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The effects of *Rosmarinus officinalis* essential oil on the quality changes and fatty acids of *Ctenopharyngodon idella*

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Abstract

The effects of the use of essential oil of *Rosmarinus officinalis* on the chemical quality and fatty acids of fish fillets of *Ctenopharyngodon idella* were investigated during frozen storage at -18 ºC for 6 months. Fish fillets were divided into three groups; the control (C) without rosemary essential oil, the second group with 0.2 % rosemary essential oil (A) and the third with 0.4 % (v/v) rosemary essential oil (B). According to compositional analysis of the rosemary essential oil by GC-MS, the α-pinene (31.91%) and 1, 8- cineol (14.66%) were the predominant components. Results showed that there were no significant differences of protein in group C and A, but differences were seen between A and B (p<0.05). A significant decrease in lipid was obtained throughout between groups during storage (p<0.05). There were no significant differences of pH between three groups at storage periods (p>0.05). Effect of rosemary essential oil showed that the least changed fatty acids were on polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA), respectively. Microbial results showed TVC content of fillets fish did not exceed the limit during storage period for A and B Groups (< 7 log CFU g^{-1}). Rosemary essential oil at 0.2% and 0.4 % was effective in controlling the chemical compositions and fatty acids, but sensory attributes reveal a decreasing trend in the attributes like color, odour, taste, firmness and general acceptance for two groups (p<0.05), but group A indicated better scores than Group B at the end of the storage period.

Keywords: *Ctenopharyngodon idella*, *Rosmarinus officinalis* essential oil, Chemical compositions, Fatty acids

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Introduction

Fish is considered as a valuable source of protein in the human diet. This is mainly because of their naturally existing high content of essential n-3 polyunsaturated fatty acids (PUFAs). There are numerous studies (Arts *et al*., 2001; Givens *et al*., 2006; Saldanha and Bragagnolo, 2008; Wang *et al*., 2014) on the favorable effects of n-3 polyunsaturated fatty acids from fish confirming that increased fish consumption has a role in the prevention of coronary heart diseases, especially myocardial infarction, arteriosclerosis, hypertension and other cardiovascular diseases.

Sea-foods are perhaps the most perishable foods and their shelf life is restricted by enzymatic and microbiological spoilage. Many quality control methods have been carried out for the determination of fish spoilage. Lipid oxidation is a major quality problem. Fish is more prone to lipid oxidation than meat due to the high degree of un-saturation in fish lipids (Yildiz *et al*., 2008). The susceptibility of fish oxidation depends not only on the mount of total lipids but also on the composition of fatty acids and their location in fish muscle tissue (Raeisi *et al*., 2015).

More literature review has proved that even fish stored at frozen temperature can undergo undesirable quality changes, namely off-flavor, toughness, color changes, due to rancidity, protein denaturation and so forth. Consequently, employing natural preservations, antimicrobial, antioxidant substances, and stabilizers in the products formulations seems quite logical (Mejlholm and Dalgaard 2002; Mahmoud *et al*., 2004; Özyurt *et al*., 2011; Ojagh *et al*., 2014). The heightened demands by consumers for better quality and improved freshness of food products have given rise to the development and implementation of natural products. Essential oils (EOs) are natural herbs antioxidants and antimicrobials agents that have been recognized for many years. They have been applied in many fish species in showing antimicrobial and antioxidant activities against food-borne pathogens and extending the shelf life of the fish (Ozogul and Ucar, 2012).

Rosemary (*Rosmarinus officinalis*) is a plant species of the Labiatae family, and its major and most active extract components (e.g., carnosol, carnosic acid, carnosol, rosmarinic acid etc.) have been proved to act against cancer and inflammation diseases in experimental animals and humans (Tironi *et al*., 2011). The effects of pre-treatment of rosemary extract and essential oil on the quality of fish have been reported by several authors (Ozogul *et al*., 2010; Uçak *et al*., 2011; Li *et al*., 2012; Abdollahi *et al*., 2014). However, the effects of treatment of rosemary essential oil on the quality of frozen fillets of grass carp (*C. idella*) have not been reported so far.

Thus, the aim of this study is to investigate the effect of different levels of rosemary essential oil in combination with freezing process on the quality of grass carp.

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Materials and methods

Plant material

R. officinalis was collected in Alborz Province of Iran and identified by Institute of Medicinal Plants, Medical University of Tehran, Iran.

Extraction of essential oil

Air-dried aerial parts of the plant were subjected to steam distillation for 2 h using Clevenger-type apparatus. The essential oil yield of the air-dried material was analyzed by gas chromatography (GC; Thermoquest 2000, Finnigan MassLab Group, Manchester, UK). The chromatograph was equipped with DB5 capillary column (30×0.25 mm ID×0.25-µm film thickness) and the data were acquired under the following conditions: initial temperature 50 ºC; program rate 2.5 ºC; final temperature 265 ºC and injector temperature 250 ºC. The carrier gas was helium and the split ratio was 1:120. The mass spectrometry (MS) was run in the electron ionization mode, using an ionization energy of 70 eV. Constituents of the oil were identified and confirmed by comparing the experimental gas chromatographic retention indices and MS fragmentation pattern with those of the manufacturer's database (WILEY 2001data software) and literature data (Roomiani *et al*., 2013).

