Original Research

Effective Role of Pomegranate Peel Extract on Quality of Tilapia Fillets During Storage

Eman T. Al-Sokary [1], Gehan I. E. Ali [1*], Mona E. Abass [1], Shereen A. Yassin [2], and Engy F. El-bahy [2]

1. Biochemistry, Nutritional Deficiency Diseases and Toxicology, Kafr el sheikh Regional Laboratory, Animal Health Research Institute, Agricultural Research Center (ARC), Egypt.
2. Food Hygiene, Kafr el sheikh Regional Laboratory, Animal Health Research Institute, Agricultural Research Center (ARC), Egypt.

Correspondence*
Gehan Ibrahem Elsayed, Biochemistry, nutritional deficiency diseases and toxicology, Kafr el sheikh Regional Laboratory, Animal Health Research Institute, Agricultural Research Center (ARC), Egypt.
Email: gehanibrahemali@gmail.com

Received: 01/12/2022
Accepted: 25/12/2022

ABSTRACT

For the methanolic pomegranate peel extract (MPPE), sixty Nile tilapia fish fillet samples (5 cm x 10 cm) were randomly divided into four groups. The fish fillet samples were dipped for 60 sec (ratio of fish to liquid, 1:2 (wt/vol) either in sterile water (the control group) or in sterile water containing 1, 2, or 3 % MPPE (the other groups). Then remove to drain and pack in low-density polyethylene pouches for 6 months at -18 ^{O} C. Microbiological, chemical, and sensory traits were examined monthly in control and treatment sample comparisons. The finding showed that the treatment with MPPE significantly slowed down microbial growth in the samples. The microbiological assay showed that G2, G3, and G4 reached the maximum acceptability limit in one month, while it took one month in the control group. Better oxidative stability and smaller increases in the values for PH, total volatile base nitrogen, peroxide value, and thiobarbituric acid-reactive compounds were also attained. Moreover, MPPE at 2% and 3% maintained a non-significant decrease in the values of essential AA and PUFA throughout the storage period. Therefore, dipping fish in MPPE can be thought of as a successful approach to increasing the product's overall quality and storage life.

Keywords: Pomegranate peel extract, Tilapia fillets, Microbiology, Chemical traits, Sensory traits.

INTRODUCTION

Fish is regarded as a very beneficial food because of its high protein content and low saturated fat level. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are well-known for their anti-inflammatory and cardiovascular disease-protective actions, are two omega-3 polyunsaturated fatty acids (PUFA) that are mostly found in fish **(*Vilavert et al., 2017*)**. Because of its great nutritional content and mouthwatering flavour, Nile tilapia (*Oreochromis niloticus*), one of the most common fish in Egypt, is a favourite among Egyptian diners. Moreover, the most frequently cultivated tilapia species worldwide is the Nile tilapia **(*Salem, 2015*).**

Fish, on the other hand, is a perishable food that spoils more quickly than other muscle foods. Microbial deterioration is aided by higher protein and moisture levels, but the presence of polyunsaturated fatty acids causes lipid and protein oxidation. These alterations have a negative impact on the sensory quality, nutritional content, and consumer acceptability, resulting in a shorter shelf life of muscle food **(*Madane et al., 2019*)**.

Fish are preserved via frozen storage, which helps to prevent or minimize biochemical changes that happen during storage. However, because fish muscle contains a lot of proteins and unsaturated fatty acids, freezing fish does not completely halt the microbial and chemical processes that cause fish quality to deteriorate **(*Berizi et al., 2016).***

Preservatives are used to keep the superior quality attributes of fish for longer and to improve the shelf life of frozen fish. Synthetic chemicals are commonly employed to prevent such alterations and reduce the development of harmful substances ***(Chauhan et al., 2019)***. Natural preservatives made from various agricultural and culinary wastes. On the other hand, food processors have recently started looking into them because they not only include antimicrobials and antioxidants, but are also widely available, affordable, and environmentally friendly. Numerous plant parts, including fruits, roots, bark, and leaves, as well as their byproducts, have also been found to be a rich source of naturally occurring bioactive substances (polyphenolic, dietary fiber, and flavonoids) that not only aid in the inhibition of oxidative changes (antioxidants), but also aid in the suppression of microbial growth (antimicrobials), thereby preventing a number of diseases ***(Madane et al., 2020).*** Also, consumers all over the world prefer natural preservatives, which are regarded as safe and have beneficial health effects, to

synthetic compounds, which are harmful and pose health hazards ***(El-Hadary and Taha, 2020).***

Punica granatum (Punicaceae), a tiny tree that is frequently planted in the Mediterranean region, produces pomegranates. The pomegranate (*Punica granatum L.*) is a fruit that is high in phytochemicals like tannins and other phenolic compounds and has a wide range of medicinal properties. The biological capabilities of various extracts or chemicals from various parts of this plant have been discovered to include antioxidant ***(Iqbal et al., 2008),*** antibacterial ***(Al-Zoreky, 2009)***, and antifungal ***(Dahham et al., 2010)*** activity. The peel of a pomegranate contains roughly 50% of the total fruit weight ***(Al-Said et al., 2009),*** and it is an inedible by-product of the pomegranate juice process ***(Gullon et al., 2016).*** Nevertheless, compared to the juice, the fruit peel has larger levels of polyphenol chemicals and stronger biological properties ***(Li et al., 2006).*** It could be used to increase the phenolic content of people's diets through functional food ingredients, food additives, nutraceuticals, and supplements ***(Gullón et al., 2020).***

Pomegranate peel extract (PPE), which has a high concentration of phenolic components such as punicalagin, punicalin, gallic acid, and ellagic acid ***(Wafa et al., 2017)***, has good antioxidant and antibacterial effects. Numerous studies have investigated PPE's preservation effects on poultry and beef products ***(Lytou et al., 2018)*** as well as its antioxidant properties on fish meat during frozen storage ***(Özalp Özen and Soyer., 2018).*** It has been demonstrated that pomegranate fruit peel extracts can stop the growth of a number of foodborne pathogens, including *L. monocytogenes*, *S. aureus, E. coli*, *Y. enterocolitica*, and *B. cereus* ***(Agourram et al., 2013).***

Therefore, the objective of the present work was to evaluate the effects of adding various levels of pomegranate peels extract, as natural preservatives, on quality of Nile tilapia fillet during frozen storage.

MATERIALS AND METHODS

Pomegranate peel powder preparation We bought mature pomegranate fruit from the market that had no obvious external cuts or rotting (Kafr Elsheikh city). Pomegranate fruits were chopped manually to separate the arils and peel, and their edible sections were meticulously separated in order to obtain the pomegranate peel. Using a sharp knife, the pomegranate peel was sliced into little pieces measuring 2 x 2 cm. It was then thoroughly cleaned with distilled water before being dried in the air for 24 hours before being baked at 40 °C for 48 hours in a ventilated oven. The dried pieces were ground into a fine powder in a heavy-duty food grinder, filtered through a 24-mesh sieve, and kept in a refrigerator at 4 °C. **Preparation of extraction** Peels that had been finely powdered (5 g) were combined with 300 ml of 80% methanol and mixed for 2 minutes in a Waring blender. Each mixture was then left, in the dark, at room temperatures for 1 h prior to filtration (Whatman No. 1) and centrifugation at 3500rpm for 10 min at 5˚C. When necessary, extracts of 80% methanol (ME) were kept at –20 ºC prior to analysis. Other sets of extract (ME) were concentrated to dryness under reduced pressure at 40 ºC to determine yields (%) per original materials. Extract was kept at -20 °C prior to analysis ***(Shiban et al., 2012).*** **Determination of total phenolic content: Antioxidant assay: 1-Determination of DPPH radical scavenging activity** the method of *Melendez and Capriles (2006)* was used to estimate the ability of PPE to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. With vigorous shaking, 1 ml of the extraction was combined with 1 ml of the DPPH (500 M). A UV spectrophotometer (PG Instruments Ltd T80+ UK) was used to measure the reaction mixture's absorbance at 517 nm after it had been let to stand at room temperature in the dark for 20 minutes. The following equation was used to determine the antioxidant activity.

$$\text{Antioxidant activity:} \quad \frac{\text{1-Abs sample 517 nm}}{\text{Abs Control 517 nm}} \quad \text{X 100}$$

