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Effect of black cumin oil (*Nigella sativa* L.) on fresh fish (*Barbus grypus*) fillets during storage at 2 ± 1 °C

Emine OZPOLAT[1]*, Muhsine DUMAN[1]

Abstract

It is important that the methods and materials used for to lengthen the shelf-life of food are simple, inexpensive and safe. For these reasons, herbal additives like cumin oil (*Nigella sativa* L.) have been preferred recently. Thus, the present study focuses on the influence of black cumin oil on chemical, microbiological and sensory quality of fish (*Barbus grypus*) fillets during storage at 2 ± 1 °C. Acceptability scores for sensory quality of all described treatment groups decreased with storage time. Defined limits for mesophilic bacteria and Total volatile basic nitrogen (TVB-N) were reached after 21st days for the control group, after 24th days for with 0.2% black cumin oil treated group and after 28th days for with 0.4% and 0.6% black cumin oil treated groups. No difference was found among groups during storage in terms of defined thiobarbituric acid (TBA) values. Consequently, it was found that black cumin oil treated groups had longer shelf-life and higher sensory quality than the untreated control group.

Keywords: black cumin oil; *Barbus grypus*; *Nigella sativa*; shelf life.

Practical Application: Addition of black cumin oil in fish fillets resulted in longer shelf life and this method could be simple, commercially and safety.

1 Introduction

Biological composition of fresh aquatic food makes them immensely perishable and leads to fast declines in their quality. Also, these products are not able to be storaged for a long time under normal refrigeration conditions due to enzymatic activity and microbiological spoilage. Alternative methods have been studied to increase the shelf-life of stored fresh fish and to increase the economic value of these products to be able to satify the increasing demands and to decrease energy costs related to these products (Ashie et al., 1996; Kykkidou et al., 2009). Therefore, alternative sources of safe, effective and acceptable natural preservatives need to be explored. The use of natural antimicrobials such as organic acids, essential oils, plant extracts, and bacteriocins could be good alternatives to ensure food safety (Burt, 2004; Holley & Patel, 2005; Emir Coban, 2010; Negi, 2012; Emir Coban & Ozpolat, 2013). *Nigella sativa* L., is known as black cumin or black seed. *Nigella sativa* L. is a member of the *Ranonculaceae* family and native to some parts of the Mediterranean region. Dried black cumin seeds have been used in salads, breads, cheese, coffee and tea (Ustum et al., 1990; Arici et al., 2005; Ramadan, 2007). Black cumin oil has been shown to demonstrate antibacterial (Arici et al., 2005; Hanafy & Hatem, 1991; Hassanien et al., 2013), antifungal (Khan et al., 2003) and antioxidant (Burits & Bucar, 2000; Luther et al., 2007) activities.

The freshwater fish shibuta (*Barbus grypus* Heckel, 1843), is a cyprinid which is found along the Euphrates and Tigris Rivers in Turkey, Syria and Iraq and is abundant and commercially important (Oymak et al., 2008). It reaches a maximum size of 2 m and 60 kg (Zivotofsky & Amar, 2006).

Limited data, however, are available with regard to the application of black cumin oil for extension of shelf life and quality during cold storage of fish. Hence, the aims of the present work were to determine the effects of black cumin oil on the shelf life of fresh *Barbus grypus* fillets during storage at 2 ± 1 °C.

2 Materials and methods

2.1 Sample preparation

Black cumin oil (100% oil) was purchased commercially from the Mecidefendi Company (Izmir, Turkey). *Barbus grypus* weighing 7-8 kg/fish was caught during the period of January-February 2014 in the Keban Dam Lake. Fish were transported to the laboratory with ice in boxes. The fish were immediately gutted, filleted and washed. The fish fillets were divided into four treatment groups; control samples without added cumin black oil and samples treated with cumin black oil at a rate of 0.2, 0.4 and 0.6% oil (volume/weight of fish flesh; v/w). Aproximately 1.5-2 kg/fish were in each of treatment groups. Cumin black oil was added to the surface of fish samples in appropriate volumes using a micropipette, followed by mildly massaging the oil onto each sample using a gloved hand. Treatment groups were vacuum packed (high barrier nylon polyethylene bags) and stored at 2 ± 1 °C until analysis on days 1, 3, 6, 9, 12, 15 18, 21, 24 and 28. The study was carried out in duplicate.