Sample preparation

40 fresh aqua-cultured grass carp (*C. idella*, average weight and length: 700± 30.00 g and 40±3.00 cm) were supplied from the aquaculture farm of Khuzestan Province of Iran. The fish samples were put in an ice box with ice and transferred to the laboratory. Then, the fish samples were beheaded, gutted and filleted by hand and washed with cold water carefully and dehydrated.

Rosemary essential oil was added onto the surface (two sides) of each fillet using a pipette so as to achieve a 0.2% and 0.4% oil volume per fish flesh weight (v/ w), respectively. Fillets were then massaged by hand for uniform distribution of the essential oil and samples were left for 4-5 minutes at room temperature. Fillets samples were randomly divided into three treatment groups: the control (C) without rosemary essential oil, the second group with 0.2 % rosemary essential oil (A) and the third with 0.4 % rosemary essential oil (B). Then each fillet was put in a separated polyamide/polyethylene bag and packed. After packaging, first the fillets were frozen by air blast method for 24 h at -30 ºC and then stored in -18 ºC for 6 months. Analysis of frozen fish fillets was carried out immediately after frozen (1, 2, 3, 4, 5, 6 months) storage at -18 ºC. Each time a total of six samples from each treatment were randomly drawn for analysis. The samples were thawed in a refrigerator overnight and a certain amount was taken from all six samples and homogenized in a mixer, then the final sample was taken from the homogenized mixture. The experiment was conducted with three replications

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and the mean was estimated. All analyses were conducted in triplicates.

Chemical composition

Determination proximate compositions

The moisture content of flesh was determined by drying to constant weight at 102-105 ºC for 20 to 24 h according to the AOAC standard method (AOAC, 2005).

Crude ash was determined after heating the sample overnight at 550 ºC (AOAC, 2005).

Crude protein content was determined by the Kjeldahl method (AOAC, 2005), employing the 6.25 conversion factor. The fat content of *C. idella* was determined by a solvent extraction (Submersion) method for fat (crude) in meat and meat products (AOAC, 2005).

Fatty acids analysis

Total lipid was extracted according to the AOAC standard method (AOAC, 2000). Lipid extracts were then saponified with 0.5 N methanolic NaOH and further transesterified with BF3 in methanol (AOAC, 2000). The fatty acid methyl esters (FAMEs) were analyzed on a Gas Chromatography (GC 1000, DANI Instrument, Switzerland) equipped with a flame ionization on a SGE Sil Tite mini-union (30 m×0.25 mm i.d.). The carrier gas was helium. The temperature profiles were as follows: initial temperature, 175 ºC; heating rate, 1 ºC min^{-1}; final temperature, 220 ºC (final time, 20 min); injector temperature, 250 ºC and detector temperature, 270 ºC. The fatty acids were identified by comparison of the retention times with those of standard (C19:0, Sigma) purified fatty acids (Taheri *et al*., 2012). Each sample was replicated three times and its average was calculated.

Microbiological analysis

Samples from each of groups (triplicate) were taken to estimate total viable count (TVC). Ten grams of fish fillets were mixed with 90 mL water 9/ 1000 (9g NaCl $1000mL^{-1}$ distilled water). From the 1: 10 $dilution^{-1}$ other decimal dilutions were prepared. Total Viable Count (TVC) was determined by using pour plate method and Plate Count Agar as medium according to ISO 8443 (2003). Then, all plates were prepared in triplicate and incubated for 2 days at 30 ºC.

Statistical analysis

The results were presented as mean± SD. Data from the different quality parameters were subjected to one-way ANOVA and differences among the means were determined for significance at $p<0.05$ using Duncan's multiple range test by using SPSS (version 20.0 for Window, SPSS, Inc., Chicago, IL, USA).

Results

Chemical composition of essential oil

The relative quantitative values of rosemary essential oil are presented in Table 1. The most important constituents of the rosemary essential oil were alpha-pinene (31.91%) and 1, 8- cineole (14.66%), respectively.

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Table 1: Essential oil composition of *Rosmarinus officinalis* identified by GC-MS.

Compound	Retention time	Percentage
Beta-pinene	9.04	1.55
1,8- cineole	11.04	14.66
Camphene	8.20	7.50
Camphor	15.67	8.68
Borneol	16.70	8.90
Verbenone	18.49	5.74
Alpha-pinene	7.77	31.91
Trans-caryophyllene	27.08	1.42
Isobornyl acetate	21.52	3.21
Alpha-Terpineol	17.63	1.97
Linalool	13.69	2.19
Beta-myrcene	9.44	3.90

Proximate composition

Groups (C), (A) and (B) of chemical composition; moisture (C: 73.70 to 20.20%; A: 72.98 to 21.42%; B: 76.00 to 19.87%), lipid (C: 4.50 to 3.11%; A: 6.17 to 3.90%; B: 7.00 to 5.22%), protein (C: 21.22 to 8.24%; A: 20.40 to 9.80%; B: 22.60 to 12.87%) and ash (C: 0.58 to 0.48%; A: 0.54 to 0.60%; B: 0.52 to 0.67%) of frozen fillets of *C. idella* stored prepared from the first to the sixth month are mentioned in Table 2.

