



Original Article

The effects of *Juniperi fructus* essential oil and vacuum packing on the shelf life of rainbow trout fillets during storage at 2±1°C



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Article Info

Abstract



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We hypothesized that the combined effect of vacuum packaging and *Juniperi fructus* essential oil addition would increase shelf life. Six different treatments were tested. The effects of the different concentrations of *J. fructus* essential oil (0%, 0.3% and 0.6%) and packing method (non-vacuum and vacuum) on the fish (*Oncorhynchus mykiss*) fillets of stored 4±1 °C were investigated in terms of its microbiological (mesophilic aerobic bacteria and yeast-mold), chemical (pH, total volatile alkaline nitrogen (TVB-N), thiobarbituric acid (TBA value)) and sensory quality. The results showed that *J. fructus* essential oil had a positive significant effect on quality parameters ($p < 0.05$). In conclusion, based primarily on sensory, TVB-N and mesophilic bacteria data the shelf-life of fresh rainbow trout was 4 days (non-vacuum packaged), 13 days (vacuum packaged), 19 and 28 days treated with *J. fructus* oil (0.3 and 0.6%, v/w) under vacuum packaged, respectively. *J. fructus* essential oil application and vacuum packaging; extended the shelf life of fish fillets by an average of 15 days. The combined use of *J. fructus* essential oil and packaging techniques could form the basis for new studies.

Keywords: *J. fructus*, Fish, Vacuum Packaging, Microbiological Quality, Essential Oil

1. Introduction

Fish has high biological value due to its amino acids, unsaturated fatty acids, vitamins and minerals. Its carbohydrate content is very low. Therefore, the pH value remains above 6.0 in the post-mortem period. This is important for the growth of microorganisms. Normally; healthy and fresh fish muscle is sterile. However, there are microorganisms, depending on the water conditions in the gills and intestines of the fish. After the fish die, these microorganisms pass into the muscle and can easily deteriorate due to pH, water activity, soft tissue structure, and the presence of non-protein nitrogenous compounds. Various methods are used to extend the shelf-life of fish and fishery products [1-3].

These are different packaging, technological methods and preservatives, etc. The shelf life quality of seafood products can be increased with vacuum packaging technique [4, 5]. Moreover, the microbial ecology of food basically depends on the environment, used equipment, food type, handling practices, processing, packaging and storage temperature [6, 7]. In the vacuum packaging, the air from the package is removed and sealed airtight. Due to the lack of oxygen, the growth and proliferation of aerobic and mesophilic microbes are limited [8, 9].

Today, many methods have been developed for the pre-

servation of foods. Among these methods, natural, economical and easy-to-use organic acids, essential oils, plant extracts and bacteriocins stand out [10, 11]. *J. fructus* L. plant is one of the drugs registered in some codex and pharmacopeias today. According to many codexes, both drugs are obtained from *Junipents communis* L. plant. To date, juniper essential oil has only been used in traditional medicine. But, today its many features, antimicrobial effect, etc. and its use in the food industry is being investigated [12-14].

Rainbow trout, live in lakes, streams and rivers, consuming zooplankton, followed by insects, crustaceans and other fish as they grow. Rainbow trout have been cultured for hundreds of years, and the most widely farmed trout in the world [15-17]. There are studies on the quality characteristics of rainbow trout during various storage conditions [16-22]. When these studies are examined; It has been observed that rainbow trout is an oily fish and its quality can deteriorate quickly. This degradation is due to microorganisms and lipid oxidation. Spoilage time for rainbow trout; the moment of capture depends on the stomach contents at the time of death and seasonal changes [16-23].

There is limited data on the application of *J. fructus* oil during the preservation of fish for the purpose of prolonging the shelf life and quality. The aims of the present

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investigation were to assess the *J. fructus* oil and different packaging (air and vacuum) on the shelf life of refrigerated ($4\pm 1^\circ\text{C}$) trout fillets (*O. mykiss*) by evaluating certain chemical, microbiological and sensory parameters.

