH might be attributed to the enzymatic degradation of the fish muscles and the production of volatile basic alkaline components (e.g., ammonia and trimethylamine) by spoilage bacteria **(Ruiz and Moral, 2001)**. Additionally, the enzymatic degradation of ATP causes the liberation of inorganic phosphate and ammonia that might lead to changes in pH value **(Sikorski et al., 1990)**. The gradual increase in pH values was noticed in propolis-treated samples (6.53±0.04 for 0.5% EEP) after 9 days of chilling storage. The control samples were rejected at 6th day, while propolis-treated groups showed had acceptable pH values according to **(EOS, 2005)** to the 12th day of storage for 0.5% treated groups and the 15th days of storage for 1 and 1.5% propolis-treated samples, respectively **(EOS, 2005)**. These results might be due to the presence of phenolic compounds in propolis extracts, which might enhance the microbial inhibition and inhibit the activity of the endogenous proteases **(Harris et al., 2001)**. Similar results have been reported by **(Suárez et al., 2014)** who reported an increase in the pH values after chilling storage.

3.2.2. TVBN value

The TVBN is one of the most widely used indices of quality for refrigerated fish products. It indicates the formation of nitrogenated compounds produced by the activity of proteolytic bacteria and native proteases in stored fish products **(Kilincceker et al., 2009; Kostaki et al., 2009)** . The changes in TVBN values for the tilapia fillets samples during the storage period were presented in Figure 2b. The mean values of TVBN in control, 0.5, 1, and 1.5% treated groups were 4.40, 4.25, 4.14, and 4.05 mg/100g, respectively at zero storage time with no significant differences (P>0.05). A gradual increase in TVBN values were noticed during storage and significant encouraging effects of propolis were observed (P< 0.05) as compared to control samples. The increase in TVBN levels were greater in the control samples compared to the tilapia fillets treated with propolis groups. The highest TVBN values were noticed in the control groups which exceed the maximum acceptable value (25 mg/100 g; **EOS, 2005**) after 6 days of storage (Figure 2b). While the treatments with propolis 0.5, 1, and 1.5% maintained acceptable levels of 24.94, 25.04, and 23.71 mg/100 g, respectively to the 9 and 12 days of storage.

These findings might be due to the antimicrobial activity of the propolis related to the main components (phenolic compounds) found in propolis **(Harris et al., 2001),** which reduced the capacity of decomposition bacteria during storage period that is considered as the main reason for purification of fish due to oxidative deamination of non-protein nitrogen compounds **(Kilincceker et al., 2009)**. Similar results have been obtained by **Duman and Ozpolat (2014),** they reported significant statistical differences (P < 0.05) between the vacuum-packed fresh Shibuta (*Barbus grypus*) fillets samples treated with 0.1, 0.3, and 0.5% (v/w) of water extract of propolis compared with the control after 24 days of

storage at 2 ±1^{0}C. Also, the results were comparable to the findings of **(Suárez, et al. 2014**). They treated Cachama fillets with ethyl alcohol (96%) as the control, 0.8% EEP, 1.2% EEP, and liquid smoke and stored for 0, 8, 16 and 24 days at 4^{o}C under vacuum packaging. The results showed high values of TVBN for the liquid smoke treatment and the low values for the EEP treatments (P<0.05).

3.2.3. Peroxide values (PV) and thiobarbituric acid values (TBA)

Both PV and TBA are employed as an index for lipid oxidation in fish and its products. Fish and fishery products of good quality would have TBA value less than 4.5 mg malonaldehyde (MDA)/kg, otherwise it will probably have undesirable smell and rancid taste **(EOS, 2005)**. Changes in TBA values of tilapia fillets samples were presented in Figure 2C. The initial TBA value of control and propolis treated groups 0.5, 1, and 1.5 % were of 0.58± 0.04, 0.56 ±0.04, 0.51±0.04, and 0.46± 0.09 mg MDA/kg. The PV values (Figure 2E) were 0.74 ± 0.16, 0.71±0.15, 0.67±0.15, and 0.56 ±0.05 milliequivalent peroxide/kg for 0, 0.5, 1, and 1.5% EEP treatments, respectively. There were no significant differences (P < 0.05) between control and treated samples at zero day. At the 6th day of chilled storage, the control groups had PV (7.85±0.42 milliequivalent peroxide/ kg) and TBA (4.97±0.27 mg malonaldehyde/kg) mean values, which exceeded the permissible limit recommended by **(EOS, 2005)**. However, gradual significant increase in PV and TBA values were observed with storage time. The raise in PV and TBA values were very slow in the groups treated with propolis extract compared to control samples, which prolonged the shelf-life of samples.

