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Effects of natural compounds of some plants on microbial contamination and sensory quality of fish fillet during refrigeration.

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ABSTRACT

During storage, the fish product can absorb moisture from its surroundings, promoting the development of microorganisms. During storage, the fish product can absorb moisture from its surroundings, promoting the development of microorganisms. Thus, the effects of thyme, marjoram, turmeric extracts and their combinations on microbiological profile, chemical parameters (pH, TVN and TBA) as well as organoleptic traits were studied during refrigerated storage (0 ± 1 °C) over a period of 12 days. Nile tilapia fish fillets were divided into 7 groups; according to plant extracts alone or combined. The first group was (T1) control; T2 marjoram; T3 thyme; T4 marjoram + thyme; T5 turmeric; T6 turmeric + marjoram; and T7 turmeric + thyme (1.5% v/w from each extract). The obtained results indicated that the turmeric alone and combined treatment of plant extracts of marjoram, thyme, and turmeric slowed down the microbial growth and delayed the chemical changes, kept the sensory attributes, and enhanced the shelf life of the Nile tilapia fish fillet during refrigerated storage. Moreover, the results proved that the control samples were unacceptable due to spoilage on the 6th day of storage. The plant extracts can inhibit the microbial growth and improve the chemical and sensory attributes and subsequently enhance the shelf life of such examined samples during refrigerated storage.

1. INTRODUCTION

Fish and fish products are important exporters of healthy diet because they contain high quality protein (Shaltout and Hashim 2002; and Hassan et al. 2019). Compared to other meats, fish meat has a lower percentage of saturated fat (Hassan et al. 2014; Edris et al., 2017; and Saad et al. 2022). Fresh fish is easily perishable once it has been caught due to endogenous enzymes and rapid microbial growth that are present in fish naturally or from contamination such as the type of environment they are from, the time of year they are harvested, and how they are prepared, and the materials used in preparation (Mei et al. 2019).

Bacterial contamination may be considered the most important cause of fish spoilage (Noor et al., 2013). Aquatic habitat and transportation problem is mainly responsible for the microbial contamination in fish (Sarkar et al. 2020).

Natural preservatives can be used to prevent the growth of bacteria or lipid oxidation in fish during storage; however, customers are frequently curious about the use of preservatives in food, and why it is important given its nutritional value, flavors, and texture to prolong its shelf life (Ghaly et al. 2010). Fish spoilage may occur due to the growth of fungi on the fish, rotting, discoloration, altering the quality and flavor of the fish and as well as significant economic loss (Ahvenainen 2003).

Fat oxidation can be divided into enzymatic and microbial oxidation, both of which can lead to a serious decrease in

qualities. It has also been shown that lipid oxidation products enhance protein denaturation, modulation of protein electrophoresis properties. Therefore, lipid oxidation leads to reduced fish acceptance by consumers (Beck 2014).

Extracts of the medical plants have antimicrobial activity, they may have positive benefits, and generally do not cause any health problems for the handler and consumer (Oral et al. 2008).

The objective of this trial is to investigate the antimicrobial and antioxidant actions of extracts of turmeric (*Curcuma longa*), thyme (*Thymus vulgaris*), and marjoram (*Origanum majorana*).

2. MATERIAL AND METHODS

2.1. Preparation of plant extracts:

Leaves of thyme, marjoram and turmeric were purchased from the Harraz Company in Cairo and prepared according to (Kothari et al. 2012).

2.2. Preparation of tilapia fish fillet:

Raw fresh tilapia fish were purchased from a local market shop and stored on ice before and transported to the laboratory. Tilapia fish were manually filleted and skinned. Fillets (100g) were cut from the fish under good hygienic and sanitary conditions to prevent any cross contamination.

