Antimicrobial Role and Sensory Influence of Oregano Essential Oil on the Shelf-Life of Atlantic Cod Loins

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Abstract
Oregano essential oil is predominantly utilized in the application of food preservation. However, the antimicrobial activity of oregano essential oil on the Atlantic cod is still unclear. In the present study, we evaluated the antimicrobial effect of oregano essential oil on the shelf-life of Atlantic cod and sensory properties under different treatments. The study showed that the shelf life of the thawed cod loins with oregano essential oil decreased by two days compared to the untreated group. The finding of the antimicrobial activity of oregano essential oil was in agreement with previous studies, but it does not prolong the shelf life of cod loins significantly.
Key words: *Atlantic cod, oregano, essential oil, shelf-life, sensory evaluation*

Anotacija
Raudonėlių eterinis aliejus vis dažniau naudojamas maistui konservuoti. Tačiau raudonėlių eterinio aliejaus antimikrobinis aktyvumas Atlanto menkėje vis dar neaiškus. Šiame tyrime įvertintas raudonėlių eterinio aliejaus poveikis Atlanto menkių nugarinių galiojimo laikui ir juslinėms savybėms taikant skirtingus apdorojimo būdus. Tyrimas parodė, kad Atlanto menkių nugarinės su raudonėlių eteriniu aliejumi galiojimo laikas sumažėjo dviem dienomis, palyginti su kontroline grupe. Remiantis tyrimo rezultatais galima teigti, kad raudonėlių eterinis aliejus pasižymi antibakterinėmis savybėmis tik tam tikromis sąlygomis.
Reikšminiai žodžiai: *Atlanto menkė, raudonėlių eterinis aliejus, galiojimo laikas, juslinis įvertinimas.*

Introduction

In recent years, due to increasingly negative consumer perceptions of synthetic preservatives, interest in essential oils and their application in food preservation has been amplified (Chouhan, Sharma, & Guleria, 2017). The properties of essential oils are extensively studied both in livestock and poultry farming, as well as in human nutrition and the preservation of food quality. The essential oil is used in the food industry to effectively reduce the oxidation of lipids and inhibit the growth of foodborne pathogens to control and prevent foodborne diseases. Therefore, essential oil has the potential not only to increase the safety and biological value of foodstuffs but also to extend their shelf life. The application of the essential oil on the raw material must be modified according to the food structures in order to improve the effectiveness of the bioactive compounds (Hyldgaard, Mygind & Meyer, 2012). The antimicrobial activity of essential oils depends on their chemical composition and the concentration of bioactive components. Essential oils such as basil, sage, rosemary, oregano, or the maidens are effective against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella* spp. (De Martino, De Feo, & Nazzaro, 2009). The bioactive compounds of the essential oils degraded the surface protein of the bacterial cell wall. The structure of gram-positive bacterial cell walls allows hydrophobic molecules to enter the cells efficiently and act on the cell wall and cytoplasm (Nazzaro *et al.,* 2013). Phenolic compounds in essential oils show antimicrobial activity against gram-positive bacteria. At low concentrations, they can interfere with enzymes involved in the energy production of gram-positive bacteria, and at higher concentrations, they can denature the proteins (Gutierrez, Barry-Ryan & Bourke, 2008).

Fish are highly susceptible to spoilage, which can be caused by chemical reactions and microbial growth (Gram & Dalgaard, 2002). The lipid oxidation and microbial growth in fish during storage can be controlled by synthetic or natural preservatives; however, consumers are often

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concerned about the use of artificial preservatives in food. Spices and their essential oils are the most efficient natural antioxidants and antimicrobial agents and have been used to preserve food for centuries (Mahmoud *et al*., 2006).

Various previous studies showed the influence of different essential oils on the shelf life of fish products. Researchers from Algeria and Turkey (Bensid *et al*., 2014) conducted a study in which they attempted to investigate the effect of ice-containing thyme (0.04% w/v), oregano (0.03% w/v), and clove (0.02% w/v) extracts on the quality parameters of anchovy. The researchers concluded that the test groups with herbal extracts reduced microbial proliferation compared to the control group. The sensory evaluation also showed that anchovies with herbal extracts had a shelf life of 12 days and control groups of 9 days. Scientists from Greece (Goulas & Kontomin, 2007) studied the combined effect of modified atmosphere packaging (MAP: 40% CO_2/30% O_2/30% N_2) and oregano essential oil on the shelf-life of lightly salted cultured sea bream (*Sparus aurata*) fillets stored under refrigeration. They found that the addition of oregano oil in MAP salted samples produced a distinct but pleasant flavor and contributed to a considerably slower process of fish spoilage, given that the fillets treated with 0.8% (v/w) oregano oil were still sensory acceptable after 33 days of storage. The preservative effect was enhanced with the increase of the oregano oil concentration. The effect of salt, oregano essential oil and packaging on fresh rainbow trout fillets was evaluated during storage at 4°C (Frangos *et al*., 2010). The addition of salt extended the product's shelf-life by nine days, whereas the combination of salt and oregano (0.2% v/w resulted in a significant shelf-life extension of trout fillets (11-12 days) according to sensory data, as compared to the control sample under aerobic conditions.