Thiobarbituric acid and PV were in the acceptable limit for 0.5% propolis treated-groups with mean values of 6.80±0.26 mg MDA/kg of PV and 4.45±0.6 milliequivalent peroxide/ kg of TBA at the 9th day of storage. But it exceeded the permissible limit and rejected at the 12th days of storage. The lowest TBA and PV values were obtained from 1.5 and 1% propolis treated-samples with mean values 5.49±0.64 and 6.76±0.28 milliequivalent peroxide for PV and 3.96±0.30 and 4.49±0.10 mg MDA/kg for TBA, respectively. These groups of samples had the longest shelf-life until the 15th day of storage because it exceeded the permissible limit recommended by **EOS (2005)**. These findings might be due to the antioxidant properties of propolis extract due to its richness of caffeic acid and its derivatives (CAPE and DMAC), galangin, kaempferol, and quercetin. Results reported herein were in agreement with that of **Duman and Ozpolat (2014)** and **Hassanin and Eldaly (2013)**.

3.2.4. Trimethylamine-nitrogen concentration (TMA-N)

Trimethylamine-nitrogen concentration (TMA-N) is a spoilage index of food, particularly in marine fishes. TMA-N is derived from trimethylamine oxide (TMAO), which is critical for osmoregulation in marine fish. During spoilage, TMAO is reduced by enzymes to TMA **(Kilnic et al., 2008)**. The TMA-N values of Tilapia fillets samples were presented in Figure 2d. The initial mean values of TMA content ranged from 0.84±0.08, 0.82±0.01, 0.79±0.07, and 0.75±0.07 mg/100g for control, 0.5, 1, and 1.5 % EEP, respectively. Then the values were significantly increased with storage time. At the 6th day of chilled storage, there were significant differences (P<0.05) between control and EEP-treated samples and the control group exceeded permissible limit recommended by **EOS (2005)**. But TMA-N was within the acceptable limits (9.88±0.13 mg/100 g) for the 0.5% EEP-treated groups till 9th day of storage and it exceeded the limits and spoiled at the 12th day of storage. The quality of fish samples was extended to 12

days in storage after treatment with 1 and 1.5 % EEP with mean values 9.99±0.08 and 8.98±0.20 mg/100g, respectively but samples were rejected after 15 days in storage.