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2.3. Experimental design:

The fish fillets were divided into 7 groups. The first group was (T1) control; T2 majourium; T3 thyme; T4 majourium + thyme); T5 turmeric; T6 turmeric + majourium; and T7 turmeric + thyme (1.5%v/w from each extract). The fish fillets of each group were dipped in each plant extract treatment and left for 30 minutes at room temperature. All samples were placed into different plastic bags and labeled and were stored at 0 ± 1 °C for 12 days. Samples were conducted in triplicate. Control was dipped in sterilized distal water where, treated, and untreated fish fillet subjected to analyses every 3 days as following:

1- Determination of Aerobic plate count (APC) according to (APHA, 1999).

2-Determination of total mould counts by pour plating method according to ISO (21527-1:2008)

3- Determination of pH value according to the technique recommended by (Pearson, 2006).

4- Determination of total volatile nitrogen (TVN) was recommended by Food and Agriculture Organization FAO (1980).

5- Determination of Thiobarbituric Acid Number (TBA) according to the method adopted by Pikul et al. (1989)

6-Sensory evaluation

Sensory evaluation of fillets was performed by a sensory panel composed of 15 experienced members to evaluate the raw fillets based on the color, flavor, texture and overall acceptability (Siah and Tahir, 2011)

2.4. Statistical Analysis:

It was applied according to Feldman et al. (2003), the analysis of variance (ANOVA) test was used to statistically assess the results.

3. RESULTS

Total bacterial counts in tilapia fillet treated with plant extracts of T2; T3; T4; T5; T6 and T7 are shown in Figure (1). The initial aerobic plate counts in control samples of tilapia fillet were 3.66 log cfu/g increases to 6.40 log cfu/g on sixth day. However, addition of plant extracts significantly reduced total bacteria count, especially in T5 and T6 groups to 3.26 log cfu/g and 3.47 log cfu/g at 12 days, respectively. While the initial count plant extracts of T2; T3; T4 groups were 3.66 log cfu/g increase at 12th day to 4.41; 5.75; 4.33 log cfu/g, respectively. ICMS (1986) stated that the upper acceptability limit of total viable bacterial count in fresh fish is $7 \log_{10}$ CFU/g flesh, and $6 \log_{10}$ CFU/g is the maximum permissible limit of Aerobic plate count recommended by Egyptian organization for standardization (EOS) (2005) in chilled fish. Furthermore, Parallel Food Testing in the European Union (1995) stated that in a recent European study by consumers, fish was

assumed “not to be in a good enough condition to be stored for long” when total plate count was 10^6 CFU/g.

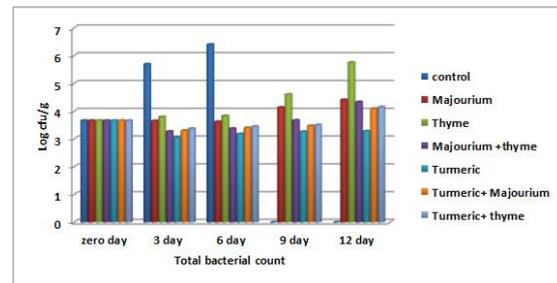


Figure 1 Aerobic plate count in *Tilapia niloticus* fillet at refrigerator temperature (0 ± 1 °C).

Mould and yeast count in tilapia fillet treated with plant extracts of T2; T3; T4; T5; T6 and T7 are shown in Figure (2). The initial total mould and yeast counts in control samples (T1) was 3.52 log cfu/g that increased to 5.84 log cfu/g after 6 days. Thus, turmeric extract had slightly increased in count 5.84 log cfu/g at sixth day. Thus, turmeric extract had slightly increased in count 3.70 Log cfu/g than initial count. The differences in the total bacterial count levels among the other groups plant extract T2; T3; T4; T6 and T7 addition were 4.2; 4.41; 4.17; 4.6 and 4.38 Log cfu/g at the end of preservation.