2. Materials and methods

Rainbow trout average weight of 200 grams was obtained from a local fisherman (Elazig, Turkey). It was carried to the laboratory by paying attention to the cold chain (in ice-cold water). *J. fructus* oil (100% oil) was purchased commercially from the Dogan Company (Istanbul, Turkey). After the fish were washed and cleaned, their skin was taken, sliced (100 ± 5 g each), they were divided into six groups. The samples were prepared the fish-processing laboratory of the Faculty of Fisheries of Firat University, Turkey. *J. fructus* oil was added to the four groups (0.3% and 0.6% (v/wt)) and control groups without oil. The ratios of *J. fructus* essential oil used; are determined by considering the relevant literature [5, 21]. *J. fructus* oil was added on the surface of fish samples in appropriate volumes by using a micropipette, followed by mild massage (with a small kitchen brush) of the oil for each sample. Polyethylene (LDPE/PA/LDPE, 75 μm in thickness, having an oxygen permeability of 52.2 $\text{mL m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$ and a water vapor permeability of 2.4 $\text{g m}^{-2} \text{ day}^{-1}$ at 0% relative humidity) bags were used for packaging. The samples were placed in these bags and the vacuum packages were closed using a vacuum packaging machine (Boxer 42, Henkelman, Den Bosch, the Netherlands) and non-vacuum packages were carefully closed by hand.

Six different treatments were tested: C1; (without *J. fructus* essential oil, non-vacuum packaging), C2; (without *J. fructus* essential oil, vacuum packaging), A1; (added *J. fructus* essential oil 0.3%, non-vacuum packaging), A2; (added *J. fructus* essential oil 0.3%, vacuum packaging), B1; (added *J. fructus* essential oil 0.6%, non-vacuum packaging), B2; (added *J. fructus* essential oil 0.6%, vacuum packaging).

In total, an average of 15 kg of fish was used for each repetition. For each group, an average of 2.5 kg of fish was packaged as average 100 ± 5 grams and used on the analysis days. The prepared packages were stored $4\pm 1^\circ\text{C}$ until analysis on days 1, 4, 7, 10, 13, 16, 19, 22, 25, 28 and 31. The study was carried out in duplicate.

2.1. Chemical Analysis

For chemical analysis, muscles were cut from the same part of the fish (from the dorsal part of the fish). TBA value, pH and TVB-N and analyses were performed according to the relevant literature, respectively [24-26]. The pH values were measured by using a pH meter (Thermo Scientific Orion 3-Star Benchtop, Cambridge, UK). The value of TBA value was determined to evaluate the oxidation stability during storage and the results were expressed as mg of malondialdehyde/kg (mg MDA/kg) fish muscle. TVB-N; It is the separation of volatile bases by water vapor distillation and the determination of these separated bases by titration with 0.1 N acid.

2.2. Microbiological Analysis

In accordance with the relevant literature, the required dilutions (10^{-1} - 10^{-8}) (with sterile 0.1% peptone water) of the samples were prepared. Plate count agar (PCA,

LAB010, LabM, Lancashire, UK) was used for mesophilic aerobic bacteria and incubated at 35°C for 2 days [25, 26]. Yeast and mould were enumerated on Rose Bengal Chloramphenicol agar (LAB036, LabM, Lancashire, UK) incubated at 25°C for 5 days [29]. Microbiological analyzes were performed in triplicate. Results are expressed as a logarithm of colony forming units (log cfu) per gram of sample [27, 28].

2.3. Sensory Evaluation

Sensory evaluation was made by a panelist group of five people. Panelists are experienced and trained in sensory evaluation. During the sensory evaluation, attention was paid to ensure that environmental conditions such as light, humidity and temperature were standard (Sensory analyses were performed under controlled conditions in individual booths. Care was taken to always do it at the same room temperature, light and time). The samples (with 20 grams of each group, cleaned, filleted and packaged) were evaluated as raw without cooking with five quality parameters consisting of color, odor, appearance, mucus and structure. The overall acceptability values are obtained by averaging these five parameters. Sensory assessment was applied by modifying Kurtcan & Gonul [30] and Fernández-Fernández et al. [31]. Panelists were asked to evaluate on a 5-point hedonic scale ranging from very poor (1) to very good (5) where: 1 – very poor, 2 – poor, 3 – normal, 4 – good and 5 – very good.