Table 2: Changing in chemical composition of *Ctenopharyngodon idella* in different treatment of rosemary essential oil during -18 ºC (means±SD). Different script letters characterize different in each raw (a-d) for different times (p<0.05) of storage and in each column (A-C) for different treatments (p<0.05).

	Month					
Composition	**1**	**2**	**3**	**4**	**5**	**6**
Protein						
C	21.22 ± 3.09^{Ad}	21.05 ± 3.00^{Ac}	15.88 ± 1.66^{Ab}	13.82 ± 1.58^{Aa}	10.30 ± 0.09^{Aa}	8.24 ± 0.08^{Aa}
A	20.40 ± 2.66^{Ad}	19.25 ± 2.00^{Be}	16.10 ± 1.68^{Ac}	14.29 ± 1.59^{Abc}	12.50 ± 0.09^{Aab}	9.80 ± 0.09^{Aa}
B	22.60 ± 4.00^{Ad}	22.95 ± 4.02^{Ce}	20.12 ± 2.08^{Abc}	18.73 ± 1.77^{Bbc}	15.63 ± 1.58^{Bab}	12.87 ± 0.09^{Ba}
Lipid						
C	4.50 ± 0.07^{Ad}	4.90 ± 0.07^{Ad}	4.70 ± 0.07^{Acd}	3.80 ± 0.02^{Abc}	3.20 ± 0.01^{Aab}	3.11 ± 0.01^{Aa}
A	6.17 ± 0.09^{Bb}	6.27 ± 0.09^{Bb}	6.00 ± 0.09^{Bb}	6.80 ± 0.08^{Bb}	5.20 ± 0.02^{Bb}	4.90 ± 0.01^{Bb}
B	7.00 ± 0.09^{Bb}	7.06 ± 0.09^{Bb}	7.10 ± 0.09^{Bb}	7.10 ± 0.09^{Cb}	6.50 ± 0.08^{Cb}	6.22 ± 0.07^{Ca}
Ash						
C	0.58 ± 0.003^{Abc}	0.66 ± 0.004^{Bc}	0.60 ± 0.004^{Abc}	0.53 ± 0.002^{Aab}	0.50 ± 0.002^{Aab}	0.48 ± 0.002^{Ab}
A	0.54 ± 0.003^{Aa}	0.58 ± 0.003^{Aa}	0.55 ± 0.003^{Aab}	0.56 ± 0.003^{Ab}	0.58 ± 0.003^{Bb}	0.60 ± 0.004^{Bb}
B	0.52 ± 0.002^{Aa}	0.39 ± 0.001^{Aa}	0.56 ± 0.003^{Be}	0.60 ± 0.004^{Bb}	0.64 ± 0.004^{Cb}	0.67 ± 0.006^{Cb}
Moisture						
C	73.70 ± 1.66^{Ac}	73.39 ± 1.61^{Ac}	33.42 ± 0.07^{ABb}	26.44 ± 0.06^{Bab}	21.33 ± 0.06^{Ab}	20.20 ± 0.01^{Ab}
A	72.89 ± 1.30^{Ac}	73.90 ± 1.77^{Ac}	33.58 ± 0.08^{Bb}	28.52 ± 0.07^{Bab}	22.35 ± 0.06^{Aa}	21.42 ± 0.06^{Aa}
B	70.00 ± 1.11^{Ab}	69.60 ± 1.10^{Ab}	26.56 ± 0.06^{Aa}	21.11 ± 0.05^{Aa}	20.91 ± 0.01^{Aa}	19.87 ± 0.01^{Aa}
pH						
C	6.17 ± 1.11^{Aa}	6.37 ± 1.25^{Aa}	6.02 ± 1.00^{Aa}	6.30 ± 1.00^{Aa}	6.00 ± 1.00^{Aa}	6.21 ± 1.00^{Aa}
A	6.29 ± 1.19^{Aa}	6.18 ± 1.05^{Aa}	6.22 ± 1.00^{Aa}	6.18 ± 0.99^{Aa}	6.20 ± 0.99^{Aa}	6.16 ± 0.99^{Aa}
B	6.99 ± 0.18^{Aa}	7.00 ± 0.16^{Aa}	6.81 ± 1.00^{Aa}	6.95 ± 2.00^{Aa}	6.88 ± 2.00^{Aa}	6.70 ± 0.18^{Aa}

The results of evaluations of protein content showed that group B (0.4% essential oil) had the highest protein content and the group C (Control) had

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the lowest. No significant differences (p>0.05) were observed between groups in the 1st month. After the 2nd month, significant differences (p<0.05) were found between Group C and B (p<0.05).

