



Effect of Rosemary Extract and Vitamin E on Lipid Peroxidation and the Quality During Chilling and Frozen Storage of Fried Nile Tilapia Fillets (*Oreochromis niloticus*)

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ABSTRACT

This study was carried out to investigate the effect of antioxidant of Rosemary extract (R.E.) and vitamin E on the quality of fried fillets of Nile tilapia during chilling and frozen storage. Fillets of Nile tilapia were treated with (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% then stored for 5, 10 and 15 days at $4 \pm 1^\circ\text{C}$ and for three months at $-18 \pm 2^\circ\text{C}$. Then chemical tests including Peroxide value (PV), Thio-barbituric acid (TBA), Tri-methylamine-nitrogen (TMA-N) and Total volatile base-nitrogen (TVB-N) were done to evaluate the preservative effect of (R.E.) and vitamin E during storage. The TBA and PV increased in all treatments due to lipid peroxidation. Results showed that TMA-N, TVB-N, value of (R.E.) and vitamin E treated samples were significantly lower than those of the control samples ($P < 0.05$). Results of our investigation revealed that R.E. retarded oxidative changes in chilling and frozen fried fillets of Nile tilapia whereas R.E. 0.1%, 0.2% and vitamin E 0.1% were not as effective as R.E. 0.3% on oxidative stability. Best oxidation inhibition results on chilling and frozen fried fillets of Nile tilapia was obtained when employing of R.E. The obtained results also showed that there was a significant ($p < 0.05$) enhancement in sensory quality attributes of fried fillets of Nile tilapia; samples treated with R.E. and vitamin E. **Conclusion**, The tested R.E. had a high effectiveness as antioxidative and antimicrobial should be utilized for extending the shelf-life through retarded the spoilage and enhancing quality attributes of fried fillets of Nile tilapia during chilling and frozen storage.

Keywords: Rosemary Extract, Antioxidant, Lipid Peroxidation, Fish, Nile Tilapia, Fillets

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INTRODUCTION

The quality attributes of meat products deteriorate, because the lipid oxidation during processing and storage. Lipid peroxidation is responsible for development of primary and secondary peroxidation products, reduction in nutritional quality, as well as changes in flavor (Maqsood and Benjakul 2011a), that can be precipitated health hazards and economic losses in terms of inferior product quality (Naveena *et al.*, 2008; Gema, *et al.*, 2018). Lipid peroxidation is a rather complex process whereby unsaturated fatty acid fraction of membrane phospholipids are oxidized, and hydroperoxides are formed which are further susceptible to oxidation or decomposition to secondary peroxidation products, as short-chain aldehydes, ketones, and other oxidized compounds which may adversely affect the overall quality and acceptability of meat products and fish meat.

Antioxidants are compounds that are capable of donating hydrogen (H·) radicals (Masuda and others 2001), (Jun *et al.*, 2019) for pairing with available free radicals to prevent the propagation reaction stage during the oxidation process. This effectively minimizes rancidity, retards lipid peroxidation, without any damage to the sensory or nutritional properties, resulting in maintaining quality and shelf-life of fish and meat products. Also, intrinsic factors are available in live muscle to prevent lipid peroxidation. These factors are often lost after slaughtering during conversion of fish muscle to meat, primary/secondary processing, handling, or storage of meat products, necessitating further supplementation with extrinsic antioxidants. For this reason, synthetic antioxidants, such as rosemary extract, were extensively used to delay, retard, or prevent the lipid oxidation by scavenging chain-carrying peroxy radicals or suppressing the formation of free radicals. However, because of the concern over the safety of these synthetic compounds, extensive work is being carried out to find novel and naturally occurring compounds to delay the peroxidation degradation of lipids, improve quality, and maintain the nutritional value of foods (Ciriano and others 2009, 2010)(Dimitrios *et al* 2007). Thus, natural antioxidants have greater application potential in the fish meat industry due to the consumers' acceptability over the synthetic antioxidants. However, the application of plant extracts, spices, herbs, and essential oils with antioxidant effects is still distant for the major reasons of limited data about their effects in different fish meat products.

Pan-frying is a popular method of preparing fatty fish, since it is fast, convenient, and can generate typical characteristics of color, flavor, texture, and palatability, which is highly appreciated by

consumers. The high temperatures generated from pan-frying may breakdown EPA and DHA through lipid oxidation. Also, studies suggest that the heat from pan-frying, not only increase the oxidation of fatty acids on the surface, but also inside fish meat. Kin *et al*(2019).

Lipid oxidation is one of the most important factors responsible for quality deterioration of fish during both refrigerated and frozen storage (Serdaroglu and Felekoglu, 2005). The investigated studies show that freezing is one of the best methods for long-term fish maintenance (Verma and Sriker, 1994; Vidya Sagar Reddy and Sriker, 1996 and Aubourg *et al.*, 2005). Freezing prevents microbial spoilage and helps to reduce fat oxidation but cannot prevent it. One of the appropriate methods to access this target is using additives such as antioxidants. The use of antioxidants is emerging as an effective methodology for controlling rancidity in oils and food (Pazos *et al.*, 2005; Rostamzad *et al.*, 2011 and Taheri *et al.*, 2012). The application of synthetic and natural antioxidants to control lipid oxidation in sea foods is well established (Khan *et al.*, 2006).