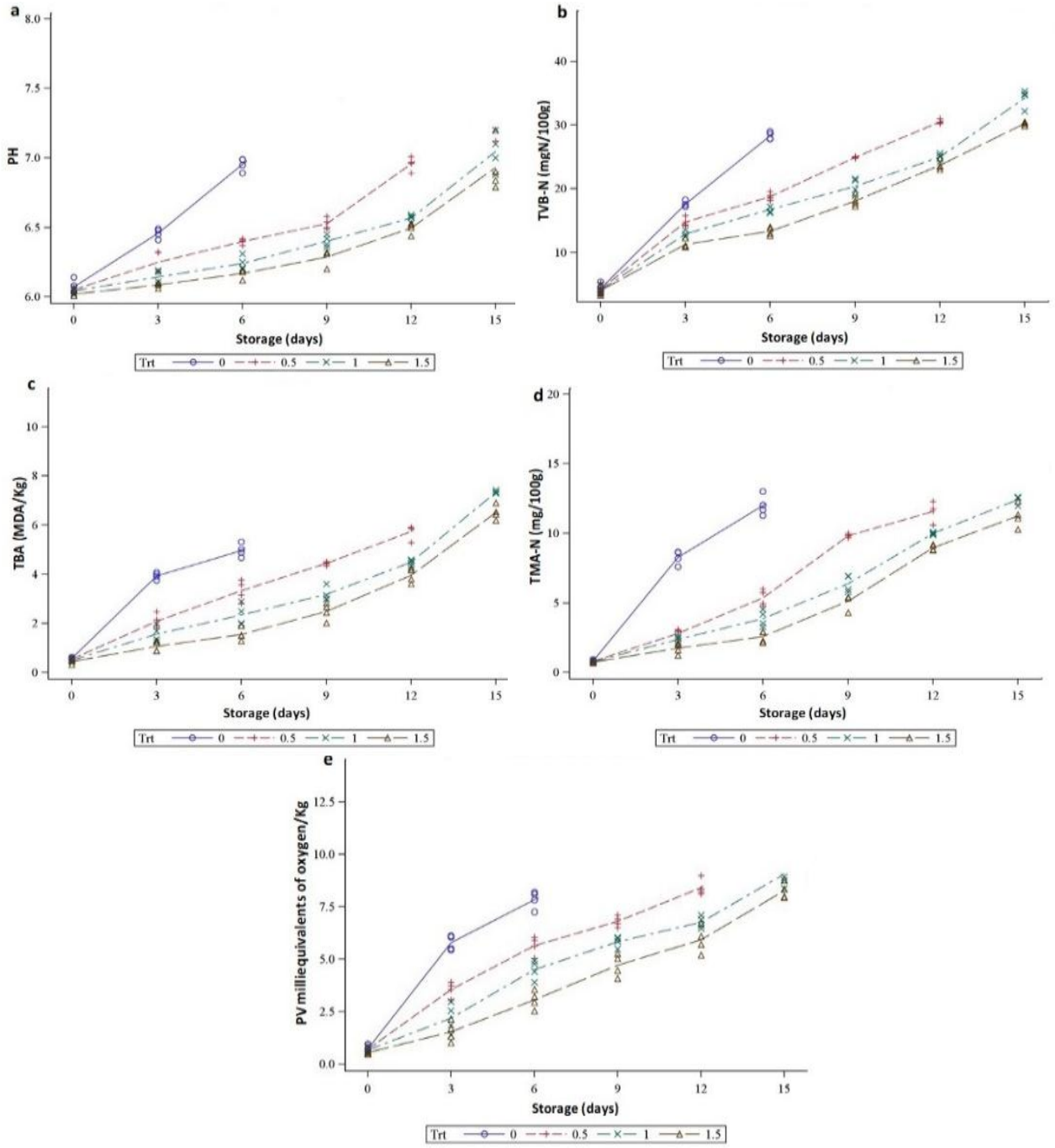

Fig 2. Changes in pH (a), TVB-N (b), TBA (c), TMA (d), and PV (e) index values of tilapia fillets samples that were treated with 0, 0.5, 1, and 1.5% (v/w) of EEP after 0, 3, 6, 9, and 12 days of storage at 4 $\pm 1^0$C.

3.3. Microbiological examination

3.3.1. Aerobic plate count (APC)

Annually, about one-third of the world's food is spoiled as a result of the microbial activity. Therefore, total number of microorganisms: aerobic plate counts (APC) has been used in mandatory seafood standards **(Lund et al., 2000)**. **ICMSF (1986)** stated that the upper acceptability limit of the total viable bacterial count in fresh fish is 7 $\log_{10}$ cfu/g flesh. In current study, results of the changes in APC with storage time were concluded in Figure 4a. The initial APC mean values were 5.94±0.05, 5.94±0.05, 5.93±0.05, and 5.92±0.05 $\log_{10}$ cfu/g for 0, 0.5, 1, and 1.5% EEP-treated groups of fillets which increased progressively ($P < 0.05$) with storage time. Mean APC values were 7.12±0.08 and 7.08±0.02 $\log_{10}$ cfu/g after 6 and 12 day in chilling storage (which was the time of production of off-odors and unacceptable taste) for 0 and 0.5% propolis- treated groups. However, the 1 and 1.5% of EEP-treated groups of fish had enhanced shelf-life till the 15th day of storage with 7.28±0.01 and 7.11±0.2 $\log_{10}$ cfu/g, respectively. From previous values the control group was rejected microbiologically on the 6th day of storage while the 0.5% propolis-treated groups were good (microbiological acceptance) until 9th day of storage at 4ºC. Meanwhile, the 1 and 1.5% propolis-treated groups had extended microbiological shelf-life until the 12th day of chilling storage compared to the control and the permissible limits of APC recommended by **ICMSF (1986)**.

