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The Effects of *Zataria multiflora* Boiss Essential Oil and Nisin on Chemical Characteristics of Rainbow Trout Fillet Stored at 4 °C

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Abstract This study evaluated the effects of *Zataria multiflora* essential oil (EO) and nisin on fresh rainbow trout fillets during storage at 4 °C. Treatments included the following: A (control samples without EO and nisin), E_1 (treated samples with 0.2% EO), E_2 (treated samples with 0.4% EO), N (treated samples with 150 IU nisin/g), E_1N (treated samples with 0.2% EO and nisin) and E_2N (treated samples with 0.4% EO and nisin). Chemical and oxidation changes were investigated in this study as the functions of treatment and storage time. E_1N and E_2N had better effects on oxidation changes and maintaining values of peroxide value and thiobarbituric acid than A, E_1, E_2 and N treatments. Lower total volatile base nitrogen was found in E_2N than in other treatments during storage time.

Keywords Rainbow trout · Shelf life extension · Essential oil · Nisin · Chemical attributes

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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 high final pH [31]. Rancidity caused by oxidation of fish lipids is one of the major problems encountered in fish processing due to producing the off-flavor and reducing their nutritional value. Many efforts have been carried out for supplying fresh fish according to consumers' demand [18]. Different methods (i.e., vacuum packaging, control packaging atmosphere, oxygen absorbers) have been developed to prevent fish products from lipid oxidation [19]. Meanwhile, 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 [1, 5, 34]. In this situation, using natural additives such as essential oils and nisin has been studied on shelf life of different foods [7, 23], 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 due to their active phenolic compounds (i.e., carvacrol, thymol) which possess antibacterial, antioxidant, antiviral and antimycotic properties [7, 8, 22]. *Zataria multiflora* Boiss belongs to *Lamiaceae* family [13] is extensively used as a flavor ingredient in a wide variety of food in Iran and possesses antioxidant properties that able to inhibit linoleic acid oxidation [32, 33]. Nisin is a polypeptide bacteriocin produced by certain strains of *Lactococcus lactis*. Antioxidant effect of nisin together with cinnamon has been

reported on northern snakehead fish fillets [23]. With regard to fish, including rainbow trout, to our knowledge, there are no studies in the literature on the antioxidant effects of *Z. multiflora* Boiss essential oil and nisin on shelf life. Thus, the aim of the present work was to determine the chemical and antioxidant changes of refrigerated rainbow trout fillets, using either nisin or *Z. multiflora* Boiss essential oil and their combination during storage at 4 °C.

Materials and Methods

Fish Samples and Preparation

Fresh aquacultured rainbow trout, that is, *Onchorhynchus mykiss*, with the average weight and length of 400 g and 270 mm, respectively, were obtained from an aquaculture farm located on Sari (Mazandaran, Iran) during May, 2010. The fish were delivered to the laboratory within 20 min of harvesting and packed in insulated boxes containing ice. The fish were then eviscerated, headed and filleted by hand (each fillet was 100 g in weight, approximately). The fillet samples were then randomly divided into six treatments lots, namely: A (control samples without nisin and EO), E_1 (treated samples with 0.2% EO), E_2 (treated samples with 0.4% EO), N (treated samples with 150,000 IU nisin/kg), E_1N (treated samples with nisin and 0.2% EO) and E_2N (treated samples with nisin 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 obtain homogenous distribution of EO. Nisin (Serva, Heidelberg, US) was also injected by syringe in two sides of fillets. All samples after vacuum packaging (Henkelman, 200 A, Netherland) were stored at 4 °C. Sampling was carried out on day: 0, 3, 6, 9, 12 and 15 of storage. *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 the Clevenger-type apparatus [30]. 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 × 0.25 mm ID × 0.25 μm film thickness). In addition, helium was used as the carrier gas with a flow rate of 0.8 ml/min. The oven was programmed at an initial temperature of 50 °C for 5 min. Temperature was increased to 240 °C with a rate of 3 °C/min and then increased to 300 °C with a rate of 15 °C/min and held for 3 min. The injector temperature was set at 290 °C. The MS was run in the electron ionization mode set at 70 eV. The ion source temperature was maintained at 220 °C [30].

