Effects of black cumin (*Nigella sativa*) oil on ammonia and biogenic amine production in rainbow trout

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**ABSTRACT**

In the present study, 0.00% (control), 0.10%, 0.40%, 0.70%, 1.00% and 1.30% black cumin oil was added to rainbow trout feed and the fish were fed for 120 days. Following the feeding period, the harvested fish were stored in the refrigerator at ±2 ºC, in Styrofoam boxes filled with ice, for 23 days. During the storage period, the effects of black cumin oil on ammonia and biogenic amine production in rainbow trout were examined. An increase was observed in the ammonia content during the storage period, with the most substantial increase observed in the control group and the least substantial increase observed in the 1.30% black cumin oil group. The biogenic amine level increased during the storage period. A significant increases was observed in the tyramine level during the storage period (<3.08 mg/100 g), and significant differences were observed between the groups in terms of spermidine, spermine, histamine, and tyramine content (p<0.05). The results of the present study suggest that the addition of black cumin oil reduced ammonia and biogenic amine production in rainbow trout.

**Key words:** Ammonia, Black cumin oil, Histamin, Rainbow trout.

**INTRODUCTION**

Rainbow trout (*Oncorhynchus mykiss*), a member of the *Salmonidae* family, is of high commercial importance and is widely consumed in Europe. This species is extensively cultured in various countries, owing to its rapid growth and high nutrient content.

Biogenic amines are present in fish meat, following deterioration caused by microbial activity. The detection of biogenic amines, particularly histamine, is of considerable importance, since they can be toxic to humans depending on their concentration. The enzymatic decarboxylation of amino acids in fish leads to the formation of several amine compounds. Decarboxylase enzymes require free amino acids as substrate. For this reason, amino acids are released and biogenic amine is produced during deterioration or decomposition process of fish due to bacterial production, amino acid decarboxylation activity, and proteolysis activity (Eitenmiller and De Souza, 1984).

The chemical structure of biogenic amines varies, and can either be aliphatic (putrescine, cadaverine, spermine, and spermidine), aromatic (tyramine and 2-phenylethylamine), or heterocyclic (histamine and tryptamine) (Silla-Santos, 1996). Among these, histamine, putrescine, cadaverine, tyramine, tryptamine, 2-phenylethylamine, spermine, and spermidine are considered important biogenic amines found in food (Shalaby, 1996). Spermine, agmatine, and cadaverine may occur naturally in foods, and not necessarily as a result of bacterial contamination (Bragadottir, 2001). The putrescine content in fish is higher than that of spermine and spermidine (Bardocz, 1995).

Biogenic amine formation in fish depends on the levels of free amino acids, the presence of microorganisms that can decarboxylate amino acids and the environmental conditions required for microbial growth and development (Silla-Santos, 1996). Biogenic amine production also varies depending on microbial flora, food additives, temperature, humidity, fermentation, and packaging conditions (Draisci et al. 1998).

One of the most important objectives of aquaculture is to have maximum yield with minimum cost and time. A variety of feed additives is used to increase aquaculture performance, protect fish health, and positively affect the quantity and quality of animal produce. Some approaches used to achieve this objective can lead to certain positive or negative effects on the quality of fish meat.

The components in black cumin are known to exert pharmacological effects such as antibacterial, antifungal, antiviral, anti-inflammatory, antihistaminic, antioxidant, antiprotozoan, and immunostimulant properties. Black cumin is used in the treatment of many conditions such as asthma, hypertension, diabetes (type II), and is known to alleviate inflammation, cough, bronchitis, headaches, eczema, flu, fever, and dizziness (Altinterim, 2010).

Supplementation of black cumin to fish feed has shown positive effects on the growth rate of fish and has led
to lower microbial activity during storage (Öz, 2013). The use of natural products to increase the growth rate of fish has immense potential for applications in the future. However, it is necessary to determine what changes black cumin causes in fish meat and what effects it may have on biogenic amine levels during storage. To our knowledge, this is the first study to address this issue.

MATERIALS AND METHODS

Fish sampling: The feeding activities were performed for 120 days at a private trout farm (Öz Trout Production Facility, Adana, Turkey). In all, 2700 rainbow trout (O. mykiss) were used for the study. Eighteen concrete pools with dimensions of 4 × 1 × 1.20m, each containing 150 fish, were used. For the duration of the trial period, the fish were fed twice a day with fish feed containing different proportions of black cumin oil (0.00%, 0.10%, 0.40%, 0.70%, 1.00%, and 1.30%).