To prevent and delay the quality changes caused by lipid oxidation in foods and seafood, various synthetic antioxidants have been used (Benjakul *et al.* 2005). However, with growing concerns regarding the safety of synthetic antioxidants, natural antioxidants have been suggested as safe alternative to synthetic antioxidants to retard oxidative processes and to improve the keeping quality of fish. This ability is due to their potential as free radical scavengers which may terminate radical chain reactions and display antibacterial effects against bacterial pathogens (Singh *et al.*, 2005) and (Sarabi *et al.*, 2017). The bacteriostatic effects of spices may occur through two mechanisms: the delay and partial inhibition of DNA and proteins synthesis (Feldberg *et al.*, 1988). Spices and herbs have been added to food since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (Nakatani, 1994). Furthermore, certain spices and herbs were used as green materials, plant extracts; essential oils (EOs) and powders for prolong the storage life of foods by preventing rancidity through their antioxidants activity or through bacteriostatic or bactericidal activity, also to food-borne pathogenic bacteria (Shelef *et al.*, 1980). Essential oils (EOs) contain a mixture of compounds, which includes terpenes, alcohols, acetones, phenols, acids, aldehydes, and esters and mainly used as food flavorings or functional components in pharmaceuticals (Corbo *et al.*, 2009)

Among natural antioxidants, rosemary (*Rosemarinus officinalis*) has been used

successfully as an antioxidant in different kinds of fish species Sardine (*Sardine pilchardus*) by Serdaroglu and Felekoglu (2005), Escolar (*Lipidocybium flavobrunium*) by (Sarabi *et al.*, 2017) and Tilapia (*Oreochromis niloticus*) by Ibrahim and EL- Sherif (2008).

Rosemary (*Rosmarinus officinalis*) oil is well known as a common spice which extensively used in the food industry. Antioxidant efficiency of rosemary extract or oil is due to high content of phenolic compounds; monoterpenes (eteric olis), diterpene phenols (carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methylcarnosate), phenolic acids (rosmarinic acid), flavonols and triterpene acids (ursolic acid, oleanolic acid, butilinic acid) which break free radical chain reactions by hydrogen atom donation (Riznar *et al.*, 2006). The anti-oxidative effect of rosemary is based on its phenolic diterpenes, creosol and carnosinic acid as well as rosmanol, epirosmanol and iso-rosmanol (Inatani *et al.*, 1983; Schwarz *et al.*, 1992).

Many studies were carried out to prove the effect of combination of refrigeration and essential oils on extending the shelf-life of fish and fish products (Zakipour and Divband, 2012).

Therefore, this study was carried out to investigate the effect of Rosemary extract and vitamin E on improve the quality and extending the shelf-life of fried Nile tilapia (*Oreochromis niloticus*) during chilling storage at $4\pm 1^{\circ}\text{C}$ for 15 days and fillets of frozen storage up to 3 months by determination of physico-chemical, microbial and sensory quality criteria so, shelf-life periodically during storage period.

MATERIALS AND METHODS

Samples collections and preparation:

Nile tilapia (*Oreochromis niloticus*) was used in this study. An initial batch was directly obtained from special farm in Benghazi. The fish samples were transferred to the laboratory of department of food science and technology (Public Health - Benghazi University). All of the fish (32.200 kg.) were immediately washed with tap water. The head, scales and all fins of the fish were removed using a sharp knife. Therefore, the fish were washed again and soaked in tap water for one hour and dressed in a fillets style weighting of each fillet was approximately 90 ± 10 g, then fillets were divided into 5 groups. Samples of the first group were left untreated (control) and dipped in edible coating solution (without any antioxidant) and packaged in polyethylene bags. Coating agents including (onion powder, starch, gluten, salt, garlic powder, isolated soy protein, sodium caseinate,

lime juice, blend spice, white pepper, red pepper, sodium ascorbate and whole egg) were obtained from local market, Fillets belonging to the second, third and fourth groups were dipped into edible coating solutions containing 0.1%, 0.2% and 0.3% of (R.E.) respectively, whereas the fifth groups were dipped in edible coating solution containing 0.1% vitamin E.

All tilapia fillets groups were left at room temperature and then pan-fried using an electrical fryer pan in frying oil (without antioxidant) heated at 170°C for 4 minute, then drained in a basket to remove excess oil and then packed and chilling storage at $4\pm 1^{\circ}\text{C}$ for 15 days and frozen storage up to 3 months at -18°C to evaluate the effects of antioxidant of RE and vitamin E during the storage. Samples were randomly drawn for analysis at every 5, 10 and 15 days through chilling storage and at every one month periods through frozen period. For all five groups of tilapia fillets analyses were carried out after the chilling and freezing process. For each type of fillets, five different batches ($n=5$) were considered and analyzed separately in order to achieve the statistical analysis.

Plant Extract:

Rosemary (*Rosemarinus officinalis* L.) was dried, extracted by ethanol (80%) in the laboratory of department of food science and technology (Public Health - Benghazi University), and then extract was concentrated by rotary evaporation and freeze drying system.

Lipid oxidation measurements:

Peroxide value (PV) was determined in the lipid extract according to the method described by AOAC (2000). Results are expressed as milliequivalents oxygen per kg lipid (meq O₂/kg lipid). Thio-barbituric acid (TBA) was determined calorimetrically by the Porkony and Dieffenbacher method as described by Kirk and Sawyer (1991). Results are expressed as mg malonaldehyde/kg (mg MAL/kg) fish muscle. on base total oxidation, including primary and secondary oxidation products. Which is a combination of PV and TBA (Totox value = $2PV + TBA$).