2.4. Statistical Analysis

The SAS program was used in the statistical evaluation of the data. Here, both the differences between the groups and the statistical significance levels of the changes according to time (Fisher's least significant difference test) (0.05) were examined [32]. All results were expressed as mean \pm SD in each group.

3. Results

The pH of the rainbow trout at the beginning of the storage (1. day) was 6.0. pH values did not show significant changes during the storage period ($p>0.05$). No difference ($p>0.05$) was observed throughout the storage period between *J. fructus* oil-treated groups (Figure 1).

In the current study, the initial TVBN values in rainbow trout fillet were determined as 11.82 mg/100 g flesh and increased with time of storage in all groups (Figure 2). These values reached above 25 mg/100 g in non-vacuum

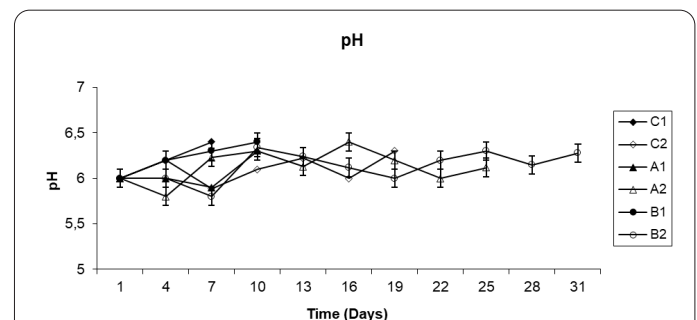


Fig. 1. Changes in pH values of rainbow trout fillets during storage at $4\pm 1^\circ\text{C}$. C1: Control 1= 0% (Without oil, Air packaged (non-vacuum packaging)), C2: Control 2= 0% (Without oil, Vacuum packaged), A1: 0.3% Oil, Air packaged (non-vacuum packaging), A2: 0.3% Oil, Vacuum packaged, B1: 0.6% Oil, Air packaged (non-vacuum packaging), B2: 0.6% Oil, Vacuum packaged.

packaging groups (C1, A1) except the group B1 on day 7. Other groups (C2, A2 and B2) exceeded the late consumption limit according to the amount of oil used. It was determined that the packaging type and *J. fructus* oil usage had a significant effect on TVB-N ($p < 0.05$).

TBA value in the different treatments during storage is shown in Figure 3. Significant differences were observed between vacuum-packaged and non-vacuum-packaged groups with respect to TBA value ($p < 0.05$). TBA value increased from the initial value (1.34 mg/kg) to the storage in the end of 5.67, 5.18, 5.63, 3.87, 3.74 and 3.74 for C1, A1, B1, C2, A2 and B2 respectively.

The counts of all determined microbiological indicators were significantly ($p < 0.05$) affected by the application of the two packings and *J. fructus* oil. The number of total aerobic mesophilic bacteria count was higher in control groups (C1 and C2) than that of *J. fructus* oil and vacuum packaged groups ($p < 0.05$) (Figure 4). The highest total aerobic mesophilic bacteria count (7.84 log cfu/g) was determined on day 7 during the storage time of the non-vacuum without oil group (C1).

In the study, yeast mold was determined as 1.32 log cfu/g fish fillet. The amount showed a statistically significant increase during storage in all groups (Figure 5). At the end of storage, yeast-mold counts for C1, A1, B1, C2, A2 and B2 reached 5.23, 4.85, 4.72, 5.73, 5.18 and 4.12 log cfu/g, respectively.

The sensory quality of raw (without cooking) *O. mykiss* flesh was evaluated on the 1., 4., 7., 10., 13., 16., 19., 22.,

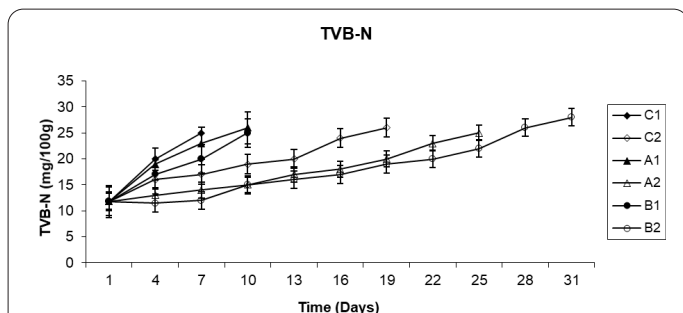


Fig. 2. Changes in TVB-N values of rainbow trout fillets during storage at 4 ± 1 °C. C1: Control 1= 0% (Without oil, Air packaged (non-vacuum packaing)), C2: Control 2= 0% (Without oil, Vacuum packaged), A1: 0.3% Oil, Air packaged (non-vacuum packaing), A2: 0.3% Oil, Vacuum packaged, B1: 0.6% Oil, Air packaged (non-vacuum packaing), B2: 0.6% Oil, Vacuum packaged.