The fish, which were 90 grams at the beginning of the study, were harvested at 260, 270, 272, 265, 290, and 285 grams. After 120 days of feeding, the harvested fish were stored in the refrigerator at ±2 °C for 23 days, in Styrofoam boxes filled with ice.

Biogenic amine analysis was carried out following the method described by Özogul et al. (2002).

Sample preparation for biogenic amine analysis: Fish muscle (5 g) was taken from the dorsal part of the fish fillet without skin and transferred to a 50-mL centrifuge tube. The sample was then homogenised using the Ultra-Turax with 20 mL 6% TCA for 3 min, centrifuged using a Hettich 32R centrifuge (Tutlingen, Germany) at 11400 g for 10 min at 4 °C and filtered through Whatman No. 1 filter paper (Maidenstone, UK). The aliquot (about 20 mL) was brought to 50 mL with distilled water and was stored in a freezer (-18 °C) for no longer than 8 weeks until derivatisation.

Derivatisation procedure: A stock solution was prepared by dissolving 2% benzoyl chloride in acetonitrile to enhance the reaction with amines. For derivatisation of standard amine solutions, 50 µL was taken (2 mL for extracted fish samples) from each free base standard solution (10 mg mL-1). One millilitre of 2 M sodium hydroxide was added, followed by 1 µL benzoyl chloride (2%), and mixed on a vortex mixer for 1 min. The reaction mixture was left at room temperature (24 °C) for 20 min. The benzoylation was stopped by adding 2 mL of saturated sodium chloride solution, and the solution was extracted twice with 2 mL of diethyl ether. The upper organic layer was transferred into a clean tube after mixing and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 500 µL of acetonitrile and 5 µL aliquots were injected into the HPLC.

Apparatus and columns: A Shimadzu Prominance HPLC apparatus (Shimadzu, Kyoto, Japan) equipped with a SPD-M20A diode array detector, two binary gradient pumps (Shimadzu LC-10AT), auto sampler (SIL 20AC), column oven (CTO-20AC) and valve unit FCV-11AL with a communication bus module (CBM-20A) was used. The column was a reverse-phase, ODS Hypersil, 5µ, 250 9 4.6 mm (Phenomenex; Macclesfield, Cheshire, UK). Oven temperature was 30 °C, and mobile phase was acetonitrile and HPLC-grade water. Chromatographic separation was carried out using continuous gradient elution with acetonitrile (eluant A) and HPLC-grade water (eluant B). The gradient was started at 40% acetonitrile and was then increased to 70% in 15 min. The total separation was 20 min and gradient was run for 20 min to ensure full separation. The injection volume was 10 µL.

Statistical analysis: The mean value and standard deviation were calculated from the data obtained from the three samples for each treatment for each specific storage time. Data were subjected to analysis of variance and Duncan’s multiple range tests using the SPSS version 13.0 statistical package (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Changes in ammonia and biogenic amines during the storage period of rainbow trout fed with black cumin oil added to the feed were recorded (Table 1). The initial ammonia content in rainbow trout fillets was 3.15–3.56 mg/100 g, whereas it increased to 14.08–19.37 mg/100 g at the last day of the storage. The most substantial increase was observed in the control group, and the least substantial increase observed in the sixth group.

A previous study on the storage of rainbow trout found that the initial ammonia content in trout meat was 16.97 mg/100 g, and this level increased to 116.33 mg/100 g at the end of the storage period (Kus, 2012). The initial ammonia level in trout fed with natural feed was 11.16 mg/100 g, whereas it was 16.99 mg/100 g in trout fed with commercial feed. Özogul et al. (2008) reported that the initial ammonia level (0.02 mg/100 g) in white grouper stored at 4 °C reached 1.76 mg/100 g at the end of the storage period. Kamari (2007) reported the initial ammonia level in catfish fillets to be 1.07 mg/100 g and that this level fluctuated throughout the storage period. In the same study, the maximum ammonia level was observed on the 18th day of storage (2.5 mg/100 g), at which point the fish were not suitable for consumption. In the present study, fish that received feed to which black cumin oil was added, showed a reduction in ammonia increase during the storage period. The most substantial increase was observed in the control group, whereas the least substantial increase was observed in the 1.30% black cumin oil group.