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[1] Department of Fisheries Techniques and Fish Processing Technology, Fisheries Faculty, Firat University, Elazig, Turkey
*Corresponding author: emineozpolat@hotmail.com

2.2 Microbiological analysis

A sample of 10 g (each package) was taken aseptically from each sample, transferred to a stomacher bag and 90 ml of sterilized peptone water (Buffer Peptone Water, LAB M) was added. The mixture was homogenized for 2 min with a stomacher (Stomacher 400, Lab. Blender, London, UK). Samples (0.1 ml) of serial dilutions of fish fillets homogenates were spread on the surface of agar plates. Total psychrophilic and mesophilic aerobic bacteria were determined on Plate Count Agar (PCA, Merck 1.05463) after incubation at 7 °C for 10 days and 30 °C for three days, respectively. Lactic acid bacteria were determined on MRS Agar (Merck 1.10660) after incubation at 28 °C for two days. *Enterobacteriaceae* was determined on Violet Red Bile Glucose Agar (Oxoid CM 485) after incubation at 37 °C for 1 day. Yeasts and molds were enumerated on Rose Bengal Chloramphenicol Agar (RBC, Merck 1.00467) after incubation at 25 °C for five days. Microbiological data were transformed into the log of the number of colony-forming units (cfu/g) (Halkman, 2005).

2.3 Chemical analysis

The pH of samples was measured according to an AOAC method (Association of Official Analytical Chemists, 1990). Ten gram samples (a package from each groups) were homogenized with 90 ml deionised water and the pH was measured with a digital pH meter (EDT.GP 353). Thiobarbituric acid (TBA) was determined according to the method of Tarladgis et al. (1960). TBA content was expressed as mg of malondialdehyde (MDA)/kg of fish flesh. Total volatile basic nitrogen (TVB-N, mg/100g fish flesh) was determined acording to the method described by Varlik et al. (1993).

2.4 Sensory analysis

Eight experienced panelists, who were academic staff and trained in sensory descriptors for fish flesh, evaluated the sensory quality of samples. The package from each treatment groups have been opened and evaluated the sensory quality. The sensory assessment of raw fish flesh was conducted using the Quality Index Method developed by Bonilla et al. (2007) with some modifications. The scheme consisted of six quality parameters (texture, mucus, leachate, odor, colour, brightness). A demerit score, which ranged from 0 to a maximum of 3, where 0 represented the best quality and a higher score (3) indicated poorer quality, was used in evaluations. Fish samples (25 g) were cooked individually in a microwave oven at full power (1600 W) for 2 min and served hot to panelists. The sensory evaluations of cooked fish flesh were assessed according to the method of Amerine et al. (1965). Panelists were asked to score odour, taste, colour and texture of fish using a 1-10 acceptability scale (scale: 9-10, excellent; 7-8, good; 5-6, limit of acceptable; ≤5, unacceptable). All the sensory evaluation were averaged.

2.5 Statistical analysis

All analytical determinations were on days 1st, 3th, 6th, 9th, 12th, 15th 18th, 21th, 24 th and 28 th. Experiments were replicated twice on different occasions with different fish samples. Each sample was analyzed three times and the mean calculated. Data were subjected to analyses of variance. The Tukey's honest significant difference procedure was used to test for differences between means ($p<0.05$) using SAS 6.1 (SAS Institute Inc., Cary, NC) (SAS Institute, 1999).

3 Results and discussion

3.1 Assessment of microbiological parameters

Changes in microflora of *Barbus grypus* flesh affected by different concentration of black cumin oil addition during storage period are shown in Figure 1.

