Description of the Protective Feature of Oregano Essential Oil Sold as a Commercial Site on Rainbow Trout

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Abstract

The essential oil extracted from plants has a shelf-life effect. This study was carried out to determine the difference of natural oregano essential oil, which is considered to be a potential food additive, from commercially available, and its effects on rainbow trout containment by using low amounts of this essential oil. The changes in rainbow trout samples were analyzed by applying commercially available oregano essential oil at a low rate (RT1: 1% and RT2: 2%), by looking at the following parameters. When the microbial load, sensory properties, pH, TVB-N, TBA numbers are examined in terms of thyme essential oil application and day relationship, the limit to be consumed in samples with 1% oregano essential oil is (RT1), on average, between the 3rd and the 6th days. It is between the 3rd and 9th days in the samples applied with 2% oregano essential oil (RT2). According to the microbial load results, the rainbow trout sample (RT: control) exceeded the consumable limit on day 6th and showed the highest value (p <0.05). In addition, it has been proven with the TVB-N and TBA values that the samples applied with essential oil are of good quality. In a sense, the shelf life is stabilized thanks to the oregano essential oil. As soon as the oregano essential dosage was too much, the protective effect increased.

Key Words: Commercial oregano essential oil, food preservation, preservative effect, quality, Rainbow trout

INTRODUCTION

Food rotting involves alterations in a food product that make it inconsumable from a sensory qualities. Spoilage can be caused by chemical differences (enzymatic, oxidation), physical differences or microbial growing and metabolism in the foodstuff. The main cause of spoilage of raw and minimally processed meat and fish is microbial growth, which...
can manifest itself in the event of textural changes, visible growth (slime), and the appearance of unpleasant odors and unwanted flavors. Because of the short shelf life of fresh fish, the increase in one-day shelf times significantly reduces food losses (Haute et al., 2017). New technologies have emerged to protect fish quality in terms of developing the economy and turning consumers towards preserved foods (Mexis et al., 2009).

Today, the demand for herbal medicinal products is increasing due to their healing therapeutic potential, safe toxicological profile and improved microbial safety (Fraj et al., 2019). The oils, spices and extracts from herbs have been used for centuries to improve the sensory properties and to prolong shelf life of foods. (Botsoglou, 2003; Karabagias et al., 2011; Barreto et al., 2016; Boskovic et al., 2020).

Natural antimicrobial compounds are applied to extend the shelf life of foods. It has been proven by studies that essential oils containing bioactive substances are used as food additives, natural antimicrobial agents and coatings that protect the nutritional values of foods. Derived from Origanum vulgare L., thyme essential oil is recognized for its oxidation-inhibiting and antimicrobial properties (Hosseini et al., 2016).

In recent years, scientific studies in food science and technology have focused on the antimicrobial properties of essential oils in relation to their use in these food bio-preservation systems. Food bio-preservation is a frequently accepted system, referred to as a natural practice that can increase shelf life and ensure food microbial safety. (Carmo et al., 2008). In this current topic, O. vulgare essential oil is cited as an interesting source of antimicrobial compounds for use in food preservation (Carmo et al., 2008).

Plant essential oils extracted from thyme (O. vulgare) have antimicrobial and antioxidant activities thanks to the phenolic compounds it contains. The studies have demonstrated the effectiveness of films combined with thyme essential oil in inhibiting the microbial growth and synthesis of microbial metabolites was as effective as yeasts and molds, including pathogenic bacteria (Cardoso et al., 2017). Carvacrol, the main thyme component, makes up more than 50% of the total fat composition. Carvacrol is recognized by the U.S. Food and Drug Administration (FDA) as a safe (GRAS) as a food additive that generally demonstrates non-hazardous and non-toxic (United States Federal Regulations Regulation number 172,515) (Fraj et al., 2019).

Oregano essential oil has been investigated for its antimicrobial and antioxidant properties in foods that are sold, such as all small, bovine animals and seafood, both treated and unprocessed. The positive results obtained support the possibility of applying Origanum vulgare essential oil as an alternative antimicrobial agent in food preservation systems.