Measurement of antioxidant activity by ABTS method: Antioxidant activity measurements were assessed using the bleaching of radical cations produced by ABTS. The radical cation was created by reacting ABTS (60 ml) with MnO2 (3 ml, 25 mg/mL) in phosphate buffer solution. ABTS is an acronym for 2,20-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) (10mM, pH 7,5 mL). The solution was centrifuged and filtered after it had been shaken for a while. At lmax734 nm, the absorbance of the resultant green blue solution (ABTS radical solution) was measured (A control). After adding (20ml of 1 mg/mL) solution of the tested material in spectroscopic grade Me OH/buffer (1:1 v/v) to the ABTS solution, the absorbance (A test) was measured. The decrease in the absorbance is expressed as % inhibition which calculated from this equation: Percentage inhibition = $[(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$. Ascorbic acid (20 ml, 2 mM) solution was used as standard antioxidant (positive control). Blank sample was run using solvent without ABTS ***(Badria et al., 2007)***

Bleomycin-dependent DNA damage: The bleomycine is a common class of glycopeptide antibiotics that utilized as anticancer medications. For evaluating the pro-oxidant effects of dietary antioxidants, the bleomycin assay has been utilized. Bleomycin, an anticancer antibiotic, binds iron ions and DNA. When heated with thiobarbituric acid (TBA), the bleomycine iron complex breaks down DNA, producing a pink chromogen. Antioxidants compete with DNA when sufficient reducing agents are added, which reduces chromogen production between the damaged DNA and TBA molecules ***(Gutteridge et al., 1981)***

Determination of SOD-like activity: The ***Bridges and Salin (1981)*** method was used to look into the activity that is similar to superoxide dismutase (SOD). This approach is based on the fact that SOD inhibits the superoxide anion produced by the xanthine/xanthine oxidase system from reducing nitro blue tetrazolium (NBT). In dimethylsulphoxide, the free (HAPT) ligand or its complexes with Mn (II), Co (II), Zn (II), Fe (III), and

UO2 (II) were produced (DMSO). For comparison, the native horseradish superoxide dismutase (HR SOD) activity has also been measured.

Preparation and treatment of fish samples: A total of 30 freshly killed Nile tilapia (*Oreochromis niloticus*) fish were purchased from a local fish market in kafr-Elshiekh city with an average weight of 500–550 g. The fish were transported within 2 hrs. to the laboratory on ice. Each fish was meticulously hand-filled after being gutted and gently cleansed with tap water. After removing the head and bone, each fish yielded two skin-on fillets. sixty Nile tilapia fish fillet samples (5 cm x 10 cm) were randomly divided into four groups. The fish fillet samples were dipped for 60 sec (ratio of fish to liquid, 1:2 (wt/vol) either in sterile water (the control group) or in sterile water containing 1, 2, or 3 % MPPE (the other groups). The dipping solutions were kept at 22°C, which is room temperature. After the fillets were taken out and left to drain for two hours at 20 °C on a metal net that had already been sanitized, the samples were each bagged separately in low-density polyethylene, followed by six months of monthly chemical, microbiological, and sensory investigation while being kept at -18 °C.

A- Chemical examination

1- **Determination of pH:** PH value was determined by using an electrical pH meter (Adwa PH meter AD11, Romania).
2- **Determination of Total Volatile Nitrogen (TVB-N) according to *(AOAC, 1990).***
3- **Determination of Thiobarbituric acid (TBA) according to (*Gray, 1978*).** Determination of Peroxide value: Peroxide value analysis of each treatment (mili/eqi O_2/kg fish fat) was determined using the method described by AOAC *(Mc Faddin 2000).*
4- **Evaluation of amino acids profile by High performance amino acid analyzer: according to *AOAC (2012).***
5- **Evaluation of fatty acids profile by Gas Chromatography with FID detector: *AOAC, (2000)*.**
   a. **Lipid Extraction:**
   b. **Methylation of Lipid:**
   c. **Separation of fatty acid methyl ester:**

B- Microbiological examination

Ten grams of fish meat were aseptically transferred to a stomacher bag containing 90 mL of water that contained 0.1% peptone. Under sterile conditions, the fish meat was homogenized for 60 seconds using a stomacher to produce a 1/10 dilution. To count total bacteria, *pseudomonads, enterobacteria*, lactic acid bacteria, and yeast, serial dilutions were made. After incubation at 35 °C for 48 h, colony-forming units were counted to determine the number of aerobic counts on plate count agar (PCA), and the results were represented as log10 CFU/g. On cephaloridin fucidin cetrimide agar (CFC), *pseudomonads* were counted and cultured at 30 °C for 48 hours. *On violet red bile glucose agar (VRBGA), enterobacteria were counted and cultured at 30 °C for 24 hours.* On de Man Rogosa Sharpe agar (MRS) and (Sabouraud dextrose agar), lactic acid bacteria and a mold count were counted, respectively. The incubation periods were 48 hours at 37 °C and 7 days at 25 °C, respectively. The number of bacterial colonies was measured in cfu/g.

C- Sensory evaluation

17 trained panelists assessed the anterior portion of each fish sample on a four-point scale: 1 = extremely like, 2 = moderately like, 3 = neither like nor dislike, and 4 = dislike. The panelists ate fish on a regular basis and had never experienced any allergy symptoms. Color, odor, texture, appearance, and overall acceptability were all evaluated by the panelists ***(Berizi et al., 2016).***

Statistical analysis: Each measurement was made three times for each group, with the mean values and standard errors being recorded in each instance. ANOVA (analysis of variance) and mean comparisons using the least significant difference were performed using SPSS (Statistical Package for the Social Sciences) to assess the significance of differences between mean values (LSD). P-values less than 0.05 were statistically significant.

RESULTS

Antioxidant activity: -

The antioxidant activities (ABTS + scavenging ability) of PPE were found to be 84.6% (TEAC; mmoltrolox eq./100 g dw). The inhibition % of DPPH was 87.5%, Bleomycin dependent DNA damage was 0.081, and SOD inhibition % was 69.1% (Tables 1- 4).

Table (1) DPPH activity of pomegranate peel extract

No.	Compounds	% Inhibition
*	Ascorbic-acid	91.4%
1	Extract	87.5%
	Total phenolic content	152.6 ± 3.42 mgGAE/g

Table (2) ABTS activity of pomegranate peel extract

No.	Method	ABTS Abs(control)-Abs(test)/Abs(control)*100	
	Compounds	Absorbance of samples	% inhibition
	Control of ABTS	0.512	0
*	Ascorbic-acid	0.060	88.3%
1	Extract	0.079	84.6%

Table (3) Bleomycin dependent-DNA damage activity of PPE:

No.	Methods	Bleomycin dependent-DNA damage
	Compounds	Absorbance of samples
	Ascorbic-acid	0.058
1	Extract	0.081

Table (4) SOD activity of PPE:

No.	Sample	Δ through 5 min	% inhibition
	Control	0.415	0%
	L-Ascorbic acid	0.090	78.3%
1	Extract	0.128	69.1%

Effect of PPE on pH of fish samples: The pH results for samples from the various treatment groups are shown in Table (5). Fish samples from the control and PPE treatments had pH values of 6.23±0.09, respectively, on day 0, with no noticeable changes ($P < 0.05$). After six months of storage, the samples' pH levels significantly increased ($P < 0.05$). The pH of the samples treated with 3% PPE was the lowest at the end of the storage period, whereas the pH of the control group was the highest.

Table (5) Changes in PH values of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 ° C.

Storage period	Control	1% PPE	2%PPE	3%PPE
First month	6.4±0.06 a	6.4±0.06 a	6.3±0.03 ab	6.2±0.03 b
Second month	6.5±0.03 a	6.4±0.03 b	6.4±0.03 b	6.3±0.06 c
Third month	6.8±0.03 a	6.6±0.06 b	6.4±0.03 c	6.3±0.03 d
Forth month	7±0.09 a	6.7±0.03 b	6.5±0.09 bc	6.4±0.07 c
Fifth month	7.2±0.09 a	6.8±0.1 b	6.6±0.06 bc	6.5±0.09 c
Sixth month	7.3±0.03 a	6.9±0.03 b	6.7±0.09 bc	6.6±0.03 c

Means within the same row of different letters are significantly different at ($P < 0.05$).

Effect of PPE on TVB-N of fish samples: The TVB-N values of the treated and control groups changed significantly ($P < 0.05$) from the fourth month of storage to the end of storage (Table 6). The initial value of TVB-N in fresh fish was 7.57± 0.13 mg/100 g. In the sixth month of storage, the TVB-N levels in the control group were 24.23±0.56 mg/100 g, whereas they were 17.58±0.41, 16.63±0.41, and 15.04±0.036 mg/100 g for samples treated with 1, 2, and 3% MPPE, respectively.