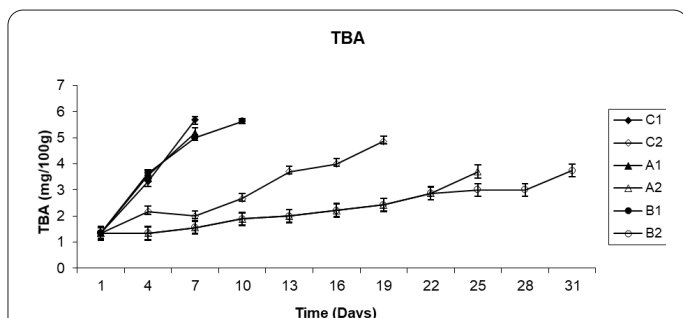


Fig. 3. Changes in TBA values of rainbow trout fillets during storage at 4 ± 1 °C. C1: Control 1= 0% (Without oil, Air packaged (non-vacuum packaing)), C2: Control 2= 0% (Without oil, Vacuum packaged), A1: 0.3% Oil, Air packaged (non-vacuum packaing), A2: 0.3% Oil, Vacuum packaged, B1: 0.6% Oil, Air packaged (non-vacuum packaing), B2: 0.6% Oil, Vacuum packaged.

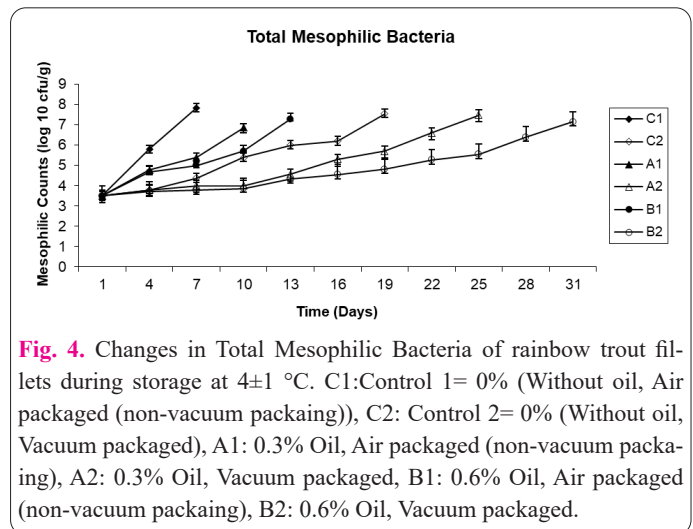


Fig. 4. Changes in Total Mesophilic Bacteria of rainbow trout fillets during storage at 4 ± 1 °C. C1: Control 1= 0% (Without oil, Air packaged (non-vacuum packaing)), C2: Control 2= 0% (Without oil, Vacuum packaged), A1: 0.3% Oil, Air packaged (non-vacuum packaing), A2: 0.3% Oil, Vacuum packaged, B1: 0.6% Oil, Air packaged (non-vacuum packaing), B2: 0.6% Oil, Vacuum packaged.

