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Antioxidant effect of rosemary extract and BHT on the quality of coated fried Escolar *(Lipidocybium flavobrunium)* fish fillets during frozen storage

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Abstract

This study was carried out to investigate the effect of Rosemary extract (RE) and Butyrate hydroxyl toluene (BHT) on the quality of fried Escolar *(Lipidocybium flavobrunium)* fish fillets during frozen storage. Escolar fish fillets were treated with RE 0.1%, 0.2%, 0.3% and BHT 0.1% then stored at -18°C up to 5 months. Then chemical tests including Peroxide value (PV), Thio-barbituric acid (TBA), Tri-methylamine-nitrogen (TMA-N), Total volatile base-nitrogen (TVB-N), Totox and acid value, were done to evaluate the preservative effect of RE and BHT during storage. The PV, TBA and Totox increased in all treatments due to lipid oxidation. The results showed that TMA-N, TVB-N, value of RE and BHT treated samples were significantly lower than those of the control samples ($P<0.05$). Results of our investigation revealed that rosemary extract retarded oxidative changes in frozen coated fried Escolar fish fillets whereas RE 0.1%, 0.2% and BHT 0.1% were not as effective as RE 0.3% on oxidative stability. Best oxidation inhabitation results on frozen coated fried Escolar fish fillets was obtained when employing a 0.3% of rosemary solution.

Introduction

Deep-fat frying is one of the most commonly used methods for the preparation and manufacture of foods throughout the world (Che Man *et al*., 1999). Nearly one-half of all lunch and dinner food orders in restaurants include one or more deep-fried items (Tsaknis *et al*., 1999). During deep-fat frying, the oil is exposed to elevated temperature in the presence of air and moisture. A number of chemical reactions, including oxidation and hydrolysis, occur during this time, as do changes due to thermal decomposition (Stevenson *et al*., 1984). Lipid oxidation in muscle foods can be initiated by non-enzymatic and enzymatic reactions (Akhtar *et al*., 1998). 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; 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; 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). Researchers are using antioxidants to prevent or reduce fat oxidation. Also they use synthetic antioxidants like Butylate hydroxyl-toluene (BHT), Butyrate hydroxyl-anisole (BHA) and Tertiary butyl hydroquinone (TBHQ) to solve oxidation problems. But nowadays synthetic antioxidants are known as carcinogen and mutating agents. So it is tried to replace natural antioxidants instead of artificial ones (Aubourg *et al*., 2004; Pourashouri *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) and Tilapia *(Oreochromis niloticus)* by Ibrahim and EL- Sherif (2008). 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). The aim of the present study was to investigate the effect of Rosemary extract and Butylate hydroxyl-toluene on the quality of fried Escolar *(Lipidocybium flavobrunium)* fish fillets during frozen storage up to 5 months.

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Materials and Methods

Sample preparation

Escolar (*Lipidocybium flavobrunium*; 20 individual fish) were caught in September 2011 as import from Vietnam to Iran. The frozen fish samples were transferred to the laboratory of department of food science and technology (Isfahan University). Then fish samples were topless, gutted and filleted (175 fillet particles) by hand and washed by cold water carefully. The weight of each fillet was105±10 g, Then the 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, whereas BHT was obtained from Sigma Chemical Co. (St. Louis, Mo. USA). Fillets belonging to the second, third and fourth groups were dipped into edible coating solutions containing 0.1%, 0.2% and 0.3% of RE respectively, whereas the fifth groups were dipped in edible coating solution containing 0.1% BHT. The reason to choose 0.1% is that first we started the experiment by small range percentages (0.01%~0.05%) on test extract but the result was similar to control sample so we increased by 10. Also more than 0.3% was rejected because taste of rosemary extract is tangibility bitter, according to the decision of trained panelists. All fish fillets groups were left at room temperature and then fried using an electrical fryer pan (Moulinex brand) in frying oil (without antioxidant) heated at 170°C for 6 minute, then drained in a basket to remove excess oil and then packed and frozen at -18°C. The coated fish fillet samples were packed in polyethylene bags. The samples were stored for 5 months in -18°C to evaluate the effects of antioxidant of RE and BHT during the frozen storage. Samples were randomly drawn for analysis at every one month periods. For all five groups of fish fillets analyses were carried out after the freezing process (0th month storage at -18°C), and after 1, 2, 3, 4 and 5 months of storage at -18°C. For each kind 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 Isfahan laboratory of food science and technology 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 O^2/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. The totox value calculated according to the definition by (Shahidi and Zhong, 2005) 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.

Statistical analysis

Data from the different quality parameters were subjected to two-way ANOVA and differences among the means were determined for significance at ($p<0.05$) using Duncan's multiple range test by using SAS 9.2 software.