3.3.2. The psychrotrophic bacterial count

The psychrotrophic bacteria are major group of microorganisms responsible for aerobically spoilage of stored fresh fish at chilled temperatures **(Gram et al., 2002)**. Psychrotrophs including *Bacillus cereus* and *Pseudomonas* spp which their presence or contamination in food lead to food poisoning and/or spoilage **(Jay, 2000)**. At zero storage time, mean values of psychrotrophic bacteria of fish samples were 4.84±0.08, 4.84±0.08, 4.83±0.08, and 4.82±0.08 $\log_{10}$ cfu/g for 0, 0.5, 1, and 1.5% propolis-treated groups, respectively (Figure 3b). There was no significant effect related to the addition of propolis at zero time in control and treated group samples. Psychrotrophic bacteria count were significantly different between control and treated groups ($P<0.05$) starting from the 3rd day of storage. After 9 days in storage, the psychrotrophic mean values of control and 0.5% propolis-treated groups were 6.17±0.13 and 6.60±0.08 $\log_{10}$ cfu/g, respectively. The highest reduction in psychrotophic counts was achieved after treating fish fillets with 1 and 1.5% EEP with mean values of 5.95±0.10 and 6.06±0.09 $\log_{10}$ cfu/g, respectively at the 12th day of chilling storage. Similar results have been reported by **Duman and Ozpolat (2014)** where they stated significant for the total viable bacterial count and psychrotrophic bacteria between the vacuum-packed fresh Shibuta (*Barbus grypus*) fillets samples treated with and without the water extract of propolis.

3.3.3. The Enterobacteriaceae count (EBC)

Results of Enterobacteriaceae counts in fish samples that were treated with or without EEP were presented in Figure 3c. Fish spoilage is affected seriously by the Enterobacteriaceae levels **(Lindberg et al., 1998)**. As shown in Figure 3c, the counts of Enterobacteriaceae was not significantly different between control and propolis extract treatments at zero day of storage. The mean counts were 2.41±0.08,

2.40±0.07, 2.39±0.10, and 2.38±0.09 log_{10} cfu/g for control, 0.5, 1, and 1.5% treatments, respectively. There was a significant decrease (P<0.05) in Enterobacteriaceae count of all treated groups than those of control group after 12 days in storage with mean values of 3.07±0.15 and 3.08±0.11 log_{10} cfu/g for the control and 0.5% EEP-treated groups at the 6th and the 12th day of storage, respectively. Such results were relatively high, and samples were spoiled as correlated from the sensory evaluation results. The highest reduction of the EBC was achieved by dipping fish fillets in 1 and 1.5% of the EEP with mean numbers of values 2.71±0.09 and 2.60±0.08 log_{10} cfu/g, respectively after 12 days in chilling storage.

3.3.4. Staphylococcal total count

Results of Staphylococcal total microbial count in the presence or the absence of various concentration of EEP treated-samples were showed in Figure 3d. It was found that all propolis-treated samples exerted significant antibacterial action on the growth of Staphylococcal. As the concentration of EEP in treated samples is increased, the antibacterial action against the test organism is enhanced. In the control group, the viable count of Staphylococcal was increased from 3.55±0.09 to 4.27±0.29 log_{10} cfu/g after 0 and 6 days of storage, respectively at 4±1ºC. After 6 days in storage, off-odors and unacceptable taste was reported from the sensory evaluation. There was a significant decrease (P< 0.05) in the staph count between all EEP-treated groups and control group. After 12 days of the storage, the staph mean number was 4.23±0.10 log_{10} cfu/g for fillet treated with 0.5% of EEP, which was relatively high, and samples were rejected. The highest reduction in the staph counts was achieved by treating fish samples with 1 and 1.5% of EEP with mean numbers of 3.82±0.06 and 3.57±0.05 log_{10} cfu/g after the 12th day of chilling storage. These results were in agreement with **Chang et al. (2005)** and **Rahman et al. (2010)** who reported the effective reduction of STAPH numbers by propolis ethanolic extract. They stated that reduction in numbers of bacteria was related to the active ingredients in propolis (phenolic compounds, cinnamic, flavonoids, aromatic acids, and benzopyrenes, which are documented with strong antimicrobial activity **(Hegazi et al., 2000; Ahn et al., 2007; Tosi et al., 2007; Kumar et al., 2008; Probst et al., 2011; Campos et al., 2014).**

