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SHORT COMMUNICATION

The Determination of Microbiological Properties of Rainbow Trout (*Oncorhynchus mykiss*) Applied with Black Cumin Oil in Different Concentrations

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ABSTRACT

In this study, the effect of black cumin oil in different concentrations (1%, 1.5% and 2%) was examined on the microbiological quality of rainbow trout (*Oncorhynchus mykiss*) fillets. The study groups were separated in four as control group (A) without containing cumin black oil, group (B) containing 1% black cumin oil, group (C) containing 1.5% black cumin oil, and group (D) containing 2% black cumin oil. The microbiological changes of (total mesophilic aerobic bacteria, total psychrophile aerobic bacteria, *Enterobactericeae* bacteria and yeast-mould) of the samples were determined in every three days period. The count of TMAB was determined exceeded the acceptable limit value (7 log cfu/g) on the 9th day in A group samples. Groups with essential oil were below this value during storage. The highest count of TPAB count at the end of the storage, 8.03±0.02 log cfu/g in group control (A), the lowest 3.05±0.09 log cfu/g in D group. It was concluded that black cumin oil added in different concentrations had positive microbiological effects on trout fillets.

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Introduction

Today, the increasing number of female employees, workload, advancing technology, and different habits of taste necessitate the catering industry. Aquaculture products can be made to ready for consumption by processing in different ways, as in other foodstuffs (Ovayolu, 1997; Metin, 2001; Kılınççeker et al., 2009; Can, 2011; Kılınççeker, 2014).

By consuming processed and consumed seafood, the product is protected, benefit more from the products, job opportunities are increased, the residues are brought to the economy, the consumer is provided with ease, the product is given a different flavor and these products are also economically utilized (Oğuzhan et al., 2006; Kılıç, 2016).

Synthetic and natural additives have been used good alternatives to ensure food safety many years. Consumers prefer natural additives due to the detrimental effects of synthetic additives health (Pizzale et al., 2002; Duman et al., 2012; Kuş, 2012; Mutlu and Bilgin, 2016; Emir Çoban et al., 2018; Oğuzhan Yıldız, 2019). For this reason, the use of natural plant extracts/oils that have antimicrobial and antioxidant effects has become widespread (Akarsu, 2016).

Black cumin (*Nigella sativa* L.) is a plant from *Ranunculaceae* family (buttercup family) which can be found in many countries, notably as East Mediterranean countries. Black cumin oil is widely used in several fields such as cosmetics, food and drug industry. It is one of the most frequently used oils in the fields of health and food. In our country, the oil is widely produced in Afyon, Burdur, Konya and

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Isparta provinces. 13 different species of black cumin is produced in our country and it is mostly consumed as a spice (Bourgou et al., 2012; Rooney and Ryan, 2005; Kar et al., 2007; Bulca, 2014; Kılıç, 2016).

The nutrient content of the black seed is composed of 20.8% crude protein, 3.7% ash, 7.0% moisture, 34.8% lipids, and 33.7% carbohydrate (Öz et al., 2017). Black cumin has been reported to posses natural antioxidant, antibacterial and antifungal effects and has been used as a food preservative because of its antioxidant and antimicrobial effect (Yimer et al., 2019). Black cumin oil is highly effective in some pathogenic Gram-positive and Gram-negative bacteria (Öz et al., 2017).

Arıcı et al., (2005) investigated the antimicrobial activity of black cumin oil. They found that black cumin oil addition improved the microbiological quality. Özpolat and Duman (2016) studied the antimicrobial properties of black cumin oil during storage at 2± 1°C and they found that black cumin oil reduced the growth of bacteria. This study aims to examine the effects of black cumin oil in different concentrations for prevention or controlling microbial growth in rainbow trout (*O. mykiss)* fillets.

Materials and Methods

Sample Preparation

The black cumin oil used in the study was procured from a private firm in Ardahan province. Rainbow trouts, 250-300 g on average weight, were obtained from a trout farming business in the Şavşat district of Artvin province and were transferred to the laboratory in cold chain conditions within the ice. Their head was cut, internal organs were removed and made into fillets. Two fillets were obtained from each fish. Then, the fillets were separated in four as control group (without added cumin black oil-A), added 1% black cumin oil (B), added 1.5% black cumin oil (C) and added 2% black cumin oil (D). A total of 8 fish samples were in each of treatment groups. Black cumin oil was applied to both sides of the fillets with a brush in appropriate volumes. It was first applied to one surface of the fillet and then to the other surface after it dried slightly (5 minutes). The fillets were then placed in foam plates and covered with stretch film and stored under refrigerator conditions (4° C ± 1). The microbiological analyses of the fillets were examined on the 0, 3, 6 and 9 days of storage. The study was conducted with two replications.

