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The Effects of Coating and *Zataria multiflora* Boiss Essential Oil on Chemical Attributes of Silver Carp Fillet Stored at 4ºC

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Abstract: The effects of *Z. multiflora* essential oil and coating on fresh silver carp fillets during storage at 4ºC were evaluated in present study. Treatments included the following: A (control samples, without coating and EO), E_1 (treated samples with 0.2% EO), E_2 (treated samples with 0.4% EO), C (coated samples), E_1C (treated by coating and 0.2% EO) and E_2C (treated by coating and 0.4% EO). The initial pH, TVB-N, PV and TBA content was 6.67, 7.75 mg N/100 g, 1.03 meq O_2/kg and 0.19 mg/kg, respectively. Both treatments and storage at 4ºC has significant ($P<0.05$) effects on aforementioned factors. *Z. multiflora* Boiss essential oil and coating were effective in retarding the production of primary and secondary lipid oxidation and microbiological activity.

Keywords: Silver carp, shelf life extension, essential oil, coating, chemical attributes

Introduction

Fish are very susceptible to both microbiological and chemical deterioration, due to large amounts of free amino acids, volatile nitrogen bases, highly unsaturated fatty acids and higher final pH (Razavi Shirazi, 2001). Chemical, enzymatic and microbial activity caused to loss of fish quality during storage (Özogul *et al*., 2006; Özyurt, 2009). Lipid oxidation is one of the major problems encountered in fish processing which have high content of polyunsaturated fatty acids. Synthetic antioxidants have been widely used as food additives to provide protection against oxidative degradation and to prolong the storage stability of foods. According to some reports, these compounds have possible toxic properties to human health and environment and can exhibit carcinogenic effects in living organisms (Stich, 1991, Ames, 1983; Baardseth, 1989). Many efforts have been done to reduce these activities for supplying fresh fish according to consumers' demand (Hassan, 2002). In this situation, using natural additives such as essential oils has been studied on shelf life of different food (Burt, 2004), to develop natural preservative with high antioxidant and antibacterial effect that could extent the shelf life of fish.

Recently, increasing attention has been focused on the use of natural antioxidants, such as essential oils. Essential oils possess antibacterial, antioxidant, antiviral and anti-mycotic properties (Burt, 2004), due to their active phenolic compounds, i.e. carvacrol, thymol (Burt, 2004; Dormana *et al*., 2003; Lee *et al*., 2005). *Z. multiflora* Boiss. With the local name of Avishan-e-Shirazi in Iran is a plant belonging to the Lamiaceae family (Gandomi *et al*., 2009), is extensively used as a flavor ingredient in a wide variety of food in Iran. This plant possesses carvacrol, thymol as main phenolic compounds and p-cymene as main non-phenolic compounds, respectively (Sharififar *et al*., 2007). The essential oil of this plant recognized as safe by EC. These compounds have antioxidant properties and are able to inhibit linoleic oxidation (Sharififar *et al*., 2007; Shaffiee *et al*., 1997). *Z. multiflora* essential oil has also been for antimicrobial purposes in food (Gandomi *et al*., 2009; Rahnema *et al*., 2009).

Edible coating can extend the shelf life of foods by functioning as solute, gas and vapour barriers (Kester and Fennema, 1986; Baldwin *et al*., 1995; Krochta and De Mulder-Johnston, 1997; Miller and Krochta, 1997; Debeaufort *et al*., 1998) and reducing the oil uptake during frying. Also, breaded foods are popular worldwide (Yazdan *et al*., 2009). Silver carp (*Hypophthalmichthys molitrix*) is the most important species in the poly-culture system in Iran. It is sold mainly as whole fresh fish and newly in fillet form in

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markets according to consumers' demand.

With regard to fish, including silver carp, to our knowledge, there are no studies in the literature on the antioxidant effects of *Z. multiflora* Boiss essential oil, on shelf life. Thus the aim of the present work was to determine the chemical and antioxidant changes of refrigerated silver carp fillets, using either edible coating or *Z. multiflora* Boiss essential oil and their combination during storage at 4ºC.