Results

Lipid oxidation

Lipid oxidation development was measured according to the PV formation (primary oxidation compounds) and TBA (secondary oxidation compounds). Changes in PV values of control and all treatments (RE 0.1%, 0.2%, 0.3% and BHT 0.1%) during frozen storage at -18°C for 5 months are shown in (Table 1). Initial PV values of control, RE 0.1%, 0.2%, 0.3% and BHT 0.1% treatments were found 1.81, 1.43, 1.38, 1.31 and 1.54 meq O_2/ kg and increased to 12.89, 7.71, 6.47, 5.71 and 8.69, respectively. All samples showed an increased PV value in fried Escolar fillets when the 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

Table 1. Changes of Peroxide values (meq O_2/Kg oil) during frozen storage of coated fried Escolar fillets that were pretreated under different conditions, (means ± SD) at -18°C up to 5 months

Treatment	Storage time (months)					
	0	1st	2nd	3rd	4th	5th
Control	1.81±0.09^{aF}	5.42 ± 0.12^{aE}	7.59 ± 0.11^{aD}	9.52 ± 0.13^{aC}	11.76± 0.12^{aB}	12.89± 0.15^{aA}
BHT-0.1%	1.54±0.08^{bF}	3.92 ± 0.12^{bE}	5.63 ± 0.08^{bD}	6.95 ± 0.07^{bC}	7.76 ± 0.07^{bB}	8.69 ± 0.09^{bA}
RE-0.1%	1.43±0.13^{cF}	3.23 ± 0.06^{cE}	4.97 ± 0.07^{cD}	6.31 ± 0.09^{cC}	6.94 ± 0.09^{cB}	7.71 ± 0.07^{cA}
RE-0.2%	1.38± 0.05^{dF}	2.76 ± 0.07^{dE}	4.12 ± 0.05^{dD}	5.19 ± 0.04^{dC}	5.62 ± 0.04^{dB}	6.47 ± 0.06^{dA}
RE-0.3%	1.31±0.03 ^{eF}	2.28 ± 0.11^{eE}	3.43 ± 0.07^{eD}	4.36 ± 0.06^{eC}	5.15± 0.06^{eB}	5.71 ± 0.08^{eA}

Means in column with different small letters indicate significant differences ($p<0.05$) among treatments and means in row with different capital letters indicate significant differences ($p<0.05$) as result of frozen storage. (SD, Standard Division)

Table 2. Changes of Thio-barbituric acid (mg mal/kg fish muscle) during frozen storage of fried coated Escolar fillets that was pretreated under different conditions, (means ± SD) at -18°C up to 5 months

Treatment	Storage time (months)					
	0	1st	2nd	3rd	4th	5th
Control	0.25±0.03^{aF}	0.54 ± 0.06^{aE}	0.82 ± 0.08^{aD}	1.11 ± 0.11^{cC}	1.46 ± 0.13^{aB}	1.89± 0.15^{aA}
BHT-0.1%	0.23±0.05^{aF}	0.46 ± 0.08^{bE}	0.72 ± 0.08^{bD}	0.99 ± 0.13^{cC}	1.28 ± 0.07^{bB}	1.64 ± 0.09^{bA}
RE-0.1%	0.18±0.06^{bF}	0.38 ± 0.04^{cE}	0.61 ± 0.07^{cD}	0.86 ± 0.11^{bC}	1.14 ± 0.09^{cB}	1.32 ± 0.07^{cA}
RE-0.2%	0.16±0.03^{bcF}	0.33 ± 0.07^{dE}	0.53 ± 0.05^{dD}	0.76 ± 0.08^{abC}	0.96 ± 0.07^{dB}	1.12 ± 0.08^{dA}
RE-0.3%	0.14±0.04 ^{cF}	0.27 ± 0.05^{eE}	0.43 ± 0.07^{eD}	0.58 ± 0.16^{aC}	0.72± 0.06^{eB}	0.84 ± 0.08^{eA}

Means in column with different small letters indicate significant differences ($p<0.05$) among treatments and means in row with different capital letters indicate significant differences ($p<0.05$) as result of frozen storage. (SD, Standard Division)

found is samples contained 0.1% BHT during storage period. Samples contained 0.3% of RE showed the lowest rate of peroxide formation. Samples can be arranged in ascending order as follows: those contained 0.3%, 0.2%, 0.1% of RE and 0.1% of BHT and then control sample.