Comparison of the amounts of lipid showed that there was a significant differences between the 3 groups (p<0.05). Group C had the lowest amount of lipid and group A had the highest (Table 2). The low lipid content in group C and A was recorded in the sixth month and the highest lipid content was recorded in the second and fourth months (p<0.05).

Statistical analysis showed there were no significant differences between moisture of different months of storage of group B (p>0.05) but there were differences between group C and B (p<0.05). During the time between months 1 to 6, the moisture content in the three groups decreased.

Table 2 shows changes in pH values in fish fillets of grass carp in different groups. No significant difference in pH values were observed during storage (p>0.05). The pH values of the studied fish fillets increased and then decreased during storage period; however this mentioned pattern was not observed in all of the samples.

Fatty acids changes

Different individual fatty acids were identified and quantified in *C. idella* muscle; results obtained throughout a 6-month frozen storage time in fillets of fish are shown in Table 3. The changes in fatty acids profiles during storage in different treatments in frozen conditions were statistically significant especially in months of 2, 3 and 4 (p<0.05).

In the last months, there were no significant differences among the fatty acid profiles obtained in samples (p>0.05).

Table 3: Changes in the fatty acid profiles of stored grass carp (*Ctenopharyngodon idella*) up to 6 months at - 18 ºC (means± SD; n=3; p<0.05; % of total lipids; ND: not detected).

Treatment	Fatty acid	1	2	3	4	5	6
C	C12:0	0.21±0.002^{a}	0.19±0.001^{a}	0.11±0.001^{a}	ND	ND	ND
B		0.14±0.001^{a}	0.10±0.001^{a}	ND	ND	ND	ND
A		0.29±0.002^{a}	0.23±0.001^{a}	0.13±0.001^{a}	ND	ND	ND
C	C14:0	5.57±0.99^{a}	3.08±0.10^{b}	2.34±0.08^{a}	2.07±0.07^{a}	1.65±0.001^{a}	1.10±0.001^{a}
B		5.34±0.92^{a}	2.68±0.07^{b}	2.71±0.07^{a}	2.52±0.07^{a}	1.32±0.001^{a}	1.28±0.001^{a}
A		3.85±0.11^{a}	3.56±0.11^{b}	3.20±0.10^{a}	2.87±0.10^{a}	2.41±0.07^{a}	1.84±0.001^{a}
C	C16:0	33.20±2.33^{c}	ND	ND	ND	ND	ND
B		29.20±2.11^{c}	27.70±2.00^{c}	15.16±1.22^{b}	9.70±1.00^{b}	4.30±0.88^{b}	1.50±0.001^{a}
A		30.40±2.21^{c}	27.20±2.00^{c}	16.30±1.66^{b}	8.30±0.99^{b}	5.60±0.99^{b}	2.20±0.07^{b}
C	C18:0	3.70±0.11^{b}	2.93±0.08^{a}	2.36±0.08^{a}	1.87±0.001^{a}	1.23±0.001^{a}	0.86±0.001^{a}
B		5.36±0.92^{b}	3.19±0.10^{a}	2.56±0.07^{a}	1.86±0.001^{a}	1.01±0.001^{a}	ND
A		3.12±0.11^{b}	2.96±0.07^{a}	2.03±0.07^{a}	1.56±0.001^{a}	1.32±0.001^{a}	1.10±0.001^{a}
C	C22:0	2.48±0.001^{a}	1.24±0.001^{a}	0.89±0.001^{a}	ND	ND	ND
B		2.98±0.001^{a}	0.23±0.001^{a}	ND	ND	ND	ND
A		2.16±0.09^{a}	0.70±0.001^{a}	ND	ND	ND	ND
C	C16:1	17.28±1.55^{b}	13.28±1.20^{b}	10.60±1.01^{a}	7.56±0.80^{a}	4.47±0.80^{a}	2.54±0.07^{a}
B		14.77±1.33^{b}	13.59±1.30^{b}	11.40±1.01^{a}	9.76±1.00^{a}	5.78±0.99^{a}	1.98±0.001^{a}
A		13.87±1.21^{b}	13.40±1.21^{b}	10.70±1.01^{a}	8.56±0.99^{a}	5.87±0.99^{a}	2.76±0.07^{a}
C	C18:1n-9	28.46±2.00^{c}	32.87±2.20^{c}	29.52±2.00^{b}	23.33±1.88^{b}	17.40±1.33^{a}	14.30±1.24^{b}
A		32.30±2.20^{c}	25.38±1.88^{c}	29.24±2.00^{b}	24.74±1.88^{b}	18.30±1.33^{a}	13.50±1.00^{a}
B		37.23±3.00^{c}	33.75±2.40^{c}	30.21±2.00^{b}	25.40±1.99^{b}	20.31±1.33^{b}	14.67±1.24^{b}