Material and Methods

Fish samples and preparation

Fresh aqua-cultured silver carp (*H. molitrix*, with average weight and length of 1000 g and 350 mm) were obtained from a warm water aquaculture farm located on Sari (Mazandaran, Iran) during May, 2010. Fish were delivered to the laboratory within 20 minute of harvesting, packed in insulated boxes containing ice. The fish were eviscerated, headed and filleted (approximately each fillet of uniform 21×9 cm^2 and weight 100 g) by hand. Skin and spiny bones were removed as possible as by hand from fillets. Fillet samples were randomly divided into six treatments lots including: A (control samples, without coating and EO), E_1 (treated samples with 0.2% EO), E_2 (treated samples with 0.4% EO), C (coated samples), E_1C (treated by coating and 0.2% EO) and E_2C (treated by coating and 0.4% EO). Each lot was repeated three times. Essential oil was added onto the surface (two sides) of each fillet and gently massaged by hand to homogenous distribution of EO. All samples after vacuum packaging (Henkelman, 200 A, Netherland) were stored at 4ºC. Sampling was carried out on day: 0, 3, 6, 9, 12, 15 and 18 of storage.

Coating was prepared according to Yazdan *et al.* (2009). Battering ingredients (wheat flour 30%, corn flour 10% and cold water 60% (w/v) were mixed thoroughly for 5 min, manually. The batter was distributed on all surfaces of fillets in a thin layer and allowed to excess batter dripping off. Battered fillets were then coated with bread crumbs (Shirin Pad co., Iran) before storing at refrigerator. *Z. multiflora* was collected from Shiraz (Fars province, Iran) and was identified by Medical Plants' Herbarium of Jehad Daneshgahi, Tehran, Iran. The air-dried aerial parts were subjected to steam distillation for 4 h using Clevenger-type apparatus (Rahnema *et al*., 2009). The extracted essential oil was analyzed by GC-MS (Agilent, model 6890 GC and model 5973 mass detector, America). Separation of active compounds of *Z. multiflora* EO was achieved on a HP-5MS (30 m x 0.25 mm ID x 0.25 μm film thickness). Helium was used as carrier gas with a flow rate of 0.8 ml/min. The oven was programmed at an initial temperature of 50ºC for 5 min. Temperature was increased to 240º with a rate of 3ºC/ min and then increased to 300º with a rate of 15º/min and held for 3 min. The injector temperature was set in 290ºC. The MS was run in the electron ionization mode set at 70 eV. The ion source temperature was maintained at 220ºC (Rahnema *et al*., 2009).

Chemical analysis

Proximate composition

Crude protein was calculated by converting the nitrogen content determined by Kjeldahl's method (AOAC, 2005). Crude lipid was determined by ether extraction using a Soxhlet method (AOAC, 2002). The moisture content was determined by drying the meat in an oven at 105ºC until a constant weight was obtained (AOAC, 2002). Ash content was determined by drying the samples in a furnace at 550 ºC for 12 h (AOAC, 2002)

pH determination

The pH value was recorded using a pH meter (Multiline P4, WTW). 5 g of fish sample was homogenized thoroughly with 45 ml of distilled water for 30 s and homogenate was used for pH determination.

Biochemical changes

Total volatile basic nitrogen (TVB-N) was determined using the method of AOAC (Association of Official of Analytical Chemists, 2002). TVB-N content was expressed as mg TVB-N/100 g fish muscle. Peroxide value (PV) was determined using the method of Egan *et al*. (1997). Thiobarbituric acid (TBA) was determined according the method used by Egan *et al*. (1997). PV and TBA content were expressed as meq per 1000 g and mg/kg, respectively.

Statistical analysis

All measurements were replicated three times for each lot and mean values ± standard deviations were reported for each case. The data were analyzed using the one and two-way analysis of variance test (ANOVA). The one-way ANOVA was used to analyze the effect of treatments on the control and also time of storage on each treatment and two-way ANOVA was used to analyze the effect of treatments and time of storage on fish samples. The Turkey's test was used for mean comparison when a significant variation was found by the ANOVA test. The significance of

results was at 5%. The software used was Minitab, release 14.

Results

According to GC-MS analysis, the main phenolic and non-phenolic active compounds in *Z. multiflora* essential oil were as: thymol (59.50%), p- cymene (13.60%), carvacrol (5.6%) and γ-terpinene (4.3%). The quantity of these compounds can be vary due to harvesting season, plant age, soil, climate, geographical sources, herb drying method and extraction method (Shafiei and Javidan, 1997; Bagamboula *et al*., 2004; Shraififar *et al*., 2007).