Changes in TBA values of control and all treatments (RE 0.1%, 0.2%, 0.3% and BHT 0.1%) during frozen storage at -18°C for 5 months are shown in (Table 2). All samples showed an increased TBA value in fried Escolar fillets when the frozen storage increased ($P<0.05$). Significant differences were found in TBA values between treatment groups at the first month. The TBA values of RE treatments were significantly lower than the control and BHT after one month of storage ($p<0.05$). The TBA value was 0.54±0.06 (mg MAL/kg oil) for the control samples at first month, After 3 months of storage more differences were found in TBA values between the control and RE treatments.

The Totox values of fried coated Escolar fillet treatments are given in Table 3. The Totox value is a measure of the total oxidation, including primary and secondary oxidation products. In the beginning of frozen storage period (0th month) Totox vale of control, RE 0.1%, 0.2%, 0.3% and BHT 0.1% treatments were found to be 3.87, 3.04, 2.92, 2.76 and 3.31 and increased to 27.73, 16.93, 14.15, 12.35 and 19 respectively, at the end of frozen storage time at -18°C. All samples showed an increased Totox value in fried Escolar fillets when the frozen storage increased ($P<0.05$). There were significant differences in control and all treatments during frozen storage in 5 months ($P<0.05$).

Total volatile basis nitrogen (TVB-N)

Increasing mean values of TVB-N were observed with longer storage periods (Table 4). Initial TVB-N values of control, RE 0.1%, 0.2%, 0.3% and BHT 0.1% treatments were found to be 16.64, 16.30, 16.15, 15.97 and 16.48 mg/100g and increased to 18.55, 17.72, 17.45, 16.96 and 17.86, respectively.

Table 3. Changes of Totox values during frozen storage of coated fried Escolar fillets that were pretreated under different conditions, (means ± SD) at -18°C up to 5 months

	Storage time (months)					
Treatment	0	1st	2nd	3rd	4th	5th
Control	3.87±0.08^{aF}	11.38±0.18^{aE}	16.00±0.17^{aD}	20.15±0.28^{aC}	24.97±0.64^{aB}	27.73±0.11^{aA}
BHT-0.1%	3.31±0.08^{bF}	8.30±0.10^{bE}	11.98±0.016^{bD}	14.89±0.11^{bC}	16.80±0.19^{bB}	19.00±0.21^{bA}
RE-0.1%	3.04±0.13^{cF}	6.84±0.09^{cE}	10.05±0.12^{cD}	13.24±0.12^{cC}	15.02±0.21^{cB}	16.93±0.18^{cA}
RE-0.2%	2.92±0.14^{cdF}	5.85±0.04^{dE}	9.77±0.05^{dD}	11.14±0.18^{dC}	12.20±0.18^{dB}	14.15±0.14^{dA}
RE-0.3%	2.76±0.11^{dF}	4.82±0.07^{eE}	7.28±0.09^{eD}	9.30±0.16^{eC}	11.02±0.12^{eB}	12.35±0.08^{eA}

Means in column with different small letters indicate significant differences ($p<0.05$) among treatments and means in row with different capital letters indicate significant differences ($p<0.05$) as result of frozen storage. (SD, Standard Division)

Table 4. Changes of TVB-N values (mg/100g sample) during frozen storage of coated fried Escolar fillets that were pretreated under different conditions, (means ± SD) at -18°C up to 5 months

	storage time (months)					
Treatment	0	1st	2nd	3rd	4th	5th
Control	16.64±0.59^{aC}	16.97±0.56^{aBC}	17.24±1.08^{aA-C}	17.63±0.88^{aAB}	17.97±0.73^{aA}	18.55±0.66^{aA}
BHT-0.1%	16.48±0.65^{aC}	16.79±0.74^{bBC}	16.95±0.58^{abA-C}	17.41±0.46^{bA-C}	17.59±0.48^{bAB}	17.86±0.75^{bA}
RE-0.1%	16.30±0.47^{bB}	16.48 ±0.61^{cB}	16.69±0.67^{bcAB}	16.97±0.70^{bcAB}	17.33±0.64^{bcA}	17.72±0.35^{cA}
RE-0.2%	16.15±0.69^{bcC}	16.29±0.95^{cBC}	16.46±0.48^{cdA-C}	16.63±0.76^{cdAB}	16.98±0.86^{cA}	17.45±0.48^{cdA}
RE-0.3%	15.97±0.91^{cD}	16.15±0.90^{dCD}	16.31±0.29^{dB-D}	16.43±0.68^{dA-C}	16.74±0.48^{cAB}	16.96±0.91^{dA}

Means in column with different small letters indicate significant differences ($p<0.05$) among treatments and means in row with different capital letters indicate significant differences ($p<0.05$) as result of frozen storage. (SD, Standard Division)