Table (6) Changes in Total volatile basic nitrogen values (TVBN) (mg/100g) of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 ° C

Storage period	Control	1% PPE	2%PPE	3%PPE
0 day	7.57±0.13a	7.57±0.13a	7.57±0.13a	7.57±0.13a
First month	7.87±0.049 a	7.78±0.075 a	7.71±0.07 a	7.63±0.15 a
Second month	9.75±0.036 a	9.75±0.045 a	9.74±0.04 a	9.76±0.035 a
Third month	12.04±0.034 a	12.01±0.067 a	12.04±0.040a	12.03±0.056a
Forth month	16.82±0.34 a	13.13±0.072b	13.06±0.047 b	12.42±0.22 c
Fifth month	21.26±0.71a	15.63±0.34 b	15.05±0.032 b	13.96±0.039 c
Sixth month	24.23±0.56 a	17.58±0.41 b	16.63±0.41 b	15.04±0.036 c

Means within the same row of different letters are significantly different at (P < 0.05)

Effect of PPE on TBARS and Peroxide value of fish samples:

Tables (7) and (8), which compare the TBA contents and peroxide values of tilapia fish with and without various storage procedures, are provided. Table (7) shows the peroxide value results. At day 0, the peroxide value of the experimental and control groups was 1.93±0.008 mEq/kg ($P < 0.05$) where this value increased during frozen storage. After second month, the 3% PPE group showed a substantial rise ($P < 0.05$). Control (18.43±1.03) and 3% PPE (9.42±0.05) had the highest and lowest values, respectively, at the end of storage. During the storage period, the peroxide values of 3% PPE were lower than those of the other groups ($P < 0.05$).

The TBARS values at 0 day was 0.18±0.005 mg of malondialdehyde/kg in all groups ($P < 0.05$). Meanwhile, TBARS values of all samples increased as the storage period increased ($P < 0.05$). Up to three months, there was no significant difference between the groups. However, after three months, the experimental groups' TBARS levels were considerably lower than the control group ($P < 0.05$). The control and 3% PPE groups had the greatest (2.71±0.13) and lowest (0.99±0.008) TBARS levels at the end of storage, respectively.

Table (7) Changes in thiobarbituric acid-reactive substances (TBARS) (mg MA/kg) of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 ° C

Storage period	Control	1% PPE	2%PPE	3%PPE
0 day	0.18±0.005 a	0.18±0.005a	0.18±0.005a	0.18±0.005 a
First month	0.47±0.018 a	0.48±0.005 a	0.47±0.014 a	0.48±0.017 a
Second month	0.60± 0.015 a	0.58± 0.015 a	0.58±0.02 a	0.57±0.02 a
Third month	0.79±0.014 a	0.79±0.008 a	0.78±0.014 a	0.79±0.008 a
Forth month	1.53±0.073 a	1.09±0.036 b	0.93±0.008 c	0.85±0.008 c
Fifth month	2.16±0.12 a	1.31±0.005 b	0.98±0.005 c	0.91±0.008 c
Sixth month	2.71±0.13 a	1.76±0.015 b	1.11±0.014 c	0.99±0.008 c

Means within the same row of different letters are significantly different at (P < 0.05)

Table (8) Changes in peroxide value (PV) (Meq/kg) of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 ° C

Storage period	Control	1% PPE	2%PPE	3%PPE
0 day	1.93±0.008 a	1.93±0.008 a	1.93±0.008 a	1.93±0.008 a
First month	3.33±0.17 a	3.29±0.16 a	3.32±0.15 a	3.31±0.17 a
Second month	7.74±0.14 a	7.71±0.14 a	7.73±0.15 a	7.70±0.14 a
Third month	12.04±0.04 a	8.93±0.037 b	8.55±0.04 c	8.00±0.06 d
Forth month	14.5±0.27 a	9.61±0.11 b	9.49±0.08 b	8.70±0.08 c
Fifth month	16.37±0.74a	10.32±0.063 b	10.05±0.18 bc	9.05±0.12 c
Sixth month	18.43±1.03 a	12.52±0.61 b	10.94±0.14 bc	9.42±0.05 c

Means within the same row of different letters are significantly different at (P < 0.05)

Evaluation of amino acids profile by High performance amino acid analyzer:

In the present study the amino acid (lysine and leucine) all over the storage period in groups treated with PPE 2% and 3% remain at higher levels 3.11±0.01 and 3.29±0.03, respectively, than the control group, which recorded 1.91±0.04 at the end of storage period.

PPE 2% and 3% maintain non-significant decrease in the values of essential amino acids throughout the 6 month of the storage period (Table 9).

Table (9): Changes in amino acids profile of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 ° C

Amino acid		Storage period					
		1	2	3	4	5	6
Proline	Control	0.57±.0035Ab	0.55±0.029Ab	0.46±0.035 Bb	0.35±0.023Cb	0.32±0.022Cb	0.26±0.023 Cc
	1%PPE	0.69±0.07Aab	0.59±0.06ABab	0.52±0.014 Bab	0.36±0.029Cb	0.36±0.03 Cab	0.34±0.029Cbc
	2% PPE	0.75±0.042Aa	0.65±0.024ABab	0.59±0.038 Ba	0.42±0.05 Cb	0.39±0.03Cab	0.38±0.03Cab
	3% PPE	0.78±0.05Aa	0.68±0.01ABa	0.62±0.04 Ba	0.56±0.06BCa	0.44±0.03Ca	0.453±0.03Ca
Phenyl alanine	control	2.05±0.04 Ac	1.86±0.02 Ac	1.33±0.11Bc	1.16±0.23BCc	1.02±0.18CDc	0.92±0.03Dc
	1%	2.54±0.05 Ab	2.48±0.07ABb	2.17±0.01BCb	2.03±0.16CDb	1.79±0.12DEb	1.49±0.13 Eb
	2%	2.73±0.03 Aa	2.68±0.05Aab	2.52±0.08ABa	2.25±0.13Bab	2.25±0.12Ba	1.72±0.19Cab
	3%	2.79±0.05 Aa	2.77±0.04Aa	2.73±0.03Aa	2.53±.14ABa	2.44±0.12Ba	2.06±0.04 Ca
Lysine	Control	3.92±0.03 Ad	3.54±0.12Bd	3.33±0.06BCd	3.15±0.04Cc	2.72±0.12Dc	2.24±0.05Ed
	1%	4.16±0.05 Ac	4.06±0.04Ac	3.98±0.06ABc	3.76±0.13BCb	3.60±0.12CDb	3.37±0.01Dc
	2%	4.50±0.21 Ab	4.43±0.67Ab	4.14±0.03Bb	4.05±0.04Ba	3.81±0.03Cab	3.60±0.09 Cab
	3%	4.80±0.09Aa	4.61±0.33Ba	4.38±0.04Ca	4.26±0.03Ca	3.92±0.04Da	3.87±0.05 Da
Leucine	Control	3.29±0.03 Ac	3.17±0.03Ac	2.95±0.04Bd	2.66±0.09Cc	2.11±0.02Dd	1.91±0.04Ed
	1%	3.88±0.06Ab	3.75±0.09Ab	3.43±0.05Bc	3.33±0.11BCb	3.13±0.03 Cc	2.91±0.03Dc
	2%	4.09±0.05Ab	3.92±0.03Bb	3.68±0.02Cb	3.48±0.03Db	3.27±0.02Eb	3.11±0.01Fb
	3%	4.38±0.12Aa	4.25±0.07Aa	4.03±0.02Ba	3.94±0.02 Ba	3.71±0.01Ca	3.59±0.02Ca
Isoleucine	Control	1.36±0.07 Ad	1.30±0.05ABc	1.17±0.03BCc	1.10±0.01Cc	0.95±0.02Dc	0.82±0.06 Dc
	1%	1.71±0.03 Ac	1.86±0.01Ab	1.64±0.11Ab	1.46±0.04 BCb	1.38±0.04BCb	1.24±0.03 Cb
	2%	1.90±0.05Ab	1.75±0.03Bb	1.60±0.02Cb	1.51±0.02 Cb	1.38±0.04Db	1.30±0.03 Db
	3%	2.07±0.04 Aa	1.97±0.04ABa	1.89±0.05BCa	1.76±0.06CDa	1.64±0.06DEa	1.52±0.04Ea
Methionine	Control	1.45±0.05 Ac	1.24±0.05Bc	1.12±0.05Bd	0.94±0.02Cd	0.82±0.04CDc	0.69±0.02Dc
	1%	1.64±0.05 bc	1.57±0.04Ab	1.40±0.02Bc	1.28±0.03 Cc	1.16±0.03Db	1.05±0.03 Db
	2%	1.80±0.05Ab	1.67±0.02Bb	1.59±0.04Bb	1.39±0.02Cb	1.23±0.01Db	1.08±0.02 Eb
	3%	2.03±0.09 Aa	1.93±0.05ABa	1.82±0.05Ba	1.62±0.02 Ca	1.53±0.01CDa	1.41±0.01 Da

Evaluation of fatty acids profile by Gas Chromatography with FID detector:

In this study, EPA + DHA⁄C16 ratio has been decreased during frozen storage. Among saturated fatty acids that increased with increasing freezing period as related to the palmitic acid, significant difference at ($P < 0.05$) were observed between the control group that was (8.18± 0.12) and 2 % and 3% PPE, which recorded (5.13±0.08) and (4.25±0.08), respectively.