Proximate composition

Proximate analysis results (Day 0 after treatments) are presented in Table 1. The results are in good agreement with those reported by Asgharzadeh *et al*. (2010). The protein content of control samples in present study was lower as compared with protein content of silver carp in Asgharzadeh *et al*. (2010) report and wild silver carp (Ali *et al*., 2006), which is attributed to nutrition differences. Significant increases (P<0.05) in protein content were observed in coated samples (C, E1C and E2C), which subsequently decreased the moisture content of these samples (Table 1). These differences could be due to effect of battering and breading ingredients.

Table 1. Proximate analysis of sliver carp fillet after different treatments

Composition	Proximate analysis (%)					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Coated (C)	Coated + 0. 2% EO (E1C)	Coated + 0.4% EO (E2C)
Total lipid	4.94 ± 0.44 ^{a}	4.94 ± 0.52 ^{a}	4.91 ± 0.48 ^{a}	4.89 ± 0.5 ^{a}	4.91 ± 0.42 ^{a}	4.91± 0.57 ^{a}
Total protein	14.35 ± 0.53 ^{b}	14.38 ± 0.41 ^{b}	14.36 ± 0.51 ^{b}	18.73 ± 0.56 ^{a}	18.60 ± 0.54 ^{a}	19.02± 0.59 ^{a}
Moisture	77.74 ± 0.45 ^{a}	77.74 ± 0.50 ^{a}	77.74 ± 0.52 ^{a}	73.37 ± 0.58 ^{b}	73.40 ± 0.54 ^{b}	73.44± 0.47 ^{b}
Ash	0.97 ± 0.02 ^{b}	0.98 ± 0.01 ^{b}	0.99 ± 0.01 ^{b}	1.09 ± 0.02 ^{a}	1.09 ± 0.02 ^{a}	1.09± 0.01 ^{a}

Values are means and S.D. of triplicate; Means with the same letter within a row were not significantly different at P<0.05 level.

pH value

The changes in pH of silver carp fillets as a function of treatments and storage time are shown in Table 2. The initial pH of control samples on day 0 was 6.67 indicating the freshness of fish samples. Higher pH value (P<0.05) was observed for coated samples in day 0 (Table 2). In contrast, the samples treated by *Z. multiflora* Boiss essential oil (E1, E2, E1C and E2C) showed lower pH values as compared with control samples. The above reported pH values was lower as compared with raw and unwashed samples of silver carp reported by Asgharzadeh *et al*. (2010). During storage at 4ºC, the pH of all treated and untreated samples increased significantly (P<0.05). The higher (P<0.05) pH values were observed for control samples followed by 0.2% and 0.4% *Z. multiflora* Boiss essential oil. In contrast, lower pH values were recorded in coated and coated with essential oil treatments. The increase of pH values during the storage period may be attributed to the production of basic compounds such as ammonia, trimethylamine as well as other biogenic amines by fish spoilage bacteria (Boskou and Debevere, 2000; Goulas and Kontominas, 2007). The two-ways ANOVA analysis showed the significant (P<0.05) effects of treatments and during of storage on ph values.

Table 2. Changes in pH in different treatments during storage at 4ºC

Days	pH					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Coated (C)	Coated + 0.2 % EO (E1C)	Coated + 0.4 % EO (E2C)
0	6.67 ± 0.02 ^{abC}	6.65 ± 0.03 ^{bE}	6.62 ± 0.02 ^{bF}	6.73 ± 0.03 ^{aB}	6.64 ± 0.02 ^{bC}	6.64 ± 0.04 ^{bB}
3	6.88 ± 0.08 ^{aC}	6.92 ± 0.02 ^{aD}	6.75 ± 0.06 ^{bE}	6.75 ± 0.04 ^{bB}	6.73 ± 0.01 ^{bC}	6.75 ± 0.02 ^{bB}
6	7.39 ± 0.17 ^{aB}	6.93 ± 0.07 ^{bD}	6.87 ± 0.03 ^{bD}	6.62 ± 0.06 ^{cB}	6.75 ± 0.05 ^{bcC}	6.79 ± 0.03 ^{bcB}
9	7.53 ± 0.03 ^{aB}	7.11 ± 0.03 ^{bC}	7.09 ± 0.02 ^{bC}	6.68 ± 0.11 ^{dB}	6.91 ± 0.03 ^{cB}	6.80 ± 0.06 ^{cdB}
12	7.53 ± 0.03 ^{aB}	7.33 ± 0.07 ^{aB}	7.36 ± 0.07 ^{aB}	6.64 ± 0.16 ^{bB}	6.82 ± 0.12 ^{bBC}	7.00 ± 0.27 ^{aAB}
15	7.79 ± 0.07 ^{aA}	7.76 ± 0.07 ^{aA}	7.70 ± 0.04 ^{aA}	7.36 ± 0.07 ^{bA}	7.25 ± 0.05 ^{bA}	7.25 ± 0.05 ^{bA}

Values are means and S.D. of triplicate; Means with the same small letter in a raw were not significantly different at P<0.05 level in different treatment. Means with the same capital letter in a column were not significantly different at P<0.05 level during storage at 4ºC.