The average initial number of mesophilic bacteria for all group samples (3.2 log cfu/g) indicates that fisher were of acceptable quality. Control, 0.2%, 0.4% and 0.6% black cumin oil added samples exceeded the value of 7 log cfu/g for mesophilic bacteria considered as the upper acceptability limit (International Commission on Microbiological Specifications for Foods, 1978) for fresh marine species on days 21, 24, 28 and 28 of storage, respectively (Figure 1a). The average psychrophilic bacteria count for the fillets was 3.77 log cfu/g. The number of psychrophilic bacteria increased with storage time, and in control group after the 12th day the count was higher than in all of treatment groups ($p<0.05$) (Figure 1b). The count of lactic acid bacteria increased during the storage period and on the 15th day the difference among groups (control and 3 treatment groups) was statistically significant ($p<0.05$). The rate of increase in lactic acid bacteria was the highest in control group ($p<0.05$) (Figure 1c). The initial Enterobacteriaceae count in fillets was 1.3 log cfu/g. During storage, the Enterobacteriaceae count increased in all groups ($p<0.05$). The rate of increase in Enterobacteriaceae in treatment groups was lower than the control group and the treatment groups with 0.4% and 0.6% black cumin oil had a lower rate of increase in Enterobacteriaceae than the treatment group with 0.2% oil ($p<0.05$) (Figure 1d). Initial yeast and mold levels were determined as 1.77 log cfu/g. The increase in yeast and mold counts in all groups was significant after the 12th day ($p<0.05$). On the15th day of storage, the rates of increase in yeast and mold in the control and 0.2% treatment groups were higher than 0.4% and 0.6% groups ($p<0.05$) (Figure 1e). With all these results, it was determined that black cumin oil addition improved the microbiological quality and increased the shelf-life of fresh *Barbus grypus* fillets In terms of microbiological quality (mesophilic bacteria, psychrophilic bacteria, lactic acid bacteria, Enterobacteriaceae and yeats and mold) similar results of black cumin oil usage were reported (Arici et al., 2005; Hassanien et al., 2013). These studies showed (Arici et al., 2005; Hassanien et al., 2013) black cumin oil addition improved the microbiological quality.

3.2 Assessment of chemical parameters and pH

Figure 2 shows the average values of the chemical parameters and pH values analysed on each sampling day. The initial pH value of the fish flesh was 5.96. pH increased significantly with storage time in all groups. The increase of pH values during the storage period may be attributed to the accumulation of ammonia and organic amine compounds and bacterial growth (Bensid et al., 2014; Schormüller, 1968). The control group had a higher ($p<0.05$) pH value than other groups only on day 24 of storage. No difference