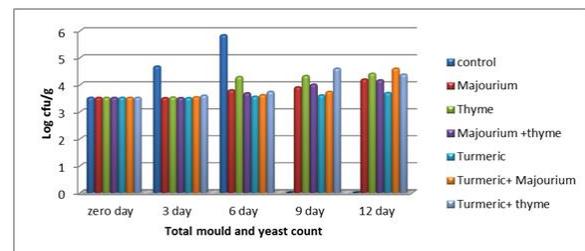


Figure 2 Mould and yeast count in *Tilapia niloticus* fillet at refrigerator temperature (0 ± 1 °C).

The results in Table (1) showed that the mean pH value was significantly higher in the control group at 3rd days and 6th days, respectively, when compared with the other groups. Moreover, the plant extracts; T2; T3; and T3 groups significantly increased pH when compared with the T5; T6 and T7 groups at the 3rd and 6th days. Meanwhile, on the 9th and 12th days, the pH value was significantly higher in the majourium and thyme groups when compared with the other groups. Regarding results of pH between periods, there was a significant increase in the pH value in the control group at 3rd days when compared with first days. Moreover, all plant extract addition groups at 6th, 9th, and 12th days significantly decreased pH when compared with the same groups at 1st and 3rd days. Egyptian Organization for Standardization (EOS, 2005) had reported the critical limits of pH for chilled fish portion which should not be more than 6.5.

Table 1 Effect of different treatment on pH of the fish fillet stored at 0 ± 1 °C.

Treatment	Mean pH				
	1 st day	3 rd day	6 th day	9 th day	12 th day
Control (T1)	5.8±0.3 ^{ax}	6.4±0.02 ^{cy}	7±0.2 ^{zz}	Spoiled	
Majourium (T2)	5.8±0.3 ^{ax}	5.8±0.02 ^{bx}	5.4±0.06 ^{cy}	5±0.06 ^{cy}	4.5±0.1 ^{bz}
Thyme (T3)	5.8±0.3 ^{ax}	5.8±0.02 ^{bx}	5.3±0.02 ^{cy}	5±0.04 ^{cy}	4.3±0.1 ^{az}
Majourim + Thyme(T4)	5.8±0.3 ^{aw}	5.8±0.02 ^{bw}	5.5±0.1 ^{cx}	4.4±0.05 ^{by}	4.2±0.1 ^{az}
Curcum (T5)	5.8±0.3 ^{av}	5.5±0.2 ^{aw}	4.4±0.02 ^{bx}	4.3±0.3 ^{ay}	3.8±0.03 ^{cz}
Curcum + Majourim(T6)	5.8±0.3 ^{av}	5.4±0.1 ^{aw}	4.6±0.1 ^{bx}	4.2±0.03 ^{cy}	3.7±0.2 ^{cz}
Curcum + Thyme (T7)	5.8±0.3 ^{av}	5.3±0.02 ^{aw}	4.6±0.04 ^{bx}	4.2±0.05 ^{cy}	3.7±0.01 ^{cz}

Table (2) shows the effect of different plant extracts on total volatile basic nitrogen (TVBN) (mg/100 g). The mean TVBN value was significantly higher in the control group on the 3rd day and 6th days, respectively, when compared with the other groups. Moreover, the plant extracts; T2 or T3

groups significantly increased TVBN when compared with the others plant extract treated groups at 3rd, 6th, 9th, and 12th days. According to EOS (2005) the examined fish fillet samples were accepted as their values are lower than the maximum permissible limits (30 mg/100 g).

Table 2 Effect of plants extracts on TVN (mg/100g) in the fish fillet at 0 ±1°C.

