

Quality enhancement of frozen Nile tilapia fillets using rosemary and thyme oil

[Melhoria da qualidade de filletes de tilápia do Nilo congelados com óleo de alecrim e tomilho]

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ABSTRACT

The food industry and the frozen fish sector in particular have benefitted greatly from advancements in food processing technologies. This study investigated the effect of adding natural antioxidants such as rosemary and thyme oil to frozen fillets of Nile tilapia (*Oreochromis niloticus*) in order to preserve their quality for consumers. Fillets were treated with rosemary and thyme at two concentrations (1% and 1.5%) and then were stored at 4°C. Samples were analyzed over 4 days for bacteriological (aerobic plate count, psychotropic count, and coliform count), chemical (determination of pH, thiobarbituric acid reactive substances-TBARS, and total volatile base nitrogen-TVB-N), and sensory quality examination (color, texture, and odor). Significant differences ($P<0.05$) were observed among different groups in terms of aerobic plate count, psychotropic count, and coliform count during the storage. Moreover, pH, TVB-N, and TBARS mean values in the treated groups were lower than those in the untreated group. The best sensory quality was obtained at the highest concentrations (1.5%) of thyme and rosemary oil.

Keywords: Nile tilapia, rosemary, thyme, shelf life, TBARS, TVB-N

RESUMO

A indústria de alimentos e o setor de peixes congelados, em particular, se beneficiaram dos avanços nas tecnologias de processamento de alimentos. Este estudo investigou o efeito da adição de antioxidantes naturais, como óleo de alecrim e tomilho, a filletes congelados de tilápia do Nilo (*Oreochromis niloticus*), a fim de preservar sua qualidade para os consumidores. Os filés foram tratados com alecrim e tomilho em duas concentrações (1% e 1,5%) e, em seguida, foram armazenados a 4°C. As amostras foram analisadas durante 4 dias para análises bacteriológicas (contagem de placas aeróbicas, psicotrópicas e coliformes), químicas (determinação do pH, substâncias reativas ao ácido tiobarbitúrico-TBARS e nitrogênio base volátil total-TVB-N) e exame da qualidade sensorial (cor, textura e odor). Diferenças significativas ($P<0,05$) foram observadas entre os diferentes grupos na contagem aeróbia de placas, contagem psicotrópica e contagem de coliformes durante o armazenamento. Além disso, os valores médios de pH, TVB-N e TBARS nos grupos tratados foram inferiores aos do grupo não tratado. A melhor qualidade sensorial foi obtida nas maiores concentrações (1,5%) de tomilho e óleo de alecrim.

Palavras-chave: tilápia do nilo, alecrim, tomilho, prazo de validade, TBARS, TVB-N

INTRODUCTION

Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758), a species native to Africa and the Middle East, is one of the most cultivated and consumed

fish in the world. With world production in aquaculture of approximately 3.2 million tons, China being its main producer, which contributes more than a third of the total global production (Prabu *et al.*, 2019). In Egypt, the country

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increases in fish production through aquaculture grew from 92,500 tons in 1971, to more than 1097,544 t in 2013, with most of this growth taking place in the Nile Delta region. Despite the pressure on water, Egypt has the largest aquaculture industry in Africa with a market value of over \$2.18 billion (CAPMAS, 2014). The industry provides about 75.46 % of the country's fish production (GAFRD, 2013), which influences strong growth in annual per capita consumption of 8.5 to 15.5kg during the period between 2009 and 2012 (GAFRD, 2009, 2012).

Fish and shellfish are particularly prone to experiencing a rapid degradation in quality as a result of the action of deteriorating bacteria that are favored with the combination of high moisture and nutrient content with a tendency for a higher pH, that leads to a rapid spoiling of seafood products after harvest (post-mortem) and is what classifies seafood products as a highly perishable item (Li *et al.*, 2012). As such, it is essential to minimize the deterioration process in order to have effective processing systems in place immediately after harvest and transport to minimize loss of fish quality and optimize the quality of the final product. The way in which this is normally achieved in the seafood processing sector is through the use of standardized techniques approved by the industry, based on protocols of critical points along the fish production chain (Pal *et al.*, 2016).

