

Study of Some Medicinal Plants on Chemical Composition of Rainbow Trout Fillets after Exposure with *Aeromonas hydrophila*

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Abstract: In this study, the effects of three medicinal plants essential oils including *Satureja bachtiarica*, *S. khuzestanica* and *Myrtus communis* were studied on the quality and composition of rainbow trout fillets after inoculation of *Aeromonas hydrophila*. The essential oils were added to gamma sterilized fillets at concentrations of 20, 40 and 80 $\mu\text{g g}^{-1}$ for *M. communis*, 0.125, 0.25 and 0.5 $\mu\text{g g}^{-1}$ for *S. bachtiarica* and 0.06, 0.12, 0.25 and 50 $\mu\text{g g}^{-1}$ for *S. khuzestanica* in sterile condition. Fish fillets were then inoculated with *A. hydrophila* (10^3 g^{-1}) and were kept in 4°C in sterile bags. The results revealed that there was no statistical difference in the examined factors at the beginning of the experiment. At the end of the experiment, although there was not statistical difference between fat, ash and humidity level; protein, FFA, TVN and TBA contents were statistically different ($p \leq 0.05$). The lowest level of FFA and TVN was observed in the samples contained 8 $\mu\text{g/g}$ *M. communis* and 0.125 $\mu\text{g/g}$ *S. bachtiarica* showing significant difference with other groups ($p \leq 0.05$). It is concluded from the results of this study that the studied essential oils have relative effect on spoilage indexes including TVB-N, FFA and TVB, however it seems that *M. communis* is more effective.

Key words: Fish spoilage • Rainbow trout • Medicinal plant essential oil

INTRODUCTION

Rainbow trout is one of the most widely cultured fish species all over the world. The annual production of this species has been rapidly increased during last decade in Iran as it reached 131000 tons/yr in 2012 [1]. At the present time, rainbow trout is the most popular and most consumed fish in Iran.

Fish are highly susceptible to both microbial and chemical spoilage rather than other foods due to the higher amount of polyunsaturated fatty acids. Since the shelf life of fish is lower than other kind of meat, it is necessary to develop methods to keep initial characteristics of fish and to avoid undesired alterations.

When unsaturated fatty acids are exposed to air with high temperature, spoilage process immediately begins, resulting in undesirable taste, color, odor and

loss of nutritional values [2]. Lipid oxidation results in production of toxic compounds such as hydroperoxides, aldehydes, ketones which change the taste and odor in a short time.

To retain the primary quality of fish, chemical preservatives, such as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT), have been widely used. Numerous studies have also been done on using natural compounds to enhance the quality and shelf life of fish [3]. Plant and their derivatives are receiving great attention as safe preservatives extending the shelf-life of fish and other aquatic products [2]. Plant extracts not only have antioxidant activity, but also contribute desirable taste and flavor to the product.

Satureja bachtiarica, *S. khuzestanica* and *Myrtus communis* are well known for their antibacterial and antioxidant activities mainly due to phenolic compounds and carvacrol.

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The aim of this study was to evaluate the effect of *Satureja bachtiarica*, *S. khuzestanica* and *Myrtus communis* essential oils on the quality and composition of rainbow trout fillets in 4°C [4].

MATERIALS AND METHODS

Sample Preparation: The rainbow trout fish samples were obtained from a local fish farm. The samples were gutted, washed and cut into chunks (75 g). In the laboratory, essential oils was added to gamma sterilized fillets at concentrations of 20, 40 and 80 µg g⁻¹ for *M. communis*, 0.125, 0.25 and 0.5 µg g⁻¹ for *S. bachtiarica* and 0.06, 0.12, 0.25 and 50 µg g⁻¹ for *S. khuzistanica* in sterile condition. In the next stage, fish fillets were inoculated with *Aeromonas hydrophila* (10³ g⁻¹) and were kept in 4°C in sterile bags. Each treatment was included in tri-replicates.

Biochemical Analysis: Physical and chemical evaluations were carried out at the beginning of the experiment (day 0) and at the end of the experiment (day 18).

