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Efficacy of Parsley Oil on Bacteriological Quality and Their Deteriorative Changes of Retail Tilapia Fillets

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Abstract | The present study aimed to investigate the effects of Parsley Essential Oil (PEO) on the chemical parameters (pH, TVN, and TBA) and bacteriological quality of fish fillets during refrigerated storage. So, 100 fillets of Nile tilapia were bought and split into 4 groups: the first group was used as a control, and the second, third, and fourth groups were given different amounts of PEO (1, 2, and 3%), respectively, to see how they affected different chemical parameters, the amount of biogenic amines, and the bacteria levels while the fish was stored in the fridge at 4°C for 8 days. Results of the study show that adding different amounts of PEO (1, 2, and 3%) to fish fillets improved their taste and texture. After treating the fillets with 3% PEO, pH levels were significantly ($p< 0.05$) dropped by 22.06%, TVN levels dropped by 62.55%, and TBA levels dropped by 82.75%. The most significant reduction in biogenic amines was observed in tyramine, which decreased by 89.04%. This was followed by reductions in putrescine, cadaverine, and histamine, respectively, at the end of the experiment using 3% PEO. Regarding the microbiological status, the results were dose-dependent, showing that higher concentrations of PEO, especially 3%, led to greater reduction percentages. The highest reduction, reaching 100%, was observed in the counts of Enterobacteriaceae and coliforms. The study concluded that using parsley essential oils at varying concentrations, particularly at 3%, could extend the shelf life of fish up to 8 days during refrigeration, specifically due to to their antioxidant and antibacterial properties.

Keywords: Parsley oil-Fish fillet, Biogenic amine, Enterobacteriaceae, Coliform

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INTRODUCTION

Fish plays a vital role in providing humans with essential dietary protein, contributing to more than 20% of the global average per capita consumption of animal proteins (Zhang *et al*., 2020). Being highly perishable, fish requires careful storage and handling to maintain its quality and freshness until cooking and consumption. The tilapia fish is one of the world's most widely farmed fish species. Its popularity in aquaculture makes it a relevant subject for re-

search aimed at improving fish quality and shelf life, and it was selected for this study because of its economic relevance.

Monitoring the fish muscle's pH indicates the physical changes occurring in the fish muscle throughout storage (Izumi, 2012). Also, volatile amines like total volatile nitrogen and trimethylamine give fish their unique smell and taste. These amines stay in the fish for several days after it is caught and are thought to be an important way to judge their quality (Etienne, 2005).

An important group of nitrogenous compounds called biologic amines are made when amino acids are decarboxylated or when aldehydes and ketones are aminated and transaminated. They are produced by raw material enzymes and microbial decarboxylation of amino acids (Visciano *et al*., 2020). Several genera of bacteria in the Enterobacteriaceae family, such as Citrobacter, Klebsiella, Escherichia, Proteus, Salmonella, and Shigella, have been linked to the production of large amounts of putrescine, cadaverine, and histamine in meats and fish products (Erdag *et al*., 2018). Additionally, Vibrionaceae, lactic acid bacteria, species of Pseudomonas, and Clostridia may also play a role in this process (Feddern *et al*., 2019). Histamine is the most significant and extensively researched amine among all biogenic amines, and it holds critical toxicological importance because it serves as the causative agent of scombroid fish poisoning and food intolerance (Comas-Basté *et al*., 2020). Furthermore, cadaverine and putrescine have been found to enhance histamine toxicity (Ruiz-Capillas and Herrero, 2019). Histamine and tyramine can lead to vasoactive and psychoactive health issues, including nausea, headaches, skin rashes, and variations in blood pressure, as well as allergic reactions (Doeun *et al*., 2017).

Microbial growth is the primary contributor to fish deterioration, significantly impacting the quality of fresh or lightly preserved varieties. Initially, fish muscles are sterile, but they become contaminated by microbes from the skin post-mortem. The high activity in the water, the low acidity (pH > 6), and the large amount of non-protein nitrogen compounds in fish all help microbes grow quickly, which changes the taste, texture, appearance, and smell of the fish in a bad way (Sperber and Doyle, 2010).