Post-mortem fish spoilage is triggered by biological reactions including lipid oxidation, enzymatic activity within the fish tissues and metabolic action at the microbial level especially for psychotropic, mesophilic aerobic bacteria and coliforms. These reactions result in a considerably reduced shelf-life for fish and seafood products in general (Arashisara *et al.*, 2004). The breakdown of lipids, in particular, a chain of reactions that are set off by the hydrolysis of fatty acids is the principal culprit in reducing the shelf life of seafood products (Hosseini *et al.*, 2010). In conjunction with lipid breakdown, undesirable microorganisms start to colonize the seafood leading to the typical off-flavor and rancidity associated with spoiled foods. At this point, such food item is deemed unfit for human consumption (Mielnik *et al.*, 2008).

Given the principal role of lipid oxidation in fish spoilage, there has been considerable interest in

finding suitable antioxidants to use in slowing down this process. Some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been developed and applied to processed food in recent decades but their use has not been widespread due to concerns around their safety and their links to cancer (Dassarma *et al.*, 2017; Farag *et al.*, 2006). As such, the focus has now shifted to finding natural antioxidants that can be obtained from various plant sources (Reddy *et al.*, 2005; Xu *et al.*, 2017).

There are ubiquitous natural sources of antioxidants found in fruits, vegetables and oilseeds but also in common herbs and spices such as sage and oregano (Pokorny *et al.* 2001; Xu *et al.* 2017). The antioxidant properties of condiments with a high phenolic content such as the herbs rosemary, oregano, sage and thyme have now been firmly established. Extracts from these herbs have been successfully used in the place of the synthetic antioxidants such as BHA and BHT (Ahn *et al.*, 2007; Kin *et al.*, 2019).

The antioxidant capacity of rosemary is attributed to three phenolic diterpenoids (carnosic acid, carnosol and rosmarinic acid), but many other components (rosmanol, epirosmanol, isorosmanol, rosmaridiphenol, rosmadial, rosmariquinone, carvacrol, carvone, cymene, cineole, fenchone, limonene, terpinene and thymol) are expected to contribute to its antioxidative and antimicrobial properties (Fu *et al.*, 2007). Rosemary oil is touted for its antimicrobial and antioxidant properties but is also popular due to its affordability and wide availability. It is believed that the phenols found in rosemary oil protect against microbes by impeding bacterial cell function and DNA synthesis (Belantine *et al.*, 2006).

The addition of essential oils showed a positive effect on the product shelf-life; and in particular, rosemary essential oil produced a remarkable effect. A treatment with 8.5ml rosemary oil/kg fish patty could effectively retard microbial growth, delay chemical deterioration, maintain or improve sensory attributes, and extend the shelf life of fish patty *Xiphophorus maculatus* (Günther, 1866) samples for 14 days during refrigerated storage (Guran, 2015). As with rosemary, the herb thyme is also commonly found throughout the Mediterranean region and is now

also commonly cultivated in temperate regions (Alçiçek, 2011). Two of the key extracts found in thyme, thymol and carvacrol, are hydrophobic compounds that damage bacterial cells by increasing their permeability to adenosine triphosphate (ATP) molecules (Burt, 2004).

Previous studies revealed that thyme (*Zataria multiflora* Boiss) retarded oxidative changes in frozen cobia (*Rachycentron canadum*) fillets, whereas thyme 250 ppm was not as effective as thyme 500 ppm in inhibiting lipid oxidation. So, the essential oils and extracts from *Zataria multiflora* Boiss are a natural source of antioxidants that can be used in the fish fillet industry (Taheri *et al.*, 2013). This is particularly crucial for seafood products that tend to have a naturally shorter shelf life. The present study investigated the use of rosemary and thyme oil in improving the quality of frozen Nile tilapia (*Oreochromis niloticus*) fillets by helping them retain their freshness attributes.

MATERIALS AND METHODS

Fifteen samples of frozen tilapia fillets (weighing from 400 to 500g) were purchased from different fish markets in the city of Tanta in the Egyptian Nile delta. The samples were then wrapped in sterile polyethylene bags and transferred to the laboratory in an insulated icebox for further processing. Rosemary and thyme oils were purchased from the oil extraction unit of the Egyptian National Research Center.