EPA values recorded 0.99± 0.02 at the first month then decreased to 0.45 ± 0.03 at the end of storage period but with using 2% and 3% PPE EPA values maintained high at the end of storage period 0.89±0.02 and 0.97±0.02, respectively. Also, DHA values recorded significant decrease from 4.15±0.04 to 0.57±0.11 in the control group but the 2% and 3% PPE groups showed no significant decrease as compared to the first month of storage (4.05±0.16) (Table 10).

Table (10): Changes in fatty acid profile of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 ^{O}C

Fatty acid		Storage period 1	2	3	4	5	6
palmitic	Control	2.53±0.23Fa	3.43±0.04Ea	5.25±0.06Da	6.51±0.08Ca	7.19±0.03Ba	8.18±0.11Aa
	1%PPE	2.24±0.03Fab	2.78±0.04Eb	3.53±0.07Db	4.53±0.06Cb	5.23±0.15Bb	6.17±0.16Ab
	2% PPE	2.03±0.05Fb	2.44±0.03Ec	3.06±0.06Dc	4.04±0.03Cc	4.52±0.07Bc	5.13±0.08Ac
	3% PPE	1.28±0.03Fc	1.73±0.04Ed	2.43±0.05Dd	3.09±0.12Cd	3.85±0.06Bd	4.25±0.07Ad
Arachidonic	control	2.10±0.02Ab	1.96±0.05Bc	1.83±0.02Cc	1.53±0.02Dc	0.95±0.01Ed	0.074±0.02Fd
	PPE1%	2.28±0.02Aab	2.17±0.03ABb	2.06±0.02BCb	1.93±0.03Cb	1.74±0.05Dc	1.40±0.12Ec
	PPE2%	2.41±0.02Aab	2.23±0.03Bb	2.20±0.03Bb	2.09±0.05Ba	1.92±0.04Cb	1.78±0.09Cb
	PPE3%	2.52±0.20Aa	2.35±0.03ABa	2.24±0.09ABCa	2.19±0.07BCa	2.13±0.02BCa	2.01±0.01Ca
EPA	control	0.99±0.02Ad	0.94±0.03Ac	0.78±0.008Bb	0.70±0.004 Cd	0.55±0.02Dc	0.45±0.03Ec
	PPE1%	1.12±0.02Ac	1.09±0.02Ab	1.08±0.06Aa	0.95±0.03Bc	0.91±0.01Bb	0.85±0.02Bb
	PPE2%	1.18±0.005Ab	1.12±0.003Bb	1.08±0.03Ba	0.998±0.01Cb	0.92±0.01Db	0.89±0.02Dab
	PPE3%	1.25±0.01Aa	1.22±0.01ABa	1.17±0.01Ba	1.12±0.01Ca	1.04±0.03Da	0.97±0.02Ea
DHA	control	4.15±0.04Ad	3.94±0.05Ad	2.68±0.09Bd	1.98±0.12Cd	1.23±0.15Dc	0.57±0.11Eb
	PPE1%	4.45±0.02Ac	4.36±0.03Ac	4.16±0.04Bc	4.06±0.02Bc	3.92±0.04Cb	3.79±0.09Ca
	PPE2%	4.86±0.05Ab	4.56±0.05Bb	4.37±0.03Cb	4.28±0.04Cb	4.07±0.03Db	3.89±0.08Ea
	PPE3%	5.53±0.05Aa	5.29±0.05Ba	4.92±0.04Ca	4.55±0.01Da	4.41±0.03Ea	4.05±0.16Fa

Effect of PPE on microbiological examination:

The average bacterial counts in the present study were significantly reduced compared to the zero day (P < 0.05) after freezing storage period. Variations in the values of total mesophilic count (TMC) of different treatments during freezing storage are shown in Table (11), In all treatments, the total mesophilic count (TMC) decreased during the first 2 months of storage (*P* < 0.05). The total mesophilic count (TMC) tended to increase after the second month of storage. Untreated samples had a significantly increase (*P* < 0.05) than treated samples. Total mesophilic count (TMC) in the 2%PPE and 3%PPE groups (4.23±2.79 and 3.99±3.20) were considerably lower than the control and 1% PPE groups (4.66±2.74 and 4.41±2.82) at the end of storage (P < 0.05).

Table (12) shows the changes in *Enterobacteriaceae* count levels over time during frozen storage. When compared to the values in the control group, treatment with 1, 2, and 3% PPE reduced *Enterobacteriaceae* counts (*P* < 0.05). Increased PPE concentrations increased the antibacterial activity of PPE against *Enterobacteriaceae* considerably (P < 0.05). The samples treated with 3% PPE showed the greatest reduction in bacterial growth, followed by the 2% and 1 % PPE. When compared to the growth of bacteria in the other experimental groups, 3% PPE dramatically reduced the growth of Enterobacteriaceae (*P* < 0.05).

The changes in counts of lactic acid bacteria (LAB) during the storage period for the treated and untreated fish groups are given in Table (13).

During frozen storage, LAB counts in 2%PPE and 3% PPE were considerably lower than in the control group. The highest (1.88±0.53) and lowest (1.25±0.07) LAB counts were found in the control and 3% PPE groups, respectively, at the end of storage.

Variations in the values of *pseudomonads* counts in fish samples during the frozen storage are showed in Table (14). During the storage period, the number of *pseudomonad* bacteria reduced in all treatments (*P* < 0.05). The samples treated with 3% PPE had the lowest pseudomonad bacterial counts at the end of the storage period when compared to

the control ($P < 0.05$). At the end of the storage period, the *pseudomonad* bacterial counts of the controls and samples treated with 1, 2, and 3% PPE were 2.77±1.14, 2.47±0.54, 2.14±0.76, and 1.91±0.52 log CFU/g, respectively.

The variations in values of yeast count in fish samples during the frozen storage are shown in Table (15). Yeast levels decreased in all treatments after 6 months of storage. Treatment with different concentrations of PPE reduced yeast counts compared to the control group ($P < 0.05$).

Table (11) Changes in total mesophilic bacterial count of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 ° C

Storage period	Control	1% PPE	2%PPE	3%PPE
0 day	4.74±2.89a	4.74±2.89a	4.74±2.89a	4.74±2.89a
First month	3.98±2.46 a	3.71±2.54 b	3.47±1.51 c	3.14±2.23 d
Second month	3.32±1.86 a	2.97±1.14 b	2.84±1.11 c	2.66±0.97 d
Third month	4.23±2.64 a	3.53±1.93 b	3.39±2.36 c	2.99±1.20 d
Forth month	4.46±3.07 a	4.17±2.89 b	3.99±2.56 c	3.50±2.38 d
Fifth month	4.59±2.90 a	4.32±2.51 b	4.04±2.72 c	3.83±2.25 d
Sixth month	4.66±2.74 a	4.41±2.82 b	4.23±2.79 c	3.99±3.20 d

Means within the same row of different letters are significantly different at (P < 0.05)

Table (12) Changes in *Enterobacteriaceae* count of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 ° C

Storage period	Control	1% PPE	2%PPE	3%PPE
0 day	3.50±2.38 a	3.50±2.38 a	3.50±2.38 a	3.50±2.38a
First month	3.27±2.4 a	3.15±1.67b	3.07±1.64 b	2.99±1.11b
Second month	3.17±2.25 a	2.93±1.41b	2.84±0.76 bc	2.65±1.88c
Third month	3.08±1.91 a	2.82±0.94b	2.71±1.25c	2.49±0.76d
Forth month	2.97±1.52 a	2.75±1.20b	2.68±1.45c	2.36±0.94d
Fifth month	2.91±1.46 a	2.63±1.36 b	2.55±1.64 bc	2.25± 1.20c
Sixth month	2.88±1.64 a	2.46±0.52 b	2.27±1.36 c	2.02 ±0.24d

Means within the same row of different letters are significantly different at (P < 0.05)

Table (13) Changes in Lactic acid bacteria count of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 o C