The investigation into natural preservatives is growing due to consumer concerns about the potential health risks associated with synthetic preservatives. Essential oils, aromatic plant extracts, contain bioactive components with various biological properties, including antioxidant, insecticidal, and antimicrobial effects (Saleh et al., 2019). Parsley, a member of the Umbelliferae family, is extensively cultivated across various regions, and its essential oil has garnered attention for its potential as a natural preservative. Parsley essential oil is extracted from all parts of the plant, with a focus on the leaves. It has a flavor that reminds us of a fresh herb. Recent research has identified its bioactive constituents, including α-pinene, D-limonene, myristicin, and oleic acid. These constituents contribute to the antioxidant properties of parsley essential oil, as reported by Abdellatief *et al*. (2017) and Farag *et al*. (2021). The high FRAP and DPPH radical inhibition values of parsley essential oil (PEO) show that it is a strong antioxidant. Additionally, it shows significant antibacterial activity. Myristicin (36.15%), apiole (20.97%), α-pinene (15.47%), and α-pinene (10.43%) are the main ingredients in parsley essential oil. Other important ingredients are alkyltetramethoxybenzene (6.45%), limonene (4.74%), and elemicin (2.74%) (Marin *et al*., 2016). Furthermore, the use of PEO successfully suppressed the generation of biogenic amines in fish samples (Özogul *et al*., 2010). However, the effects of PEO in extending the shelf life of the fish received less attention.

The current study aimed to investigate the effect of parsley oil on maintaining quality parameters (pH, TVN, and TBA), biogenic amine content, and bacteriological status in tilapia fillets during refrigerated storage.

MATERIALS AND METHODS

Preparation of Parsley Essential Oil (PEO) According to Karimi *et al*. (2014)

500 grams of dried leaves and seeds of parsley were purchased from a reputable grocery store in Damanhour City and were prepared at the lab of food hygiene, Animal Health Research Institute, Damanhur branch, by dipping in 400 ml of cold distilled water in a volumetric flask (2L) in hydrodistilled water for 3 hours using a Clevenger-type apparatus that is connected to a water condenser placed over a heating mantle. The parsley's essential oil yield can range from 0.5% to 2% of the dry weight. The oils of parsley used in this study were in pure state and were stored in a sealed dark amber glass bottle at 4°C until subsequent tests. The extracted parsley oils were added to fish fillet at three different concentrations (1, 2, and 3%).

Collection, Preparation, and Treatment of Fish Fillet with PEO According to Khalafalla *et al*. (2015).

About 60 Nile tilapia (*Oreochromis niloticus*) fish samples (average weight 350-500 g) were purchased from a fish market in Damanhour City, Egypt, on the same day of harvesting. Each sample was gutted, deheaded, cleaned, and filleted into two pieces of about 100 g weight per piece (60 fish fillet samples). The samples were then wrapped in sterile polyethylene bags and transferred directly to the laboratory in a sterile icebox for further preparation within 2 hours after purchase. The samples were divided into

4 groups; the 1st was untreated control (C), and 3 groups were dipped in three concentrations of PEO (1, 2, and 3%) for 10 minutes inside a refrigerator, then the dipping solution was discarded. Treated fish fillet samples were labeled and aerobically packaged in triplicates inside fiber dishes, then stored at 4 ˚C in the refrigerator. The treated groups were subjected to chemical and microbiological assessment at day zero (within 2 h after treatment) and then periodically every 2 days until decomposition (0, 2nd, 4th, 6th, and 8th). The experiment replicates three times.

Sensory Evaluation of Fish Fillets According to Kenar *et al*. (2010)

For each concentration, seven adults who were trained but unaware of the experimental approach were given 100 ± 10 grams of fish fillet. The samples were coded with a specific number, and the panelists were asked to rate the overall acceptance while they were fresh (uncooked). After that, both the control fish fillet and the treated fillets with varying concentrations of parsley essential oil (PEO) were cooked and presented to the panelists for sensory evaluation. To cleanse their palate between samples, the panelists drank warm water, and a 9-point scale was utilized to assess each attribute, where a score of 9 corresponded to "very good quality," scores of 7-8 to "good quality," and a score of 6 to "sufficient quality." The value equal to 5 represented the acceptability threshold, while a score of 1-4 corresponded to "unacceptable quality." For each fillet sample, the total sensory evaluation score (the average of sensory quality attributes) was calculated and submitted.

Chemical Analysis of Treated Fish Fillet

Potential of hydrogen ion concentration (pH) measurement according to EOS, 63-11/ (2020): Ten milliliters of neutralized distilled water and 10 grams of fish samples were mixed together. After shaking the mixture continuously at room temperature for 10 minutes, it was left to stand. The pH value was then measured using a pH electrical meter (Bye Model 6020, USA). The pH meter was calibrated using two buffer solutions with accurately defined pH values (alkaline pH 7.01, acidic pH 4.01). Neutralized water was used to clean the pH electrode, which was then introduced to the samples after the temperature correction system was adjusted.