Volatile amine formation:

Total volatile base-nitrogen (TVB-N) values were measured by the direct distillation method according to Goudlas and Kantians (2005). The results are expressed as mg TVB/N 100 g-1 muscle. Tri-methylamine-nitrogen (TMA-N) values were determined by means of the picrate method, as previously describe by (Aubourg *et al.*, 2007). This involves the preparation of a 5% (w/v) trichloroacetic acid extract of fish muscle. The results are expressed as mg TMA/N 100 g-1 muscle.

Organoleptical evaluation:

Samples were organoleptically evaluated for colour, taste, flavour and overall acceptability. A group of 10 staff members of department of food science and technology (Public Health - Benghazi University), as judges checked the organoleptic properties of the samples and grades ranged from zero to 10 (Teeny and Miyachi, 1972) as mentioned in Table (1).

Table 1: Description of organoleptic properties scores.

Score	Description	Score	Description
10	Ideal	4	Fair
9	Excellent	3	Poorly fair
8	Very good	2	Poor
7	Good	1	Very poor
6	Fairly good	0	Repulsive
5	Acceptable		

Statistical analysis:

Three replications of each trial were performed. Moisture, protein, fat, ash, total volatile bases nitrogen (TVBN), trimethylamine nitrogen (TMAN), thiobarbituric acid (TBA), peroxide value (PV), and sensory data were analyzed using ANOVA and means were separated by Duncan' test (1955) at a probability level of $P < 0.05$ (SAS, 2000).

RESULTS AND DISCUSSION**Chemical composition of Raw tilapia fillets and treated fried fillets of Nile tilapia:**

Proximate composition of raw tilapia fillets, untreated and treated fried fillets with vitamin E and Rosemary extract during storage. Mean values for the proximate chemical composition of fresh raw tilapia fillets and storage coated fried fillets untreated and treated are given in Table 2. For raw tilapia fillets; the moisture, crude protein, fat, ash and carbohydrates contents were 77.33, 84.47, 8.00, 5.99 and 1.54%, respectively. Meanwhile, the corresponding values in fried fillets of tilapia treated with Rosemary extract 0.3% and Vitamin E 0.1% were 61.50- 62.92, 82.31-83.57, 9.44-9.15, 5.34-5.71 and 2.91- 1.57% (on dry weight basis), respectively. From these results, it could be observed that due to frying of tilapia fillets, the moisture content was significantly reduced ($P < 0.05$), while fat was significantly increased ($P < 0.05$). Frying increased fat content, possibly as a result of both moisture content losses and absorption of some frying sun flower oil inside the tissues. It is clear from the present results that parameters chemical composition in frying of fillets of tilapia were not affected by inclusion of the plant essential oils as there were no significant different ($p < 0.05$) between their means and those

of control. These results are nearly accordance with obtained by Elagba *et al.* (2010).

It could be observed that chilling and frozen storage of fried fillets of tilapia resulted in significant changes ($p > 0.05$) of gross chemical composition compared with the fried fillets of tilapia at zero time. In general, by storage at chilling at $4 \pm 1^\circ\text{C}$ and freezing at $-18 \pm 2^\circ\text{C}$, the moisture, protein and ash were slowly reduced. It might be assumed that with drip separated during thawing of freezing samples, some losses of moisture, protein and ash were occurred, as reported by Wen-Hsin and Lillard (1998); Puwastien *et al.* (1999); Santerre *et al.* (2000), Musaiger (2008); Erkan *et al.* (2010) and Ansorena *et al.* (2010).

Changes in peroxide value (PV) and thiobarbituric acid (TBA):

Lipid oxidation development was measured according to the PV Changes in PV values of control and treatments RE (0.1%, 0.2%, 0.3%) and Vitamin E (0.1%) during chilling storage at $4 \pm 1^\circ\text{C}$ for 15 days and frozen storage at -18°C for 3 months are shown in (Table 3). Initial PV values of control, (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% treatments were found 5.81, 5.42, 5.37, 5.29 and 5.52 meq O_2/kg and increased to 11.98, 8.94, 6.87, 5.63 and 9.96, respectively after chilling storage period and increased to 11.81, 8.71, 7.63, 5.54 and 9.84 respectively after freezing storage period.

All samples of fried fillets of tilapia showed an increase in PV value in when the chilling and frozen storage increased ($P < 0.05$). Control samples showed highest formation rate of peroxide value in compare with all the treatments and after that, the highest rate was found is samples contained 0.1% Vitamin E during storage period. Samples contained 0.3% of RE showed the lowest rate of peroxide formation.

As shown in (Table 4). Initial TBA values of control, (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% treatments were found 0.34, 0.27, 0.23, 0.20 and 0.32 meq O_2/kg and increased to 1.31, 0.95, 0.86, 0.69 and 1.17 respectively after chilling storage period and increased to 1.2, 0.82, 0.73, 0.62 and 1.05 respectively after freezing storage period.

There were increased TBA value in fried tilapia fillets when the chilling and frozen storage period increased ($P < 0.05$). The TBA values of RE treatments were significantly lower than the control and Vitamin E after 5 days of chilling storage and one month of frozen storage ($p < 0.05$). After storage more differences were found in TBA values

between the control and RE treatments. The increment in TBA and PV during storage could be resulted from lipid oxidation; these results are in harmony with those obtained by Darweash (1996); Raju *et al.* (1999) and Aro *et al.* (2000).

The peroxide value of a sample indicates the concentrations of peroxides and hydro-peroxides

that are produced during the early stages of lipid oxidation. The peroxide values are monitored for a sample and when it sharply increases, it indicates the end of the shelf life for that sample. The main use of a peroxide value is to determine the quality of oil sample (Kaya *et al.*, 1993).