Total volatile basic nitrogen

The changes in TVB-N of silver carp fillets as a function of treatment and storage time are shown in Table 3. The initial (day 0) TVB-N value of control samples was 7.75 mg N/100 g, which was significantly (P<0.05) higher than E1 and E2 samples and lower than C, E1C and E2C samples at day 0. TVB-N values increased progressively with time of storage at 4ºC for all treatments and reached to 35.68, 31.37, 30.67, 30.13, 29.48 and 29.16 for control, E1, E2, C, E1C and E2C samples, respectively. The TVB-N content in fish muscle is not only different between species but also is variable in same species due to age, sex, season and environment (Razavi Shirazi, 2001). Although some researchers concluded that TVB-N is not a good quality index for fish (Mexis *et al*., 2009), but it could be used as a quality index. Because the increases in TVB-N content of fish samples during storage is directly to the activity of spoilage bacteria and endogenous enzymes (Özogul *et al*., 2004; Goulas and Kontominas, 2007). TVB-N is composed of different compounds including ammonia, methylamine, dimethylamine as well as trimethylamine (Razavi Shirazi, 2001) which are products of activity of spoilage bacteria and endogenous enzymes.

Table 3. Changes in TVB-N values in different treatments during storage at 4ºC

Days	TVB-N (mg/100 g wet samples)					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Coated (C)	Coated + 0. 2% EO (E1C)	Coated + 0.4% EO (E2C)
0	7.75 ± 0.06 ^{bF}	7.31 ± 0.07 ^{cF}	7.15 ± 0.04 ^{dF}	8.22 ± 0.10 ^{aF}	8.14 ± 0.01 ^{aF}	8.23 ± 0.03 ^{aF}
3	10.09 ± 0.08 ^{cE}	10.40 ± 0.04 ^{aE}	10.17 ± 0.07 ^{bF}	10.38 ± 0.03 ^{aE}	10.30 ± 0.05 ^{abE}	10.28 ± 0.07 ^{abE}
6	16.83 ± 0.16 ^{aD}	16.52 ± 0.06 ^{bD}	16. 04 ± 0.03 ^{cD}	14.50 ± 0.02 ^{dD}	13.70 ± 0.16 ^{eD}	13.46 ± 0.07 ^{eD}
9	24.21 ± 0.27 ^{aC}	22.52 ± 0.20 ^{bC}	22.32 ± 0.08 ^{bC}	21.18 ± 0.44 ^{cC}	19.20 ± 0.08 ^{dC}	18.34 ± 0.09 ^{eC}
12	30.79 ± 0.39 ^{aB}	27.57 ± 0.09 ^{bB}	26. 54 ± 0.05 ^{cB}	24.62 ± 0.13 ^{dB}	23.63 ± 0.15 ^{eB}	23.29 ± 0.13 ^{eB}
15	35.68 ± 0.40 ^{aA}	31.37 ± 0.16 ^{bA}	30.67 ± 0.11 ^{cA}	30.13 ± 0.07 ^{cA}	29.48 ± 0.25 ^{dA}	29.16 ± 0.06 ^{dA}

Values are means and S.D. of triplicate; Means with the same small letter in a raw were not significantly different at P<0.05 level in different treatment. Means with the same capital letter in a column were not significantly different at P<0.05 level during storage at 4ºC.

TVB-N values of control samples reached the upper acceptability limit set by the EU (EEC, 1995) for TVBN values of fish (35 mg N/100 g of fish flesh) at day 15 of storage, while for other treated samples, TVB-N content was still lower than upper acceptability limit (Table 3). The lower TVB-N content in samples treated by *Z. multiflora* Boiss essential oil may be attributed to the antibacterial properties of essential oil and more specifically to its phenolic constituents such as carvacrol and thymol. The antibacterial properties and preservative effect of this essential oil agrees with the same effect of other essential oils such as oregano EO (Burt, 2004; Goulas and Kontominas, 2007). The two-ways ANOVA analysis showed that both treatments and storage at 4ºC has significant (P<0.05) effects on TVB-N values.