Determination of total volatile nitrogen "TVN" according to EOS: 63-9/ (2006): In a clean distillation flask, 300 ml of distilled water and 10 grams of fish samples were thoroughly mixed. The resulting mixture was then enriched with two grams of magnesium oxide and an anti-foaming agent. Subsequently, 25 ml of 2% boric acid and a few drops of the indicator were added to a 500 ml receiving flask, and the receiver tube was positioned so that it extended below the boric acid solution. Within 10 minutes, the distillation flask reached boiling temperature, and distillation continued for an additional 25 minutes. Following this, a titration of TVN (total volatile nitrogen) against H2SO4 M 0.1 was carried out until a pink color appeared. TVN was calculated according to the following formula:

TVN/l00grams = (mls H2 So4 n 0.1 for sample – ml H2 So4 n 0.1 for blank) x 14

Determination of thiobarbituric acid number "TBA" according to EOS: 63-10/ (2006): The test relies on measuring malonaldehyde (MDA) as a byproduct of lipid peroxidation. To summarize, 50 ml of distilled water was mixed with ten grams of prepared fish samples and transferred to a distillation flask. An antifoaming agent and 50 ml of diluted hydrochloric acid were then added to the flask. Within 10 minutes of reaching boiling, the distillation flask was heated to distill 50 ml of diluted hydrochloric acid. Subsequently, 5 ml of the distilled solution was placed in a covered tube, and 5 ml of prepared thiobarbituric acid was added. The tube was covered, placed in a water bath, boiled for 35 minutes, and then cooled with water for 10 minutes. The sample's absorbance at 538 nm was measured using a spectrophotometer (UNICAM 969AA Spectronic, USA).

TBA value = absorbance of sample x 7.8 (malonaldehyde (mg)/ kg).

Determining the Concentration of Biogenic Amines using HPLC

Using the method suggested by Pinho *et al*. (2001) and Magwamba *et al*. (2010), four biogenic amines were found in chilled fish that had been treated with different amounts of parsley oil. These were histamine (HIS), tyramine (TYR), putrescine (PUT), and cadaverine (CAD).

Amine extraction: 25 g of treated fish samples were homogenized with 125 ml of 5% trichloroacetic acid for 3 min. using a warning blender. Then, we achieved filtration using Whatman No. 1 filter paper and transferred 10 ml of the filtrate into a glass tube containing 4 g of NaCl and 1 ml of 50% NaOH. We then sacked and extracted three times, using 5 ml of n-butanol: chloroform (1:1 v/v), before stopping and sacking vigorously for 2 minutes. Finally, we centrifuged for 5 minutes at 3000 rpm. The upper layer was transferred to a 50-ml separating funnel using a disposable Pasteur pipette. The organic extracts (upper layer) were mixed with 15 ml of n-heptane, which was then extracted three times with 0.2 ml of n-HCL. The n-HCL layer was then collected in a glass plug tube. Solution was evaporated just to dryness using a water bath at 95˚C with the aid of air currents.

Formation of dansyl amines: 100 µl of each stock standard solution was transferred to a vial (50 ml) and dried under vacuum. About 0.5 ml of saturated NaHCO3 solution was added to the residue of the sample extract (or the standard), the vial was stoppered, and its content was carefully mixed to prevent loss due to spattering. Carefully, a 1.0 mL of dansyl chloride solution was added and mixed thoroughly using a vortex mixer. The reaction mixture was incubated at 55° for 45 min., then 10 ml of distilled water was added to the reaction mixture and the vial was stopped and shaken vigorously using the Vortex mixer. Three times, 5 mL of diethyl ether was used to extract dansylated biogenic amines. Again, the vial was stopped, and then shacked carefully for 1 minute, and the ether layers were collected in a culture tube using a disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35 °C in a dry bath with the aid of air current. The obtained dry film was dissolved in 1 mL of methanol, and then 10 µl was injected in HPLC.

Bacteriological examination of treated fish fillet with PEO

The fish fillet treated with PEO underwent a bacteriological examination.

We weighed 10 grams of treated fish fillet samples in a clean environment and mixed them for one minute in a lab blender with 90 ml of 0.1% sterile peptone water (Oxide CM0009) to make a 1:10 original dilution. Ten-fold serial dilutions up to 106 were made to cover the range of sample contamination that was expected. Plates with plate count agar were kept at 30 °C for 48 hours to get an idea of the total aerobic bacterial count, which is what ISO 4833:2003 says must be done. For psychrotrophic counting, surface plating on plate count agar was used, and the plates were incubated at 6.5 °C for 10 days as required by ISO 17410:2019. As per ISO 21528-2 (2017), violet red bile glucose agar medium was used to count enterobacteriaceae, and plates were kept at 37°C for 24 hours. Coliforms were counted on violet red bile agar medium (VRB), and the plates were kept at 37°C for 48 hours, as required by ISO 7218/(2007).