There are many studies in the literature on the antibacterial and antioxidant properties of thyme essential oil and its subsequent effect on the shelf life of foods. However, there is very little data on the effect of thyme essential oil on the shelf life of fish and fish products (Goulas and Kontominas, 2007). While some scientists have observed that O. vulgare L. extends shelf life (Goulas and Kontominas, 2007; Mexis et al., 2009; Klein et al., 2013; Hosseini et al., 2016), there is a lack of information on both the effects of commercially available oils and their effects on shelf life by keeping their concentrations to the lowest levels (Hosseini et al., 2016). This study was done to detect this work was done to detect the effects of both the use of oregano essential oil available in the market and its low amount on fish quality.

MATERIAL AND METHODS

Raw Material

Fresh fish (n=36) of the species Oncorhynchus mykiss with a weight of approximately 2.5-3 kg and their fillets from 1.3-2 kg and 30-35 cm tall. were obtained from local fish markets in Elazig. Fishes was brougt to the Firat University Biology laboratory in styrofoam boxes in ice and made into fillets the same day. Oreganum (100% oil) was purchased from the Kalsec Company (33-03, 864286). The study was conducted in three replicates.

Oncorhynchus mykiss Fillets Preparation and Packaging

The fillets were made into fillets without skin removing and they washed with plenty of water. The trout fillets were divided in to three groups in total; the control sample without oreganium oil, Oreganium oils at ratio of 1 % and 2 % oil (v/w = volume/weight of fish flesh) were used to the trout fillets. Oreganium essential oils was added on both sides of each trout fillet using a micropipette. Then it was massaged with a sterile glove and was immediately started the packaging process. Prepared fish fillets was packaged...
under aerobic conditions. For packing, Ziplock bags made from LDPE plastic material was used and each fillet bag was classified as 50-60 g. They were stored at about 4±1°C until necessary assessments were performed to determine chemical, microbiological and sensorial during shelf life on days 0th, 3th, 6th, 9th, and 12th.

**Microbiological Assessment**

On each sampling day, each fish fillets (10 g each) were taken from each experimental group and transferred into the sterile stomacher bags. Moreover, serial dilutions of homogenized fillets samples were made and then 0.1 mL of each dilution was added with pipette to the surface of Plate Count Agar (LAB149, 125801/093) plates for evaluation of aerobic bacteria (AB) incubated at 30 ± 1 °C for 3 days and psychrophile bacteria (PB) at 5 ± 1 °C for 7 days. For lactic acid bacteria, 0.1 ml of each dilution was added with pipette on to the surface of de Man Rogosa Shape agar (LABM093, 123839/254) and the plaques with approximately 2-3 mm MRS medium. After the samples were cultured to to Potato Dextrose Agar medium (PDA – LABM098, 123328/229) in plaques, incubated at 25 ± 1 °C for 4-5 days, the Yeast and Mould colonies were counted (Harrigan, 1998).

**Chemical Assessment**

The pH values of all samples were measured using a pH meter (Thermo Scientific Orion 3-Star, UK) (AOAC, 2002). TVB-N (Total volatile basic nitrogen- mg 100g⁻¹) analysis was performed according to the method reported by Connell and Shewan, (1979). TBA analysis (Thiobarbituric acid-mg MA kg⁻¹) of the experimental fillets was determined with the spectro-photometric method by Tarladgis et al., (1960).

**Organoleptic Assessment**

The Organoleptic assessment of fish fillets was done by a five-member trained panel from the university. To conduct organoleptic assessment, the samples were baked in a baking at 10 minutes at 180 °C. Evaluation has been made in terms of general acceptability. Panellists evaluated general acceptability of the samples on a hedonic scale (9-point) ranging from distaste extremely (1) to like extremely (9) (Lawless and Heymann, 2010).

**Statistical Assessment**

The statistical datas of in this study determined with the IBM SPSS 22.0 soft ware package program. The statistical comparisons (p<0.05) were performed among all groups storage days with variance analysis (One-way Anova) (Duncan,1955) multiple range test.

**RESULTS AND DISCUSSIONS**

**Microbiological Assessment**

Microbiological analysis has shown that oregano essential oil is a potential preservative in reducing the proliferation of microorganisms (aerobic bacteria, psychrophyle bacteria, lactic acid bacteria and yeast-mould) that shorten the shelf life of rainbow trout fillets.