Peroxide value (PV)

PV values of treated and control samples of silver carp are presented in Table 4. The initial PV value of control samples at day 0 was 1.03 meq O_2/kg. This amount was lower as compared with raw and unwashed samples of silver carp reported by Asgharzadeh *et al*. (2010). Lower PV value was observed in samples treated by *Z. multiflora* Boiss essential oil at day 0 (Table 4). PV values increased significantly (*P*<0.05) with time of storage at 4ºC for all treatments. Significant lower (*P*<0.05) PV value was observed for treated samples during the storage period at 4ºC. The two-ways ANOVA analysis showed that both treatments and storage at 4ºC had significant (P<0.05) effects on PV values.

The PV value is an index of lipid oxidation measuring primary oxidation products. Fish are very susceptible to both microbiological and chemical deterioration, due to their chemical composition (Goulas and Kontominas, 2007). Storage of food

Table 4. Changes in PV values in different treatments during storage at 4ºC

Days	PV (meq per 1000g)					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Coated (C)	Coated + 0.2% EO (E1C)	Coated + 0.4% EO (E2C)
0	1.03 ± 0.05 ^{aF}	0.93 ± 0.05 ^{aF}	0.91 ± 0.07 ^{aF}	1.04 ± 0.03 ^{aF}	0.86 ± 0.13 ^{aF}	0.92 ± 0.08 ^{aF}
3	3.12 ± 0.02 ^{aE}	2.88 ± 0.12 ^{bE}	2.85 ± 0.04 ^{bE}	2.68 ± 0.01 ^{cE}	2.46 ± 0.07 ^{dE}	2.40 ± 0.04 ^{dE}
6	6.54 ± 0.03 ^{aD}	5.41 ± 0.14 ^{bD}	5.41 ± 0.09 ^{bD}	5.19 ± 0.23 ^{bcD}	5.09 ± 0.11 ^{cD}	4.81 ± 0.01 ^{dD}
9	8.12 ± 0.02 ^{aC}	7.49 ± 0.05 ^{bcC}	7.44 ± 0.07 ^{bcC}	7.51 ± 0.01 ^{bC}	7.35 ± 0.10 ^{cC}	7.14 ± 0.03 ^{dC}
12	10.30 ± 0.05 ^{aB}	9.24 ± 0.13 ^{bB}	8.70 ± 0.13 ^{cB}	9.11 ± 0.10 ^{bB}	8.87 ± 0.04 ^{bcB}	8.69 ± 0.06 ^{cB}
15	13.3 ± 0.10 ^{aA}	11.29 ± 0.07 ^{bA}	10.64 ± 0.12 ^{cA}	10.58 ± 0.25 ^{cdA}	10.41± 0.03 ^{cdA}	10.26 ± 0.03 ^{dA}

Values are means and S.D. of triplicate; Means with the same small letter in a raw were not significantly different at P<0.05 level in different treatment. Means with the same capital letter in a column were not significantly different at P<0.05 level during storage at 4ºC.

products is accompanied by oxidation of unsaturated fatty acids (Misharina and Polshkov, 2005). This process is more important in sea foods due to higher poly-unsaturated fatty acid content. It is accepted that hydroperoxides (first product of oxidation) are an intermediate, which is itself odorless, but when breaks down to smaller molecules which do produce the off-flavor (Hamilton, 1994). Degradation of products formed during oxidation of unsaturated fatty acids is followed by the formation of low-molecular-weight volatile compounds, which account for foreign shades of odor and flavor (Misharina and Polshkov, 2005).

The results of present study indicate that *Z. multiflora* Boiss essential oil and coating are effective in retarding the production of primary lipid oxidation. Similar results were obtained by Mexis *et al*. (2009) and Ojagh *et al*. (2010). The major protective effect of *Z. multiflora* Boiss essential oil is owed to its carvacrol, thymol content and them antioxidant effects in free radical scavenging (Mexis *et al*., 2009). Similar results were obtained by Mexis *et al*. (2009) and Ojagh *et al*. (2010). PV value was recorded as 13.3 meq O_2/kg for control samples at day 15 that was in the range of upper acceptability recommended by Huss (1995) (10-20 meq O_2/kg), while the treated samples has significantly (P<0.05) lower PV value at day 15.