Chemical Analysis

Proximate Composition

Crude protein was calculated by converting the nitrogen content determined by Kjeldahl's method [3]. Crude lipid was determined by ether extraction using a Soxhlet method [4]. The moisture content was determined by drying the meat in an oven at 105 °C until a constant weight was obtained [4]. Ash content was determined by drying the samples in a furnace at 550 °C for 12 h [4].

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 [4]. 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. [9]. Thiobarbituric acid (TBA) was determined according the method used by Egan et al. [9]. PV and TBA contents were expressed as meq per 1,000 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

Proximate Composition

Proximate analysis results (Day 0 after treatments) are presented in Table 1. The protein and lipid content of raw samples were 16.39 and 5.37%, respectively. The results are in good agreement with those reported by González-Fandos et al. [15]. The protein content of control samples in present

study was slightly higher as compared with protein content of rainbow trout reported by González-Fandos et al. [15].

pH Determination

The changes in pH of rainbow trout fillets as a function of treatments and storage time are shown in Table 2. The initial pH of control samples on day 0 was 6.79 indicating the freshness of fish samples. Higher pH value ($P < 0.05$) was observed for E_2N samples in day 0 (Table 2). In contrast, the E_1, E_2, N, E_1N samples showed lower pH values as compared with control samples. The above reported pH values were higher as compared with raw samples of rainbow trout reported by González-Fandos et al. [15] and also minced rainbow trout reported by Nykänen et al. [26]. During storage at 4 °C, the pH of all treated and untreated samples increased significantly ($P < 0.05$). Significant higher ($P < 0.05$) pH values were observed for control samples followed by nisin-treated samples at the end of experiment. In contrast, lower pH values were recorded in treatments with essential oil. The two-ways ANOVA analysis showed the significant ($P < 0.05$) effects of treatments and during of storage on pH values.

Determination of Total Volatile Basic Nitrogen

The changes in TVB-N of rainbow trout fillets as a function of treatment and storage time are shown in Table 3. The

Table 1 Proximate analysis of rainbow trout fillet after different treatments

Composition	Proximate analysis (%)					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Nisin (N)	Nisin + 0. 2% EO (E1 N)	Nisin + 0.4% EO (E2 N)
Total lipid	5.37 ± 0.00^{AB}	5.39 ± 0.00^{A}	5.29 ± 0.00^{C}	5.34 ± 0.04^{B}	5.32 ± 0.02^{BC}	5.29 ± 0.00^{C}
Total protein	16.39 ± 0.00^{C}	16.54 ± 0.04^{A}	16.48 ± 0.00^{B}	16.49 ± 0.00^{AB}	16.44 ± 0.04^{BC}	16.52 ± 0.02^{AB}
Moisture	77.03 ± 0.04^{A}	76.99 ± 0.00^{AB}	76.89 ± 0.00^{B}	76.94 ± 0.00^{B}	76.94 ± 0.04^{B}	76.90 ± 0.00^{B}
Ash	0.91 ± 0.01^{B}	0.91 ± 0.02^{B}	0.91 ± 0.00^{B}	0.98 ± 0.00^{A}	0.97 ± 0.03^{A}	0.98 ± 0.01^{A}

Values are means and SD of triplicate; means with the same letter within a row were not significantly different at $P < 0.05$ level