Table 2: Proximate composition of fried fillets of tilapia that were pretreated by Vitamin E and Rosemary extract (R.E.) on dry weight basis.

Source	moisture	Protein *	Fat *	Ash *	Carbohydrates *	Energy (calories)
Raw fish fillets	77.33 ± 0.2 ^a	84.47± 0.03 ^a	8.00 ± 0.04 ^b	5.99 ± 0.01 ^a	1.54 ± 0.02 ^b	416.04± 0.03 ^b
Vitamin E	62.92±0.04 ^b	83.57±0.20 ^a _b	9.15±0.04 ^{ab}	5.71±0.07 ^a	1.57±1.03 ^b	422.91±0.08 ^a
R.E.(0.1)	62.39±0.06 ^b	82.59±0.07 ^b	9.34±0.01 ^a	5.36±0.03 ^a	2.71±0.07 ^a	425.26±0.04 ^a
R.E.(0.2)	61.79±0.07 ^{bc}	82.42±0.09 ^b	9.39±0.03 ^a	5.32±0.01 ^a	2.87±0.05 ^a	425.67±0.03 ^a
R.E.(0.3)	61.50±0.05 ^{bc}	82.31±0.06 ^b	9.44±0.05 ^a	5.34±0.06 ^a	2.91±0.08 ^a	425.84±0.05 ^a

^{a-bc}Means within a raw with the different superscript are significantly different (p<0.05). Values are expressed as Mean ± SE.

Table 3. Changes of Peroxide values (meq O₂/kg Oil) during Cold and frozen storage of fried tilapia fillets that pretreated by Rosemary extract and Vitamin E for 15 days at 4±1°C and for three month at -18±2°C.(On wet weight basis).

Treatment	Storage Period						
	Fried(0)	Chilling storage period			Frozen Storage period		
		5 Days	10 Days	15 Days	1month	2month	3month
Control	5.81±0.7 ^a	8.09±0.03 ^a	10.09±0.02 ^a	11.98±0.02 ^a	7.92±0.04 ^a	9.90±0.02 ^a	11.81±0.01 ^b
Vitamin E	5.52±0.6 ^a	6.53±0.01 ^b	9.58±0.03 ^b	9.96±0.04 ^b	6.42±0.01 ^b	8.52±0.01 ^b	9.84±0.03 ^b
R.E. (0.1)	5.42±0.02 ^a	5.83±0.03 ^c	7.79±0.02 ^c	8.94±0.03 ^c	5.73±0.07 ^c	7.32±0.07 ^c	8.71±0.02 ^c
R.E. (0.2)	5.37±0.04 ^a	5.61±0.02 ^c	6.68±0.05 ^d	6.87±0.04 ^d	5.44±0.05 ^c	6.56±0.02 ^d	7.63±0.04 ^d
R.E. (0.3)	5.29±0.03 ^a	5.39±0.01 ^c	5.54±0.02 ^e	5.63±0.02 ^e	5.31±0.06 ^c	6.45±0.02 ^d	5.54±0.01 ^e

^{a-e}Means within a raw with the different superscript are significantly different (p<0.05). Values are expressed as Mean ± SE.

Table 4. Changes of Thio-barbituric acid (mg mal/kg fish muscle) during Cold and frozen storage of fried fillets of tilapia that were pretreated by Vitamin E and Rosemary extract (R.E.)for 15 days at 4±1°C and for three month at -18±2°C.(On wet weight basis).

Treatment	Storage time						
	Chilling Storage time				Frozen Storage time		
	Fried(0)	5days	10days	15days	1 month	2 month	3 month
Control	0.34±0.02 ^a	0.74±0.03 ^a	1.07±0.2 ^a	1.31±0.03 ^a	0.63±0.03 ^b	0.91±0.02 ^a	1.2±0.02 ^a
Vitamin E	0.32±0.06 ^a	0.67±0.07 ^{ab}	0.91±0.03 ^a _b	1.17±0.02 ^a _b	0.52±0.08 ^a	0.78±0.05 ^{ab}	1.05±0.05 ^{ab}
R.E. (0.1)	0.27±0.04 ^{ab}	0.53±0.03 ^b	0.79±0.02 ^b	0.95±0.04 ^b	0.44±0.03 ^a _b	0.67±0.06 ^b	0.82±0.06 ^b
R.E. (0.2)	0.23±0.04 ^a _b	0.51±0.02 ^b	0.68±0.05 ^b _c	0.86±0.03 ^b _c	0.39±0.05 ^a _b	0.59±0.04 ^{bc}	0.73±0.05 ^b _c
R.E. (0.3)	0.20±0.03 ^b	0.42±0.05 ^c	0.56±0.2 ^c	0.69±0.01 ^c	0.31±0.05 ^b	0.47±0.07 ^c	0.62±0.02 ^c

^{a-c}Means within a raw with the different superscript are significantly different (p<0.05). Values are expressed as Mean ± SE.