Statistical Analysis

To statistically analyze the data, we used the statistical analysis system (SAS, 2014), Cary, USA, Version 9.3 software. The mean and standard deviation (SD) of the organoleptic, chemical, and bacteriological parameters were displayed. Tukey's Kramer (HSD) post-hoc test ($p > 0.05$) and a nested procedural model ($p<0.05$) were used to compare significant means.

RESULTS AND DISCUSSION

As shown in Table 1, the control fish fillet sample was completely spoiled after the sixth day of storage at 4 °C. Ivanov *et al.* (2015) reported that the fillet sample with a total sensory evaluation score lower than 3 was evaluated as unacceptable for consumption. The fish fillet samples that were treated with different amounts of parsley essential oil (1%, 2%, and 3%) were more well-received overall during the whole cold storage period. On the eighth day of the experiment, the 3% PEO treatment got the highest score of 6.33, which is higher than the acceptable level of 5. In contrast, the control group scored only 3.33 on day 4, which is unacceptable. Among the treated fish fillet samples, those treated with 3% parsley demonstrated the highest acceptability, while the samples treated with 1% parsley essential oil (PEO) had the lowest acceptability compared to the control samples. Our findings showed that PEO enhanced the overall acceptability of fish fillets, with the results varying depending on the concentration; these findings were consistent with previous research. According to Riel *et al.*, (2017), the different concentrations of parsley extract powder (1.07, 2.14 g, and 4.29 g/kg sausage) improved the overall acceptance of the treated sausages during storage. In addition, Badee *et al.* (2020) reported that parsley essential oil at concentrations of 600 ppm advanced sensory characteristics and improved the wholesomeness of the beef burger during refrigeration storage.

Table 1: Pattern of general acceptance of *Tilapia niloticus* fillet treated with variant concentrations of Parsley oils throughout chilling storage duration at 4°C.

Storage period	Control	Overall acceptance		
		PEO 1.0 %	PEO 2.0 %	PEO 3.0 %
Zero day	7.33 ± 0.57^{Ea}	7.34 ± 0.57^{Ea}	8.33 ± 0.57^{Ca}	9.00 ± 0.0^{Aa}
2nd day	5.66±0.05^{Fb}	6.00 ±1.00^{Eb}	7.66 ± 0.02^{Cb}	8.34 ±0.56^{Ab}
4th day	3.33± 0.03^{Hc}	5.66± 0.04^{Ec}	6.67± 0.03^{Cc}	8.00± 01.0^{Ac}
6th day	1.66± 0.01^{Hd}	4.66± 0.03^{Ed}	6.00± 0.00^{Cd}	6.66± 0.01^{Ad}
8th day	1.00± 0.00^{Ge}	4.35± 0.57^{De}	5.34± 0.05^{Be}	6.33± 0.57^{Ae}

** Means carrying different superscript Capital letter on the same row are significantly different (P<0.05).*
***Means carrying different superscript small letter on the same column are significantly different (P<0.05).*

The data in Table 2 shows that the chilled fish samples that were treated with different amounts of parsley essential oil (PEO) always had lower pH values than the control samples during the experiment. This was especially true at a concentration of 3%, which led to a higher reduction percentage of up to 22.06% by the eighth day of chilled storage. While the pH of the control chilled fish samples

Table 2: Pattern and reduction percent of pH, TVB-N and TBA values of Tilapia niloticus fillet treated with variant concentrations of Parsley oil throughout chilling storage duration at 4°C.