Storage period	Control	1% PPE	2%PPE	3%PPE
0 day	2.78±0.57 a	2.78±0.57 a	2.78±0.57 a	2.78±0.57 a
First month	2.65±0.52a	2.38±0.51b	2.27±0.75c	2.20±0.51d
Second month	2.51±0.58a	2.17±080b	2.11±0.76c	1.94±0.31d
Third month	2.32±0.1a	2.07±0.72b	1.90±0.43c	1.79±0.18d
Forth month	2.14±0.42a	1.93±0.43b	1.82±0.41c	1.63±0.07d
Fifth month	1.98±0.1a	1.86±0.31b	1.72±0.1c	1.46±0.06d
Sixth month	1.88±0.53a	1.77±0.1b	1.64±0.32c	1.25±0.07d

Means within the same row of different letters are significantly different at (P < 0.05)

Table (14) Changes in pseudomonad count of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 o C

Storage period	Control	1% PPE	2%PPE	3%PPE
0 day	3.32±1.11 a	3.32±1.11 a	3.32±1.11 a	3.32±1.11 a
First month	3.23±2.07 a	3.11±1.75 b	2.99±1.76 c	2.94±1.25 c
Second month	3.04±1.52 a	2.97±1.45 b	2.86±1.32 c	2.77±0.75 d
Third month	2.95±0.76 a	2.83±1.17 b	2.69±0.94 c	2.59±0.76 d
Forth month	2.91±2.09 a	2.67±1.15 b	2.46±0.08 c	2.08±0.76 d
Fifth month	2.83±1.14 a	2.59±1.15 b	2.27±0.79 c	1.94±0.22 d
Sixth month	2.77±1.14 a	2.47±0.54 b	2.14±0.76 c	1.91±0.52 d

Means within the same row of different letters are significantly different at (P < 0.05)

Table (15) Changes in yeast count of tilapia fillets treated with different concentrations of pomegranate peel extract (PPE) during 6 months of storage at -18 o C

Storage period	Control	1% PPE	2%PPE	3%PPE
0 day	2.98±1.11 a	2.98±1.11 a	2.98±1.11 a	2.98±1.11 a
First month	2.94±0.58a	2.91±1.18a	2.77±1.74b	2.55±1.64c
Second month	2.86±1.20a	2.75±1.32b	2.53±1.46c	2.20±0.98d
Third month	2.81±0.94a	2.51±1.52b	2.25±0.56c	2.08±0.76d
Forth month	2.76±1.71a	2.43±1.08b	2.07±0.57c	1.91±0.31c
Fifth month	2.69±1.39a	2.34±1.15b	1.96±0.16c	1.88±0.43c
Sixth month	2.63±1.36a	2.17±0.75b	1.91±0.08c	1.69±0.18c

Means within the same row of different letters are significantly different at (P < 0.05)

Sensory evaluation

Changes in the sensory attributes values (color, odor, texture, appearance, and total acceptability) in different tilapia fish fillets groups are given in Table (16). The quality of attributes in all samples was reduced following 6 months of storage, this was noticed by panelists. Over a freezing period, the Highest and lowest scores were obtained for satisfying color PPE classes of 1 % and 3%, respectively. Where it is observed that increasing PPE concentration decreases color quality. The color quality was better in the 1% PPE treated samples than that in the 2% and 3% PPE experimental groups.

The odor and textural quality of samples was suitable with considerable decreases in the unfavorable odors in 1% PPE, 2% PPE, and 3% PPE groups during the 5th month 1.71±0.005, 1.71±0.01, and 1.72±0.01 and 6th month 1.87±0.008, 1.83±0.008, and 1.84±0.01, respectively of storage period ($P < 0.05$) compared with control group 2.03±0.06 and 2.21±0.04 during the 5th and 6th month, respectively. The texture quality of samples was suitable during the 5th and 6th months of storage in 1% PPE, 2% PPE and 3% PPE groups ($P < 0.05$).

During the storage, the overall likeability grade in the 1% PPE treated fish samples was higher than those of the other PPE groups. The total likeability in different concentrations of PPE fish samples groups was improved. However, this was only significant in the 1% PPE group ($P < 0.05$).

Table (16). Effect of pomegranate peel extract treatment on the sensory scores of tilapia fillets during 6 months of storage at -18 °C

Storage time	treatment	Color	odor	texture	Total acceptability	appearance
First month	Control	1.38±0.015 a	1.45±0.006 a	1.69±0.008 a	2.04±0.02 a	2.16±0.01 a
	1%PPE	1.93±0.012 b	1.44±0.008 a	1.69±0.003 a	1.95±0.05 a	2.13±0.008 a
	2%PPE	1.94±0.012 b	1.47±0.005 a	1.69±0.01 a	2.36±0.008 b	2.16±0.01 a
	3%PPE	1.94±0.008 b	1.46±0.01 a	1.72±0.005 a	2.41±0.005 b	2.15±0.008 a
Second month	Control	1.63±0.008 a	1.42±0.01 a	1.72±0.008a	2.06±0.01 a	2.21±0.005 a
	1%PPE	2.02±0.005 b	1.44±0.005 a	1.69±0.01 a	2.03±0.01 a	2.22±0.008 a
	2%PPE	2.04±0.005 bc	1.44±0.008 a	1.69±0.008a	2.40±0.008 b	2.21±0.008 a
	3%PPE	2.06±0.015 c	1.44±0.005 a	1.70±0.01 a	2.41±0.008 b	2.23±0.005 a
Third month	Control	1.72±0.02 a	1.52±0.01 a	1.79±0.005 a	2.07±0.04 a	2.34±0.005 a
	1%PPE	2.11±0.01 b	1.52±0.005 a	1.57±0.008 b	2.15±0.02a	2.33±0.004 a
	2%PPE	2.15±0.008 c	1.52±0.01 a	1.76±0.008 a	2.43±0.01 b	2.33±0.01 a
	3%PPE	2.17±0.008 c	1.54±0.005 a	1.77±0.008 a	2.46±0.005 b	2.33±0.008 a
Forth month	Control	1.90±0.005 a	1.61±0.01 a	2.01±0.01 a	2.29±0.02 a	2.53±0.005 a
	1%PPE	2.12±0.003 b	1.62±0.01 a	1.62±0.01 b	2.14±0.008 b	2.52±0.004 a
	2%PPE	2.13±0.01 b	1.62±0.005 a	1.64±0.008 b	2.24±0.005 b	2.52±0.003 a
	3%PPE	2.12±0.008 b	1.63±0.02 a	2.02±0.02 a	2.29±0.008a	2.52±0.008 a
Fifth month	Control	2.11±0.003 a	2.03±0.06 a	2.11±0.008 a	2.43±.0.02	2.57±.0.005 a
	1%PPE	1.93±0.02 b	1.71±0.005 b	1.73±0.008 b	2.22±0.005 b	2.51±0.02 b
	2%PPE	2.11±0.008 a	1.71±0.01b	1.75±0.01 b	2.26±0.005 b	2.51±0.005 b
	3%PPE	2.13±0.008 a	1.72±0.01 b	1.75±0.008 b	2.25±0.008 b	2.58±0.005 a
Sixth month	Control	2.15±0.02 a	2.21±0.04 a	2.24±0.01 a	2.50±0.01 a	2.65±0.005 a
	1%PPE	1.98±0.01 b	1.87±0.008 b	1.83±0.008 b	2.31±0.01 b	2.20±0.12 b
	2%PPE	2.15±0.005 a	1.83±0.008 b	1.84±0.008 b	2.48±0.01 a	2.63±0.005 a
	3%PPE	2.17±0.01 a	1.84±0.01 b	1.85±0.01 b	2.49±0.008 a	2.65±0.008 a

Likability scores: 1 like extremely, 2 like moderately, 3 neither like nor dislike, 4 dislike. Different letters representing significant difference in each month ($P < 0.05$).