Chemical analysis of the samples (moisture, ash, crude protein and fat content) was carried out according to Association of Official Analytical Chemists [5]. Chemical changes in fish were evaluated by determination of total volatile nitrogen, thiobarbituric acid value and free fatty acid levels [6]. In each analysis, an average of three replicates was recorded.

Statistical Analysis: The mean values of different treatments in days 0 and 18 were compared using Duncan test. The differences were considered statistically significant in the case of P ≤ 0.05.

RESULTS

The results of this study revealed that there is no statistical difference in the examined factors at the beginning of the experiment (Table 1). At the end of the experiment, although there was not statistical difference between fat, ash and humidity level; protein, FFA, TVN and TBA contents were statistically different (p ≤ 0.05) (Table 2).

Table 1: Chemical analysis of the samples in day 0.

	Ash (%)	Crude protein (%)	Fat (%)	FFA (mEq/L)	Humidity (%)	TBA (mg)	TVN (mg/ 100g)
2µg/g <i>M.communis</i>	1.04 ± 0.04 ^a	20.63 ± 1.55 ^a	3.53 ± 0.35 ^a	1.19 ± 0.26 ^a	73.36 ± 1.40 ^a	0.08 ± 0.01 ^a	8.60 ± 0.56 ^a
4 µg/g <i>M.communis</i>	0.85 ± 0.06 ^a	19.9 ± 0.51 ^a	3.18 ± 0.44 ^a	1.07 ± 0.21 ^a	73.53 ± 1.55 ^a	0.08 ± 0.01 ^a	8.60 ± 0.86 ^a
8 µg/g <i>M.communis</i>	0.99 ± 0.11 ^a	19.67 ± 1.38 ^a	3.81 ± 0.27 ^a	1.02 ± 0.25 ^a	74 ± 1.15 ^a	0.08 ± 0.01 ^a	8.16 ± 1.45 ^a
0.125 µg/g <i>S. bachtiarica</i>	1.04 ± 0.23 ^a	20.04 ± 0.15 ^a	3.36 ± 0.43 ^a	0.97 ± 0.37 ^a	74.80 ± 1.08 ^a	0.09 ± 0.02 ^a	9.38 ± 0.74 ^a
0.25 µg/g <i>S. bachtiarica</i>	0.91 ± 0.23 ^a	20.42 ± 0.49 ^a	3.11 ± 0.16 ^a	1.02 ± 0.18 ^a	74.6 ± 1.04 ^a	0.09 ± 0.006 ^a	8.78 ± 0.32 ^a
0.5 µg/g <i>S. bachtiarica</i>	1.04 ± 0.23 ^a	18.95 ± 0.54 ^a	3.63 ± 0.50 ^a	1.29 ± 0.16 ^a	72.7 ± 1.38 ^a	0.08 ± 0.02 ^a	9.70 ± 1.90 ^a
0.06 µg/g for <i>S. khuzistanica</i>	0.86 ± 0.12 ^a	19.4 ± 0.70 ^a	3.85 ± 0.87 ^a	1.12 ± 0.36 ^a	72.66 ± 0.73 ^a	0.012 ± 0.06 ^a	9.40 ± 0.96 ^a
0.12 µg/g for <i>S. khuzistanica</i>	1.06 ± 0.15 ^a	20.23 ± 1.40 ^a	4.02 ± 0.80 ^a	1.56 ± 0.12 ^a	75.06 ± 0.73 ^a	0.10 ± 0.01 ^a	10.25 ± 0.92 ^a
0.25 µg/g for <i>S. khuzistanica</i>	0.92 ± 0.01 ^a	19.8 ± 1.41 ^a	4.42 ± 0.45 ^a	1.34 ± 0.43 ^a	73.70 ± 1.08 ^a	0.09 ± 0.01 ^a	10.50 ± 1.23 ^a
0.5 µg/g for <i>S. khuzistanica</i>	0.89 ± 0.05 ^a	19.57 ± 2.65 ^a	3.75 ± 0.13 ^a	0.95 ± 0.12 ^a	75.2 ± 0.96 ^a	0.10 ± 0.01 ^a	10.83 ± 1.77
Control	0.93 ± 0.17 ^a	19.91 ± 1.11 ^a	3.71 ± 0.27 ^a	1.01 ± 0.38 ^a	74.4 ± 0.35 ^a	0.09 ± 0.07 ^a	9.37 ± 0.51 ^a

Table 2: Chemical analysis of the samples in day 18.