The collected samples were divided into five groups (one sample for each group in three replicates): group 1: untreated control (C); group

2: treated with 1% rosemary oil (R1); group 3: treated with 1.5% rosemary oil (R2); group 4: treated with 1% thyme oil (T1); and group 5: treated with 1.5% thyme oil (T2). These concentrations were used to know the effects of lower and higher concentrations of rosemary and thyme oil on frozen tilapia fillet quality and were chosen based on previous researches (Khalafalla *et al.*, 2015).

Each group of the treated and control fillets was packed separately after 30 minutes. Fillet packages were then labeled and stored at 4°C in a refrigerator shelf. The treated groups were examined on the same day (after one hour of treatment) to evaluate their sensory attributes and bacteriological and chemical analyses were carried out on them. A periodical examination was then carried out every two days for the treated and control fish fillets, while sensory evaluation was carried out every day until the spoilage of all the samples. This trial was replicated two more times during different weeks with the same steps and the same five samples in each trial.

Five panelists were assigned to evaluate the sensory attributes of tilapia fillet samples. The fillet samples were coded, and the panelists were not informed about the experimental approach. Panelists that were selected were experienced in the sensory evaluation of various food products. The panelists were asked to evaluate the samples of each group for color, texture, and odor as outlined in (Table 1). The descriptions of sensory properties and how to rate a sample for the particular sensory property were on overall acceptability standard.

Table 1. Overall acceptability standard of the examined samples of frozen Nile tilapia (*Oreochromis niloticus*) fillets (Mohammed, 2013)

Overall acceptability	Color	Texture	Odor
(5) Highly acceptable	(5) Very light creamy white	(5) Very hard	(5) Very good fishy
(4) Acceptable	(4) Light	(4) Hard	(4) Good
(3) Middle	(3) Acceptable	(3) Middle	(3) Middle
(2) Unacceptable	(2) Dark	(2) Soft	(2) Bad
(1) Rejected	(1) Very Dark	(1) Very Soft	(1) Very bad offensive

The chemical analysis included pH, thiobarbituric acid-reactive substances (TBA-RS), and total volatile basic nitrogen (TVB-N). The pH was determined according to the method described by (Pearson, 2006), TBA-RS was estimated using technique developed by (Pikul *et al.*, 1989), and

TVB-N was measured according to the method recommended by (FAO, 1980). For bacteriological examination, the samples of tilapia fillets were prepared according to the technique recommended by (Sampling..., 1986). Aerobic plate count at 37°C and psychotropic

count at 4°C were performed according to techniques recommended by (ICMSF, 1990). Nutrient agar medium was used to culture aerobic and psychotropic bacteria. The conventional method of coliform counting using violet red bile agar developed by (Feng *et al.*, 2002) was used. Data analyses were carried out in SPSS version 20 and were expressed as means±standard error (SE). Comparison of means between different treatments was done using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Table 2 summarizes the observed changes in sensory attributes between the control and treatment groups. On Day 0, the mean score of sensory attributes was 'very acceptable'. Lower scores were attributed to R1 and T1 samples as a

result of the dark discoloration, soft texture and bad odor. R2 and T2 samples had higher scores in terms of acceptable color, medium texture, and acceptable odor. The results show a strong relationship between the concentration of rosemary oil and the color, texture and odor of the samples. High concentrations of rosemary oil are associated with strong antibacterial and antioxidant attributes. However, the high rosemary oil concentration also comes with a strong aroma which has an adverse effect on the overall sensory parameters, as reported by Gammariello *et al.* (2008). Conversely, as noted by Yildiz (2016) the concentration of 1% thyme oil was seen as most acceptable. This suggests using a large concentration of thyme oil would be acceptable. At a 1% concentration, microbial activity and TVB-N are reduced whilst flavor is enhanced.