Increase of PV in control samples in contrast with all treatments showed development of off-flavor is one of the major effects of lipid oxidation (Fagan *et al.*, 2003 and Sahari *et al.*, 2009) and at the further stage of lipid peroxidation; changes in color and nutritional value are observed (Sahari *et al.*, 2009). In control samples PV < 20 meq O₂/kg were obtained at the end of the period. However, all treatments (RE 0.1%, 0.2%, 0.3% and Vitamin E 0.1%) showed a progressive but slow increase (P < 0.05) with storage period. The lowest PV was observed for fried fillets of tilapia treated with RE 0.3%. According to the results, it is concluded that RE treatments had significant effect on delaying lipid oxidation. Similar to our findings Serdaroglu and Felekoglu (2005) reported the anti-oxidative effect of rosemary extract for sardine (*Sardina pilchardus*). Increase in PV values of fried and chill-reheated samples also reported by Nikoo *et al.* (2010) which indicated that lipid oxidation took place during frying and reheating process. As reported by Al- Saghir *et al.* (2004) in addition of heat treatment, the kind of cooking oil also can alter the peroxide value. Similar to our results, Nessrien Yasin and Abou-Taleb (2007) reported that PV value increased in semi fried mullet fillets of tilapia during cold storage. At the end of storage period, lowest TBA value was recorded as 5.54 mg MAL/ kg oil for the RE 0.3% treatment. TBA values indicated that control samples had more rancid than samples treated with RE, throughout the storage period.

Treatment with containing of synthesis antioxidant (Vitamin E), showed lower antioxidant effect in comparison with all of the concentrations of rosemary. Similar results were found for the same process by Nessrien Yassin and Abotaleb (2007) that they studied on antioxidant effect of thyme and marjoram on semi fried mullet fish fillet in 4°C storage. These results are in agreement with those reported by Ibrahim and EL-Sherif (2008).

Totally, the results showed that usage of RE and Vitamin E had positive influence on delaying lipid oxidation and increasing shelf-life of tilapia fillets (P < 0.05). TBA values indicated that control samples and samples with added Vitamin E were more rancid than samples treated with RE throughout the storage time. Frying and chilled storage followed by reheating also increased the TBA value, indicating that the secondary products of oxidation increased during the procedure.

Although the lipid oxidation occurred during frying or subsequent storage and reheating, TBA value were lower than acceptability level for human consumption reported by Nikoo *et al.* (2010). The level of 7-8 mg MAL/kg oil is the limit of acceptability of TBA. Results showed that RE

0.3% was the most effective antioxidant. Similar results were reported by Serdaroglu and Felekoglu (2005) on Sardine mince and Nikoo *et al.* (2010) on Kutum.

Changes in total volatile bases nitrogen (TVBN):

Production of total volatile bases nitrogen (TVBN) and increment in trimethylamine nitrogen (TMAN) in fish muscles during storage could be used as indicator of bacterial activity. TVBN and TMAN are considered a valuable tool in the evaluation of fish quality during storage because it's rapid accumulation in muscles under storage conditions. Increasing mean values of TVB-N were observed with longer storage periods (Table 5). Initial (TVBN) values of control, (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% treatments were found 13.77, 13.43, 13.28, 13.1 and 13.61 meq O₂/ kg and increased to 14.21, 13.56, 13.20, 13.12 and 13.98, respectively after chilling storage period and increased to 14.1, 13.44, 13.10, 12.91 and 13.88 respectively after freezing storage period.

All samples showed an increased TVB-N value in coated fried fillets of tilapia when the chilling and frozen storage period increased (P < 0.05), there were Significant differences between control and all treatments during storage period (P < 0.05) whereas significant difference was recorded between treatment groups (RE 0.1%, 0.2%, 0.3% and Vitamin E 0.1%) during storage period (P < 0.05), at the end of storage time the lowest TVB-N level was found in samples treated with RE 0.3%. In general, as the concentration of RE increased, the TVB-N value was decreased.

The TVB-N content quantifies a wide range of basic volatile compounds (ammonia, methylamine, di-methylamine, tri-methylamine, and etc) that should be produced as a result of microbiological activity during the chilling storage or arise from the thermal breakdown of endogenous compounds during cooking (Rodriguez *et al.*, 2008). In comparison with the values reported by (Nessrien Yassin and Abotaleb, 2007), formation of TVB-N was slightly higher for all treatments from the beginning until the end of period of storage. Increase in TVB-N during storage can be due to increase of released ammonia from amination of adenosine monophosphate or histamine (Sahari *et al.*, 2009).

In this study, increase in TVB-N level may be a result of ammonia releasing and other volatile amines from muscular damaged tissue. The increment in TVB-N content during storage period may be due to bacterial activity. However, the breakdown occurred in fillet proteins in rosemary extract was of low rate and might be due to antimicrobial agent of rosemary extract (Ibrahim

and EL-Sherif, 2008). The level of TVB-N in freshly caught fish is generally between 5 and 20 mg N/100g muscle. However, the levels of 30-35 mg N/100g muscle are considered the limit of

acceptability for ice-stored cold water fish (Zarei *et al.*, 2011). In our study TVB-N value for all treatment groups did not exceed 15 mg/100g sample.

Table 5. Changes of TVB-N values (mg/100g sample) during Cold and frozen storage of coated fried fillets of tilapia that were pretreated by Vitamin E and Rosemary extract (R.E.) for 15 days at 4±1°C and for three month at -18±2°C.(On wet weight basis).