	Ash (%)	Crude protein (%)	Fat (%)	FFA (mEq/L)	Humidity (%)	TBA (mg)	TVN (mg/ 100g)
2µg/g <i>M.communis</i>	0.86 ± 0.14 ^a	20.2 ± 0.90 ^{ab}	4.21 ± 0.35 ^a	3.06 ± 0.66 ^{ab}	72.66 ± 1.3 ^a	0.03 ± 0.01 ^{ab}	86.47 ± 1.43 ^{bcd}
4 µg/g <i>M.communis</i>	0.82 ± 0.06 ^a	20.06 ± 1001 ^{ab}	3.37 ± 0.60 ^a	3 ± 0.20 ^{ab}	72.49 ± 0.75 ^a	0.06 ± 0.066 ^e	61.65 ± 6.75 ^{ab}
8 µg/g <i>M.communis</i>	0.94 ± 0.09 ^a	18.8 ± 0.50 ^a	3.60 ± 0.40 ^a	2.73 ± 0.64 ^a	73.23 ± 1.22 ^a	0.03 ± 0.06 ^{ab}	57.56 ± 2.65 ^a
0.125 µg/g <i>S. bachtiarica</i>	1.02 ± 0.02 ^a	20.46 ± 0.75 ^{ab}	3.58 ± 0.47 ^a	2.61 ± 1.01 ^a	74.08 ± 0.99 ^a	0.14 ± 0.01 ^c	56.94 ± 2.74 ^a
0.25 µg/g <i>S. bachtiarica</i>	0.96 ± 0.06 ^a	19.13 ± 0.50 ^{ab}	3.87 ± 0.27 ^a	3.82 ± 0.24 ^{abc}	74.6 ± 0.9 ^a	0.33 ± 0.03 ^d	59.46 ± 2.16 ^a
0.5 µg/g <i>S. bachtiarica</i>	1.04 ± 0.11 ^a	20.3 ± 1.11 ^{ab}	3.89 ± 0.13 ^a	2.96 ± 0.22 ^{ab}	72.93 ± 0.66 ^a	0.11 ± 0.003 ^{bc}	67.42 ± 6.77 ^{ab}
0.06 µg/g for <i>S. khuzistanica</i>	0.91 ± 0.07 ^a	19.7 ± 0.36 ^{ab}	3.33 ± 0.23 ^a	5.37 ± 2.05 ^{bc}	72.56 ± 2.07 ^a	0.03 ± 0.004 ^{bc}	82.12 ± 8.97 ^{abc}
0.12 µg/g for <i>S. khuzistanica</i>	0.89 ± 0.14 ^a	20.33 ± 0.96 ^{ab}	3.59 ± 0.49 ^a	5.38 ± 0.84 ^{bc}	74.56 ± 1.48 ^a	0.11 ± 0.004 ^a	80.84 ± 10 ^{bcd}
0.25 µg/g for <i>S. khuzistanica</i>	1.03 ± 0.02 ^a	20.06 ± 1.95 ^{ab}	3.43 ± 0.79 ^a	5.53 ± 1.36 ^{abc}	72.46 ± 1.25 ^a	0.01 ± 0.005 ^d	86.77 ± 5.66 ^d
0.5 µg/g for <i>S. khuzistanica</i>	0.98 ± 0.21 ^a	19.60 ± 1.47 ^b	4.19 ± 0.35 ^a	4.32 ± 0.45 ^{abc}	71.76 ± 1.62 ^a	0.37 ± 0.02 ^{ab}	108.54 ± 7.96 ^{cd}
Control	0.90 ± 0.05 ^a	22.10 ± 0.90 ^{ab}	3.83 ± 0.28 ^a	4.44 ± 0.51 ^c	71.83 ± 1.6 ^a	0.02 ± 0.002 ^d	99.81 ± 9.49 ^{cd}