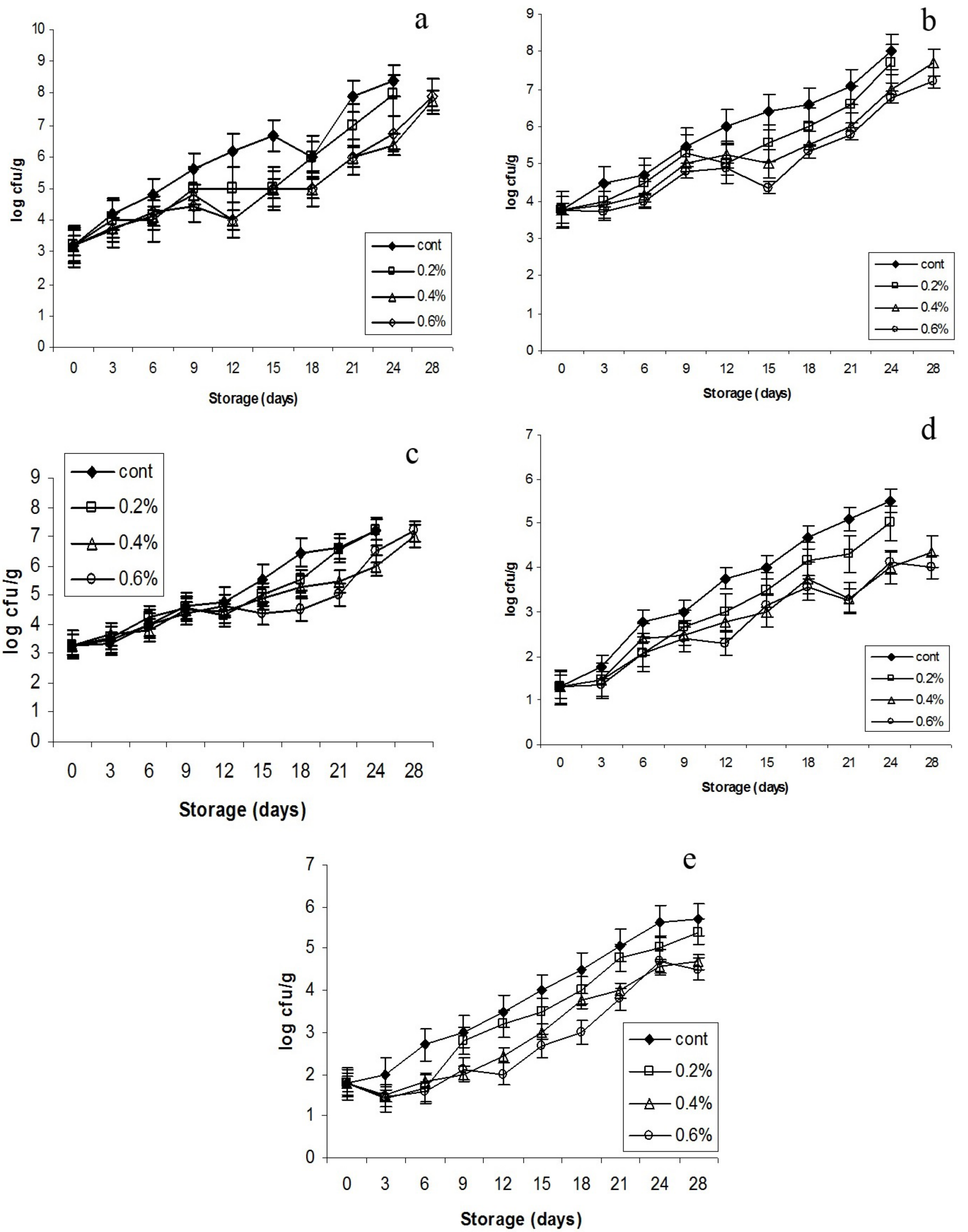

Figure 1. Changes in the counts of (log_{10} CFU/g) (a) mesophilic bacteria; (b) psychrophilic bacteria; (c) lactic acid bacteria; (d) Enterobactericeae; (e) yeats and mold: in the *Barbus grypus* flesh different concentration of black cumin oil addition during storage at 2 ± 1 °C.

(p>0.05) was observed throughout the storage period between black cumin oil treated groups (Figure 2 a).

The TVB-N is a measure of the ammonia, trimethylamine and dimethylamine formed from the breakdown of nucleotides and from the deamination of amino acids by microorganisms (Contreras-Guzman, 2002; Oliveira et al., 2010). According to Huss (1995) the acceptable TVB-N level is 35-40 mg N/100g. At the begining of storage TVB-N values for fish fillets were determined at 18.2 mg/100 g for fish flesh. The TVB-N values were significantly different between control and treatment groups ($p<0.05$) on 21st and 24th days of storage. The values of TVB-N increased during the storage period and exceeded the acceptable limit on the 21th day for the control group, 24th day for 0.2% black cumin oil added group, 28th day for 0.4% and 0.6% black cumin oil added groups ($p<0.05$). These results demonstrate that black cumin oil was effective in reducing the TVB-N values in the fish flesh (Figure 2b).

The thiobarbituric acid (TBA) test is generally used as a measure of lipid oxidation (Yu et al., 2002). TBA values among treatments followed similar increasing trends with storage period, but values were all less than 0.5 mg malonaldehyde/kg). All values were were lower than the limit value (5 mg malonaldehyde/kg) during the storage period. It was determined that there was no significant differences in TBA values for the control and treatment groups during the study period ($p>0.05$).

3.3 Assessment of sensory analysis

The sensory quality of raw and cooked *Barbus grypus* flesh was evaluated 2 days after fish were caught and on the 3rd, 6th, 9th, 12th, 15th, 18th, 21st and 24th days of storage. Depending upon sensory analysis, the treatment groups with 0.4% and 0.6% black cumin oil had the longest shelf-life with 24 days. The group treated with 0.2% oil and the control group had 21 and 18 days long shelf-life, respectively. In addition, the treatment groups were highly preferred by panelists due to their desirable odour. The results of the sensory assesment indicated that shelf-life of fish flesh was affected by black cumin oil addition. (Figure 3). Similar results were seen in different studies in which positive effects of plant extracts (such as, thyme, oregano, clove and rosemary) on food (Ozyurt et al., 2011; Bensid et al., 2014).