Table 2. Overall acceptability of the examined samples of frozen Nile tilapia (*Oreochromis niloticus*) fillets according to (Mohammed, 2013)

Treatment	Storage time (days)					
	0	1	2	3	4	5
Control	5	4	2	1	-	-
R1	5	4	3	2	2	1
R2	5	4	4	3	3	2
T1	5	4	3	2	2	1
T2	5	4	4	3	3	2

Ghaly *et al.* (2010) reported a low acceptance of stored fresh fish primarily as a result of metabolites accumulating. The longer samples were stored for, the lower the acceptability of the samples with the lowest score observed on Day 6. In contrast, samples in the control group were deemed as unacceptable as early as Day 4 of storage. This suggests that antioxidants may play a role in prolonging fish product shelf life by an average of 2 days. The control group in the study by Soto-Valdez *et al.* (2015) witnessed a high degree of loss in firmness whereas samples treated with antioxidants were firmer. This is likely due to the abundance of residual lipids which delayed biological interactions between protein and lipids. Taheri (2013) reported a delay in the development of unfavorable attributes when using thyme oil on frozen samples.

The control samples were significantly more acidic than the treatment groups (Table 3). This is

in line with the findings by Yildiz (2016) where samples treated with thyme oil were less acidic. This is also in line with findings by Ozogul *et al.* (2017) where the acidity of control samples increased with storage time. This increase in acidity could be attributed to increased production of ammonia and similar compounds by bacteria typically associated with fish spoilage (Zakipour Rahimabadi and Divband 2012). The fifth treatment group in this study was associated with the lowest pH values. The highest acceptable limit, as recommended by Egyptian Standards (ES, 2009) is a pH of 6.5. The control group and group R1 were above the 6.5 limits on Day 2 and 4 following storage. However, these samples were still within the 'acceptable' range in terms of sensory attributes. The results of this study are in line with the study reported by Ozyurt *et al.* (2009) of fish decomposing at pH values greater than 7.1.

Table 3. Chemical analysis of frozen Nile tilapia fillets

Analysis	Treatment	Storage time (days)		
		0	2	4
pH	Control	5.91±0.02 a	6.76±0.06 a	-
	R1	5.89±0.02 a	6.25±0.03 b	6.62±0.03 a
	R2	5.86±0.02 ab	6.13±0.03 c	6.36±0.04 b
	T1	5.87±0.02 ab	6.18±0.04 bc	6.40±0.05 b
	T2	5.83±0.02 b	6.06±0.02 dc	6.20±0.04 c
TVB-N (mg/kg)	Control	2.37±0.04 a	19.15±0.52 a	-
	R1	2.23±0.04 ab	11.69±0.28 c	19.12±0.69 a
	R2	2.13±0.06 bc	9.73±0.17 d	16.50±0.27 b
	T1	2.16±0.06 bc	10.07±0.23d	16.93±0.33 b
	T2	2.07±0.05 c	8.12±0.10 e	14.43±0.41c
TBARS (mg%)	Control	1.21±0.05 a	4.14±0.12 a	-
	R1	1.14±0.04 ab	2.94±0.12 c	4.13±0.08 a
	R2	1.06±0.03 bc	2.18±0.12 d	3.72±0.10 b
	T1	1.08±0.03 bc	2.38±0.14 d	3.85±0.08 b
	T2	1.01±0.03 c	1.86±0.11 d	3.24±0.10 c

The mean values in the same column with different letters are significantly different ($P < 0.05$)

*Values represent the mean±standard error

There was an increase in TBARS values across all treatments and control groups that increased with the duration of time stored. Table 3 shows the observed changes in TBARS values for the tilapia fillets. TBARS values greater than 3-4 mg of MDAkg⁻¹ indicates that a product has deteriorated in quality, in terms of rancidity (Frangos *et al.* 2010). The control group in this study experienced the greatest TBARS values. This can likely be explained by the breakdown of hydroperoxides (Chaijan *et al.* 2006). The fifth treatment (T2) had the lowest TBARS values which are on the line with the known antioxidant properties of thyme oil. Results from a study by Khalafalla *et al.* (2015) suggested that by adding thyme to tilapia fillets, their shelf life can be prolonged by up to 9 days versus controls. However, herein in the present study treating tilapia fillets with rosemary and thyme oil at 1% and 1.5% before storing them in the refrigerator preserved the quality attributes and prolonged the shelf-life for only two days longer than the control samples.