Treatment	Storage Period						
	Chilling Storage Period				Frozen Storage Period		
	Fried (0 time)	5 Days	10 Days	15 days	1month	2month	3month
Control	13.77±0.04 ^a	13.56±0.01 ^a	13.83±0.02 ^a	14.21±0.01 ^a	13.44±0.07 ^a	13.71±0.02 ^a	14.1±0.01 ^a
α-tocopherol	13.61±0.06 ^a	13.37±0.04 ^a	13.57±0.03 ^a	13.98±0.03 ^{ab}	13.26±0.04 ^a	13.42±0.01 ^a	13.88±0.02 ^{ab}
R.E.(0.1)	13.43±0.04 ^a	13.07±0.03 ^a	13.25±0.05 ^a	13.56±0.04 ^{ab}	12.95±0.07 ^{ab}	13.16±0.05 ^{ab}	13.44±0.03 ^b
R.E.(0.2)	13.28±0.03 ^{ab}	12.87±0.02 ^{ab}	13.04±0.02 ^a	13.20±0.05 ^{ab}	12.76±0.05 ^b	12.93±0.02 ^{ab}	13.1±0.04 ^b
R.E.(0.3)	13.1±0.05 ^{ab}	12.74±0.05 ^{ab}	12.86±0.02 ^{ab}	13.12±0.02 ^{ab}	12.62±0.02 ^b	12.78±0.04 ^b	12.91±0.03 ^{bc}

^{a-bc}Means within a row with the different superscript are significantly different (p<0.05). Values are expressed as Mean ± SE.

Table 6. Changes of TMA-N values (mg/100g sample) during chilling and frozen storage fried fillets of tilapia that were pretreated by Vitamin E and Rosemary extract (R.E.) for 15 days at 5±1°C and for three month at -18±2°C. (On wet weight basis).

Treatment	Storage Period						
	Chilling Storage Period				Frozen Storage Period		
	Fried(0)	5 Days	10 Days	15 Days	1month	2month	3month
Control	2.35±0.03 ^a	2.59±0.01 ^a	2.69±0.01 ^a	2.79±0.02 ^a	2.46±0.01 ^a	2.57±0.03 ^a	2.68±0.02 ^a
Vitamin E	2.10±0.01 ^a	2.31±0.03 ^a	2.42±0.02 ^a	2.50±0.03 ^a	2.20±0.02 ^a	2.27±0.02 ^a	2.39±0.03 ^a
R.E. (0.1)	1.71±0.05 ^{ab}	1.93±0.02 ^{ab}	1.99±0.04 ^{ab}	2.07±0.04 ^{ab}	1.83±0.03 ^a	1.87±0.04 ^{ab}	1.96±0.04 ^{ab}
R.E. (0.2)	1.49±0.03 ^{ab}	1.75±0.05 ^{ab}	1.82±0.03 ^{ab}	1.87±0.02 ^{ab}	1.64±0.04 ^{ab}	1.69±0.02 ^{ab}	1.78±0.02 ^{ab}
R.E. (0.3)	1.38±0.04 ^b	1.60±0.04 ^{ab}	1.68±0.02 ^{ab}	1.76±0.05 ^{ab}	1.45±0.02 ^{ab}	1.54±0.01 ^{ab}	1.65±0.05 ^{ab}

^{a-b}Means within a row with the different superscript are significantly different (p<0.05). Vitamin E values are expressed as Mean ± SE.

Changes in trimethylamine nitrogen (TMAN):

Amine formation in coated fried fillets of tilapia during storage period was also measured by the TMAN content (Table 6). Initial (TMA-N) values of control, (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% treatments were found 2.35, 1.71, 1.49, 1.38 and 2.10 meq O₂/ kg and increased to 2.79, 2.07, 1.87, 1.76 and 2.50, respectively after chilling storage period and increased to 2.68, 1.96, 1.78, 1.65 and 2.39 respectively after freezing storage period.

All samples showed an increased TMA-N value in fried fillets of tilapia when the storage period

increased (P<0.05). There were Significant differences between control and all treatments in storage period (P<0.05) whereas significant difference was recorded between treatment groups (RE 0.1%, 0.2%, 0.3% and VitaminE 0.1%) during storage period (P<0.05). At the end of storage time the lowest TMA-N level was found in samples treated with RE 0.3%.

Concerning TMA-N its content slightly increased may be due the conversion of TMAO oxide to TMA (Ibrahim and El-Sherif, 2008). TMA-N is often used as an index in assessing the shelf-life and keeping quality of sea food products because

rapidly accumulates in the muscle under cold and frozen conditions. The TMA-N production in fish tissue during cold storage could be used as an indicator of bacterial activity and it is an accepted deterioration measure. The pungent odor of spoiled fish has been often related to TMA-N tissue levels, also with the number of spoiling organisms present in many fish species and the rejection limits is usually from 5 to 10 mg TMA/N 100 g⁻¹ muscle (Zarei *et al.*, 2011). Our results were far below the limit of acceptability of 10-15 mg TMA/N 100 g⁻¹ (Selmi and Sadok, 2008) in all treatments during cold and frozen storage period. TMA formation in the actual fried samples can be explained by means of two different pathways: (1) As a result of TMAO bacterial catalysis breakdown during the chilled storage, and (2) TMA can be produced from TMAO by thermal breakdown during the frying process (Rodriguez *et al.*, 2008). From the TMA-N results, the samples treated with 0.3% RE have higher effect on the bacterial growth in fish samples during cold and frozen storage than other treatments. The application of natural antioxidant (rosemary) in coating layer of fillets of tilapia stored under cold conditions resulted in a decreased microbial population compared to control samples as proved by several other studies on the essential oils of natural antioxidants (Nessrie Yasin and Abou-Taleb, 2007). Similar to our results, Ibrahim and El-Sherif (2008) reported that TMA-N levels of rosemary extract added to fillets of tilapia were significantly lower than control samples after 4 months storage at -18°C.