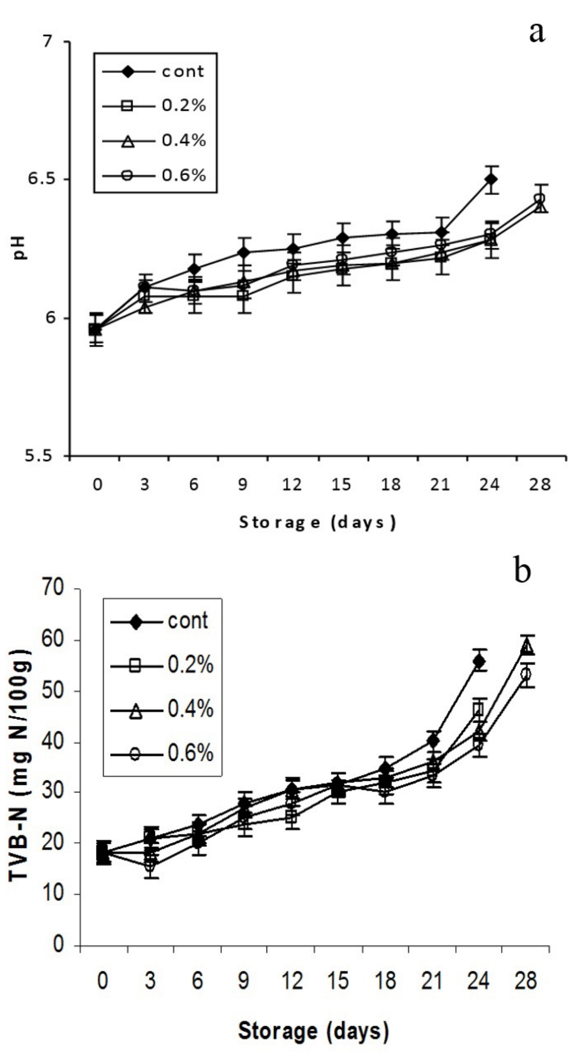

Figure 2. pH (a) and TVB-N (b) in the *Barbus grypus* flesh different concentration of black cumin oil addition during storage at 2 ± 1 °C.

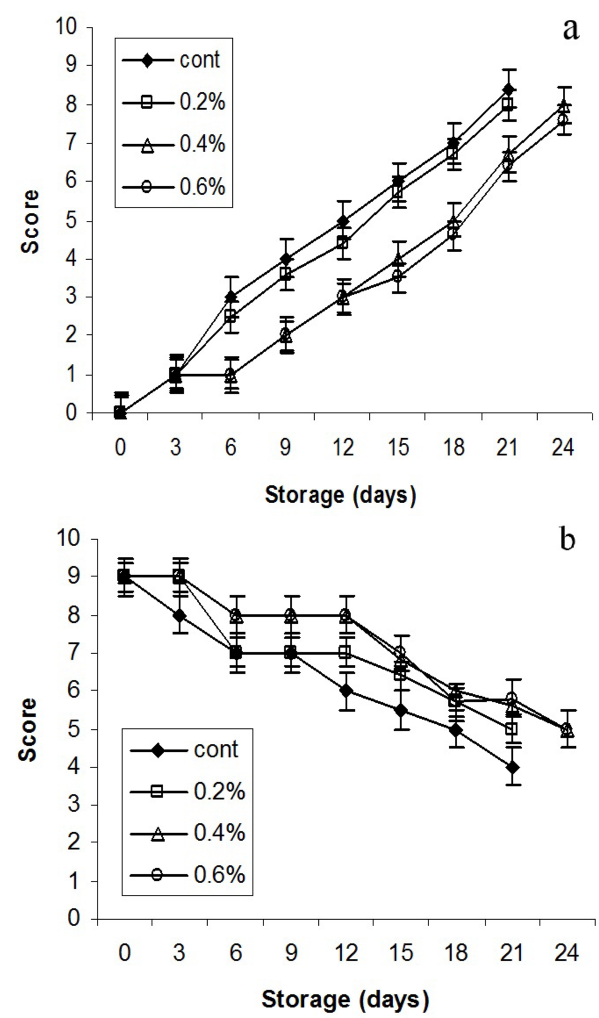

Figure 3. Sensory assessment of raw (a) and cooked (b) *Barbus grypus* flesh different concentration of black cumin oil addition during storage at 2 ± 1 °C.

4 Conclusion

The results of this study suggest that black cumin oil no adverse effect of fish fillets and black cumin oil dose of 0.6% more longer shelf life and higher sensory quality than the other threated dose (0.2% and 0.4%). Thus, black cumin oil has been usable of fish fillets as additive.

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