Offord *et al.* (1997) reported the high antioxidant property of rosemary oil and attributed it to compounds such as carnosol (carnosic acid). Similarly, Marino *et al.* (1999) reported high antioxidant content in thyme oil, which they attributed to the abundance of phenols such as carvacrol. TBARS values of all samples at the termination of the study did not exceed the

acceptable limit of 4.5 mg/kg suggested by ES (ES, 2009).

TVB-N values increased in line with storage periods as displayed in (Table3). TVB-N values increased significantly for all values as the duration of storage increased. TVB-N values of all samples at the termination of the study did not exceed the acceptable limit of 30 mg% suggested by ES (ES, 2009). The highest TVB-N values were observed in the control group whereas the lowest values were observed in the samples treated with thyme oil (T2). Similarly, Nowzari *et al.* (2013) also found higher TVB-N values in the control group of rainbow trout (*Oncorhynchus mykiss*) fillets than groups treated with essential oils over a period of 16 days. Ozogul *et al.* (2017) also reported a significantly higher increase in TVBN values in the control groups of rainbow trout fillets than groups treated with nano emulsions containing herb oils (rosemary, laurel, thyme and sage) for 24 days. Khalafalla *et al.* (2015) found similar lower TVBN values in Nile tilapia fillets treated with thyme extract (0.5%) and rosemary extract (1.5%) and attributed this effect for the antibacterial effect of thyme and rosemary extracts thereby decreasing ammonia production by bacteria. Fish flesh provides an ideal environment for bacterial growth.

As such, bacterial activity results in fish spoilage during storage (Souza *et al.* 2010). Yıldız (2016)

reported that rainbow trout treated with thyme oil stayed fresher for longer. In addition, Hosseini *et al.* (2016) who investigated the effect of oregano (*Origanum vulgare* L.) essential oil (OEO; 1.2% w/v) on shelf-life extension of rainbow trout (*Oncorhynchus mykiss*) fillet found positive association between TVB-N content and freshness.

The microbiological analysis of the groups in this study is summarized in (Table 4). Significant differences were found during the storage of tilapia fillets. At the start of the study, control samples featured higher counts of organisms than the treatments. In contrast, the R1 group had the

lowest psychotropic counts at the start of the study. The greatest antimicrobial activity was observed at the start of the study for the R1, R2, T1, and T2 groups. In agreement with our results, Duman *et al.* (2012) also observed a significant reduction in aerobic and psychotropic bacterial growth in samples treated with rosemary and thyme oils. This can be attributed to the antimicrobial properties of natural compounds from rosemary and sage tea (Kenar *et al.*, 2010). Similarly, Ozogul *et al.* (2011) reported that addition of rosemary oil led to lower bacterial growth during storage and reduced biochemical changes.

Table 4 Bacteriological analysis of frozen *Nile tilapia* fillets (CFU/g).

Microorganism	Treatment	Storage time (days)		
		0	2	4
Aerobic plate count	Control	$1 \times 10^8 \pm 5 \times 10^7$ a	$2 \times 10^8 \pm 0$ a	-
	R1	$7 \times 10^7 \pm 6 \times 10^7$ b	$2 \times 10^8 \pm 5 \times 10^7$ a	$2 \times 10^9 \pm 0$ a
	R2	$6 \times 10^7 \pm 2 \times 10^7$ b	$1 \times 10^8 \pm 5 \times 10^7$ b	$6 \times 10^8 \pm 2 \times 10^8$ b
	T1	$6 \times 10^7 \pm 2 \times 10^7$ b	$2 \times 10^8 \pm 5 \times 10^7$ a	$2 \times 10^9 \pm 5 \times 10^8$ a
	T2	$3 \times 10^7 \pm 9 \times 10^6$ c	$1 \times 10^8 \pm 4 \times 10^7$ b	$1 \times 10^9 \pm 6 \times 10^8$ c
Psychotropic count	Control	$3 \times 10^7 \pm 2 \times 10^7$ a	$4 \times 10^7 \pm 1 \times 10^7$ a	-
	R1	$2 \times 10^6 \pm 1 \times 10^6$ c	$3 \times 10^7 \pm 1 \times 10^7$ b	$2 \times 10^8 \pm 0$ a
	R2	$2 \times 10^7 \pm 2 \times 10^7$ a	$2 \times 10^7 \pm 2 \times 10^7$ c	$2 \times 10^8 \pm 2 \times 10^8$ a
	T1	$3 \times 10^7 \pm 2 \times 10^7$ b	$3 \times 10^7 \pm 2 \times 10^7$ b	$7 \times 10^7 \pm 3 \times 10^7$ b
	T2	$2 \times 10^7 \pm 2 \times 10^7$ a	$3 \times 10^7 \pm 1 \times 10^7$ b	$2 \times 10^8 \pm 5 \times 10^7$ a
Coliform count	Control	$8 \times 10^6 \pm 6 \times 10^6$ a	$3 \times 10^7 \pm 1 \times 10^7$ a	-
	R1	$5 \times 10^6 \pm 3 \times 10^6$ a	$2 \times 10^7 \pm 5 \times 10^6$ b	$1 \times 10^8 \pm 4 \times 10^7$ a
	R2	$2 \times 10^6 \pm 8 \times 10^5$ b	$3 \times 10^6 \pm 1 \times 10^6$ c	$7 \times 10^7 \pm 2 \times 10^7$ b
	T1	$5 \times 10^6 \pm 0$ a	$2 \times 10^7 \pm 1 \times 10^7$ b	$1 \times 10^8 \pm 5 \times 10^7$ a
	T2	$4 \times 10^6 \pm 2 \times 10^6$ ab	$2 \times 10^7 \pm 1 \times 10^7$ b	$1 \times 10^8 \pm 4 \times 10^7$ a