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Table 3 continued:							
C	C18:2n-6	8.07±0.99[a]	9.86±1.00[b]	8.50±0.99[a]	7.30±0.80[a]	5.60±0.99[a]	4.32±0.70[a]
B		12.36±1.00[b]	9.65±1.00[b]	8.78±0.98[a]	7.60±0.80[a]	5.80±0.99[a]	4.90±0.70[a]
A		14.00±1.25[b]	9.64±1.00[b]	8.45±0.98[a]	7.20±0.80[a]	6.11±0.99[a]	4.70±0.70[a]
C	C18:3n-3	2.77±0.08[b]	2.32±0.08[a]	1.90±0.001[a]	1.42±0.001[a]	0.95±0.001[a]	ND
B		3.17±0.10[b]	2.96±0.08[a]	2.12±0.08[a]	1.76±0.001[a]	1.25±0.001[a]	0.94±0.001[a]
A		3.05±0.10[b]	2.51±0.08[a]	2.35±0.08[a]	1.78±0.001[a]	1.62±0.001[a]	1.20±0.001[a]
C	C20:4n-6	0.29±0.001[a]	ND	ND	ND	ND	ND
B		0.32±0.001[a]	ND	ND	ND	ND	ND
A		0.25±0.001[a]	ND	ND	ND	ND	ND
C	C20:5n-3	2.00±0.07[a]	0.53±0.001[a]	ND	ND	ND	ND
B		1.68±0.001[a]	0.29±0.001[a]	0.29±0.001[a]	0.26±0.001[a]	0.26±0.001[a]	0.15±0.001[a]
A		1.06±0.001[a]	0.42±0.001[a]	0.33±0.001[a]	0.27±0.001[a]	0.26±0.001[a]	0.26±0.001[a]
C	C22:6n-3	1.66±0.001[a]	0.88±0.001[a]	0.88±0.001[a]	0.81±0.001[a]	0.67±0.001[a]	0.51±0.001[a]
B		1.04±0.001[a]	0.99±0.001[a]	0.99±0.001[a]	0.75±0.001[a]	0.77±0.001[a]	0.78±0.001[a]
A		1.40±0.001[a]	1.08±0.001[a]	1.00±0.001[a]	0.88±0.001[a]	0.89±0.001[a]	0.89±0.001[a]

Different script letters characterize different in each raw (a-c) for different times of storage (p<0.05).

Palmitic acid (C16:0) and myristic acid (C14:0) were the major fatty acids among the SFAs during storage and lauric acid (C12:0) and Stearic acid (C18:0) were also in minimum in value (Table 3). Expect for the first and second month, significant differences were observed among the SFA (saturated fatty acids) during frozen storage (p<0.05). There were significant differences among MUFA contents during the 6 months (p<0.05). Oleic acid (C18:1n-9) and palmitoleic acid (C16:1) were in the maximum value of fish tissue as compared to other fatty acids. There were significant differences among PUFA contents during 6 months (p<0.05). It is noticeable that linoleic (C18:2n-6) was predominant in the total n-6 polyunsaturated fatty acids in fillets of grass carp and linolenic acid (C18:3n-3) was the major total n-3 polyunsaturated fatty acids in fillets of grass carp.

Table 4: Changes in fatty acids series of stored *Ctenopharyngodon idella* up to 6 months at -18 ºC in different treatments (means± SD, n=3); p<0.05; % of total lipids.

		1	2	3	4	5	6
$\sum$ SFA	C	44.16±1.66[a]	7.44±0.08[a]	5.70±0.06[a]	3.94±0.02[a]	2.88±0.02[a]	1.96±0.01[a]
	A	41.02±1.60[a]	33.90±0.66[a]	20.43±0.44[b]	14.08±0.[22a]	6.63±0.05[a]	2.78±0.01[a]
	B	38.82±0.66[a]	34.65±0.66[a]	21.66±0.44[b]	12.73±0.09[a]	9.33±0.09[a]	5.14±0.06[a]
$\sum$ MUFA	C	45.74±1.88[a]	46.15±1.89[a]	40.12±1.60[b]	30.89±0.55[b]	21.78±0.44[b]	16.48±0.33[b]
	A	47.07±2.00[a]	38.97±0.66[a]	40.64±1.60[b]	34.50±0.66[b]	24.80±0.55[b]	15.48±0.28[b]
	B	51.10±2.15[a]	47.15±1.90[a]	40.91±1.60[b]	33.96±0.66[b]	17.24±0.28[b]	17.43±0.55[b]
$\sum$ PUFA	C	14.79±0.26[a]	13.59±1.22[b]	10.40±0.16[a]	8.72±0.08[a]	6.55±0.06[a]	4.32±0.05[a]
	A	16.57±0.33[a]	13.49±1.20[b]	10.90±0.18[a]	9.36±0.09[a]	7.05±0.07[a]	5.84±0.06[a]
	B	14.76±0.26[a]	13.52±1.22[b]	10.23±0.15[a]	8.98±0.08[a]	7.72±0.07[a]	5.90±0.06[a]
$\sum$ n − 3 PUFA	C	6.43±0.06[a]	3.73±0.04[b]	3.07±0.03[a]	2.23±0.02[a]	1.62±0.01[a]	0.51±0.01[a]
	A	5.89±0.06[a]	4.24±0.06[b]	3.40±0.03[a]	2.78±0.02[a]	2.28±0.02[a]	1.87±0.01[a]
	B	5.51±0.06[a]	4.01±0.06[b]	3.68±0.04[a]	2.93±0.02[a]	2.77±0.02[a]	2.35±0.02[a]
$\sum$ n − 6 PUFA	C	10.36±0.16[a]	10.39±0.16[b]	8.50±0.08[a]	7.30±0.07[a]	5.60±0.06[a]	4.47±0.03[a]
	A	14.36±0.26[a]	9.94±0.12[b]	9.07±0.09[a]	7.86±0.07[a]	6.06±0.06[a]	5.16±0.06[a]
	B	15.31±0.28[a]	10.06±0.16[b]	8.78±0.08[a]	7.47±0.07[a]	6.37±0.07[a]	4.96±0.05[a]
n-3/ n-6	C	0.62±0.02[a]	0.35±0.01[b]	0.36±0.01[a]	0.30±0.01[a]	0.28±0.01[a]	0.11±0.01[a]
	A	0.41±0.02[a]	0.42±0.01[b]	0.37±0.01[a]	0.35±0.01[a]	0.37±0.01[a]	0.36±0.01[a]
	B	0.35±0.01[a]	0.39±0.01[b]	0.41±0.02[a]	0.39±0.01[a]	0.43±0.01[a]	0.47±0.01[a]