Table 2 Changes in pH in different treatments during storage at 4 °C

Days	pH					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Nisin (N)	Nisin + 0. 2% EO (E1 N)	Nisin + 0.4% EO (E2 N)
0	6.79 ± 0.03^{Be}	6.65 ± 0.02^{De}	6.68 ± 0.00^{CDf}	6.73 ± 0.02^{Cd}	6.65 ± 0.02^{De}	6.95 ± 0.02^{Ac}
3	7.16 ± 0.03^{Ad}	6.86 ± 0.03^{CDd}	6.82 ± 0.01^{De}	7.04 ± 0.05^{Bc}	6.91 ± 0.02^{Cd}	6.83 ± 0.02^{Dd}
6	7.34 ± 0.02^{Ac}	6.93 ± 0.02^{Cc}	6.75 ± 0.02^{Dd}	7.09 ± 0.07^{Bc}	6.88 ± 0.03^{Cd}	6.76 ± 0.01^{De}
9	7.62 ± 0.02^{Ab}	7.10 ± 0.02^{Bb}	7.08 ± 0.02^{Bc}	7.12 ± 0.02^{Bc}	7.10 ± 0.01^{Bc}	7.10 ± 0.02^{Bb}
12	7.69 ± 0.04^{Ab}	7.13 ± 0.02^{Cb}	7.16 ± 0.01^{Cb}	7.31 ± 0.02^{Bb}	7.16 ± 0.01^{Cb}	7.11 ± 0.01^{Cb}
15	7.86 ± 0.04^{Aa}	7.22 ± 0.01^{CDa}	7.27 ± 0.01^{Ca}	7.43 ± 0.02^{Ba}	7.24 ± 0.01^{Ca}	7.17 ± 0.01^{Da}

Values are means and SD of triplicate; means with the same small letter in a row 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 4 °C

Table 3 Changes in TVB-N values in different treatments during storage at 4 °C

Days	TVB-N (mg/100 g wet samples)					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Nisin (N)	Nisin + 0. 2% EO (E1 N)	Nisin + 0.4% EO (E2 N)
0	9.87 ± 0.12^{Af}	9.64 ± 0.03^{Bf}	8.08 ± 0.07^{Cf}	9.74 ± 0.03^{ABf}	9.91 ± 0.01^{Af}	8.03 ± 0.02^{Cf}
3	13.46 ± 0.25^{Ae}	12.74 ± 0.03^{Be}	11.94 ± 0.04^{Ce}	12.80 ± 0.10^{Be}	12.71 ± 0.01^{Be}	11.52 ± 0.13^{De}
6	17.02 ± 0.02^{Ad}	15.62 ± 0.58^{Bd}	14.47 ± 0.50^{Cd}	15.12 ± 0.11^{BCd}	15.09 ± 0.07^{BCd}	14.43 ± 0.57^{Cd}
9	19.15 ± 0.13^{Ac}	17.21 ± 0.09^{Bc}	17.23 ± 0.12^{BCc}	17.35 ± 0.07^{Bc}	16.87 ± 0.46^{BCc}	16.64 ± 0.37^{Cc}
12	23.17 ± 0.33^{Ab}	21.27 ± 0.18^{Bb}	20.85 ± 0.23^{BCb}	21.37 ± 0.13^{Bb}	20.67 ± 0.24^{Cb}	20.38 ± 0.26^{Cb}
15	26.80 ± 0.29^{Aa}	25.40 ± 0.25^{Ba}	25.12 ± 0.10^{Ba}	25.27 ± 0.14^{Ba}	24.61 ± 0.12^{Ca}	24.16 ± 0.06^{Ca}

Values are means and SD of triplicate; Means with the same small letter in a row 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 4 °C

initial TVB-N value of control samples was 9.87 mg N/100 g, which was significantly ($P < 0.05$) higher than E_1, E_2 and E_2N samples at day 0. TVB-N content in control and treated samples was much lower as compared with the results of Frangos et al. [12] for raw rainbow trout. TVB-N values increased progressively with time of storage at 4 °C for all treatments and reached 26.80, 25.40, 25.12, 25.27, 24.61 and 24.16 mg N/100 g for control, E_1, E_2, C, E_1C and E_2C samples, respectively. The two-ways ANOVA analysis showed that both treatments and storage at 4 °C have significant ($P < 0.05$) effects on TVB-N values.

Determination of Peroxide Value (PV)

PV values of treated and control samples of rainbow trout are presented in Table 4. The initial PV value of control samples at day 0 was 1.09 meq O_2/kg. This amount was lower as compared with raw samples of rainbow trout reported by Ortiz et al. [28]. Lower PV value was observed in other treatments especially in samples treated by *Z. multiflora* Boiss essential oil and nisin at day 0 (Table 4). PV values increased significantly ($P < 0.05$) with time of storage at 4 °C for all treatments. Significant lower ($P < 0.05$) PV value was observed for treated samples during storage at 4 °C. The two-ways ANOVA analysis showed that both treatments and storage at 4 °C had significant ($P < 0.05$) effects on PV values.