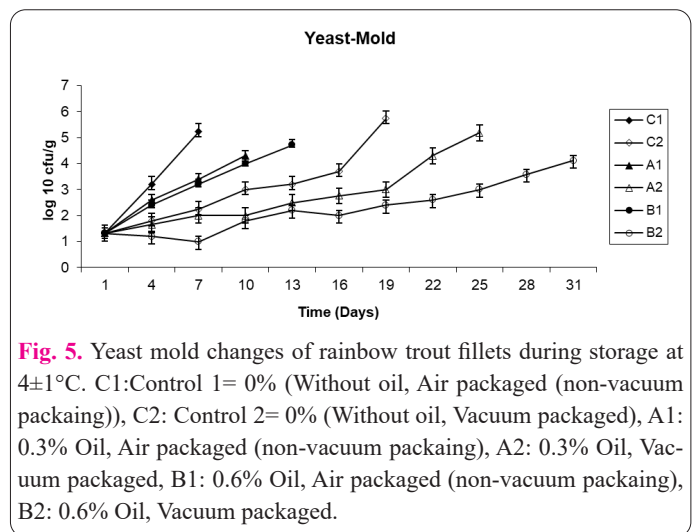


Fig. 5. Yeast mold changes of rainbow trout fillets during storage at 4 ± 1 °C. C1: Control 1= 0% (Without oil, Air packaged (non-vacuum packaing)), C2: Control 2= 0% (Without oil, Vacuum packaged), A1: 0.3% Oil, Air packaged (non-vacuum packaing), A2: 0.3% Oil, Vacuum packaged, B1: 0.6% Oil, Air packaged (non-vacuum packaing), B2: 0.6% Oil, Vacuum packaged.

25., 28. and 31. days of storage. According to the overall acceptability (average of color, odor, appearance, mucus and structure (texture)) data the observed shelf life of samples was C1=4 days, A1=7 days, B1=10 days, C2=16 days, A2= 22 days and B2=28 days (Figure 6). As the storage time increased, sensory evaluation scores decreased ($p < 0.05$).

4. Discussion

Different packaging methods and essential oil applications did not have a significant effect on pH values. In similar study [4, 5, 33] did not find significant differences between packing atmospheres (air, vacuum). The pH value changes depending on the basic nitrogenous compounds, the accumulation of alkaline compounds, autolytic activity of indigenous enzymes and bacterial growth. Fish size, water rate, seasonal, gender and stress factors are effective on pH level [34, 35]. Arashisar et al. [33] and Silva and White [36], reported that essential oil applications in foods had no statistical effect on pH. All these relevant literatures support the results in this study.

TVB-N value is an important quality criterion in fish. The TVB-N value increases as the fish spoilage. While 30–35 mg N/100 g TVB-N indicates spoilage, this value is reported as 25 mg N/100 g of fish muscle in freshwater fish. TVB-N value; It varies depending on species, season, gender, age, and stress factors [35, 37, 38]. In the study, the TVB-N value increased in all groups during storage. However, packaging method and essential oil application

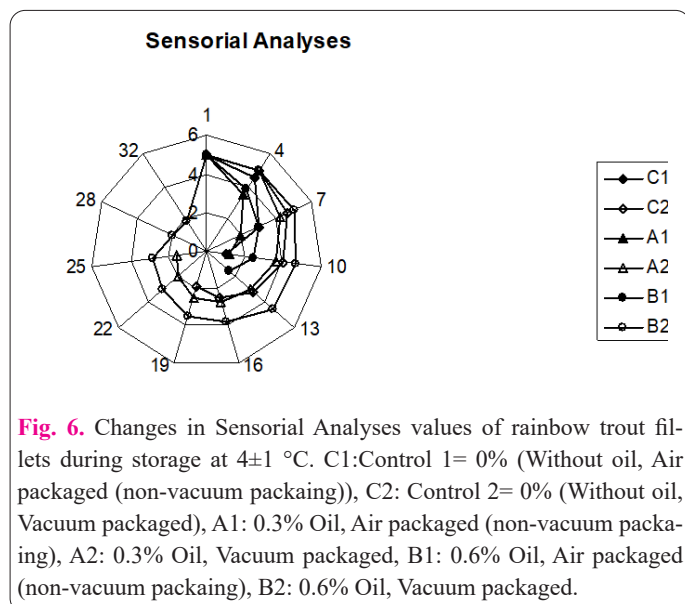


Fig. 6. Changes in Sensorial Analyses values of rainbow trout fillets during storage at 4±1 °C. C1:Control 1= 0% (Without oil, Air packaged (non-vacuum packaing)), C2: Control 2= 0% (Without oil, Vacuum packaged), A1: 0.3% Oil, Air packaged (non-vacuum packaing), A2: 0.3% Oil, Vacuum packaged, B1: 0.6% Oil, Air packaged (non-vacuum packaing), B2: 0.6% Oil, Vacuum packaged.

affected this increase (Figure 2). Many studies show similarity with our results [4, 39, 40]. It has been reported that the addition of thyme and thyme oil to sea bass does not exceed the limit of 30 mg N/100 g during storage at 0-2 °C [39]. It was reported that after treatment of carp fillets with 0.5% carvacrol and thymol (v/v), the TVB-N value was 30 mg N/100 g in storage at 5 °C [40].