The PUFA accounted for C 14.79%, A 16.57% and B groups 14.76% of the total fatty acids. A decrease was observed in the percentage of fatty acids during storage which was statistically significant (p<0.05) for

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SFA in third month, MUFA in third to sixth months and PUFA in second month of storage time. Results showed the lowest changes in profile fatty acids of *C. idella* in B group. Expect for the last month, results showed that the PUFA/SFA ratio was less than 1 (Table 4) and decrease of PUFAs, in contrast to SFA, led to a significant decrease in this ratio (p<0.05) during frozen storage. Table 3 shows the MUFAs were the most abundant fatty acids in the tissues of grass carp, accounting for 51.10% of the total fatty acids in B group.

Microbiological changes

Microbial counts on the fish fillets affected by *R. officinalis* EO are presented in Fig. 1. Initial total viable counts of fish fillets in C, A and B Groups were 3.10± 0.55 and 3.50± 0.55 log CFU g^{-1}, respectively. TVC increased with an increase in storage time (Fig. 1). TVC content of fillets fish did not exceed the limit during the storage period for A (4.82±0.11 log CFU g^{-1}) and B (4.31±0.21 log CFU g^{-1}) groups (< 7log CFU g^{-1}). The control group contained high level of TVC (7.40±0.32 log CFU g^{-1}) compared to treatment groups (p<0.05). On the other hand, there were no significant differences (p>0.05) between group A and B. Rosemary EO was effective in controlling the growth of microorganisms.