The mean values in the same column with different letters are significantly different ($P < 0.05$)

*Values represent the mean \pm standard error

Apart from group R1 on day 0, the aerobic plate count and psychotropic count for all samples exceeded the limit of 10^6 CFU/g suggested by ES (ES, 2009). This value was found by Ozogul *et al.* (2004) to have been effectively spoiled. It is important to note that the growth of psychotropic bacteria does not stop in refrigerators (Duman *et al.* 2015). Another study by Fu and Labuza (1993) reported significant increases in bacterial count associated with increased fridge storage times. Burt (2004) found antimicrobial properties in a wide variety of essential oils which could counter bacteria associated with food spoiling.

In this study, groups C, R1, R2, T1, and T2 had gradually increasing coliform counts as storage time increased. The differences found were significant as storage time increased; however,

Guran, (2015) at day 0 no difference in the coliform count was observed between groups. Group R2 had the lowest coliform count. In the existing work, the coliform count was lower in R2 than the others at Day 2 and Day 4 of storage. Previous studies have established the limiting effect of rosemary oil on coliform growth (Belantine *et al.*, 2006; Ozogul *et al.*, 2011). The counts observed in all groups exceeded the limit of 10^2 CFU/g set by ES (ES, 2009). However, the results of this study were similar to those obtained by (Olaleye and Abegunde, 2015).

Indicator microorganisms are most often used to assess food sanitation as fish and other free-swimming marine animals do not usually carry those organisms, particularly of mammalian microflora, including *Escherichia coli* and *fecal*

coliforms, their presence on processed seafood is clear evidence of contamination from terrigenous source. Thus, the presence of these organisms may not only indicate the hygienic condition under which the processing establishment operates, but also the presence of potential microorganisms that may be harmful to the consumer (Jay *et al.*, 1986). Coliforms are microorganisms that can cause food infection inducing sickness in the digestive system, and are unavoidable without proper sterilization method (Lund *et al.*, 2000). Lower results were observed in Haque *et al.* (2013) who found that the total coliforms counts in the sample varied from 9.5×10^5 to 1.03×10^5 cfu/g of the frozen samples. A high count can result in large financial losses and damage to public health. Such contamination is a result of unwashed hands and dirty equipment and water Yadav *et al.* (2006).

CONCLUSIONS

The results of this study suggest that treating tilapia fillets with rosemary and thyme oil at 1% and 1.5% before storing them in the refrigerator may preserve the quality attributes and prolong the shelf-life for up to two days longer than control samples. Strong antioxidant activity but poor antimicrobial activity was observed in all treatments. As such, it is recommended to add an antimicrobial agent in order to increase the shelf life of the products. A high concentration of thyme and rosemary oils such as 1.5% is recommended as they offered the best sensory and chemical quality.

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