Microbiological Analyses

25 g samples were taken from the fish for microbiological analyses, transferred into stomacher bags and 225 ml of sterile peptone water was added. Then the bags were homogenized in a stomacher device. Plate Count Agar (PCA) medium was prepared in order to count the total aerobic mesophilic bacteria (TAMB) and total psychrophilic bacteria (TPAB). Two parallel plantings were performed with the prepared dilutions in accordance with the spread plate method. TAMB was left for incubation at 30°C for 2 days and TPAB was left for incubation at 7°C for 7 days. Man Rogosa Sharpe Agar medium was used for the enumeration of lactic acid bacteria. The petri dishes were incubated at 30°C for 2 days for LAB count. Violet Red Bile Dextrose (VRBD) Agar was prepared for the enumeration of *Enterobacteriaceae* and was incubated at 30°C for 2 days in anaerobic conditions. Rose Bengal Chloramphenicol Agar was used for the enumeration of yeast-mould and sonar microbial enumeration was conducted after the petri dishes were left for incubation at 25°C for 5 days (Gökalp et al., 2001).

Statistical Analysis

Data were evaluated using analysis of variance (ANOVA) using the statistical package for social scientists- SPSS 22 software at significance level of 95%. The variance of significance was verified using Duncan test.

Results and Discussion

The microbiological analysis results of rainbow trout which were added black cumin oil extract in different concentrations (%1, 1.5% and 2%) were demonstrated in Table 1.

Significant increases were determined in the amount of TAMB during the preservation process ($p<0.05$). While the highest increase was observed in the control group, the lowest amount of TAMB was determined in the D sample. On the first day of storage, TAMB bacteria values were respectively determined as 4.07, 3.09. 2.87 and 2.71 log cfu/g in group A, B, C and D. The acceptable amount of TAMB and TAPB in fresh fish were determined as 7 log cfu/g by the ICMSF (1986). Samples in group A reached limit value (7 log cfu/g) on the 9th day. Groups with essential oil were below this value during storage period. Akarsu (2016) examined the amount of TAMB in thyme extracts which were obtained by applying different processing techniques (hot infusion, cold infusion, distillation and boiling) on trout fillets. As a result of the 21-days of storage, it was reported that the amount of TMAB exceeded the limit value on the 17th day in the control group and the 21st day in other groups. In the study of Andevari and Rezai (2011) in which the effects of cinnamon oil extract in different concentrations (1%, 1.5% and 2%) were examined on the quality of rainbow trout, it was stated that the amount of TAMB bacteria in cinnamon oil additive samples were lower than the control group. In the study of Uçak (2019) which was conducted with green tiger shrimp and japanese shrimp that were treated with onion peel extract, it was reported that the onion peel extract significantly slowed the bacterial growth and total viable count on the shrimps which were treated with onion skin extract was significantly lower than the control groups ($p<0.05$). Similar results were found by previous studies (Özpolat and Duman 2016; Öz et al., 2017).

An increase was observed in the amount of TPAB depending on the storage duration in all of the groups and the amount of TPAB were respectively determined as 4.36, 3.45, 3.22 and 3.05 log cfu/g in the group A, B, C and D. The determined value in the control group was higher than the other groups ($p<0.05$). Erkan et al. (2011) emphasized that the laurel and cinnamon ail applications on the bluefish were effective on the amount of TPAB. In the study of Duman et al. (2012), rosemary and thyme oil were applied on marinated crayfish and it was reported that the amount of TPAB was higher in the control

group than the essential oil added groups. Our study is similar to the findings of this research. When the TMAB and TPAB values of this study are examined, it can be stated that the essential oils have an antimicrobial effect. This study showed black cumin oil addition improved the microbiological quality.