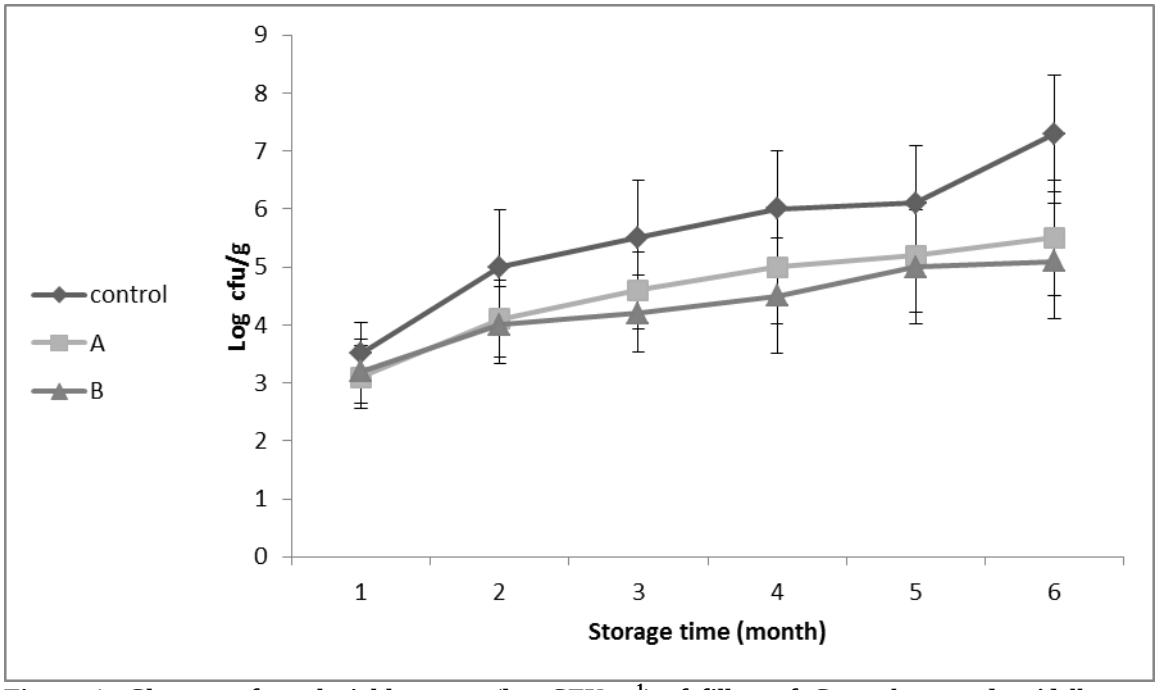


Figure 1: Changes of total viable count (log CFU g^{-1}) of fillets of *Ctenopharyngodon idella* as affected by *Rosmarinus officinalis* essential oil during storage at -18 ºC.

Sensory changes

The sensory quality of fillets of grass carp treated with rosemary essential oil were evaluated in terms of color, odor, taste, firmness and general acceptability (Table 4). The acceptability of fish and fishery products during frozen storage depends on the changes in their sensory attributes. According to the statistical analysis, there were no significant

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differences (p>0.05) in color of all groups during the storage. In the 1st, 2nd, 3rd, 4th, 5th, and 6th months of storage, differences were observed in taste (p<0.05) between the control and rosemary treated groups. An obvious deterioration was observed in the control and group B at the end of the storage period. General acceptability seems to be significantly different (p<0.05) among the control, Group A and Group B in the 5th and 6th months. Sensorial parameters of both the control and experimental groups decreased significantly during the storage period (p<0.05). Because the flavor and taste of rosemary essential oil were much stronger in Group B than in Group A, Group A was mostly preferred by the panelists. The use of rosemary essential oil improved the sensory quality of fillets grass carp. The data also indicated that fillets of treated with rosemary essential oil could be stored at -18 ºC for six months while retaining their good quality characteristics in terms of sensory assessment (p>0.05).

Table 5: Sensory attributes of frozen fillets of grass carp treated with rosemary essential oil ($X\pm S_x$: average± SD). C: control, A: 0.2% rosemary essential oil, B: 0.4% rosemary essential oil. 9: very good quality, 7–8: good quality, 5–6: acceptable, 1–4: unacceptable. Different letters in the same column for each storage days indicate significant differences.

Storage time (month)	Color	Odour	Taste	Firmness	General acceptance	Groups
	8.99±0.44^{a}	8.88±0.44^{a}	9.11±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	C
1	8.99±0.44^{a}	8.88±0.44^{a}	8.87±0.00^{a}	9.00±0.00^{a}	9.00±0.00^{a}	A
	8.99±0.44^{a}	8.89±0.44^{a}	8.20±0.00^{b}	9.00±0.00^{a}	9.00±0.00^{a}	B
	8.60±0.33^{a}	8.80±0.33^{a}	8.60±0.59^{a}	8.70±0.59^{a}	9.00±0.00^{a}	C
2	8.60±0.33^{a}	8.70±0.33^{a}	8.40±0.58^{a}	8.60±0.33^{a}	8.50±0.65^{a}	A
	8.80±0.33^{a}	8.70±0.33^{a}	8.20±0.58^{a}	8.00±0.34^{a}	8.75±0.97^{a}	B
	8.30±0.29^{a}	8.50±0.32^{a}	8.00±0.26^{a}	8.80±0.34^{a}	8.25±0.60^{a}	C
3	8.50±0.32^{a}	8.25±0.30^{a}	7.66±0.40^{a}	8.60±0.33^{a}	8.33±0.60^{a}	A
	8.40±0.32^{a}	8.50±0.32^{a}	7.00±0.40^{b}	8.00±0.41^{a}	8.00±0.00^{a}	B
	8.00±0.00^{a}	7.50±0.40^{b}	7.33±0.40^{a}	8.18±0.41^{a}	7.80±0.55^{a}	C
4	8.00±0.00^{a}	8.20±0.45^{a}	8.00±0.00^{a}	8.00±0.44^{a}	7.30±0.50^{a}	A
	8.30±0.00^{a}	8.40±0.45^{a}	6.00±0.33^{b}	8.20±0.45^{a}	7.00±0.50^{a}	B
	8.00±0.00^{a}	7.20±0.40^{a}	6.00±0.40^{a}	7.50±0.44^{a}	6.88±0.44^{ab}	C
5	7.90±0.40^{a}	8.20±0.45^{a}	7.50±0.40^{a}	7.25±0.44^{a}	6.55±0.40^{a}	A
	7.60±0.40^{a}	8.20±0.45^{a}	6.55±0.40^{b}	7.00±0.40^{a}	7.00±0.50^{a}	B
	6.00±0.40^{a}	6.50±0.40^{a}	5.50±0.40^{b}	5.25±0.40^{ab}	6.11±0.33^{ab}	C
6	7.10±0.40^{a}	7.00±0.40^{a}	6.50±0.40^{a}	6.50±0.55^{a}	7.54±0.40^{b}	A
	7.00±0.40^{a}	7.00±0.40^{a}	6.00±0.00^{b}	5.70±0.50^{a}	6.22±0.42^{a}	B

Discussion

Many studies (Zaouali *et al*., 2010; Jalali- Heravi *et al*., 2011; Roomiani *et al*., 2013) observed that the most important constituents of rosemary are 1, 8- cineole and alpha-pinene but differences were observed between them. The essential oil content of different plants vary (together with the biological active compounds contained) depending on which part of the plant it is obtained from (flower, stem, leaves, whole plant, etc.) the variety of the plant, its harvest season and the method of cultivation.