Storage period	Control	pH values					
		Parsley oil 1.0 %		Parsley oil 2.0 %		Parsley oil 3.0 %	
	Mean± SD	Mean± SD	Red %	Mean± SD	Red %	Mean± SD	Red %
Zero day	6.26 ± 0.06^{Ae}	5.99 ± 0.03^{Bc}	4.31	5.89 ± 0.01^{Cd}	5.91	5.79 ± 0.01^{Dd}	7.50
2nd day	6.51 ± 0.06^{Ad}	5.98± 0.01^{Bc}	8.14	6.06 ± 0.03^{Cc}	6.91	6.01 ± 0.03^{Dc}	7.68
4th day	6.94 ± 0.05^{Ac}	6.11 ± 0.01^{Bb}	11.95	6.08 ± 0.01^{Bc}	12.39	6.02 ± 0.05^{Cc}	13.25
6th day	7.03 ± 0.10^{Ab}	6.13 ± 0.01^{Bb}	12.80	6.11 ± 0.02^{Bb}	13.08	6.07 ± 0.03^{Cb}	13.65
8th day	7.84 ± 0.03^{Aa}	6.16 ± 0.03^{Ba}	21.42	6.13 ± 0.02^{Ca}	21.81	6.11 ± 0.08^{Ca}	22.06
TVB-N values							
Zero day	3.94 ± 0.09^{Ce}	4.03 ± 0.01^{Bd}	-2.28	4.31 ± 0.01^{Ab}	-9.39	3.11 ± 0.01 ^{De}	21.06
2nd day	5.33 ±0.04^{Ad}	5.02± 0.02^{Ba}	5.81	4.83 ± 0.09 ^{Da}	9.38	3.86 ± 0.02 ^{Ca}	27.57
4th day	7.34± 0.06^{Ac}	4.30± 0.01^{Bb}	41.41	4.25 ± 0.04^{Cc}	42.09	3.54 ± 0.01 ^{Db}	51.77
6th day	8.04± 0.03^{Ab}	4.10± 0.02^{Bc}	49.01	4.10 ± 0.02^{Bd}	9.01	3.42 ± 0.06 ^{Cc}	57.46
8th day	8.52± 0.07^{Aa}	4.09± 0.01^{Bc}	51.99	3.94± 0.03^{De}	53.75	3.19 ± 0.01^{Cd}	62.55
TBA values (mg/kg)							
Zero day	0.17 ± 0.06^{Ae}	0.17 ± 0.06^{Ab}	0.0	0.15 ± 0.06^{Bb}	11.76	0.14 ± 0.06^{Ba}	17.64
2nd day	0.21 ±0.04^{Ad}	0.19 ±0.04^{Ba}	9.52	0.17 ±0.04^{Ca}	19.04	0.15 ±0.02^{Da}	28.57
4th day	0.42± 0.03^{Ac}	0.15± 0.04^{Bc}	64.28	0.16± 0.03^{Ba}	61.90	0.13± 0.03^{Cb}	69.04
6th day	0.58± 0.03^{Ab}	0.13± 0.03^{Bd}	77.58	0.13± 0.03^{Bc}	77.58	0.10± 0.01^{Cc}	82.75
8th day	0.72± 0.06^{Aa}	0.15± 0.06^{Bc}	79.16	0.13± 0.06^{Cc}	81.94	0.09± 0.06^{Dd}	87.50

** Means carrying different superscript Capital letter on the same row are significantly different (P<0.05).*

***Means carrying different superscript small letter on the same column are significantly different (P<0.05).*

exceeded the permissible limit established by Egyptian standards (3494/2005) (not more than 6.5), on the 4th day of storage, the pH values of the chilled fish treated with PEO consistently remained within the normal range. This can be attributed to the antimicrobial properties of parsley oil, which effectively reduce the microbial load in the fish fillet. As a result, the pH of the fish is maintained within the normal range (Badee *et al*., 2020). Therefore, parsley essential oil may have a significant effect on the pH levels of chilled fish, potentially contributing to the preservation and quality of the fish during storage, as reported by Özogul *et al*. (2010).

The results shown in Table 2 show that the TVN levels of the control fish samples went up over the storage period, but they didn't go over the limit allowed by Egyptian standards (3494/2005), which say that 30 mg/100 g of TVN in fish muscle is the point at which the fish should go bad. On the other hand, the fish samples treated with various concentrations of parsley essential oil exhibited lower TVN values compared to the control samples. On the 8th day of storage, the highest reduction percentage reached 62.55%, particularly at a concentration of 3% PEO. That the TVN values dropped in samples that were treated with PEO might be because phenolic compounds and other compounds stopped meat protein from breaking down on its own (Ashour *et al*., 2014; Marn *et al*., 2016).

Table 2, shows that the control fish samples' TBA levels went up over the storage period but stayed within the acceptable range set by Egyptian standards (3494/2005), which says that the highest allowable TBA level is 4.5 MDA/kg of fish meat, indicating that the fish is still very fresh. Conversely, the fish samples treated with varying concentrations of parsley essential oil (1%, 2%, and 3%) displayed lower TBA values compared to the control samples. On the eighth day of storage, the highest reduction percentage reached 87.50%, particularly at a concentration of 3% PEO. This might be due to its antioxidant properties, which contribute to reducing lipid oxidation and preserving the quality of the fish samples throughout storage (Farag *et al*., 2021). According to Zhang *et al*., (2006), the dominant compound found in parsley essential oil (PEO) is myristicin, accounting for 32.75% of the composition. Myristicin exhibits a moderate level of antioxidant activity. The second dominant compound is apiol, comprising 17.54% of the oil. Although apiol is not the primary compound, it may contribute significantly to the antioxidant activity of PEO. Our findings agreed with Badee *et al*.,'s (2020) documentation that parsley oil at concentrations of 600 ppm lowers the TBA values of beef burgers during the chilling period for 8 days.