Treatment	TVN (mg/100g)				
	1 st day	3 rd day	6 th day	9 th day	12 th day
Control (T1)	14.3±1.5 ^{aX}	22.3±1.4 ^{bY}	27.2±0.7 ^{aZ}	Spoiled	
Majourim (T2)	14.3±1.5 ^{aX}	14±0.3 ^{cX}	13±0.3 ^{bX}	12.6±0.2 ^{aX}	12.2±0.3 ^{aX}
Thyme (T3)	14.3±1.5 ^{aX}	14±0.2 ^{cX}	13±0.5 ^{bX}	12.5±0.3 ^{aX}	12±0.1 ^{aX}
Majourim + Thyme(T4)	14.3±1.5 ^{aX}	13.3±0.2 ^{dX}	12±0.1 ^{bX}	11.4±0.1 ^{aY}	10.4±0.2 ^{bY}
Curcum (T5)	14.3±1.5 ^{aX}	12±0.3 ^{dX}	11±0.1 ^{bX}	10±0.2 ^{bY}	10±0.2 ^{bY}
Curcum + Majourim(T6)	14.3±1.5 ^{aX}	12±0.1 ^{dX}	11.4±0.2 ^{bX}	10±0.2 ^{bY}	10±0.1 ^{bY}
Curcum + Thyme (T7)	14.3±1.5 ^{aX}	11.7±0.1 ^{dX}	11.2±0.2 ^{bX}	10.2±0.2 ^{bY}	10±0.2 ^{bY}

*Means with different superscript letters in the same column are significantly different (P<0.05).

Conforming to "EOS" (2005), TBA 4.5 mg MDA / 1 kg of fish flesh were generally considered as the acceptable limit beyond that fish will usually progress a disagreeable. The mean TBA value was significantly higher in the control group at 3rd days and 6th days, respectively, when compared

with the other groups. Moreover, the plant extract of curcum alone significantly decreased TBA when compared with the other plant extract-treated groups at the 3rd, 9th, and 12th days (Table 3).

Table 3 Effect of various plants extracts on TBA (mg MDA / 1 kg) of the examined fish meat fillet stored at 0 ±1 °C.

Treatment	TBA (mg/100g)				
	zero day	3 rd day	6 th day	9 th day	12 th day
Control (T1)	0.52±0.01 ^{aX}	1.1±0.07 ^{eY}	1.6±0.01 ^{aZ}	Spoiled	
Majourim (T2)	0.52±0.01 ^{aW}	0.42±0.01 ^{cX}	0.41±0.02 ^{bY}	0.39±0.02 ^{dZ}	0.39±0.01 ^{aZ}
Thyme (T3)	0.52±0.01 ^{aW}	0.43±0.0 ^{cX}	0.40±0.02 ^{bX}	0.38±0.02 ^{dY}	0.37±0.01 ^{bZ}
Majourim + Thyme(T4)	0.52±0.01 ^{aW}	0.40±0.01 ^{cX}	0.31±0.02 ^{cY}	0.32±0.02 ^{cZ}	0.31±0.002 ^{cY}
Curcum (T5)	0.52±0.01 ^{aV}	0.38±0.002 ^{bW}	0.32±0.003 ^{cX}	0.30±0.001 ^{bY}	0.29±0.02 ^{dZ}
Curcum + Majourim(T6)	0.52±0.01 ^{aV}	0.36±0.001 ^{aW}	0.31±0.002 ^{cX}	0.29±0.01 ^{aY}	0.30±0.004 ^{cZ}
Curcum + Thyme (T7)	0.52±0.01 ^{aW}	0.36±0.001 ^{aX}	0.31±0.002 ^{cY}	0.29±0.01 ^{aZ}	0.29±0.004 ^{dZ}

*Means with different superscript letters in the same column are significantly different (P<0.05).

The effects of different plant extracts on the sensory scores of the fish fillet during storage for 12 days at (0 ± 1°C) are given in Table (4). The results observed that, overall acceptability of control (T1), (T2), (T3), (T4), (T5), (T6) and (T7) of the fish fillet at 1st day were (10, 10, 10, 10, 10, 10, and 10), respectively. Values decrease recorded a change

after 12 days of storage were (8,7,9,8; 8,7,9,8; 9,8,9,8,7; 9,9,9,9; 9,9,9,9; 9,9,9,9) to appearance, texture, odour, and overall, respectively, at the end of storage. The results stated that the control sample was unacceptable due to spoilage on the 3rd day of storage.