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Investigations on fish nutrition have shown that fatty acid profiles were affected by dietary lipid level, seasonal changes and temperature. Fresh water fish are capable of converting C18 polyunsaturated fatty acid (PUFA) to the longer chain C20 and C22 HUFA (Aubourg, 1999; Du *et al*., 2008; Murray *et al*., 2014). For this reason, fatty acids profiles in the fillets of fish reflected fatty acids profile of commercial feed (Yildiz *et al*., 2008).

The decrement in the moisture and protein indicates that protein fractions of the fillets would have undergone structural changes during frozen storage leading to the loss in the functionality like water-holding capacity and water-soluble nitrogen content. Fish are known for the loss in water-holding capacity and water- soluble nitrogen in the thaw drip during frozen storage. This can be due to percentage balance in chemical composition because of loss in the moisture content (Yeganeh *et al*., 2009). Results showed moisture and protein are decrease whereas fat content are increased in some months.

Decrement in moisture and increment in fat may be due to evaporation of water and absorption of fat. Upadhay and Das (2006) reported a decrease in moisture content in fish fillets of grass carp during frozen storage. This reduction in the amount of protein can be attributed to changes to the protein into nitrogen solution during storage time (TVN) (Kenar *et al*., 2010).

Changes in lipid content had an important role, as the index showing loss of quality and total lipid is one of the most important indexes to determine levels of frozen fish spoilage. The final reduction of total lipid in the measured samples was likely due to lipid oxidation and the effects of enzymes in lipid hydrolytic spoilage and changes to free fat acids. Further degradation results in the formation of aldehydes and ketones called secondary oxidation products (Mahmoudzadeh *et al*., 2010).

Although pH value is not a suitable index on its own to determine quality of fish, it can be useful as a guideline for quality control of fish when used with other quality parameters. Initial post mortem pH can vary from 5.4 to 7.2 depending on fish species and other factors (Grigorakis *et al*., 2003). The pH values between 6.8 and 7 were proposed as acceptance limit of fish (Varlik *et al*., 1993; Orak and Kayisoglu, 2008) and values above 7 were considered to be spoiled (Taheri and Motallebi, 2012). The changes in pH of the samples can be related to (i) the glycolysis, (ii) bacterial growth inhibitory effects of rosemary EO (iii) decomposition of amino compounds during storage time (Jasour *et al*., 2011). The pH values were shown to be more stable ($p<0.05$) in B group in comparison with other treatments that are attributed to the antibacterial property of rosemary and its effect on delaying microbial growth and amino acids decomposition (Erkan *et al*., 2011).

The maximum recommended bacterial counts for marginally acceptable quality fish and fishery products are 7 log CFU g^{-1} (ICMSF, 1986). This recommendation was met

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by our results. The obtained results showed that microbial changes in all samples were generally in agreement with sensory panel evaluations. When fish was unacceptable by panelist, total viable count was in the range 6-7 log CFU g^{-1}

TVC are used as an acceptability index for fish products because of the effect of bacteria in spoilage. When sea-foods are frozen, the microorganism present on the surface and the tissue of fish are generally inactivated. Thus, during frozen storage, microbial changes in fish are minimal. Although some microorganisms survive at low temperature, their activities are suppressed and bacterial numbers may be reduced when recommended temperatures are maintained (Ozogul *et al*., 2011)

Changes in aroma, color and flavor can be because of microorganism growth and operation (Ozogul *et al*., 2004). Similar results were obtained from the other studies (Corbo *et al*., 2009; Tokur *et al*., 2006; Uçak *et al*., 2011; Khanipour and Mirzakhani, 2013).

It was observed that treatment of grass carp with rosemary essential oil had significant effects on the quality of fillets as assessed by chemical, microbiological, sensory methods and changes of fatty acids. Phenolic constituents of rosemary essential oil can be used in commercial food products because they provide safe and high quality foods. According to the results obtained two treatments (A and B) were significantly effective in freshness stability of fish fillets when compared with the control group (C), especially at higher concentration, had the greatest effect on chemical, microbiological attributes but not on sensory attributes, so that the level of these quality indicators for the 0.2% essential oil group reached the limit of acceptability later than for the 0.4% essential oil group. These data indicate that the fillets of grass carp treated with rosemary essential oil during 6 months frozen storage were under good conditions.

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