If we make a comparison based on ICMSF, (1986), if 7 days CFU / g is an acceptable value for freshwater fish, we can say that samples prepared by treating oregano essential oil prolong the shelf life in terms of microbiology. Accordingly, rainbow trout fillets treated with 1% oregano essential oil have extended shelf life until the 9th days, and the 2% until the 12th days. When the microorganism load of rainbow trout fillets is examined in Tables 1 and 2, it is seen that the essential oil has a positive effect on fish shelf life.

According to this finding; the load of aerobic bacteria in the working groups was determined to be in the level of 3.58 ± 0.47 log cfu g⁻¹ to 7.28 ± 0.47 log cfu g⁻¹. The large amount of microorganisms shown on control samples (RT) from day 6th of storage, resulted into significant differences (p<0.05) in the total aerobic bacteria load when compared with the other part samples treated with oregano essential oil. As shown in Table 1, the load of aerobic bacteria at RT2 on the first day was lower than other rainbow trout samples during the storage period (Initial bacterial load RT; 3.58±0.47 log cfu g⁻¹ and RT1: 3.72±0.17 log cfu g⁻¹, RT2: 2.75±0.69 log cfu g⁻¹, respectively). Unacceptable aerobic bacteria load was detected on 6th in the control group (RT), and on day 12th in the other groups (RT1 and RT2). The control group had the highest load of aerobic bacteria on day 6th (RT: 7.28± 0.47 log cfu g⁻¹) while the number of bacteria in the other groups reached to the highest value 9th day, 12th day (RT1: 6.20±0.60 log cfu g⁻¹, RT2:6.14±0.76 log cfug⁻¹). The results show that as the concentration of oregano essential oil used increases (RT1: 1%.RT2: 2), that is, it decreases the
microorganism load. The using (0.8% b/v/w) oregano essential oil slowed the spoilage rate on Goulas and Kontominas (2007) modified atmosphere packaged and salted sea bream samples. Teixeira et al., (2014) was studied that is of antibacterial effect against microorganisms of garlic essential oil with fish protein films.

We believe that the 4.21±0.61 log cfu g⁻¹ and 3.64±0.09 log cfu g⁻¹ reduction in psychrophilic load on 0th is the result of essential oil treatment of the trout fillet. The control group had the highest number of psychrotrophic bacteria on day 6th (RT: 6.34±0.43 log cfu g⁻¹) while the number of bacteria in the other groups reached to the highest value in 9th day, 12th days (RT2: 6.80±0.19 log cfu g⁻¹, RT2: 6.84±0.00 log cfu g⁻¹) at the end of the storage period respectively. According to the statistical analysis, significant differences (p<0.05) were found in the control group and the other groups treatment with orreganum in different concentration for psychophilic bacteria populations throughout storage. In a study by Cardoso et al., (2017) on the protective effect of active films based on poly (butylene adipate-co-tereephthalate) treated with oregano essential oil (0.0, 2.5, 5.0, 7.5 and 10.0 g) on fish fillets, it was found that the development of total coliform, Staphylococcus aureus and psychrotrophic microorganisms decreased and showed an antimicrobial effect except for these film treated with 2.5 g oregano essential oil. In addition, it has been stated that fish fillets extend the shelf life up to 10 days under cooling. Considering the low concentration (RT1:1%, RT2: 2%) values we used, the data we found are compatible with the study conducted by Coradaso, (2017). In our study, although the concentrations of commercial essential oil were tested as low, the shelf life of the trout samples was stable. Teixeira et al., (2014) was studied that is of antibacterial effect against microorganisms of garlic essential oil with fish protein films.