Table 5. Changes of TMA-N values (mg/100g sample) during frozen storage of coated fried Escolar fillets that were pretreated under different conditions, (means ± SD) at -18°C up to 5 months

	storage time (months)					
Treatment	0	1st	2nd	3rd	4th	5th
Control	3.10±0.03^{aE}	3.21±0.07^{aD}	3.32±0.08^{aC}	3.43±0.08^{aB}	3.49±0.03^{aB}	3.58±0.06^{aA}
BHT-0.1%	2.85±0.08^{bC}	2.89±0.05^{bC}	2.95±0.03^{bC}	3.07±0.06^{bB}	3.18±0.03^{bAB}	3.24±0.07^{bA}
RE-0.1%	2.45±0.05^{cD}	2.53±0.06^{cCD}	2.58±0.04^{cBC}	2.65±0.1^{cAB}	2.71±0.04^{cAB}	2.68±0.05^{cA}
RE-0.2%	2.23±0.03^{dE}	2.34±0.04^{dD}	2.39±0.03^{dCD}	2.48±0.06^{dBC}	2.56±0.06^{dAB}	2.64±0.05^{cA}
RE-0.3%	2.12±0.02^{dB}	2.19±0.07^{eAB}	2.27±0.00^{eAB}	2.35±0.08^{eA}	2.42±0.06^{eA}	2.49±0.03^{dA}

Means in column with different small letters indicate significant differences ($p<0.05$) among treatments and means in row with different capital letters indicate significant differences ($p<0.05$) as result of frozen storage. (SD, Standard Division)

All samples showed an increased TVB-N value in coated fried Escolar fish fillets when the frozen storage increased ($P<0.05$), there were Significant differences between control and all treatments in 5 months ($P<0.05$) whereas significant difference was recorded between treatment groups (RE 0.1%, 0.2%, 0.3% and BHT 0.1%) during 5 months ($P<0.05$), 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.

Trimethylamine-nitrogen (TMA-N)

Amine formation in coated fried Escolar fish fillets during frozen storage was also measured by the TMAN content (Table 5). Initial TMA-N values of control, RE 0.1%, 0.2%, 0.3% and BHT 0.1% treatments were found to be 3.1, 2.45, 2.23, 2.12 and 2.85 mg/100g and increased to 3.58, 2.68, 2.64, 2.49 and 3.24, respectively. All samples showed an increased TMA-N value in fried Escolar fish fillets when the frozen storage increased ($P<0.05$). There were Significant differences between control and all treatments in 5 months ($P<0.05$) whereas significant difference was recorded between treatment groups (RE 0.1%, 0.2%, 0.3% and BHT 0.1%) during 5 months ($P<0.05$). However, no difference ($P>0.05$) was detected between RE 0.1% and 0.2%, treated samples at the 5th month storage. At the end of storage time the lowest TMA-N level was found in samples treated with RE 0.3%.

Discussion

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). 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; 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 O^2/kg were obtained at the end of the period. However, all treatments (RE 0.1%, 0.2%, 0.3% and BHT 0.1%) showed a progressive but slow increase (P<0.05) with frozen time, so that values above 10 were not attained even at the end of the storage. The lowest PV was observed for fried Escolar fillets 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)* mince. 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 fish fillets during cold storage.

The presence of TBA in a sample of meat indicates that lipid peroxidation has taken place. The level of TBA showed the amount of peroxidation that has already occurred (Lukaszewicz *et al*., 2004). Results showed that TBA values of the control sample increased sharply in frozen storage. This was probably due to the destruction of hydro-peroxides into secondary oxidation products, especially aldehydes in the later stages of lipid oxidation (Chaijan *et al*., 2006). Samples treated with RE showed a gradually increase in TBA values between the first and fifth month (especially treatment with RE 0.3%). However, at the end of storage period, lowest TBA value was recorded as 0.84 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 time at -18°C. The BHT treated samples had TBA values in acceptable limits after 5 months of storage; however significant differences were found in TBA values between the BHT treated samples and control samples at the end of the storage period. Treatment with containing of synthesis antioxidant (BHT), 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). Comparison among treatments revealed the order of TBA increase (P<0.05) at the end of the storage as: RE 0.3% < RE 0.2% < RE 0.1% < BHT 0.1% < BC. Totally, the results showed that usage of RE and BHT had positive influence on delaying lipid oxidation and increasing shelf-life of fillets (P<0.05). TBA values indicated that control samples and samples with added BHT were more rancid than samples treated with RE throughout the storage time at -18°C. 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.