PV value was recorded as 15.05, 12.66, 12.19, 12.98 meq O_2/kg for control, E_1, E_2 and N samples at day 15, respectively, that was in the range of upper acceptability recommended by Huss [20] (10–20 meq O_2/kg), while E_1N and E_2N samples had significantly ($P < 0.05$) lower PV value at day 15.

Determination of Thiobarbituric Acid (TBA)

The changes in TBA of rainbow trout 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 [11] and widely used for the assessment of degree of lipid oxidation [21]. MDA formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen [11]. The initial TBA value of control samples on day 0 was 0.21 mg/kg indicating the freshness of fish samples. The samples treated by *Z. multiflora* Boiss essential oil (E_1, E_2, E_1N and E_2N) and nisin (N) 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 4 °C. Both treatments and storage at 4 °C had significant

Table 4 Changes in PV values in different treatments during storage at 4 °C

Days	PV (meq per 1,000 g)					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Nisin (N)	Nisin + 0. 2% EO (E1 N)	Nisin + 0.4% EO (E2 N)
0	1.09 ± 0.00^{Ae}	0.99 ± 0.00^{Ba}	0.97 ± 0.02^{BCf}	0.98 ± 0.00^{Bf}	0.95 ± 0.00^{Cf}	0.91 ± 0.00^{Df}
3	1.81 ± 0.01^{Ae}	1.71 ± 0.03^{Ca}	1.67 ± 0.01^{CDe}	1.79 ± 0.00^{Be}	1.72 ± 0.02^{Ce}	1.63 ± 0.03^{De}
6	3.07 ± 0.01^{Ad}	2.69 ± 0.00^{BCa}	2.58 ± 0.03^{Dd}	2.72 ± 0.03^{Bd}	2.65 ± 0.04^{Cd}	2.56 ± 0.02^{Dd}
9	5.85 ± 0.05^{Ac}	5.11 ± 0.00^{Ba}	4.97 ± 0.01^{Cc}	5.12 ± 0.00^{Bc}	5.10 ± 0.00^{Bc}	4.84 ± 0.04^{Dc}
12	10.14 ± 0.00^{Ab}	9.75 ± 0.04^{Ba}	9.18 ± 0.01^{Cb}	9.71 ± 0.01^{Bb}	9.28 ± 0.03^{Cb}	8.54 ± 0.08^{Db}
15	15.05 ± 0.80^{Aa}	12.66 ± 0.28^{Ba}	12.19 ± 0.12^{Ba}	12.98 ± 0.45^{Ba}	9.63 ± 0.06^{Ca}	9.16 ± 0.05^{Ca}

Values are means and SD of triplicate; means with the same small letter in a row 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 4 °C

Table 5 Changes in TBA values in different treatments during storage at 4 °C

Days	TBA (mg/kg)					
	Control (A)	0.2% EO (E1)	0.4% EO (E2)	Nisin (N)	Nisin + 0. 2% EO (E1 N)	Nisin + 0.4% EO (E2 N)
0	0.21 ± 0.00^{Af}	0.13 ± 0.00^{Be}	0.12 ± 0.00^{BCd}	0.11 ± 0.01^{Cd}	0.09 ± 0.00^{Df}	0.07 ± 0.00^{Ef}
3	0.27 ± 0.00^{Ae}	0.24 ± 0.00^{Bd}	0.23 ± 0.00^{Cc}	0.21 ± 0.00^{Dc}	0.12 ± 0.00^{Ee}	0.11 ± 0.00^{Fe}
6	0.31 ± 0.00^{Ad}	0.24 ± 0.00^{Bd}	0.24 ± 0.00^{Bc}	0.23 ± 0.00^{Cc}	0.15 ± 0.00^{Dd}	0.13 ± 0.00^{Ed}
9	0.54 ± 0.00^{Ac}	0.48 ± 0.00^{Bc}	0.46 ± 0.00^{Cb}	0.43 ± 0.00^{Db}	0.23 ± 0.00^{Ec}	0.21 ± 0.00^{Fc}
12	0.79 ± 0.00^{Ab}	0.66 ± 0.01^{Bb}	0.64 ± 0.00^{Ca}	0.62 ± 0.01^{Da}	0.43 ± 0.01^{Eb}	0.41 ± 0.00^{Fb}
15	0.86 ± 0.00^{Aa}	0.75 ± 0.00^{Ba}	0.63 ± 0.01^{Ca}	0.62 ± 0.00^{Ca}	0.52 ± 0.01^{Da}	0.51 ± 0.00^{Da}