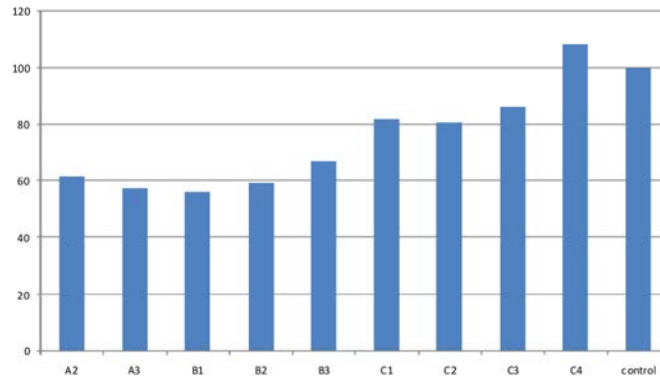


Fig. 1: TVN level in fish fillets in day 18. A1: 20 $\mu\text{g/g}$, A2: 40 $\mu\text{g/g}$, A3: 80 $\mu\text{g/g}$ *M.communis*, B1: 0.125 $\mu\text{g/g}$, B2: 0.25, B3: 0.5 $\mu\text{g g}^{-1}$ *S. bachtiarica*. C1: 0.06 $\mu\text{g/g}$, 0.12 $\mu\text{g/g}$, 0.25 $\mu\text{g/g}$, 50 $\mu\text{g g}^{-1}$ for *S. khuzistanica*

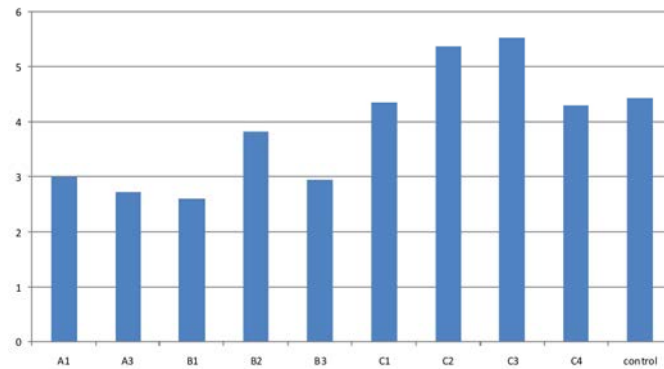


Fig. 2: Free fatty acid level in fishfillets in day 18. A1: 20 $\mu\text{g/g}$, A2: 40 $\mu\text{g/g}$, A3: 80 $\mu\text{g/g}$ *M.communis*, B1: 0.125 $\mu\text{g/g}$, B2: 0.25, B3: 0.5 $\mu\text{g g}^{-1}$ *S. bachtiarica*. C1: 0.06 $\mu\text{g/g}$, 0.12 $\mu\text{g/g}$, 0.25 $\mu\text{g/g}$, 50 $\mu\text{g g}^{-1}$ for *S. khuzistanica*