Totox values were increased as shown in Table 3. During lipid oxidation, it is often observed that PV first rises and then falls as hydro-peroxides decompose. Totox value measures both hydro-peroxides and their breakdown products, and provides a better estimation of the progressive oxidative deterioration of fats and oils (Shahidi and Zhong, 2005). Samples treated with antioxidants were significantly more effective in retarding of the rate of rancidity than control samples during the subsequent frozen storage. Rosemary at 0.3% and 0.2% concentration showed the highest antioxidant effects on lipid oxidation by lowering the Totox values than RE 0.1%, BHT 0.1% and control samples at the end of frozen storage. Comparison among treatments revealed the order of Totox values increase (P<0.05) at the end of the storage as: RE 0.3% < RE 0.2% < RE 0.1% < BHT 0.1% < control. Totally, the results showed that usage of RE and BHT had positive influence on delaying lipid oxidation (P<0.05). Similar results were reported by (Kalantari *et al*., 2010).

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 (Rodrıguez *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 mono phosphate 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 frozen storage 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 19 mg/100g sample.

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 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 mgTMA/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 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 frozen storage then other treatments. The application of natural antioxidant (rosemary) in coating layer of Escolar fish fillets 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 Tilapia fillets were significantly lower than control samples after 4 months storage at -18°C.

Conclusion

As a result of a frozen storage period of 5 months, a marked content increase was found in the PV, TBA, TMA-N, TVB-N and Totox value. However, a preserving effect on such parameters could be observed due to the Rosemary and BHT treatment. Results of our investigation revealed that rosemary extract and BHT retarded oxidative changes in coated frozen Fried Escolar fillets whereas RE 0.1%, 0.2% and BHT as not as effective as RE 0.3% on oxidative stability. The efficiency of antioxidant inhibiting lipid oxidation throughout frozen storage was in the following order: RE 0.3% > RE 0.2% > RE 0.1% > BHT > control ($P<0.05$).

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Reference

Akhtar, P., Gray, J. I., Booren, A. M. and Garling, D. L. 1998. Effect of dietary components and surface application of oleoresin RE in lipid stability of rainbow trout *(Oncorhynchus mykiss)* muscle during refrigerated and frozen storage. Journal of Food lipids 5: 43–45.

Al-saghir, S., Thurner, K., Wanger, K. H., Frisch, G. and Luf, W. 2004. Effects of different cooking procedure on lipid quality and cholesterol oxidation of farmed salmon fish *(Salmo salar)*. Journal of Agriculture Food Chemistry 52: 5290-5296.

AOAC. 2000. Official Methods of Analysis, 17th edition. Association of Official Analytical Chemists, Arlington, Washington (USA).

Aubourg, S., Perez-Alonso, F. and Gallardo, J. M. 2004. Studies on rancidity inhibition in frozen horse mackerel *(Trachurus trachurus)* by citric acid and ascorbic acids. European Journal of Lipid Science and Technology 106: 232-240.

Aubourg, S., Quitral, V., Angelica Larraın, M., Rodrıguez, A., Gomez, J., Maier, L. and Vinagre, J. 2007. Autolytic degradation and microbiological activity in farmed coho salmon *(Oncorhynchus kisutch)* during chilled storage. Food Chemistry 104: 369–375.

Aubourg, S., Rodriguez, A. and Gallardo, J. M. 2005. Rancidity development during frozen storage of mackerel *(Scomber scombrus)*: effect of catching season and commercial presentation. European Journal of Lipid Science and Technology 107: 316-323.

Chaijan, M., Benjakul, S., Visessanguan, W and Faustman, C. 2006. Changes of lipids in sardine *(Sardinella gibbosa)* muscle during iced storage. Food Chemistry 99: 83-91.

Che Man, Y. B. and Tan, C. P. 1999. Effects of natural and synthetic antioxidants on changes in refined, bleached, and deodorized Palm Olein during deep- fat frying of potato chips. Journal of American Oil Chemistry Society 76: 331–340.

Fagan, J. D., Gormley, R. T. and Huircheartaigh, M. 2003. Effect of freeze-chilling, in comparison with fresh, chilling and freezing, on some quality parameters of raw whiting, mackerel and salmon portion. Food Science and Technology 36: 647-655.

Goudlas, A. E. and Kontaminas, M. G. 2005. Effect of salting and smoking-method on the keeping quality of chub mackerel *(Scomber japonicus)*: biochemical and sensory attributes. Food Chemistry 93:511-520.

IEbrahim, S.M. and EL-Sherif, S. A. 2008. Effect of some plant extracts on quality aspects of frozen Tilapia (*Oreochromis niloticus* L.) fillets. Global Veterinaria 22: 62-66.

Inathani, R., Nakatani, H., Fuwa, H. and Seto, H. 1983. The stereochemistry of the OH group on C-7 rosmanol has been revealed to be a configuration as the structure 1 on the basis of a re-examination of the NOE analysis. Journal of Agriculture Bio Chemistry 47: 521–528.

Kalantari, F., Bahmaei, M., Ameri, M. and Shoaei, E. 2010. Effect of vegetable oil oxidation on the hydrogenation reaction process. Grasas y Aceites 61: 361-368.