Table 3: Pattern and reduction percent of some biogenic amines in Tilapia niloticus fillet treated with variant concentrations of parsley oil throughout chilling storage duration at 4°C.

Storage period	Control	Histamine					
		PEO 1.0 %		PEO 2.0 %		PEO 3.0 %	
	Mean± SD	Mean± SD	Red %	Mean± SD	Red %	Mean± SD	Red %
Zero day	18.01 ± 0.01^{Ad}	15.51 ± 0.03^{Bd}	13.88	4.51 ± 0.01^{De}	74.95	7.61 ± 0.03^{Ce}	57.74
2nd day	20.30±0.02^{Bc}	34.20 ±0.04^{Aa}	-69.47	10.10 ±0.02^{Dd}	50.24	12.30 ±0.02^{Ca}	39.40
4th day	25.0± 0.03^{Ab}	25.09± 0.04^{Ab}	-0.36	15.20± 0.03^{Ba}	39.20	9.19± 0.04^{Cb}	63.24
6th day	25.20± 0.01^{Ab}	17.59± 0.03^{Bc}	30.19	13.29± 0.03^{Cb}	47.26	8.79± 0.01^{Dc}	65.11
8th day	27.90± 0.02^{Aa}	13.0± 0.01^{Be}	53.40	11.99± 0.05^{Cc}	57.02	8.09± 0.05^{Dd}	71.00
Tyramine							
Zero day	2.51 ± 0.01^{Ad}	0.0 ± 0.0^{Be}	100	0.0 ± 0.0^{Bd}	100	0.0 ± 0.0^{Be}	100
2nd day	4.05±0.02^{Bc}	7.20 ±0.04^{Aa}	-77.77	2.51 ±0.01^{Cc}	38.02	1.20 ±0.02^{Da}	70.37
4th day	4.49± 0.01^{Bc}	6.10± 0.01^{Ab}	-35.85	3.20± 0.03^{Ca}	28.73	1.00± 0.04^{Db}	77.72
6th day	5.19± 0.01^{Ab}	4.89± 0.03^{Bc}	5.78	3.09± 0.01^{Ca}	40.46	0.90± 0.01^{Dc}	82.65
8th day	6.30± 0.01^{Aa}	4.51± 0.01^{Bd}	28.41	2.89± 0.05^{Cb}	54.12	0.69± 0.02^{Dd}	89.04
Putrescine							
Zero day	9.11 ± 0.01^{Be}	15.51 ± 0.01^{Ac}	-70.25	7.21 ± 0.02^{Cb}	20.85	1.61 ± 0.01^{De}	82.32
2nd day	12.30±0.01^{Bd}	29.09 ±0.02^{Aa}	-136.50	9.30±0.01^{Ca}	24.39	2.70±0.02^{Da}	78.04
4th day	14.49± 0.01^{Bb}	19.30± 0.01^{Ab}	-33.19	5.50± 0.03^{Cc}	62.04	2.50± 0.01^{Db}	82.74
6th day	13.30± 0.01^{Bc}	14.20± 0.03^{Ad}	-6.76	4.29± 0.01^{Cd}	67.74	1.99± 0.01^{Dc}	85.71
8th day	15.10± 0.01^{Aa}	10.99± 0.01^{Be}	27.21	3.51± 0.02^{Ce}	76.75	1.80± 0.02^{Dd}	88.07
Cadaverine							
Zero day	15.50 ± 0.01^{Bc}	20.0± 0.01^{Ac}	-29.03	10.01 ± 0.02^{Cc}	34.83	7.51 ± 0.01^{Dc}	51.54
2nd day	17.20±0.01^{Bb}	45.0 ±0.01^{Ab}	-161.62	10.10 ±0.01^{Cb}	41.27	7.90 ±0.02^{Db}	54.06
4th day	17.99± 0.01^{Ba}	29.30± 0.01^{Aa}	-62.86	13.49± 0.03^{Ca}	25.01	6.50± 0.01^{Da}	63.86
6th day	19.10± 0.01^{Aa}	18.99± 0.03^{Aa}	0.57	4.50± 0.01^{Ca}	76.43	5.80± 0.01^{Ba}	69.63
8th day	20.39± 0.01^{Aa}	12.0± 0.01^{Ba}	41.14	8.50± 0.02^{Ca}	58.31	5.30± 0.02^{Da}	74.01