Thiobarbituric acid (TBA)

The changes in TBA of silver carp fillets as a function of treatment and storage time are shown in Table 5. The TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content (Fernandez *et al*., 1997) and widely used for assessment of degree of lipid oxidation (Ibrahim Sallam, 2007). MDA formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Fernandez *et al*., 1997). The initial TBA

value of control samples on day 0 was 0.19 mg/kg indicating the freshness of fish samples. The samples treated by *Z. multiflora* Boiss essential oil (E1, E2, E1C and E2C) and coating (C) showed lower TBA values as compared with control samples (Table 5). As can be seen in Table 5, TBA value increased gradually during storage at 4ºC. Both treatments and storage at 4ºC had significant ($P<0.05$) effects on TBA values. The lowest TBA values were observed for E1C and E2C samples at day 15. The results of present study indicate that *Z. multiflora* Boiss essential oil and coating are effective in retarding the production of secondary lipid oxidation products. Goulas and Kontominas (2007) found lower contents of TBA in two MAP samples containing 0.4% and 0.8% oregano EO, indicating the strong antioxidant effect of oregano EO which acts as a radical scavenger. Ojagh *et al*. (2010) also found lower contents of TBA in rainbow trout fillet treated by chitosan coating enriched with cinnamon oil. According to Ibrahim Sallam (2007), the maximum level of TBA value indicating good quality of the fish is 5 mg/kg of tissue. TBA values of control and treated samples in present study were much lower than such proposed limits throughout the 15 days storage. Similar results were obtained by Goulas and Kontominas (2007) and Ojagh *et al*. (2010).

Table 5. Changes in TBA values in different treatments during storage at 4ºC

Days	TBA (mg/kg)					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Coated (C)	Coated + 0.2% EO (E1C)	Coated + 0.4% EO (E2C)
0	0.19 ± 0.08 ^{aC}	0.11 ± 0.02 ^{abE}	0.09 ± 0.01 ^{bE}	0.08 ± 0.00 ^{bF}	0.08 ± 0.01 ^{bE}	0.07 ± 0.01 ^{bE}
3	0.26 ± 0.01 ^{aC}	0.21 ± 0.01 ^{bD}	0.14 ± 0.03 ^{cE}	0.13 ± 0.00 ^{cE}	0.11 ± 0.01 ^{cDE}	0.11 ± 0.02 ^{cDE}
6	0.29 ± 0.01 ^{aC}	0.24 ± 0.01 ^{abD}	0.22 ± 0.05 ^{bD}	0.18 ± 0.01 ^{bD}	0.16 ± 0.02 ^{bcD}	0.14 ± 0.01 ^{cD}
9	0.52 ± 0.01 ^{aB}	0.45 ± 0.01 ^{bC}	0.31 ± 0.01 ^{cC}	0.33 ± 0.01 ^{cC}	0.27 ± 0.03 ^{cdC}	0.23 ± 0.03 ^{dC}
12	0.76 ± 0.01 ^{aA}	0.65 ± 0.02 ^{bB}	0.51 ± 0.02 ^{cB}	0.51 ± 0.02 ^{cB}	0.47 ± 0.01 ^{cB}	0.41 ± 0.01 ^{dB}
15	0.85 ± 0.04 ^{aA}	0.75 ± 0.00 ^{bA}	0.61 ± 0.03 ^{cA}	0.61 ± 0.03 ^{cA}	0.59 ± 0.04 ^{cdA}	0.52 ± 0.01 ^{dA}

Values are means and S.D. of triplicate; Means with the same small letter in a raw were not significantly different at $P<0.05$ level in different treatment. Means with the same capital letter in a column were not significantly different at $P<0.05$ level during storage at 4ºC.

Conclusion

Successful inhibition of lipid oxidation and microbial growth in refrigerated silver carp fillets were possible with *Z. multiflora* essential oil and coating either separately or in combination. Based on biochemical analysis, best result found for samples treated by coating and 0.4% EO (E_2C) which followed by E_1C. In conclusion, antioxidative capacity of the essential oil of *Z. multiflora* Boiss could be attributed to the presence of high amount of carvacrol, thymol as main phenolic compounds and p-cymene as main non-phenolic compounds. Owing to good protective features exhibited by *Z. multiflora* Boiss essential oil and edible coating, these materials could be used for active coating for fish as a safe preservative under refrigerated storage.

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