Khan, M. A., Parrish, C. C. and Shahidi, F. 2006. Effects of mechanical handling, storage on ice and ascorbic acid on lipid oxidation in cultured Newfoundland blue mussel *(Mytilus edulis)*. Journal of Food Chemistry 99: 605-614.

Kirk, R. and Sawyer, R. 1991. Pearson's composition and Analysis of Foods, 9th ed. Longman scientific and technical pp: 642-643.

Kaya, A., Tekin, A. R. and Oner, M. D. 1993. Oxidative stability of sunflower and olive oils: comparison between a modified active oxygen method and long term storage. Lebensm Wiss Technology 26: 464-468.

Lukaszewicz, M., Szopa, J. and Krasowska, A. 2004. Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants. Food Chemistry 88: 225-231.

Nessrien Yassin, M. N. and Abou-Taleb, M. 2007. Antioxidant and Antimicrobial Affects of Marjoram and Thyme in Coated Refrigerated Semi Fried Mullet Fish Fillets. World Journal Dairy and Food Science 2: 01-09.

Nikoo, M., Ghomi, M., Zakipour Rahimabadi, E., Benjakul, S. and Javadian, B. 2010. The effects of deep-frying, refrigerated storage and reheating on the fat content, oxidation and fatty acid composition of the fish Rutilus frisii kutum. Journal of Food Processing and Technology 1: 1-4.

Pazos, M., Gallardo, J. M., Torres, J. L. and Medina, I. 2005. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. Food Chemistry 92: 547-557.

Pourashouri, P., Shabanpour, B., Aubourg, S., Rohi, G. and Shabani, A. 2009. An investigation of rancidity inhibition during frozen storage of wells catfish *(Silurus glanis)* fillets by previous ascorbic and citric acid treatment. Journal of Food Science and Technology 448: 1503-1509.

Rodrıguez, A., Carriles, N., Cruzb, J. and Aubourg, S. 2008. Changes in the flesh of cooked farmed salmon *(Oncorhynchus kisutch)* with previous storage in slurry ice (-1.5ºC). Food Science and Technology 41: 1726-1732.

Rostamzad, H., Shabanpour, B., Shabani, A. and Shahiri, H. 2011. Enhancement of the storage quality of frozen Persian sturgeon fillets by using of ascorbic acid. International Food Research Journal 18: 109-116.

Sahari, M.A., Nazemroaya, S. and Rezaei, M. 2009. Fatty Acid and Biochemical Changes in Mackerel *(Scomberomorus Commerson)* and Shark *(Carcharhinus dussumieri)* fillets during frozen storage. American-Eurasian Journal of Sustainable Agriculture 3: 519-527.

Schwrtz, K. and Ternes, W. 1992. Anti-oxidative constituents of *Rosmarinus officinalis* and *salvia officinalis*. I. Determination of phenolic di-terpenes with anti-oxidative activity amongst toco-chromanols using HPLC. Z. Lebensm Unters 19: 95–98.

Selami, S. and Sadoki, S. 2008. The effect of natural antioxidant (*Thymus vulgaris* (Linnaeus)) on flesh quality of tuna (*Thunnus thynnus* (Linnaeus)) during chilled storage. Pan-American Journal of Aquatic Sciences 3: 36-45.

Serdaroglu, M. and Felekoglu, E. 2005. Effects of using rosemary extract and onion juice on oxidative stability of sardine *(Sardina pilchardus)* mince. Journal of Food Quality 28: 109-120.

Shahidi, F. and Zhong, Y. 2005. Lipid Oxidation: Measurement Methods, In: F. Shahidi (Edit.), Bailey's Industrial Oil and Fat Products, Wiley-Interscience, A John Wiley and Sons, New Jersey, P. 357.

Stevenson, S. G., Vaisey-Genser, M. and Eskin, N. A. M. 1984. Quality control in the use of deep frying oils. Journal of American Oil Chemist Society 61: 1102–1108.

Taheri, S., Motallebi, A. A., Fazlara, A., Aftabsavar, Y. and Aubourg, S. 2012. Effect of previous ascorbic acid treatment on the fatty acid profile of cobia *(Rachycentron canadum)* fillets during frozen storage. Grasas y Aceites 63: 70-78.

Tsaknis, J. Lalas, S., Gergis, V., Dourtoglou, V. and Spilitois, V. 1999. Quality changes of Moringa oleifera, variety Mbololo of Kenya seed oil during frying. Journal of Agriculture Food Chemistry 47: 4495-4499.

Verma, J. K. and Srikar, L. N. 1994. Protein and lipid changes in pink perch *(Nemipterus japonicus)* mince during frozen storage. Journal of Food Science and Technology 31: 238-240.