According to Egyptian standards (3494/2005), the most histamine that can be in 100 grams of fish meat is 10 mg. The results in Table 3 show that the amount of biogenic amines in the control fish samples went over the limit while they were being stored and increased. This means that the fish that was tested had higher histamine content. Surprisingly, on the other hand, treated fish samples with varying concentrations of parsley essential oil (1, 2, and 3%) displayed lower biogenic amines content compared to the control samples. Using different concentrations of parsley essential oil improved the levels of histamine, tyramine, putrescine, and cadaverine during chilled storage. On the eighth day of storage, the levels of histamine, tyramine, putrescine, and cadaverine all dropped by 71.0, 89.04, 88.07, and 74.01%, respectively. This was especially true at a concentration of 3%, which was below the MPLs set by Egyptian standards for histamine. This could be due to PEO's antimicrobial properties, which inhibit the growth of histamine-producing bacteria and reduce histamine levels in the treated sample. It also reduces other biogenic amines, such as tyramine, putrescine, and cadaverine, which are linked to food-borne hazards and possible health risks (Visciano *et al*., 2012). According to Cai et al. (2015), treating fish fillets with plant essential oils could lower the amount of biogenic amines present. This supports our finding that histamine levels dropped after treating the fish fillets with 1, 2, and 3% PEO, with levels of 15.51± 0.03, 4.51± 0.01, and 7.61± 0.03 mg/kg, respectively. These findings support the results of Luyun *et al*. (2015), who suggested that the addition of essential oils can lower histamine concentrations, prolong the shelf life of fish meat products, and maintain the quality of fish fillet.

The numbers in Table 4 show that the numbers of aerobic bacteria, psychrotrophic bacteria, Enterobacteriaceae bacteria, and coliform bacteria in the control samples went up over time, up until the eighth day of storage. Conversely, the fish samples treated with various concentrations of parsley essential oil (1, 2, and 3%) exhibited lower levels of bacterial count compared to the control samples. This could be attributed to the antimicrobial properties of parsley essential oil (PEO), which primarily stem from its capacity

Table 4: Pattern and reduction percent of bacteriological count (log10 cfu/g) of Tilapia niloticus fillet treated with variant concentrations of Parsley oil throughout chilling storage duration at 4°C.

Storage period	Control	Aerobic plate count					
		PEO 1.0 %		PEO 2.0 %		PEO 3.0 %	
	Mean± SD	Mean± SD	Red %	Mean± SD	Red %	Mean± SD	Red %
Zero day	6.20±0.03^{Ad}	6.11 ±0.03^{Ba}	1.45	5.96 ±0.02^{Cb}	3.87	5.51 ±0.11^{De}	11.12
2nd day	6.28±0.01^{Ac}	6.06 ±0.05^{Bb}	3.50	5.99 ±0.03^{Ca}	4.61	5.73 ±0.04^{Da}	8.75
4th day	6.29± 0.02^{Ac}	6.05± 0.03^{Bc}	3.81	5.99± 0.05^{Cc}	4.76	5.70± 0.08^{Db}	9.37
6th day	6.36± 0.02^{Ab}	6.00± 0.02^{Bd}	5.66	5.94± 0.04^{Cd}	6.60	5.66± 0.03^{Dc}	11.01
8th day	6.48± 0.03^{Aa}	5.97± 0.03^{Be}	7.87	5.84± 0.04^{Ce}	9.87	5.59± 0.05^{Dd}	13.73
Psychrotrophic count							
Zero day	4.50±0.17^{De}	4.73 ±0.03^{Ba}	-5.11	0.00 ±0.00^{Cb}	100	4.89 ±0.11^{De}	-8.66
2nd day	5.91±0.06^{Cd}	5.63 ±0.05^{Bb}	4.73	5.79 ±0.46^{Ca}	2.03	5.53 ±0.09^{Da}	6.42
4th day	6.00± 0.04^{Bc}	5.86± 0.03^{Bc}	2.33	5.70± 0.03^{Cc}	5.00	5.49± 0.06^{Db}	8.50
6th day	6.23± 0.02^{Ab}	5.85± 0.02^{Bd}	6.09	5.58± 0.09^{Cd}	10.43	5.41± 0.15^{Dc}	13.16
8th day	6.30± 0.03^{Aa}	5.84± 0.03^{Be}	7.30	5.54± 0.04^{Ce}	12.06	5.25± 0.09^{Dd}	16.66
Enterobacteriaceae count							
Zero day	4.28±0.09^{Be}	4.31 ±0.02^{Ac}	-0.70	3.86±0.08^{Ce}	9.81	3.75 ± 0.08^{Da}	12.38
2nd day	4.98±0.17^{Ad}	4.31 ±0.12^{Bc}	13.45	3.92 ±0.15^{Cd}	21.28	0.00 ±0.00^{Db}	100
4th day	5.08±0.01^{Ac}	4.39±0.17^{Bb}	13.58	4.00±0.09^{Cb}	21.25	0.00 ±0.00^{Db}	100
6th day	5.30±0.04^{Ab}	4.56 ±0.03^{Ba}	13.96	4.25± 0.03^{Ca}	19.81	0.00 ±0.00^{Db}	100
8th day	5.48±0.03^{Aa}	4.16±0.15^{Bd}	24.08	4.07± 0.11^{Cc}	25.72	0.00 ±0.00^{Db}	100
Coliforms count							
Zero day	4.33±0.07^{Ae}	4.30 ±0.07^{Ba}	0.69	3.83±0.12^{Cc}	11.54	3.80 ±0.22^{Da}	12.24
2nd day	4.41±0.06^{Ad}	4.03 ±0.16^{Bc}	8.61	0.00 ±0.00^{Ce}	100	0.00 ±0.00^{Cb}	100
4th day	5.25±0.02^{Ac}	4.01±0.17^{Bd}	23.61	3.39±0.35^{Cb}	35.42	0.00 ±0.00^{Db}	100
6th day	5.44±0.01^{Ab}	4.16 ±0.15^{Bb}	23.52	4.07±0.06^{Ca}	25.18	0.00 ±0.00^{Db}	100
8th day	5.50±0.01^{Aa}	3.96±0.24^{Be}	28.0	3.89±0.11^{Cb}	29.27	0.00 ±0.00^{Db}	100