Values are means and SD of triplicate; means with the same small letter in a row 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 4 °C

($P < 0.05$) effects on TBA values. The lowest TBA values were observed for E_1N and E_2N samples at day 15.

Discussion

Correlation between pH value and fish freshness has been reported by Abbas et al. [2]. The low muscle pH value in day 0 of iced storage reflected the good nutritional state of fish. In present study, the pH value of all treated and untreated samples increased significantly ($P < 0.05$). The increase in 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 [6, 16]. Treating the samples with nisin and essential oil reduced the pH value during storage at 4 °C.

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 [31]. Although some researchers concluded that TVB-N is not a good quality index for fish [24], but it could be used as a quality index. TVB-N values in all treated and control samples increased progressively with time of storage at 4 °C. This could be related to the activity of spoilage bacteria and endogenous enzymes [16, 29]. TVB-N is composed of different compounds including ammonia, methylamine, dimethylamine as well as trimethylamine [31] which are products of activity of spoilage bacteria and endogenous enzymes. In present study, TVB-N values of all treated and untreated samples were still lower than upper acceptability limit set by the EU [10]. The lower TVB-N content in E_1N and E_2N samples may be attributed to the antibacterial properties of essential oil and more specifically to its phenolic constituents such as carvacrol and thymol and their synergistic effect with nisin. The antibacterial properties and preservative effect of this essential oil agree with the same effect of other essential oils such as oregano EO [7, 16].

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 [16]. Storage of food products is accompanied by oxidation of unsaturated fatty acids [25]. This process is more important in sea foods due to higher polyunsaturated 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 [17]. 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 [25]. The results of present study indicated that *Z. multiflora* Boiss essential oil and nisin were effective in retarding the production of primary lipid oxidation. Similar results were obtained by Mexis et al. [24] and Ojagh et al. [27]. 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 [24].

The results of present study indicated that *Z. multiflora* Boiss essential oil and nisin were effective in retarding the production of secondary lipid oxidation products. Goulas and Kontominas [16] 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. Also, Ojagh et al. [27] found significantly lower contents of TBA in rainbow trout fillet treated by chitosan coating enriched with cinnamon oil.

According to Ibrahim Sallam [21], the maximum level of TBA value indicating good quality of the fish is 5 mg/kg of tissue. But Gill [14] has determined much lower content of TBA (range of 1–2 mg malonaldehyde kg^{-1}) in fish sample as limit of acceptability. 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 [16] and Ojagh et al. [27].

To establish the better antioxidant effect of natural essential oils, the combination of them with other preservation factors must be evaluated to determine whether there are synergistic effects. Recently, the synergistic effect between essential oils and other antimicrobial substances has been conclusively demonstrated and it has been noted that the activities of the essential oil constituents (e.g., carvacrol and thymol) are enhanced by the presence of nisin [35]. The present study showed a greater inhibitory effect of *Z. multiflora* Boiss essential oil and nisin on lipid oxidation when used together.

Conclusion

Successful inhibition of lipid oxidation and microbial growth in refrigerated rainbow trout fillets were possible with *Z. multiflora* essential oil and nisin either separately or in combination. Based on biochemical analysis, best result found for samples treated by nisin and 0.4% EO (E_2N) which followed by E_1N. 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 nisin, these materials could be used as natural protectants for fish as a safe preservative under refrigerated storage.

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