TBA value is a parameter that can measure lipid oxidation in fish and which is directly related to oxygen. Since the oxygen permeability of vacuum packaging is very low, the TBA value does not change much when the remaining oxygen in the environment is depleted. TBA values did not exceed the acceptable limit throughout the storage period. Especially in vacuum-packed groups, the increase in TBA value is lower since there is no oxygen. These findings are in agreement with the results reported for fish [22, 33, 43-46]. Cadun et al. [46], reported that the application of rosemary and thyme oil at a dose of 300 ml/L to crayfish was effective on TBA value. Fasseas et al. [47], reported that the TBA value was lower in meat treated with thyme and sage essential oils. Auburg [48] reported that the TBA values may not give actual rates of lipid oxidation since malondialdehyde can interact with other components of fish, such as nucleosides, nucleic acid, proteins, amino acids of phospholipids, and other aldehydes, which are the end products of lipid oxidation. For this reason; TBA does not imply a concrete limit of acceptability since its content can vary quite a lot depending on the kind of seafood substrate. It is better to consider it is a relative index, not an absolute.

The acceptable upper limit for seafood foods is 7 log cfu/g or 10⁶ microorganisms/g. If this value is accepted as the acceptable limit, the shelf life is approximately 32, 25, 19, 13, 10, and 7 days, respectively, of groups B2, A2, C2, B1, A1 and C1. Vacuum packaging and application of *J. fructus* oil extended shelf life. Mahmoud et al. [42] found that dipping carp fillets in carvacrol/thymol solution (1%) both reduced the initial total aerobic mesophilic bacteria and extended the shelf life from 4 days to at least 12 days at 5°C. Giatrakou et al. [49] reported that the oregano oil 0.1% v/wt extended the shelf life of swordfish fillets by 8 days, as determined by sensory and microbiological analysis. It has been emphasized in many studies that vacuum packaging and essential oil application extend the storage

period [50-52]. The antimicrobial activity of *J. fructus* was reported by Ukita & Matsuda [53].

Yeasts and molds are beneficial microorganisms that can be used in the food industry, but they can cause spoilage. They can occur as a result of cross-contamination in food production or inadequate hygiene practices. Packaging methods, pH reduction, water activity limitation, control of oxygen tension, thermal processing, and antifungal agents (antifungal preservatives) are used to prevent potential yeast and mold spoilage [52, 54]. The antifungal activity of essential oil has been reported by many authors [52, 54-57]. According to the results, the combined use of vacuum packaging and *J. fructus* oil had an inhibitory effect on the growth of yeast mold.

The purpose of sensory evaluation is to develop the product, increase sales, maintain quality, analyze marketing and determination of storage quality. The result determined by chemical and microbiological analyses should be supported by sensory. A food that is chemically and microbiologically appropriate must be also sensory appropriate [58, 59]. Sensory evaluation scores decreased as storage time increased. With vacuum packaging and *J. fructus* oil application, the decrease in sensory quality was later (Figure 6). It has been emphasized in many studies that packaging techniques and essential oil applications have a positive effect on sensory evaluations [23, 50, 52, 55, 60], but no literature has been found for the application of *J. fructus* oil.

5. Conclusion

In conclusion, based primarily on sensory, TVB-N and mesophilic bacteria data the shelf-life of fresh rainbow trout was 4 days (non-vacuum packaged), 13 days (vacuum packaged), 19 and 28 days treated with *J. fructus* oil (0.3 and 0.6%, v/w) under vacuum packaged, respectively. It has been determined that vacuum packaging and *J. fructus* oil application have positive effects on the microbiological, chemical and sensory quality of fresh fish fillets and prolong the storage period.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Emine Özpolat: Research design and supervision, perform all laboratory procedures.

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