DISCUSSION

In addition to ellagitannins, gallotannins, ellagic acids, gallagic acids, catechins, anthocyanins, ferulic acids, and quercetins, pomegranates are also high in polyphenols. These polyphenols have a variety of biological effects, including the removal of free radicals, prevention of oxidation and microbial growth, reduction of the risk of cardiovascular and cerebrovascular illnesses, and prevention of some malignancies (***Mena et al., 2011; Çalışkan and Bayazit, 2012***). The results of the antioxidant activity value of PPE were similar to that of Tunisian PPEs (21.24–29.80 mmoltrolox eq./100 g) ***(Elfalleh et al., 2011).*** The primary redox characteristics of phenolics, which enable them to function as reducing agents, hydrogen donors, singlet oxygen quenchers, and possibly even have a metallic chelating potential, are what give them their antioxidant action. Additionally, the antioxidant activity in the extract depends not only on the concentration but also on the structure and interactions of the antioxidants, thanks to synergism between them ***(Seram et al., 2006).***

As a result of microbial food decomposition, base components including TVB-N and ammonium were produced and accumulated, raising the pH *(Song et al., 2011).* The pH of the samples treated with 3% PPE was the lowest at the end of the storage period, whereas the pH of the control group was the highest. The progressive increase in pH in untreated samples could be attributed to microbial action over time the storage period. Similar findings were also reported by *Basiri et al. (2015)* and *Berizi et al. (2016).*

The reduced TVB-N levels in the fish treated with 1, 2, and 3% PPE compared to the control could be due to PPE's inhibitory effect on microbes, particularly spoilage bacteria *(Kanatt et al., 2010).* During storage time, peroxide and TBARS results were significantly lower in the groups exposed to various concentrations of PPE than in the control group and therefore had greater stability towards lipid oxidation. PPE's antioxidant action inhibits superoxide hydroxyl and peroxyl, which eventually led to fat oxidation, as previously reported by *Li et al. (2006).* Moreover, Fish muscle's fatty acids underwent

oxidation processes during storage, resulting in the formation of malonaldehyde and low molecular-weight substances including aldehydes and ketones. Pomegranate peel extract also had higher levels of proanthocyanidins, flavonoids, and total phenolics. Pomegranate peel extract contains a significant amount of polyphenols, which participate in a variety of bioactivities like scavenging free radicals and thwarting oxidation *(Caliskan and Bayazit, 2012).* PPE has metal-chelating, reducing, and radical-scavenging properties, which allowed it to slow down the oxidation of fish lipids *(Berizi et al., 2016*).

According to the majority of studies, glutamic acid, aspartic acid, lysine, and leucine are the primary amino acids found in fish. Numerous variables, such as preparations before freezing, rigor at the time of freezing, freezing rate, maximum freezing temperature, storage temperature and time, variation in storage temperature, and thawing techniques, all affect the degree of protein denaturation (***Dyer, 1951; Sikorski, 1978; Acton et al., 1983)***

Protein percent decreases with increasing duration of frozen storage; low protein levels are not preferred since long-term preservation degrades the nutritional value of fish meat. Similar observations were reported for fish samples that were frozen for sixty days **(*Arannilewa et al., 2006*).** On the other hand, in the present study the amino acid (lysine, leucine) all over the storage period in groups treated with PPE2% and 3% remain at higher levels 3.11±0.01 and 3.29±0.03, respectively, than the control group which recorded 1.91±0.04 at the end of storage period. PPE 2% and 3% maintain non-significant decrease in the values of essential amino acids throughout the 6 month of the storage period the results obtained from this study show a preservative effect on fish flesh and the amino acid content of frozen fillets fish treated with 2%and 3% PPE maintained its quality and normal during 6 months of storage.

One of the best sources of polyunsaturated fatty acids for the diet is fish. Fish sensory and nutritional qualities have been preserved in great part by freezing and frozen storage **(*Erikson, 1997*).** Eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3) are the two main PUFAs. Because it is the precursor of the 3-series eicosanoids, EPA is the most significant essential fatty acid of the n3 series in the human diet **(*Chen et al., 1995*).** With increasing freezing time, polyunsaturated fatty acids decreased in comparison to saturated fatty acids. It has been proposed that the EPA + DHAC16 ratio is a reliable indicator of lipid oxidation **(*Jeong et al., 1990).*** According to this study, has decreased during frozen storage. The same outcome was discovered in oysters ***(Jeong et al., 1990).*** This ratio has a negative association with storage period, demonstrating that oxidation mechanisms are active throughout frozen storage. DHA values recorded significant decrease from 4.15±0.04 to 0.57±0.11 in the control group but the 2% and 3% PPE groups showed no significant decrease as compared to the first month of storage (4.05±0.16) these results were due to using PPE as natural preservative. Our results are in agreement with ***(Shahidi and Naczk, 2004),*** who reported that edible film and coating with PPE has a gas barrier property and resistant to oxygen diffusion and may retard lipid oxidation and degradation of PUFA induced by auto oxidation. During frozen storage of fish oils especially in fatty fish leads to the formation of volatiles associated with rancidity ***(Pazos et al., 2005).***

The fish muscle is sterile when caught, but is quickly contaminated by surface as well as intestinal bacteria, along with contamination from the aquatic **(*Tarkhasi, 2016*).** The average bacterial counts in the present study were significantly reduced compared to the zero day ($P < 0.05$) after freezing storage period. It's possible that up to 60% of the bacteria die during the freezing procedure **(*Rahman and Valez-Ruiz, 2007*)**, which explains the lower microbiological result than the zero day. When the temperature is dropped to the freezing zone, ice crystal formation damages the cell membrane of bacteria, according to **(*Rahman and Valez-Ruiz, 2007*).** Important cell-internal chemicals such as potassium ions or RNA leak out as a result, and the microorganisms' viability suffers. Furthermore, the cells may die as a result of osmotic dehydration.

The results of bacterial and yeast count confirmed the antibacterial properties of PPE and the antibacterial quality of PPE increases as the extract concentration increases, as the best concentration in reducing microbial growth was 3% PPE, followed by the 2 and 1% groups. Our findings revealed that 3% PPE significantly lower the growth of microbes compared with the growth in the other treated groups ($P < 0.05$).

PPE polyphenols, particularly tannins, are the most abundant components in the PPE extract and have been linked to antimicrobial activity (antiviral, antifungal, and antibacterial) **(*Miguel et al., 2010*).** It's been suggested that phenolic chemicals breakdown cell wall protein, disrupt the cytoplasmic membrane, and interfere with membrane-integrated enzymes **(*Shan et al., 2007*).** It has also been claimed that tannins' antibacterial effect is owing to their capacity to precipitate proteins, causing leakage of the microorganism's cell membrane and assisting cell lysis and death ***(Endo et al. 2010). Furneri et al. (2002)*** reported that the antibacterial activity of pomegranate peels might be indicative to the presence of some metabolic toxins or broad-spectrum antibiotic compounds. There are similar reports were studied previously about antimicrobial effect of PPE (***Berizi et al., 2016*),** who investigated the effect of PPE on the quality of trout for 6 months at -18 °C. At the end of storage, the lowest bacterial counts were detected in trout to which PPE was applied. ***Zhuang et al. (2019)*** investigated that PPE prolonged the shelf-life of bighead carp fillets for about 2 days and PPE decreased the relative abundance of Acinetobacter in the middle-period of storage, and thus changed the microbial composition.

In their study of the combined impacts of chitosan and pomegranate peel extract on the general quality of rainbow trout during the frozen storage, ***Berizi et al. (2018)*** showed that the numbers of psychrophilic, LAB, and molds in the Ch + 4%PPE were reduced to 2, 3, and 0.5 log, respectively. On the other hand, ***Rosas-Burgos et al. (2017)*** found that the peel of pomegranate fruits contains antifungal and antibacterial substances such as ellagic acid and punicalagins (α and β), which could be used as a natural alternative to synthetic antimicrobial agents. ***El-Nashi et al. (2015), Agourram et al. (2013),*** examined the antibacterial activity of pomegranate peels and they discovered that pomegranate peels inhibit both gram positive and gram-negative microorganisms.

However, as the PPE concentration increased, the overall likeability decreased. The increase in unfavorable colour and smell of the samples treated with greater concentrations of PPE was unwelcome given that pomegranate peel extract is an aromatic and colored ingredient. Because PPE is used to protect fish from putrefactive bacteria and to reduce fat oxidation, sensory assessment scores declined as PPE concentrations increased, potentially as a result of unfavorable improvements. These improvements helped the PPE samples as a whole. Although the product's aromatic properties and texture were improved at the higher concentrations, there was parallel decrease in general acceptability. Finally, the results showed the greatest scores on the overall acceptance to the color and odor were seen in the 1% PPE treated samples.

CONCLUSION

As a conclusion, MPPE is recommended as a natural agent to improve chemical, microbial and sensory properties of frozen fillet fish during 6-month storage.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Acton, J. C., Ziegler, G. R., & Burge, Jr., D. L. (1983). CRC Crit. Rev. ood.sci. Nutri.N.J.,18(2),99. https://doi.org/10.1080/10408398209527360

Agourram, A., Ghirardello, D., Rantsiou, K., Zeppa, G., Belviso, S., Romane, A., ...& Giordano, M. (2013). Phenolic content, antioxidant potential, and antimicrobial activities of fruit and vegetable by-product extracts. International Journal of Food Properties, 16(5), 1092-1104. https://doi.org/10.1080/10942912.2011.576446

Al-Zoreky, N. S. (2009). Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. International journal of food microbiology, 134(3), 244-248. https://doi.org/10.1016/j.ijfoodmicro.2009.07.002

AOAC. (1990). Official Methods of Analysis of the Association of Official Analytical chemists, 3rd.ed. Washingt.