Table 1. The results of microbiological analyzes of rainbow trout samples applied with black cumin oil (log cfu/g)

Microbiological Analyzes	Group	0. day	3. day	6. day	9. day
Total Aerobic Mesophilic Bacteria	A	4.07±0.03^{a}	5.51±0.05^{b}	6.91±0.08^{c}	7.92±0.09^{d}
	B	3.09±0.14^{a}	3.80±0.14^{b}	4.75±0.12^{c}	5.21±0.02^{d}
	C	2.87±0.12^{a}	3.48±0.24^{b}	4.32±0.16^{c}	5.02±0.18^{d}
	D	2.71±0.04^{a}	3.24±0.08^{b}	4.13±0.07^{c}	4.66±0.12^{d}
Total Psychrotrophic Bacteria	A	4.36±0.06^{a}	5.86±0.05^{b}	7.02±0.02^{c}	8.03±0.02^{d}
	B	3.45±0.14^{a}	4.25±0.10^{b}	4.63±0.21^{b}	5.82±0.07^{c}
	C	3.22±0.07^{a}	3.94±0.09^{b}	4.30±0.11^{c}	5.56±0.14^{d}
	D	3.05±0.09^{a}	3.65±0.17^{b}	3.96±0.05^{b}	5.11±0.09^{c}
Lactic Acid Bacteria	A	2.16±0.06^{a}	2.92±0.09^{b}	3.31±0.14^{c}	5.03±0.12^{d}
	B	2.02±0.03^{a}	2.50±0.24^{b}	3.09±0.12^{c}	4.36±0.10^{d}
	C	2.00±0.00^{a}	2.18±0.04^{a}	2.86±0.11^{b}	3.82±0.23^{c}
	D	2.00±0.00^{a}	2.07±0.06^{a}	2.60±0.07^{b}	3.53±0.07^{c}
Enterobacteriaceae	A	2.00±0.00^{a}	2.97±0.04^{b}	3.55±0.13^{c}	4.09±0.14^{d}
	B	2.00±0.00^{a}	2.31±0.19^{a}	2.95±0.09^{b}	3.30±0.12^{b}
	C	2.00±0.00^{a}	2.08±0.07^{a}	2.78±0.12^{b}	3.12±0.07^{b}
	D	2.00±0.00^{a}	2.04±0.06^{a}	2.52±0.07^{b}	2.95±0.06^{c}
Yeast-Mould	A	2.00±0.00^{a}	2.25±0.11^{a}	2.67±0.12^{b}	3,07±0.11^{c}
	B	2.00±0.00^{a}	2.11±0.10^{a}	2.42±0.03^{b}	2.65±0.08^{c}
	C	2.00±0.00^{a}	2.00±0.00^{a}	2.33±0.07^{b}	2.51±0.05^{c}
	D	2.00±0.00^{a}	2.00±0.00^{a}	2.09±0.06^{a}	2.37±0.05^{b}

A= control; B= sample applied with 1% black cumin oil; C= sample applied with %1,5 black cumin oil; D= sample applied with 2% black cumin oil.

The highest amount of LAB was determined in the samples of the control group (A), and the lowest amount of bacteria was determined in the samples of group D of which 2% black cumin oil was added. An increase was observed in the amount of LAB in all of the groups throughout the storage duration. Duman et al. (2012) emphasized that the amount of LAB in the rosemary and thyme oil added groups were lower than the control group. Patır et al. (2015) reported that the amount of LAB in marinated trout (*O. mykiss*) with eugenol-added samples were lower than the control group. Compared to other studies, our current research results showed similar results both in terms of storage and positive effects of plant oils.

At the end of the storage process, the amount of *Enterobacteriaceae* were determined as 4.09 log cfu/g in the samples of group A, 3.30 log cfu/g in the samples of group B, 3.12 log cfu/g in the samples of group C, and 2.95 log cfu/g in the samples of group D and the difference between the groups were significant. In the study of İnanlı et al. (2018) in which the effect of chitosan coating enriched with blueberry and goji berry extracts was examined on the microbial growth of rainbow trout fillets, it was reported that the highest amount of *Enterobacteriaceae* was determined in the control group (İnanlı et al., 2018). Özpolat and Duman (2016) reported higher Enterobacteriaceae count control group and lower the treatment groups black cumin oil.

While the amount of yeast-mould increased in all of the groups depending on the storage duration, the highest value was determined in the samples of group A (3.07±0.11 log cfu/g) at the last day of the storage. Significant differences were determined between the groups throughout the storage duration ($p<0.05$). İnanlı et al. (2018) determined the highest amount of yeast-mould in the rainbow trout fillets as 5.14±0.12 log cfu/g in the samples of the control group and the lowest amount of yeast-mould in the samples which were coated with goji berry added chitosan as 3.74±0.19 log cfu/g. Our study is similar to the findings of this research.

Conclusion

When the overrall microbiological analysis results were evaluated, it can be stated that black cumin oils added with different concentrations have an antibacterial effect on the rainbow trout fillets.

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