Organoleptic evaluation:

Organoleptic evaluation scores of colour, taste, flavour and overall acceptability estimated during chilling and frozen storage fried fillets of tilapia that were pretreated by Vitamin E and Rosemary extract for 15 days at 4±1°C and for 3 month at -18±2°C are presented in Table 7.

Initial colour scores of control, (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% treatments were found 9.3,9.2,9.1,9.0, and8.9 respectively and decreased to 8.4, 8.0, 8.3, 8.5 and 8.0 respectively after chilling storage period and decreased to 8.6, 8.4,8.5,8.6 and 8.31 respectively after freezing storage period.

Initial taste scores of control, (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% treatments were found 9.3,9.3,9.2,9.1, and8.9 respectively and decreased to 8.5, 8.4, 8.3, 8.5 and 8.0 respectively after chilling storage period and decreased to 8.7, 8.5,8.0,8.5 and 8.3 respectively after freezing storage period.

Initial flavor scores of control, (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% treatments were found 9.5,9.4,9.2,9.3, and9.1 respectively and decreased to 8.5, 8.5, 8.3, 8.5 and 8.0 respectively after chilling storage period and decreased to 8.7, 8.5,8.0,8.6 and 8.3 respectively after freezing storage period.

Initial Overall acceptability scores of control, (R.E.) 0.1%, 0.2%, 0.3% and Vitamin E 0.1% treatments were found 9.0, 9.1, 9.0, 9.1, and 8.8 respectively and decreased to 8.4, 8.0, 8.2, 8.3 and 79.0 respectively after chilling storage period and decreased to 8.6, 8.4, 8.3, 8.5 and 8.2 respectively after freezing storage period.

Samples after frying directly showed the highest scores for colour, taste, flavour, and overall acceptability, respectively, compared with the samples of fillets of tilapia fried after chilling for 15 days at 4±1°C and frozen storage period for three months at -18±2°C respectively. The scores of colour, taste, flavour and overall acceptability showed a significant decreases (P<0.05) after storage periods.

Therefore, it could be concluded that, the gradual decrease in colour, taste, flavour and overall acceptability scores throughout the storage period at different temperatures, could be attributed to the protein denaturation, hydrolysis and fat oxidation, which are the major factors of changes in organoleptic properties during storage periods. These results are in agreement with those given by Darweash (1996); Schubring (1996) and Raju *et al.* (1999).

From the results obtained in the present study, it may be recommended that, the best consumption of fried fillets of tilapia is after processing directly, followed by 15 days at 4±1°C and frozen storage period for three months at -18±2°C respectively.

CONCLUSION

As a result of a chilling and frozen storage period, a marked content increase was found in the PV, TBA, TMA-N and TVB-N value. However, a preserving effect on such parameters could be observed due to the Rosemary and Vitamin E treatment. Results of our investigation revealed that rosemary extract and Vitamin E retarded oxidative changes in coated frozen Fried fillets of tilapia whereas RE 0.1%, 0.2% and Vitamin E as not as effective as RE 0.3% on oxidative stability. The efficiency of antioxidant inhibiting lipid oxidation throughout chilling and frozen storage was in the following order: RE 0.3% > RE 0.2% > RE 0.1% > Vitamin E > control (P<0.05).

Table 7. Colour and taste scores during chilling and frozen storage fried fillets of tilapia that were pretreated by Vitamin E and Rosemary extract (R.E.) for 15 days at 4±1°C and for three month at -18±2°C.

Colour							
Treatment	Fried(0)	Storage period					
		Chilling Storage period			Frozen Storage period		
		5 Days	10 Days	15 Days	1month	2month	3month
Control	9.3±0.3 ^a (E.)	8.9±0.2 ^a (V.G.)	8.7±0.1 ^a (V.G.)	8.5±0.4 ^a (V.G.)	9.0±0.5 ^a (E.)	8.9±0.3 ^a (V.G.)	8.7±0.1 ^a (V.G.)
Vitamin E	8.9±0.5 ^a (V.G.)	8.5±0.1 ^a (V.G.)	8.3±0.4 ^a (V.G.)	8.0±0.3 ^a (V.G.)	8.8±0.2 ^a (V.G.)	8.5±0.4 ^a (V.G.)	8.3±0.3 ^a (V.G.)
R.E.(0.1)	9.2±0.3 ^a (E.)	8.9±0.2 ^a (V.G.)	8.7±0.3 ^a (V.G.)	8.4±0.5 ^a (V.G.)	9.0±0.4 ^a (E.)	8.7±0.2 ^a (V.G.)	8.5±0.3 ^a (V.G.)
R.E.(0.2)	9.1±0.4 ^a (E.)	8.6±0.5 ^a (V.G.)	8.5±0.2 ^a (V.G.)	8.3±0.1 ^a (V.G.)	9.0±0.5 ^a (E.)	8.4±0.3 ^a (V.G.)	8.0±0.4 ^a (V.G.)
R.E.(0.3)	9.0±0.2 ^a (E.)	8.8±0.4 ^a (V.G.)	8.6±0.1 ^a (V.G.)	8.5±0.5 ^a (V.G.)	8.9±0.2 ^a (V.G.)	8.6±0.3 ^a (V.G.)	8.5±0.4 ^a (V.G.)