Table 4 Changes in sensory characteristics of fish fillet samples treated by plant extracts during refrigerated stored (0±1°C).

		At 0 day	3 rd day	6 th day	9 th day	12 th day
T ₁	appearance	10	4			
	texture	10	4	ND	ND	ND
	odour	10	3			
	overall	10	3.7			
T ₂	appearance	10	9	9	8	8
	texture	10	8	8	7	7
	odour	10	9	9	9	9
	overall	10	8.7	8.7	8	8
T ₃	appearance	10	9	9	8	8
	texture	10	8	8	7	7
	odour	10	9	9	9	9
	overall	10	8.7	8.7	8	8
T ₄	appearance	10	9	9	9	9
	texture	10	9	9	8	8
	odour	10	9	9	9	9
	overall	10	9	9	8.7	8.7
T ₅	appearance	10	10	10	9	9
	texture	10	10	10	9	9
	odour	10	10	10	9	9
	overall	10	10	10	9	9
T ₆	appearance	10	10	10	9	9
	texture	10	10	10	9	9
	odour	10	10	10	9	9
	overall	10	10	10	9	9
T ₇	appearance	10	10	10	9	9
	texture	10	10	10	9	9
	odour	10	10	10	9	9
	overall	10	10	10	9	9

4. DISCUSSION

Fresh fish is very sensitive to microbial contamination, Fresh fish is very fragile, and while refrigeration or freezing might lengthen its shelf life, these measures might not be sufficient to stop lipid oxidation and microbial growth. The shelf quality needs to be improved as well. It does not mean that preservatives must be added properly during storage (Erkan *et al.*, 2015). The rapid development of promising techniques is needed for the application of natural preservatives, as many herbs, plants, vegetables, and fruits extract or powders. These natural preservatives have antioxidant and antimicrobial properties in food, which have attracted more and more attention from consumers. In general, the audience will choose a food that does not contain preservatives, but if they are not available, the same consumer will choose a food that contains natural over synthetic preservatives (Carocho *et al.* 2014).

Plant extracts are frequently used in situations involving fish preservation. The antimicrobial activities of plant extracts may be due to the combined effects of polyphenol adsorption into the fungal cell membrane, membrane disruption and follow subsequent cellular contents leakage, and the generation of hydroperoxides from polyphenols. Plant extracts also inhibit lipid oxidation in food (Mei *et al.* 2019). From the obtained result, it was found that thyme, marjoram, and turmeric extracts have antimicrobial activity due to thyme and marjoram extracts, with the major bioactive compounds being thymol and carvacrol, respectively. In addition, thymol can also be found in marjoram. According to Kumar *et al.* (2006), Curcumin and phenolic compounds are primarily responsible for the turmeric extract's antibacterial and antifungal effects, and these compounds' high flavone and sulphur content may also play a role. The turmeric and combined treatments containing thyme + marjoram extracts, turmeric + marjoram and turmeric + thyme extracts showed a lower total bacterial and mould & yeast count than other treatments due to synergistic effects. Pezeshk *et al.* (2011) reported that extracts from turmeric (*Curcuma longa*) alone or in combination with shallot (*Allium cepa*) extract (1.5% each, v/w) were found to retain quality characteristics of vacuum-packaged rainbow trout (*Oncorhynchus mykiss*) during a refrigerated storage period of over 20 days.

The antimicrobial effect of *Majorana syriaca* plant extract obtained by ethyl acetate has been demonstrated in minced yellow fin tuna stored at 0°C (Al-Bandak *et al.* 2009). The application of ethanolic tomato plant extract blended with a chitosan edible coating extended, for at least 5 days, the shelf life of sierra fillets stored on ice due to their antimicrobial power (Ramírez-Guerra *et al.* 2018). Viji *et al.* (2015) recorded that the mint and citrus extracts have a role in microbiological controlling change in chill stored Indian mackerel.