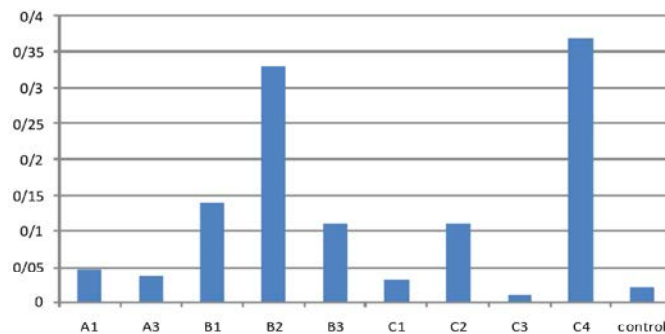


Fig. 3: TBA in fishfillets in day 18. A1: 20 $\mu\text{g/g}$, A2: 40 $\mu\text{g/g}$, A3: 80 $\mu\text{g/g}$ *M.communis*, B1: 0.125 $\mu\text{g/g}$, B2: 0.25, B3: 0.5 $\mu\text{g g}^{-1}$ *S. bachtiarica*. C1: 0.06 $\mu\text{g/g}$, 0.12 $\mu\text{g/g}$, 0.25 $\mu\text{g/g}$, 50 $\mu\text{g g}^{-1}$ for *S. khuzistanica*

The lowest level of FFA and TVN was observed in the samples contained 8 $\mu\text{g/g}$ *M.communis* and 0.125 $\mu\text{g/g}$ *S. bachtiarica* showing significant difference with other groups ($p \leq 0.05$), However TVN level in all groups was above the maximum permissible amount according to the international standards (20 mg/100g) in day 18.

TBA content was found to be lower in the fillets contained *S. khuzistanica* and *M.communis*. Although *S.*

bachtiarica could decrease TVB-N and FFA content of the samples, it was not effective on TBA (Figures 1-3).

DISCUSSION

Fish and other aquatic food are extremely perishable due to high content of water and unsaturated fatty acids. The process of spoilage takes place mainly

due to bacterial activity, oxidation and autolysis which affect the flavour, texture and the appearance of the flesh.

Oxidative rancidity is the most common form of chemical spoilage of fish. Lipids oxidation involves the reaction of oxygen with the double bonds of fatty acids. Fish meat consist of high level of polyunsaturated fatty acids (PUFA) making it highly susceptible to oxidation.

Considering the high susceptibility of fish to spoilage, using preservatives are recommended to keep the primary quality. Many studies have been done on using chemical and natural preservatives to increase shelf time of aquatic food [7].

Natural preservative such as plant extracts are recommended as food preservative with no side effect. Since antimicrobial and antioxidant properties of plant extracts and essential oils have been proven, there is an increasing request for these compounds [8].

TVN, TBA and FFA are important chemical spoilage indexes of fish meat. The thiobarbituric acid value is an index of lipid oxidation and is calculated by measuring of malondialdehyde (MDA) content of meat [9].

TBA is the index of lipid oxidation and is widely used for determination of degree of lipid oxidation. TBA is calculated based on malondialdehyde content of fish which is an initial product of lipid oxidation [4]. Total volatile bases nitrogen is an important quality index of fish. The increase in TVB-N content of fish during storage is mainly due to endogenous autolytic enzymes and the activity of spoilage bacteria [10].

In this study 3 medicinal plant essential oils (*S. bachtiarica*, *S. khuzestanica* and *M. communis*) with known antimicrobial and antioxidant activity were used to increase shelf life of rainbow trout fillets previously exposed with *A. hydrophila*. The results of the present study indicated that essential oils are relatively effective on some spoilage indexes.

The lowest value of TVB-N and FFA was found in the groups treated with *M. communis* and *S. bachtiarica* with a statistical difference comparing to the other groups showing the antimicrobial and antioxidant activity of them ($p \leq 0.05$). TBA content was found to be lower in the fillets contained *S. khuzistanica* and *M. communis*. Although *S. bachtiarica* could decrease TVB-N and FFA content of the samples, it was not effective on TBA.

The main protective effect of the above mentioned essential oils is related to high content of phenolic compounds and carvacrol [11].

The antioxidant activity of *M. communis* is reported to be correlated with the content of different phenolic

constituents [12, 13]. Many studies proved that phenolic compounds like flavonoids, phenolic acid, coumarines, lignans, hydroxycinnamates and stilbenes, have strong antioxidant activity [14]. The antibacterial and antioxidant effects of these essential oils are in agreement with the results of other studied [10,12].

The results of this study revealed that essential oils of the above mentioned plants have relative effect on spoilage indexes including TVB-N, FFA and TVB, however it seems that *M. communis* more effective.

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