Taste							
Treatment	Fried (0)	Storage period					
		Chilling Storage period			Frozen Storage period		
		5 Days	10 Days	15 Days	1month	2month	3month
Control	9.3±0.2 ^a (E.)	8.9±0.1 ^a (V.G.)	8.7±0.3 ^a (V.G.)	8.4±0.5 ^a (V.G.)	9.0±0.4 ^a (E.)	8.8±0.2 ^a (V.G.)	8.6±0.1 ^a (V.G.)
Vitamin E	8.8±0.1 ^a (V.G.)	8.6±0.4 ^a (V.G.)	8.4±0.2 ^a (V.G.)	8.0±0.5 ^a (G.)	8.7±0.3 ^a (V.G.)	8.4±0.1 ^a (V.G.)	8.1±0.2 ^a (V.G.)
R.E.(0.1)	9.3±0.4 ^a (E.)	8.5±0.2 ^a (V.G.)	8.3±0.3 ^a (V.G.)	8.0±0.5 ^a (V.G.)	8.7±0.2 ^a (V.G.)	8.6±0.1 ^a (V.G.)	8.4±0.3 ^a (V.G.)
R.E.(0.2)	9.2±0.2 ^a (E.)	8.7±0.5 ^a (V.G.)	8.6±0.3 ^a (V.G.)	8.3±0.2 ^a (V.G.)	8.9±0.1 ^a (V.G.)	8.7±0.3 ^a (V.G.)	8.5±0.2 ^a (V.G.)
R.E.(0.3)	9.1±0.3 ^a (E.)	8.8±0.1 ^a (V.G.)	8.7±0.2 ^a (V.G.)	8.5±0.2 ^a (V.G.)	8.9±0.4 ^a (V.G.)	8.8±0.5 ^a (V.G.)	8.6±0.2 ^a (V.G.)

E= Excellent. V.G.= Very good. G= Good. F.G.= Fairly good.

Table 8. Flavour and Overall acceptability scores during chilling and frozen storage fried fillets of tilapia that were pretreated by Vitamin E and Rosemary extract (R.E.) for 15 days at 4±1°C and for three month at -18±2°C.

Flavour							
Treatment	Fried(0)	Storage period					
		Chilling Storage period			Frozen Storage period		
		5 Days	10 Days	15 Days	1month	2month	3month
Control	9.5±0.3 ^a (E.)	9.2±0.3 ^a (E.)	8.9±0.3 ^a (V.G.)	8.5±0.3 ^a (V.G.)	9.4±0.3 ^a (E.)	9.1±0.3 ^a (E.)	8.8±0.3 ^a (V.G.)
Vitamin E	9.1±0.1 ^a (E.)	8.7±0.1 ^a (V.G.)	8.5±0.1 ^a (V.G.)	8.3±0.1 ^a (V.G.)	9.0±0.1 ^a (E.)	8.7±0.1 ^a (V.G.)	8.5±0.1 ^a (V.G.)
R.E.(0.1)	9.4±0.2 ^a (E.)	9.0±0.2 ^a (E.)	8.9±0.2 ^a (V.G.)	8.7±0.2 ^a (V.G.)	9.2±0.2 ^a (E.)	9.0±0.2 ^a (E.)	8.9±0.2 ^a (V.G.)
R.E.(0.2)	9.2±0.3 ^a (E.)	8.9±0.3 ^a (V.G.)	8.8±0.3 ^a (V.G.)	8.6±0.3 ^a (V.G.)	9.1±0.3 ^a (E.)	9.0±0.3 ^a (E.)	8.9±0.3 ^a (V.G.)
R.E.(0.3)	9.3±0.1 ^a (E.)	8.9±0.1 ^a (V.G.)	8.8±0.1 ^a (V.G.)	8.5±0.1 ^a (V.G.)	9.0±0.1 ^a (E.)	8.9±0.1 ^a (V.G.)	8.7±0.1 ^a (V.G.)

Overall acceptability							
Treatment	Fried(0)	Storage period					
		Chilling Storage period			Frozen Storage period		
		5 Days	10 Days	15 Days	1month	2month	3month
Control	9.0±0.1 ^a (E.)	8.9±0.1 ^a (V.G.)	8.7±0.3 ^a (V.G.)	8.4±0.5 ^a (V.G.)	9.0±0.4 ^a (E.)	8.8±0.2 ^a (V.G.)	8.6±0.1 ^a (V.G.)
Vitamin E	8.8±0.2 ^a (V.G.)	8.4±0.4 ^a (V.G.)	8.2±0.2 ^a (V.G.)	7.9±0.5 ^a (G.)	8.6±0.3 ^a (V.G.)	8.4±0.1 ^a (V.G.)	8.2±0.2 ^a (V.G.)
R.E.(0.1)	9.1±0.1 ^a (E.)	8.5±0.2 ^a (V.G.)	8.3±0.3 ^a (V.G.)	8.0±0.5 ^a (V.G.)	9.0±0.2 ^a (E.)	8.6±0.1 ^a (V.G.)	8.4±0.3 ^a (V.G.)

R.E.(0.2)	9.0±0.3 ^a (E.)	8.7±0.5 ^a (V.G.)	8.4±0.3 ^a (V.G.)	8.2±0.2 ^a (V.G.)	8.9±0.1 ^a (V.G.)	8.7±0.3 ^a (V.G.)	8.3±0.2 ^a (V.G.)
R.E.(0.3)	9.1±0.2 ^a (E.)	8.8±0.1 ^a (V.G.)	8.6±0.2 ^a (V.G.)	8.3±0.2 ^a (V.G.)	9.0±0.4 ^a (E.)	8.7±0.5 ^a (V.G.)	8.5±0.2 ^a (V.G.)

E= Excellent. V.G.= Very good. G= Good. F.G.= Fairly good.

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