The main components of oregano essential oil are carvacrol, β-fenchyl alcohol, thymol, and γ-terpinene. Hot water extract has the most potent antioxidant properties and the highest phenolic content. Carvacrol is a significant compound in oregano with a highly antibacterial ability. Thymol also inhibits gram-positive and gram-negative bacteria and has antioxidant properties (Teixeira *et al.*, 2013). Although the mechanism of oregano essential oil components has been studied in many pilot works in the past, detailed knowledge of most of the bioactive compounds and their mechanism is still lacking. However, recent studies have shown that essential oils are strong candidates to replace synthetic chemicals used by the food industry to improve quality and extend shelf life. The aim of this study is to study the effect of oregano essential oil on the sensory attributes and the shelf-life of thawed Atlantic cod (*Gadus Morhua*) loins stored under refrigerated storage conditions.

Materials and methods

Fish receiving and preparation. The research was carried out at the request of a fish processing company in order to extend the shelf life of fish products. Freshly thawed Atlantic cod (*Gadus Morhua*) loins (average individual weight of approximately 220g) were received from the company, transported to the laboratory in packages on ice, and used within 2 hours.

Oregano (Origanum vulgare) essential oil treatment.

The whole batch (84 pieces of loins) was divided into three groups. The first group was the control group, and the two others were experimental groups, which were treated with different concentrations of oregano essential oil. The essential oil used for the research was intended for food production and obtained from the company Young Living. This application method of oregano essential oil was mentioned in the previous studies (Goulas & Kontomin, 2007 and Atrea *et al.* 2009). Oregano essential oil was prepared by mixing it with canola oil to obtain the required concentrations of 0.5% and 5%. 1 ml of application was applied to the cod fillet on both sides and rubbed by hand. After preparation, the samples were placed in plastic bags and tightly closed to prevent outside air from entering and labeling. The bags with cod loins of all research groups were kept in a refrigerator at 4°C.

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Microbiological Analysis. The enumeration of microorganisms was performed for each group during the 8-day storage period. The total plate counts were determined from the prepared groups by serial decimal dilution and culturing the microorganisms in plate count agar at 30°C for 72 hours according to ISO 6887-3:2017.

Sensory evaluation. Sensory evaluation of cod loins was carried out by the six-member panel. The odor and appearance of uncooked fillets were evaluated every two days during storage at 4°C. Treated samples were evaluated against freshly thawed loins and designated a score of 5. The sample preference, which was less than the freshly thawed loins, was scored 1 to 4, where 1 = most disliked. The sample preference, which was more than the freshly thawed loins, was scored 6 to 9, where 9 = most liked. Untreated loins were also compared with untreated fresh loins and treated loins.

Statistical Analysis. Two cod loins per treatment per sampling day were analyzed. Statistical analysis of results was performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) statistical software. Analysis of variance (ANOVA) with one-way and two-way methods was performed for the comparison of sensory evaluation parameters and oregano essential oil concentration among the different experimental groups. Duncan's multiple comparison test was used. A significant difference was accepted at the values of $p<0.05$.

Results and discussion

Sensory characteristics of cod loins. In the present study, the sensory evaluation of cod loins was analyzed by following microbiological analysis. Treated loins had a distinct but acceptable pleasant odor, and sensory scores indicate that in the first two days of testing, they were "preferred" to "typical" in appearance and odor compared to the control loins (Table 1). These treated loins were considered sensory inadmissible because of spoiled fish association by the sixth day of storage and were discontinued from the further sensory analysis. The appearance and odor of untreated control loins were the same ($p<0.05$) as fresh cod loins for up to 6 days of storage.