### Table 1. Changes in microbial communities (log cfu g⁻¹) during storage of rainbow fillets (RT) and under different concentration treated with oregano essential oils (RT1, RT2)

| Storage days | The Aerobic Bacteria (log cfu g⁻¹) | The Psychrophylic Bacteria (log cfu g⁻¹) |
|--------------|----------------------------------|----------------------------------------|
|              | RT  | RT1 | RT2 | RT  | RT1 | RT2 |
| 0            | 3.58±0.47x | 3.72±0.17x | 2.75±0.69x | 4.21±0.61x | 3.78±0.08x | 3.64±0.09x |
| 3            | 5.07±0.47y | 5.14±0.53y | 3.76±0.16y | 4.85±0.01y | 4.17±0.57y | 3.77±0.12y |
| 6            | 7.28±0.47yz | 5.21±0.45yz | 5.03±0.67yz | 6.34±0.43yz | 4.80±0.10yz | 4.88±0.06yz |
| 9            | NA   | 6.20±0.60y | 6.07±0.52y | NA   | 6.22±0.52y | 5.38±0.08y |
| 12           | NA   | 7.33±0.06x | 6.14±0.76x | NA   | 6.80±0.19x | 6.84±0.00x |
| 15           | NA   | NA   | NA   | NA   | NA   | NA   |

RT: The trout samples present not oregano essential oil; RT1: 1% oregano essential oil was added to the trout samples (volume/weight of fish flesh=v/w), RT2: 2% oregano essential oil was added to trout samples and oil. The vertical change shows differences between groups. The horizontal change shows differences between days. Significance was defined at p<0.05. No significant difference was defined at p>0.05. NA: None acceptability.

### Table 2. Changes in microbial communities (log cfu g⁻¹) during storage of carp fillets (RT) and under different concentration treated with oregano essential oils (RT1, RT2)

| Storage days | Lactic Acid Bacteria (log cfu g⁻¹) | Yeast and Mould (log cfu g⁻¹) |
|--------------|----------------------------------|-------------------------------|
|              | RT  | RT1 | RT2 | RT  | RT1 | RT2 |
| 0            | 3.38±0.08x | 3.96±0.48y | 3.03±0.79x | 3.73±0.26x | 3.12±0.08x | 3.21±0.26x |
| 3            | 3.71±0.23x | 4.01±0.61x | 3.85±0.66x | 4.99±0.45x | 4.55±0.07x | 4.59±0.04x |
| 6            | 5.38±0.69x | 5.07±0.08y | 4.70±0.20y | 7.03±0.43yz | 4.61±0.06yz | 5.21±0.52yz |
| 9            | NA   | 4.62±0.07x | 5.81±0.09x | NA   | 6.07±0.12x | 5.57±0.02x |
| 12           | NA   | 5.89±0.01x | 6.47±0.66x | NA   | 6.67±0.01x | 6.03±1.40x |
| 15           | NA   | NA   | NA   | NA   | NA   | NA   |

RT: The trout samples present not oregano essential oil; RT1: 1% oregano essential oil was added to the trout samples (volume/weight of fish flesh=v/w), RT2: 2% oregano essential oil was added to trout samples and oil. The vertical change shows differences between groups. The horizontal change shows differences between days. Significance was defined at p<0.05. No significant difference was defined at p>0.05. NA: None acceptability.
The lactic acid bacteria load is shown in Table 2. The lactic acid bacteria load was determined as 3.38±0.08 log cfu g⁻¹ - 5.38±0.69 log cfu g⁻¹ in all groups during storage. There is significant difference between both groups: RT and RT1, RT2 (on 6th) (p>0.05). RT1 and RT2 has extended the shelf life of rainbow trout up to 12 th (4.62±0.07 log cfu g⁻¹) and 12 th days (6.47±0.66 log cfu g⁻¹).

The amount of yeast-mould of all the others samples has been detected during storage period (Table 2.) The yeast-mould population in RT groups was determined as 7.03±0.43 log cfu g⁻¹ in 6th day but, RT1; 6.47±0.01 log cfu g⁻¹ in 12th day, RT2; 3.38±0.08 log cfu g⁻¹. This results showed that oregano essential oils have a preserving effect. The significant differences (p<0.05) were found in the control group and the other groups treatment with oregano essential oil in different concentration for yeast-mold count throughout storage. Significant difference of yeast-mold count of samples was determined on some of the storage days (p<0.05). The pH value of samples have been no found difference statistical among groups (p>0.05). Researchers set pH 6.8 as the consumable limit and consider fish with pH values 7 and above as stale, while some of them report that fish with a pH value above 7th should be evaluated together with other characteristics, although it is common to be considered suspicious (Demirci and Oraklı, 1999).