Vidya Sagar Reddy, G. and Sriker, L. N. 1996. Effect of preprocess ice storage on lipid change of Japanese threadfin bream *(Nemipterus japonicus)* mince during frozen storage. Asian Fisheries Science 9: 109-114.

Zarei, M., Fazlara, A., Jamaledin, D. and Mojtahedi, M. 2011. Microbial and chemical quality of vacuum-

packed frozen fillet of narrow-barred spanish mackerel *(Scomberomorus commerson)* and silver promfret *(Pampus argenteus)* market in Iran. World Journal of Fish and Marine Science 3: 410-413.

Reference

Akhtar, P., Gray, J. I., Booren, A. M. and Garling, D. L. 1998. Effect of dietary components and surface application of oleoresin RE in lipid stability of rainbow trout *(Oncorhynchus mykiss)* muscle during refrigerated and frozen storage. Journal of Food lipids 5: 43–45.

Al-saghir, S., Thurner, K., Wanger, K. H., Frisch, G. and Luf, W. 2004. Effects of different cooking procedure on lipid quality and cholesterol oxidation of farmed salmon fish *(Salmo salar)*. Journal of Agriculture Food Chemistry 52: 5290-5296.

AOAC. 2000. Official Methods of Analysis, 17th edition. Association of Official Analytical Chemists, Arlington, Washington (USA).

Aubourg, S., Perez-Alonso, F. and Gallardo, J. M. 2004. Studies on rancidity inhibition in frozen horse mackerel *(Trachurus trachurus)* by citric acid and ascorbic acids. European Journal of Lipid Science and Technology 106: 232-240.

Aubourg, S., Quitral, V., Angelica Larraın, M., Rodrıguez, A., Gomez, J., Maier, L. and Vinagre, J. 2007. Autolytic degradation and microbiological activity in farmed coho salmon *(Oncorhynchus kisutch)* during chilled storage. Food Chemistry 104: 369–375.

Aubourg, S., Rodriguez, A. and Gallardo, J. M. 2005. Rancidity development during frozen storage of mackerel *(Scomber scombrus)*: effect of catching season and commercial presentation. European Journal of Lipid Science and Technology 107: 316-323.

Chaijan, M., Benjakul, S., Visessanguan, W and Faustman, C. 2006. Changes of lipids in sardine *(Sardinella gibbosa)* muscle during iced storage. Food Chemistry 99: 83-91.

Che Man, Y. B. and Tan, C. P. 1999. Effects of natural and synthetic antioxidants on changes in refined, bleached, and deodorized Palm Olein during deep- fat frying of potato chips. Journal of American Oil Chemistry Society 76: 331–340.

Fagan, J. D., Gormley, R. T. and Huircheartaigh, M. 2003. Effect of freeze-chilling, in comparison with fresh, chilling and freezing, on some quality parameters of raw whiting, mackerel and salmon portion. Food Science and Technology 36: 647-655.

Goudlas, A. E. and Kontaminas, M. G. 2005. Effect of salting and smoking-method on the keeping quality of chub mackerel *(Scomber japonicus)*: biochemical and sensory attributes. Food Chemistry 93:511-520.

IEbrahim, S.M. and EL-Sherif, S. A. 2008. Effect of some plant extracts on quality aspects of frozen Tilapia (*Oreochromis niloticus* L.) fillets. Global Veterinaria 22: 62-66.

Inathani, R., Nakatani, H., Fuwa, H. and Seto, H. 1983. The stereochemistry of the OH group on C-7 rosmanol has been revealed to be a configuration as the structure 1 on the basis of a re-examination of the NOE analysis. Journal of Agriculture Bio Chemistry 47: 521–528.

Kalantari, F., Bahmaei, M., Ameri, M. and Shoaei, E. 2010. Effect of vegetable oil oxidation on the hydrogenation reaction process. Grasas y Aceites 61: 361-368.

Khan, M. A., Parrish, C. C. and Shahidi, F. 2006. Effects of mechanical handling, storage on ice and ascorbic acid on lipid oxidation in cultured Newfoundland blue mussel *(Mytilus edulis)*. Journal of Food Chemistry 99: 605-614.

Kirk, R. and Sawyer, R. 1991. Pearson's composition and Analysis of Foods, 9th ed. Longman scientific and technical pp: 642-643.

Kaya, A., Tekin, A. R. and Oner, M. D. 1993. Oxidative stability of sunflower and olive oils: comparison between a modified active oxygen method and long term storage. Lebensm Wiss Technology 26: 464-468.

Lukaszewicz, M., Szopa, J. and Krasowska, A. 2004. Susceptibility of lipids from different flax cultivars to peroxidation and its lowering by added antioxidants. Food Chemistry 88: 225-231.