AOAC. (1990). Official Methods of Analysis of the Association of Official.

AOAC. (2000). Official Methods of Analysis of the Association of Official Analytical chemists, NO.994.12. Chapter4, 18-19.19th Edition.

AOAC. (2012). Official Methods of Analysis of the Association of Official Analytical chemists,17 Ed. 969.3 and 991.39 fatty acids in oils and fats preparation of methyl esters Boron Tri fluorid- AOAc-IUPAC Method Codex-Adopted-AOAC Method.Chapter41 ,19-20.

Arannilewa, S. T., Salawu, S. O., Sorungbe, A. A., & Ola-Salawu, B. B. (2006). Effect of frozen period on the chemical, microbiological and sensory quality of frozen Tilapia fish (Sarotherodun galiaenus). Nutrition and health, 18(2), 185-192. https://doi.org/10.1177/026010600601800210

Basiri, S., Shekarforoush, S. S., Aminlari, M., & Akbari, S. (2015). The effect of pomegranate peel extract (PPE) on the polyphenol oxidase (PPO) and quality of Pacific white shrimp (Litopenaeus vannamei) during refrigerated storage. LWT-Food Science and Technology, 60(2), 1025-1033. https://doi.org/10.1016/j.lwt.2014.10.043

Berizi, E., Hosseinzadeh, S., Shekarforoush, S. S., & Barbieri, G. (2018). Microbial, chemical, textural and sensory properties of coated rainbow trout by chitosan combined with pomegranate peel extract during frozen storage. International journal of biological macromolecules, 106, 1004-1013. https://doi.org/10.1016/j.ijbiomac.2017.08.099

Berizi, E., Shekarforoush, S. S., & Hosseinzadeh, S. (2016). Effects of methanolic pomegranate peel extract on the chemical, sensory, textural, and microbiological properties of gutted rainbow trout (Oncorhynchus mykiss) during frozen storage. Journal of Food Protection, 79(10), 1700-1706. https://doi.org/10.4315/0362-028X.JFP-16-047

Caliskan, O., & Bayazit, S. (2012). Phytochemical and antioxidant attributes of autochthonous Turkish pomegranates. Scientia Horticulturae, 147, 81-88. https://doi.org/10.1016/j.scienta.2012.08.032

Chauhan, P., Pradhan, S. R., Das, A., Nanda, P. K., Bandyopadhyay, S., & Das, A. K. (2019). Inhibition of lipid and protein oxidation in raw ground pork by Terminalia arjuna fruit extract during refrigerated storage. Asian-Australasian Journal of Animal Sciences, 32(2), 265. Doi: 10.5713/ajas.17.0882

Chen, I. C., Chapman, F. A., WEI, C. L., Portier, K. M., & O'keefe, S. F. (1995). Differentiation of cultured and wild sturgeon (Acipenser oxyrinchus desotoi) based on fatty acid composition. Journal of Food Science, 60(3), 631-635. https://doi.org/10.1111/j.1365-2621.1995.tb09844.x

Connell, J. J. (1990). Methods of assessing and selecting for quality. Control of fish quality, 2, 122-150.

Dahham, S. S., Ali, M. N., Tabassum, H., & Khan, M. (2010). Studies on antibacterial and antifungal activity of pomegranate (Punica granatum L.). Am. Eurasian J. Agric. Environ. Sci, 9(3), 273-281. https://portal.arid.my/Publications/148b7c19-cf2e-4e8.pdf

Dyer, W. J. (1951). PROTEIN DENATURATION IN FROZEN AND STORED FISH a. Journal of Food Science, 16(1-6), 522-527. https://doi.org/10.1111/j.1365-2621.1951.tb17416.x

Elfalleh, W., Tlili, N., Nasri, N., Yahia, Y., Hannachi, H., Chaira, N., Ying, M., & Ferchichi, A. (2011). Antioxidant capacities of phenolic compounds and tocopherols from Tunisian pomegranate (Punica granatum) fruits. Journal of food science, 76(5), C707-C713. https://doi.org/10.1111/j.1750-3841.2011.02179.x

El-Hadary, A. E., & Taha, M. (2020). Pomegranate peel methanolic-extract improves the shelf-life of edible-oils under accelerated oxidation conditions. Food Science & Nutrition, 8(4), 1798-1811. https://doi.org/10.1002/fsn3.1391

El-Nashi, H. B., Fattah, A. F. A. K. A., Rahman, N. R. A., & Abd El-Razik, M. M. (2015). Quality characteristics of beef sausage containing pomegranate peels during refrigerated storage. Annals of Agricultural Sciences, 60(2), 403-412. https://doi.org/10.1016/j.aoas.2015.10.002

Endo, E. H., Cortez, D. A. G., Ueda-Nakamura, T., Nakamura, C. V., & Dias Filho, B. P. (2010). Potent antifungal activity of extracts and pure compound isolated from pomegranate peels and synergism with fluconazole against Candida albicans. Research in Microbiology, 161(7), 534-540. https://doi.org/10.1016/j.resmic.2010.05.002

Erickson, M. C. (1997). Lipid oxidation: Flavor and nutritional quality deterioration in frozen foods. In Quality in frozen food (pp. 141-173). Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-5975-7_9

Badria, F. A., Ameen, M., & Akl, M. R. (2007). Evaluation of cytotoxic compounds from Calligonum comosum L. growing in Egypt. Zeitschrift für Naturforschung C, 62(9-10), 656-660. https://doi.org/10.1515/znc-2007-9-1005

Furneri, P. M., Marino, A., Saija, A., Uccella, N., & Bisignano, G. (2002). In vitro antimycoplasmal activity of oleuropein. International journal of antimicrobial agents, 20(4), 293-296. https://doi.org/10.1016/S0924-8579(02)00181-4

Gray, J. I. (1978). Measurement of lipid oxidation: a review. Journal of the American Oil Chemists' Society, 55(6), 539-546. https://doi.org/10.1007/BF02668066

Gullon, B., Pintado, M. E., Pérez-Álvarez, J. A., & Viuda-Martos, M. (2016). Assessment of polyphenolic profile and antibacterial activity of pomegranate peel (Punica granatum) flour obtained from co-product of juice extraction. Food control, 59, 94-98. https://doi.org/10.1016/j.foodcont.2015.05.025

Gullón, P., Astray, G., Gullón, B., Tomasevic, I., & Lorenzo, J. M. (2020). Pomegranate peel as suitable source of high-added value bioactives: Tailored functionalized meat products. Molecules, 25(12), 2859. https://doi.org/10.3390/molecules25122859

Iqbal, S., Haleem, S., Akhtar, M., Zia-ul-Haq, M., & Akbar, J. (2008). Efficiency of pomegranate peel extracts in stabilization of sunflower oil under accelerated conditions. Food Research International, 41(2), 194-200. https://doi.org/10.1016/j.foodres.2007.11.005

Gutteridge, J. M., Rowley, D. A., & Halliwell, B. (1981). Superoxide-dependent formation of hydroxyl radicals in the presence of iron salts. Detection of 'free'iron in biological systems by using bleomycin-dependent degradation of DNA. Biochemical Journal, 199(1), 263-265. https://doi.org/10.1042/bj1990263

J**eong, B. Y., Oshima, T., Koizumi, C., & Kanou, Y. (1990).** Lipid deterioration and its inhibition of Japanese oyster (Crasostrea gigas) during frozen storage. Nippon Suisan Gakkaishi56, 2083–2091. https://preview.kstudy.com/W_files/kiss10/71500161_pv.pdf

Kanatt, S. R., Chander, R., & Sharma, A. (2010). Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. International journal of food science & technology, 45(2), 216-222. https://doi.org/10.1111/j.1365-2621.2009.02124.x

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food chemistry, 96(2), 254-260. https://doi.org/10.1016/j.foodchem.2005.02.033

Lytou, A. E., Nychas, G. J. E., & Panagou, E. Z. (2018). Effect of pomegranate-based marinades on the microbiological, chemical and sensory quality of chicken meat: A metabolomics approach. International journal of food microbiology, 267, 42-53. https://doi.org/10.1016/j.ijfoodmicro.2017.12.023

Madane, P., Das, A. K., Nanda, P. K., Bandyopadhyay, S., Jagtap, P., Shewalkar, A., & Maity, B. (2020). Dragon fruit (Hylocereus undatus) peel as antioxidant dietary fibre on quality and lipid oxidation of chicken nuggets. Journal of food science and technology, 57(4), 1449-1461. https://doi.org/10.1007/s13197-019-04180-z