When looking at the pH value of the RT and the pH value of samples with treated the other oregano essential oil (RT1, RT2); the degradation rate of the fish samples slowed down on RT1; 3rd and RT2; 6th days thanks to the oregano essential oil (Figure 1). The low pH value slows down the degradation rate in samples with essential oil, it has stabilized the shelf life in a sense. In one study, the effect of O₂ absorbent and oregano essential oil (0.4% v/w) on the pH value of rainbow trout fillets (O. mykiss) with the emergence of protein decomposition products, the pH decreased from the initial value of 6.65-6.09 and then increased (Mexis et al., 2009). pH value It is a defining parameter to prevent multiplying of unwanted microorganisms (Catarinoa et al., 2017). In our study, In the RT1 and RT2 values are 6.91-6.58 and 6.87-6.65 between 0th and 12th days and a decrease has occurred even at low levels, which indicates that the shelf life is stable. Hosseini et al., (2016), stated that a lower increase in pH was observed (C, Fish Gelatin, Fish Gelatin+Oreganium:6.45-0. day; Fish Gelatin, Fish Gelatin+Oreganium:7.56-7.57 6.day) in trout samples with gelatin coating (4%, w/v) containing oregano (Origanum vulgare L.) essential oil (1.2% w/v). We think that the reason for the slow decrease of the pH value in trout samples is due to the use of only oreganum essential oil.

**Chemical Assessment**

The control and the treated fish samples were evaluated periodically for chemical (pH; total volatile basic nitrogen-TVB-N; thiobarbituric acid-TBA) characteristics.

The pH value of control groups was decreased on 6th day (Figure 1). Significant difference of pH value of samples was determined on some of the storage days (p<0.05). The pH value of samples have been no found difference statistical among groups (p>0.05) Researchers set pH 6.8 as the consumable limit and consider fish with pH values 7 and above as stale, while some of them report that fish with a pH value above 7th should be evaluated together with other characteristics, although it is common to be considered suspicious (Demirci and Oraklı, 1999). When looking at the pH value of the RT and the pH value of samples with treated the other oregano essential oil (RT1, RT2); the degradation rate of the fish samples slowed down on RT1; 3rd and RT2; 6th days thanks to the oregano essential oil (Figure 1). The low pH value slows down the degradation rate in samples with essential oil, it has stabilized the shelf life in a sense. In one study, the effect of O₂ absorbent and oregano essential oil (0.4% v/w) on the pH value of rainbow trout fillets (O. mykiss) with the emergence of protein decomposition products, the pH decreased from the initial value of 6.65-6.09 and then increased (Mexis et al., 2009). pH value It is a defining parameter to prevent multiplying of unwanted microorganisms (Catarinoa et al., 2017). In our study, In the RT1 and RT2 values are 6.91-6.58 and 6.87-6.65 between 0th and 12th days and a decrease has occurred even at low levels, which indicates that the shelf life is stable. Hosseini et al., (2016), stated that a lower increase in pH was observed (C, Fish Gelatin, Fish Gelatin+Oreganium:6.45-0. day; Fish Gelatin, Fish Gelatin+Oreganium:7.56-7.57 6.day) in trout samples with gelatin coating (4%, w/v) containing oregano (Origanum vulgare L.) essential oil (1.2% w/v). We think that the reason for the slow decrease of the pH value in trout samples is due to the use of only oreganum essential oil.
In the study of Boskovic et al. (2020), they stated that the bioactivity of essential oils varies at different pH values and can enter the cells more easily at low pH. This means that the essential oil maintains its protective effect at low pH.

The value of TBAs should not be less than 3 in a very high quality sample, and not more than 5 in a good sample. The consumable limit value is between 7 and 8 (Demirci and Orak, 1999). The TBA value reached its highest level after the 6th day in the control, and 4.16 ± 0.50 mg MA kg⁻¹ on the 9th day and after. From the 6th day at RT1 to 3.23 ± 0.56 mg MA kg⁻¹, from the 9th day at RT2, 3.33 ± 1.33 mg MA kg⁻¹ were at very good levels, and the fish samples that were left in the essential oil environment on the 12th day began to become stale (Figure 2).