to disrupt bacterial cell membranes. Compounds like myristicin mess up the lipid bilayer of the plasma membrane. This makes it easier for contents inside cells to leak out, which kills the cell (Swamy et al., 2016). Using different amounts of parsley essential oil had a big positive effect on the number of *Enterobacteriaceae* and coliforms that were present during chilled storage. By the second day, the 3% concentration of parsley essential oil had completely stopped the growth of all the bacteria. On the eighth day of storage, the aerobic bacterial count dropped by 13.73 percent, the psychrotrophic count dropped by 16.66 percent, the Enterobacteriaceae count dropped by 100%, and the coliform count dropped by 100%. According to our findings, parsley essential oil exhibits antimicrobial properties against the tested microorganisms. These results are consistent with the study conducted by Karimi *et al.* (2014), which reported that the essential oils derived from parsley seeds and leaves possess a more potent antibacterial effect. Their research also highlighted the ability of parsley essential oil to control pathogenic bacteria, thereby extending the shelf life and enhancing the safety of processed food products. In a study by Cai et al. (2015), it was observed that treatment with plant essential oils not only preserved the sensory quality of fish fillets during storage but also showed the ability to reduce microbial counts. Additionally, Seyyednejad *et al.* (2008) reported that parsley extract has inhibitory effects on the growth of different species of both gram-positive and gram-negative bacteria. These findings suggest that plant essential oils, including parsley extract, have the potential to act as natural antimicrobial agents and can help maintain the quality and safety of fish products. Similarly, Wong and Kitts (2006) demonstrated the antibacterial activity of parsley extract against *E. coli*.

The present study demonstrated that parsley essential oil effectively inhibits Enterobacteriaceae and coliform bacteria, particularly at a concentration of 3% PEO. This finding agrees with what Wahba *et al.* (2010) found: parsley is excellent at killing Gram-negative bacteria like Enterobacteriaceae and coliform.

CONCULSIONS AND RECOMMENDATIONS

Based on the aforementioned data, it can be concluded that 1, 2, and 3% of parsley essential oil could be used for dipping tilapia fillets to improve the sensory characteristics and prolong their shelf life for 8 days. It has been said that parsley oil can act as an antioxidant, which lowers the TBA value, TVN value, and biogenic amine content of the fillets that were treated with it. The results depended on how much parsley essential oil was used. Higher concentrations (2% and 3%) had bigger effects on lowering the number of microbes, especially Enterobacteriaceae and coliform bacteria. Therefore, parsley essential oil holds promise as a natural preservative in food to combat microbial growth and enhance shelf life.

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NOVELTY STATEMENT

The novelty of our work can be summarized as that when we studied the effect of Parsley Essential Oil (PEO) on the content of Biogenic Amines in Tilapia fish fillet, we found that the content of Biogenic Amines studied dropped after treatment with PEO. These findings are very important in maintaining the quality of fish fillet, extension of shelf life of fish meat and decreasing possible health risk.

AUTHOR'S CONTRIBUTIONS

All authors contributed equally to the study.

Conflict of Interest

The authors declare that they have no conflict of interest.

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