Regarding the pH value of fish fillets, statistically significant differences were observed in the 3-12 days period between the treatments of all groups. The lower pH in treated fish fillet samples supplemented with marjoram extract, thyme extract, marjoram + thyme extract, turmeric extracts, turmeric + marjoram extract, and turmeric + thyme extract may be due to the presence of antimicrobial ingredients in plant extracts. For the pH values of the control groups during chilling storage, an increase in pH was observed due to the presence of alkaline compounds resulting from protein decomposition. However, in this study, fish meat's 6th, 9th, and 12th-day pH concentrations being acidic may be

advantageous for the product's shelf life. Unfortunately, this pH level is detrimental for eating (Kayim and Can 2010).

TVB-N is usually used as an indicator of the quality of fish and fish products (Kilincceker *et al.* 2009). The samples treated by plant extracts (marjoram, thyme, and turmeric alone or in combination) lower of the TVB-N values due to reduced ability of microorganisms for oxidative and deamination of protein or together (Fan *et al.* 2008). The increase in the TBA values may be attributed to the autoxidation of fish fat and the formation of some TBA-reaction substances during storage in control samples. The degree of lipid oxidation is more commonly assessed using the thiobarbituric acid index (Sallam *et al.* 2007). Most plant extracts' antioxidant action has been linked to phenolic chemicals' capacity to neutralize free radicals, donate hydrogen atoms or electrons, or bind metal cations (Ojagh *et al.* 2010). Different studies have observed that turmeric is a good source of natural antioxidant and antibacterial agents (Jayaprakasha *et al.* 2002; Kumar *et al.* 2006).

For use in food, it is crucial to research how different plant extract combinations affect antimicrobial activity in low concentrations. Whole plant extracts have been shown to have stronger antibacterial activity than when separate major components are combined, suggesting that plant essential oils' lesser constituents may be working in concert (Burt, 2004).

Different concentration ranges of the supercritical fluid extraction (SFE) achieved at 450 bar and 50 °C were examined (0.05- 2.5%, g extract/100 ml medium). The samples showed similar but different degrees of inhibition of the growth of the three mycelial fungi. In the applied concentration of marjoram Soxhlet extract (2.5%) visible fungi growth was observed of each fungi examined. In previous studies, it was found that *Origanum majorana* extract inhibited the mycelial growth of *Penicillium digitatum* fungus at a concentration of 400 µl/ml, although the extract belonged to the thymol/carvacrol chemotype (Daferea *et al.* 2000).

Selmi and Sadok (2008) investigated the combined effects of thyme powder (*Thymus vulgaris*) sprinkle (0.1% w/w) and vacuum packaging on the quality of tuna (*Thunnus thynnus*) during storage at 0 °C. They discovered that thyme treatment minimized auto-oxidation of lipids, as evidenced by lower TBARS and higher PUFA values when compared to the control. Abouel-Yazeed (2019) found that the sample coated with thyme+ chitosan was the best after storage bass fish for 16 days, while the control sample had a storage period of only four days and could not be accepted by the panelists after more time. According to Pezeshk *et al.* (2011), herbal extracts' antioxidant and antibacterial properties have been found to increase the shelf life of items while preserving their quality. The favorable effects on the texture, flavor, color, and general acceptability of the fish in the last few days were amplified by the combined treatment of both turmeric and shallot extracts.

5. CONCLUSION

In conclusion, from the current study, it was obvious that the turmeric treatment and combined treatment of extracts of marjoram, thyme, and turmeric can slow up the fungal growth and be late the chemical changes, keep the sensory attributes, and enhance the shelf life of the Nile tilapia fish fillet during refrigerated storage. Therefore, turmeric treatment and combination of marjoram, thyme, and turmeric extracts can be used as secure methods for keeping fish in refrigerated storage.

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