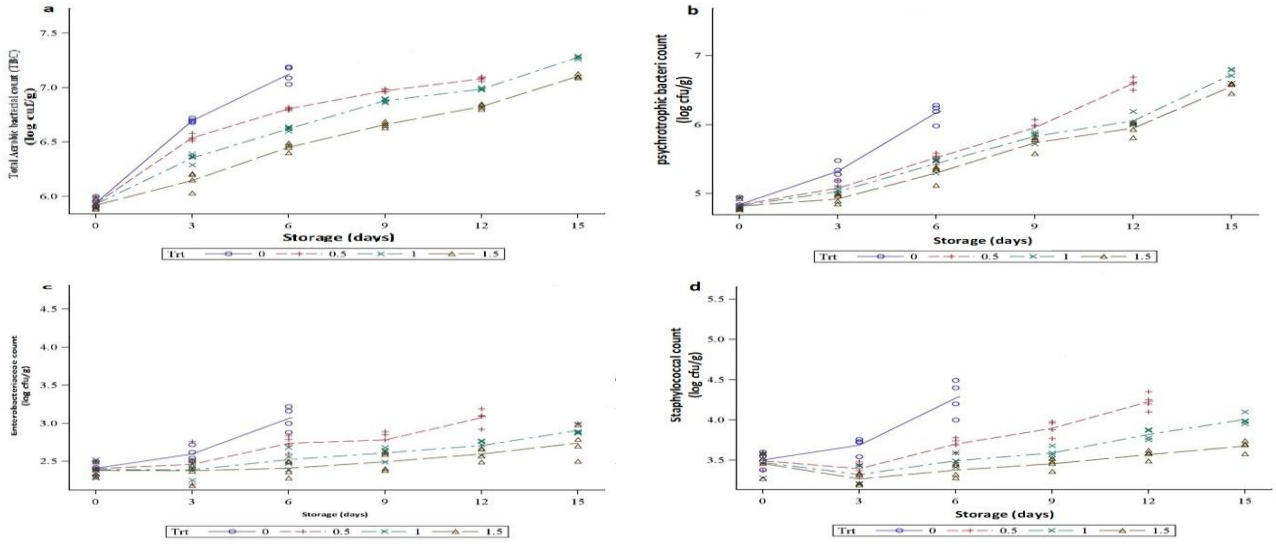

Fig 3. Changes in total viable counts (a), psychrotrophic bacteria counts (b), Enterobacteriaceae counts (c) and Staphylococcal counts (d) of tilapia fillets samples that were treated with 0, 0.5, 1, and 1.5% (v/v) of EEP after 0, 3, 6, 9, and 12 days of storage at 4 ±1^{0}C.

4. Conclusions

Based on the data obtained from the present study, it was demonstrated that dipping fish fillets in the ethanolic extract of propolis profoundly enhanced the sensory, chemical, and microbiological characteristics. The 0.5% treated samples had a shorter shelf-life compared to that samples treated with 1 or 1.5% of EEP but all three levels of EEP extended the shelf-life of sample compared to the control. Ethanolic extract of propolis can be an option in the preservation of fresh fish products as an alternative to chemical preservatives.

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تحسين جودة وفترة حفظ فيليه اسماك البلطي بإستخدام المستخلص الايثانول لصمغ النحل (البروبوليس)

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تعد الأسماك بيئة خصبة لنمو العديد من الميكروبات التي تسبب فسادها بشكل سريع وجعلها خطرا علي الصحة العامة للمستهلك لذا يعتبر حفظ الاسماك من اهم الحلول التي تتجه اليها صناعه الاسماك. وقد زاد الاهتمام باستخدام المواد الطبيعيه كمواد حافظه لتجنب الاثار الضاره للمواد الحافظه الكيميائيه من هنا أجريت هذه الدراسة على فيليه أسماك البلطي لتقييم تاثير البروبوليس (صمغ النحل) علي تحسين فترة حفظها وتحسين جودتها. وقد تم اختبار المستخلص الايثانولي للبروبوليس بتركيزات (0 ، 0.5 ، 1 ،و 1.5 %) علي الصفات الحسيه والكيميائيه وبعض الميكروبات االمسببه للمرض بعد أزمنة 0 ، 3 ، 6 ، 9 ، 12و15 يوم من التخزين علي 4±1°م. أوضحت النتائج زياده فتره الحفظ للمجموعات المعالجه بتركيزات 0.5 ، 1 ،و 1.5% من البروبوليس الي 12 يوم بالمقارنه بالمعاملة الكنترول التي فسدت بعد 6 ايام. أيضا أظهرت النتائج ان المجموعات المعالجه بالبروبوليس من شرائح الاسماك تحسنا معنويا في الصفات الكيميائيه والحسيه وتقليل معنوي لأعداد الميكروبات التي تسبب فساد الاغذية مقارنة بالمجموعه التي لم يتم معالجتها بالمستخلص. لذا ينصح بمزج الاسماك بنسب صغيرة من البروبوليس لزيادة فترة حفظها بالإضافة الي تحسين صفاتها الكيماوية والحسيه وتقليل الميكروبات الممرضه بها.