The biogenic amine levels fluctuated during the storage period. While trimethylamine and 2-phenylethylamine were not generally found in trout meat, putrescine, histamine, cadaverine, spermine, spermidine, serotonin, tyramine, and dopamine were the principal amines found in trout meat. The most commonly occurring amines, in meat from both naturally occurring and cultured rainbow trout, were dopamine, serotonin, and tyramine (Yavuzer, 2011).
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Table 1 continued............
Histamine is one of the most rarely found amines found naturally in fish muscle, owing to physiological and environmental factors (Veciana-Nogues et al., 1997). In this study, spermidine and spermine levels in trout meat were 1.13–1.43 mg/100 g and 2.53–1.86 mg/100 g, respectively, and an increase was observed in all groups during the storage period, with the lowest levels recorded in the 6th group. Spermidine and spermine levels in trout fillets stored in polyethylene packs at 3 °C for 17 days remained under 11 and 9.5 mg/kg, respectively (Krizek et al., 2011). Liu et al. (2010) reported that tyramine, spermidine, and spermine levels in tilapia fillets stored in polyethylene packs with high oxygen permeability, at 0 °C, showed fluctuations throughout the storage period, with the highest value being 15 mg/kg.

As fish meat decomposes, histamine is produced as a result of decarboxylation of histidine by bacteria (Fadhlouai-Zid et al., 2012). Histamine levels are low in freshly caught fish, usually below 0.1 mg/100 g (Auerswald et al., 2006). Histamine is one of the most rarely found amines in trout muscle. In the present study, the initial histamine level at the beginning of the storage period was 0.01–0.06 mg/100 g and it remained under 2 mg/100 g throughout the storage period. Histamine levels of 5 mg/100 g, which is the legal limit determined by the FDA in trout muscle (FDA, 1995), were not observed in any of the groups, on any day of the storage period. All groups had similar levels throughout the storage period. All groups had similar levels throughout the storage period. All groups had similar levels throughout the storage period. All groups had similar levels throughout the storage period. All groups had similar levels throughout the storage period. All groups had similar levels throughout the storage period. All groups had similar levels throughout the storage period.

Putrescine and cadaverine play an important role in terms of increasing the toxic effects of histamine. However, cadaverine is a useful index for initial deterioration stages of fish (Al Bulushi et al., 2009). Krizek et al. (2011) reported that cadaverine was not detected until the 11th day of the storage period in trout fillets stored in polyethylene packs, at 3 °C for 17 days. However, the cadaverine levels showed rapid increases starting from the 11th day of storage, with the levels reaching 38.8 mg/kg at the end of the storage period. The putrescine level, which was 3.1 mg/kg initially, increased to 29.5 mg/kg at the end of the 17-day storage period (Krizek et al., 2011). In the present study, whereas the initial putrescine level varied between 1.14 and 0.78 mg/100 g, no cadaverine was detected at the beginning of the storage period, in any of the groups. Furthermore, no significant difference in terms of putrescine production was observed at the 17th day of storage. While the lowest level of putrescine was recorded in the 3rd group, the highest level was recorded in the control group. Liu et al. (2010) reported that cadaverine level showed a gradual increase in tilapia fillets stored in aerobic packs at 0 °C and reached 42.1 mg/kg at the end of the storage period (29 days).

Significant differences were observed between the groups in terms of spermidine, spermine, histamine, and tyramine content (p < 0.05). Spermidine and spermine are amines that cadaverine is a useful index for initial deterioration stages of fish. In the present study, whereas the initial putrescine level varied between 1.14 and 0.78 mg/100 g, no cadaverine was detected at the beginning of the storage period, in any of the groups. Furthermore, no significant difference in terms of putrescine production was observed at the 17th day of storage. While the lowest level of putrescine was recorded in the 3rd group, the highest level was recorded in the control group. Liu et al. (2010) reported that cadaverine level showed a gradual increase in tilapia fillets stored in aerobic packs at 0 °C and reached 42.1 mg/kg at the end of the storage period (29 days).

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the storage period. However, the lowest histamine level was
observed in the 6th group on the 23rd day and the highest
level was observed in the control group. Krizek et al.
(2011) reported a considerably low level of histamine
(<0.4 mg/kg) in rainbow trout fillets stored at 3 °C.
Accumulation of serotonin in cold-stored rainbow trout
meat varied between 2.30 and 7.77 mg/100g. An increase
in serotonin level was observed in all groups throughout
the storage period, but no statistically significant
difference was observed between the groups (p<0.05). The
dopamine level in trout meat varied between 1.11 and 9.41
mg/100 g. Tyramine was one of the most commonly found
amines in trout meat, in this study.

The results of the present study suggest that the
addition of black cumin oil reduced ammonia and biogenic
amine production in rainbow trout.

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