Hosseini et al. (2016) stated that TBA values remained low between the 0th day and the 16th day (0.01-0.008 mg MA kg⁻¹ of flesh, in rainbow trout) and found that there was fluctuation during storage. In terms of TBA amounts, no significant differences were determined among the groups (p<0.05). Moreover, significant difference was determined between groups (RT and the other groups) on some of the measurement days (1st, 3rd, and 6th days) (p<0.05). Karaton Kuzgun and Gürel İnanlı, (2018), determined that combining thyme oil with chitosan and applying films of shelf life of luciobarbus esocinus fillets extended the by 33 days. And also they appears that TBA changes are similar to our study.

When looking at the relationship of oregano essential oil environment and day in TVB-N values (Figure 3), the highest value was observed in the control sample on the 6th day, 29.66± 0.99 mg 100g⁻¹, and on the 9th and 12th days at RT1 and RT2. Compared to the control with RT1 and RT2 groups, the reduction continued on day 3rd to 6th in medium containing both oregano essential oils.

Fish gelatin coating (4%, w/v) containing thyme (O. vulgare L.) essential oil (1.2% w/v) by Hosseini et al., (2016) under refrigerated storage (4 ± 1°C) of rainbow trout (O. mykiss) examined; The TVB-N values we obtained were found to be higher. In the TVB-N values; A significant increase was observed for control samples after day 8th and for the other samples after day 16th. The significant differences in the TVB-N amounts of the samples was seen for day 6th the control groups with the other groups (p < 0.05). Moreover, an important difference was identified between the days checked (0th, 3rd, and 6th-all groups; 9th, and 12th days-RT1,RT2) (p < 0.05). In terms of the data obtained from fish kept in RT1 and RT2, It was determined that RT1 is fresh and good between 0th and 9th days at RT2 on 0th and 6th days, and RT1 not exceeds consumable limits from 9th and 12th days on RT2. When our results are compared with the TVB-N values of Mexis et al., (2009), the highest TVB-N values of the samples with 0.4% w/v thyme essential oil and oxygen absorber added were between 10 th and 15 th days. Our TVB-N values with only essential oil added are the highest in RT1 and RT2 from 9th and 12th days. This has shown that oregano oil can be used without using oxygen absorbers, and it shows that using only oregano essential oil will positively affect the shelf life. According to the results of all these parameters, the consumption limit is between 3 and 6 days at RT1 and 3 to 9 days at RT2.
Organoleptic Assessment

The organoleptic assessment of trout fillets under stored oregano essential oils is given in Figure 4. The scores obtained in the organoleptic assessment showed that the oregano essential oil treated samples (RT1, RT2) were generally acceptability as good by the panelists, whereas the samples without only essential oil were less acceptable in terms of their sensory properties. Accordingly, the demand rate of RT2 is up to the 9th and 12th days and RT1 is up to the 9th day. RT1 (8.60±0.49, 0th on day) was demanded more due to the increase in the essential oil concentration. As a result, oregano oil has a positive effect on being preferred organoleptically. The significant differences in the generally acceptability points of the samples was observed for on day 3th on day 6th the control groups with the other groups (p<0.05). Moreover, an important difference was observed between control days (p<0.05). Thyme has also positively increased the sensory feature in trout samples due to its high aroma feature. A similar situation was observed in other studies due to this feature (Haute et al., 2017; Catarinoa, 2017).

CONCLUSION

In recent years, commercial essential oils have been of interest to reduce the growth of food spoilage microorganisms and natural foods that do not have toxic effects. Essential oils used to preserve the quality of fish and its products, which are a perishable food, stand out as they are used as a natural food preservative and do not carry a very high cost economically. As soon as the oregano essential oil dosage was too much, the positive effect on rainbow trout was further increased. In a sense, the shelf life is stabilized thanks to the oregano essential oil. Based on oregano essential oil application and day relationship, according to microbial load, sensory properties, pH, TVB-N, TBA counts generally in trout samples (RT1, RT2) containing 1% and 2% oregano essential oil 3rd and 6th show good quality.

CONFLICT OF INTEREST STATEMENT

The author declares that there is no conflict of interest in this study.

RESEARCH AND PUBLICATION ETHICS STATEMENT

The author declares that the research and publication ethics are complied with in the study.

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