Madane, P., Das, A. K., Pateiro, M., Nanda, P. K., Bandyopadhyay, S., Jagtap, P., Barba, F.J., Shewalkar, A., Maity, B.& Lorenzo, J. M. (2019). Drumstick (Moringa oleifera) flower as an antioxidant dietary fibre in chicken meat nuggets. Foods, 8(8), 307. https://doi.org/10.3390/foods8080307

Chaijan, M., Benjakul, S., Visessanguan, W., & Faustman, C. (2005). Changes of pigments and color in sardine (Sardinella

gibbosa) and mackerel (Rastrelliger kanagurta) muscle during iced storage. Food chemistry, 93(4), 607-617. https://doi.org/10.1016/j.foodchem.2004.10.03 5

McCarrell, E. M., Gould, S. W., Fielder, M. D., Kelly, A. F., El Sankary, W., & Naughton, D. P. (2008). Antimicrobial activities of pomegranate rind extracts: enhancement by addition of metal salts and vitamin C. BMC Complementary and Alternative Medicine, 8(1), 1-7. https://doi.org/10.1186/1472-6882-8-64

McFaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria, (7ndedn) Baltimore, Williams and Wilkins. https://agris.fao.org/agris-search/search.do?recordID=US201300530081

Meléndez, P. A., & Capriles, V. A. (2006). Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine, 13(4), 272-276. https://doi.org/10.1016/j.phymed.2004.11.009

Mena, P., García-Viguera, C., Navarro-Rico, J., Moreno, D. A., Bartual, J., Saura, D., & Martí, N. (2011). Phytochemical characterisation for industrial use of pomegranate (Punica granatum L.) cultivars grown in Spain. Journal of the Science of Food and Agriculture, 91(10), 1893-1906. https://doi.org/10.1002/jsfa.4411

Miguel, M. G., Neves, M. A., & Antunes, M. D. (2010). Pomegranate (Punica granatum L.): A medicinal plant with myriad biological properties-A short review. Journal of Medicinal Plants Research, 4(25), 2836-2847. http://hdl.handle.net/10400.1/6275

Özalp Özen, B., & Soyer, A. (2018). Effect of plant extracts on lipid and protein oxidation of mackerel (Scomber scombrus) mince during frozen storage. Journal of food science and technology, 55(1), 120-127. https://doi.org/10.1007/s13197-017-2847-6

Pacheco-Aguilar, R., Lugo-Sánchez, M. E., & Robles-Burgueño, M. R. (2000). Postmortem biochemical and functional characteristic of Monterey sardine muscle stored at 0 C. Journal of Food Science, 65(1), 40-47. https://doi.org/10.1111/j.1365-2621.2000.tb15953.x

Pazos, M., Gallardo, J. M., Torres, J. L., & Medina, I. (2005). Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. Food Chemistry, 92(3), 547-557. https://doi.org/10.1016/j.foodchem.2004.07.036

Pearson, D. (1981). The Chemical analysis of food, (8th Ed.). J. A Churchill, London, pp: 535. https://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1795104

Rahman, M. S., & Valez-Ruiz, J. F. (2007). Food preservation by freezing. Pages 635-665 in Handbook of Food Preservation. M. S. Rahman ed. CRC Press, Boca Raton, FL. Reichel.

Rosas-Burgos, E. C., Burgos-Hernández, A., Noguera-Artiaga, L., Kačániová, M., Hernández-García, F., Cárdenas-López, J. L., & Carbonell-Barrachina, Á. A. (2017). Antimicrobial activity of pomegranate peel extracts as affected by cultivar. Journal of the Science of Food and Agriculture, 97(3), 802–810. https://doi.org/10.1002/jsfa.7799

Bridges, S. M., & Salin, M. L. (1981). Distribution of iron-containing superoxide dismutase in vascular plants. Plant Physiology, 68(2), 275-278. https://doi.org/10.1104/pp.68.2.275

Saad, H., Bouhtoury, C.E., Pizzi, A., Rode, K., Charrier, B., & Ayed, N. (2012). Characterization of pomegranate peels tannin extractives. Ind Crop Prod 40:239–246. https://doi.org/10.1016/j.indcrop.2012.02.038

Salem, M.A.A. (2015): Studies on Some Predominant Bacterial Diseases in Some Fresh Water Fishes in Dakahlian Farms. M V Sc. Thesis, Faculty of Veterinary Medicine, Mansoura University, Egypt.

Seram, N., Schulman, R.N., & Heber, D. (2006). Pomegranates: ancient roots to modern medicine. CRC Press/Taylor & Francis, Boca Raton. https://books.google.com.eg/books?hl=ar&lr=&id=3ofMBQAAQBAJ&oi=fnd&pg=PP8&dq=Pomegranates:+ancient+roots+to+modern+medicine.+CRC+Press/Taylor+%26+Francis,+Boca+Raton.+&ots=RrlMiKyRoO&sig=XjOS1QhjrRcL4uFBE82tlEzolVg&redir_esc=y#v=onepage&q=Pomegranates%3A%20ancient%20roots%20to%20modern%20medicine.%20CRC%20Press%2FTaylor%20%26%20Francis%2C%20Boca%20Raton.&f=false

Shahidi, F., & Naczk, M. (2004). Phenolics in food and nutraceuticals. Boca Raton. FL: CRC Press. https://doi.org/10.1201/9780203508732

Shan, B., Cai, Y.Z., Brooks, J.D. & Corke, H. (2007). The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology. 117(1):112 – 9. https://doi.org/10.1016/j.ijfoodmicro.2007.03.003

Shiban, M.S., Al-Otaibi, M.M., & Al-Zoreky, N.S. (2012). Antioxidant activity of pomegranate (Punica granatum L.) fruit peels. Food and Nutrition Sciences; 3:991-996. DOI:10.4236/fns.2012.37131

Sikorski, Z. (1978). Int. J. Refrigeration, 1 (3), 173. https://doi.org/10.1016/0140-7007 (78)90094-4

Song, Y., Liu, L., Shen, H., You, J., & Luo, Y. (2011). Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (Megalobrama amblycephala). Food control, 22(3-4), 608-615. https://doi.org/10.1016/j.foodcont.2010.10.012

Tabaraki, R., Heidarizadi, E., & Benvidi, A. (2012). Optimization of ultrasonicassisted extraction of pomegranate (Punica granatumL.) peel antioxidants by response surface

methodology. Sep Purif Technol. https://doi.org/10.1016/j.seppur.2012.06.038

Tarkhasi, A. (2016). Effect of edible coating containing pomegranate peel extract on quality and shelf life of silver carp (Hypophthalmichthys molitrix) fillet during refrigerated storage. J. Food Ind. Microbiol, 2(2). doi:10.4172/2572-4134.1000112

Vilavert, L., Borrell, F., Nadal, M., Jacobs, S., Minnens, F., Verbeke, W., Marques, A., & Domingo, J.L. (2017). Health risk/benefit information for consumers of fish and shellfish: Fish Choice, a new online tool. Food Chem. Toxicol., 104, 79–84. https://doi.org/10.1016/j.fct.2017.02.004

Wafa, B.A., Makni, M., Ammar, S., Khannous, L., Ben Hassana, A., Bouaziz, M., Es-Safi, N.E., & Gdoura, R., (2017). Antimicrobial effect of the Tunisian Nana variety Punica granatum L. extracts against Salmonella enterica (serovars Kentucky and Enteritidis) isolated from chicken meat and phenolic composition of its peel extract. Int. J. Food Microbiol. 241, 123–131.

Zhuang, S., Li, Y., Jia, S., Hong, H., Liu, Y., & Luo, Y. (2019). Effects of pomegranate peel extract on quality and microbiota composition of bighead carp (Aristichthys nobilis) fillets during chilled storage. Food Microbiology, 82, 445-454.

Cite this Paper

Eman T. Al-Sokary, Gehan I. E. Ali, Mona E. Abass, Shereen A. Yassin, and Engy F. El-bahy (2023). Effective Role of Pomegranate Peel Extract on Quality of Tilapia Fillets During Storage. MJVM., Vol. (3), Issue (1) Special Issue of 2nd Sci Conference of Fac Vet Med Matrouh University, pages (19-31). **DOI:10.21608/MJVM.2022.180284.1014.**

About the Journal

Matrouh Journal of Veterinary Medicine (MJVM)
The official journal of the faculty of veterinary medicine, Matrouh University, Egypt.
Publisher: *Matrouh University, Egypt.*
ISSN (Online):2735-458X
ISSN (Print): 2735-4903
Indexed in EKB Database