Nessrien Yassin, M. N. and Abou-Taleb, M. 2007. Antioxidant and Antimicrobial Affects of Marjoram and Thyme in Coated Refrigerated Semi Fried Mullet Fish Fillets. World Journal Dairy and Food Science 2: 01-09.

Nikoo, M., Ghomi, M., Zakipour Rahimabadi, E., Benjakul, S. and Javadian, B. 2010. The effects of deep-frying, refrigerated storage and reheating on the fat content, oxidation and fatty acid composition of the fish Rutilus frisii kutum. Journal of Food Processing and Technology 1: 1-4.

Pazos, M., Gallardo, J. M., Torres, J. L. and Medina, I. 2005. Activity of grape polyphenols as inhibitors of the oxidation of fish lipids and frozen fish muscle. Food Chemistry 92: 547-557.

Pourashouri, P., Shabanpour, B., Aubourg, S., Rohi, G. and Shabani, A. 2009. An investigation of rancidity inhibition during frozen storage of wells catfish *(Silurus glanis)* fillets by previous ascorbic and citric acid treatment. Journal of Food Science and Technology 448: 1503-1509.

Rodrıguez, A., Carriles, N., Cruzb, J. and Aubourg, S. 2008. Changes in the flesh of cooked farmed salmon *(Oncorhynchus kisutch)* with previous storage in slurry ice (-1.5ºC). Food Science and Technology 41: 1726-1732.

Rostamzad, H., Shabanpour, B., Shabani, A. and Shahiri, H. 2011. Enhancement of the storage quality of frozen Persian sturgeon fillets by using of ascorbic acid. International Food Research Journal 18: 109-116.

Sahari, M.A., Nazemroaya, S. and Rezaei, M. 2009. Fatty Acid and Biochemical Changes in Mackerel *(Scomberomorus Commerson)* and Shark *(Carcharhinus dussumieri)* fillets during frozen storage. American-Eurasian Journal of Sustainable Agriculture 3: 519-527.

Schwrtz, K. and Ternes, W. 1992. Anti-oxidative

constituents of *Rosmarinus officinalis* and *salvia officinalis*. I. Determination of phenolic di-terpenes with anti-oxidative activity amongst toco-chromanols using HPLC. Z. Lebensm Unters 19: 95–98.

Selami, S. and Sadoki, S. 2008. The effect of natural antioxidant (*Thymus vulgaris* (Linnaeus)) on flesh quality of tuna (*Thunnus thynnus* (Linnaeus)) during chilled storage. Pan-American Journal of Aquatic Sciences 3: 36-45.

Serdaroglu, M. and Felekoglu, E. 2005. Effects of using rosemary extract and onion juice on oxidative stability of sardine *(Sardina pilchardus)* mince. Journal of Food Quality 28: 109-120.

Shahidi, F. and Zhong, Y. 2005. Lipid Oxidation: Measurement Methods, In: F. Shahidi (Edit.), Bailey's Industrial Oil and Fat Products, Wiley-Interscience, A John Wiley and Sons, New Jersey, P. 357.

Stevenson, S. G., Vaisey-Genser, M. and Eskin, N. A. M. 1984. Quality control in the use of deep frying oils. Journal of American Oil Chemist Society 61: 1102–1108.

Taheri, S., Motallebi, A. A., Fazlara, A., Aftabsavar, Y. and Aubourg, S. 2012. Effect of previous ascorbic acid treatment on the fatty acid profile of cobia *(Rachycentron canadum)* fillets during frozen storage. Grasas y Aceites 63: 70-78.

Tsaknis, J. Lalas, S., Gergis, V., Dourtoglou, V. and Spilitois, V. 1999. Quality changes of *Moringa oleifera*, variety Mbololo of Kenya seed oil during frying. Journal of Agriculture Food Chemistry 47: 4495-4499.

Verma, J. K. and Srikar, L. N. 1994. Protein and lipid changes in pink perch *(Nemipterus japonicus)* mince during frozen storage. Journal of Food Science and Technology 31: 238-240.

Vidya Sagar Reddy, G. and Sriker, L. N. 1996. Effect of preprocess ice storage on lipid change of Japanese threadfin bream *(Nemipterus japonicus)* mince during frozen storage. Asian Fisheries Science 9: 109-114.

Zarei, M., Fazlara, A., Jamaledin, D. and Mojtahedi, M. 2011. Microbial and chemical quality of vacuum-packed frozen fillet of narrow-barred spanish mackerel *(Scomberomorus commerson)* and silver promfret *(Pampus argenteus)* market in Iran. World Journal of Fish and Marine Science 3: 410-413.