Table 1. Sensory evaluation scores of Atlantic cod loin treated with oregano essential oil 0.5% (O 0.5) and oregano essential oil 5% (O 5) during eight-day storage at 4°C

Treatment	Odor					Appearance				
	D0 [d]	D2	D4	D6	D8	D0	D2	D4	D6	D8
Fresh	5[a]	5[a]	5[a]	5[a]	5[a]	5[a]	5[a]	5[a]	5[a]	5[a]
Control	5[a]	4.9[a]	4.8[b]	4.4[b]	3.1[b]	5.2[b]	5[a]	4.9[a]	4.6[bc]	3.8[b]
O 0.5	5.2[b]	5.1[a]	4.5[c]	ND [e]	ND	5[a]	5.1[a]	4.5[b]	ND	ND
O 5	5.3[b]	5[a]	4.6[c]	ND	ND	5[a]	5[a]	4.33[c]	ND	ND

[a-c] Means within the same column with different superscripts are significantly different ($P<0.05$).
[d] D0: Day 0, D2: Day 2, D4: Day 4, D6: Day 6, D8: Day 8
[e] ND: not determined

Microbiological analysis of cod loins. The initial total plate counts in cod loins varied from 4.36 $\log_{10}$ CFU/g in untreated samples to 4.25 $\log_{10}$ CFU/g in (O 0.5) and 4.30 $\log_{10}$ CFU/g (O 5) samples (Fig. 1). On the second day of storage, reduced growth of microorganisms was observed in treated groups ($p<0.05$) compared to the untreated group, and the difference of the total plate count between O 0.5 and O 5 was significant ($p<0.05$). On the sixth day of storage, the growth of microorganisms in treated groups increased significantly, exceeding 7 $\log_{10}$ CFU/g in the samples. The values were 7.11 $\log_{10}$ CFU/g in O 0.5 and 7.15 $\log_{10}$ CFU/g in O 5 ($p<0.05$), respectively, while the control group reached a similar value on the eighth day. The study showed that the storage time of the thawed cod loins with oregano essential oil decreased compared to the control group.

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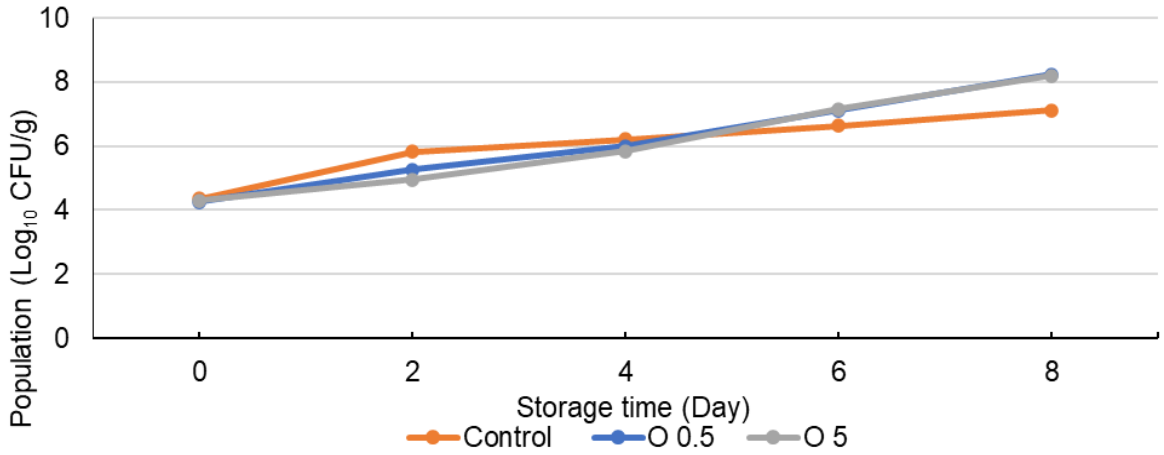

Fig. 1. Total aerobic bacterial counts on Atlantic cod loins treated with oregano essential oil during eight-day storage at 4°C

The antimicrobial activity of oregano essential oil. Although some studies indicated that oregano essential oil could provide a significant bacteriostatic/inhibition effect, the results of the present study showed that the essential oil could not preserve cod loins for the whole storage time. The antimicrobial effectiveness may be attributed to aerobic storage conditions. It agrees with another study where it was recognized that the addition of oregano essential oil affected slower microbial growth in minced meat stored in modified atmosphere packages, but no pronounced inhibition was found in aerobically stored meat (Skandamis & Nychas, 2001). Also, the performance of essential oils is improved with physical conditions, which are low temperature, low pH, and low oxygen content. In aerobic conditions, the evaporation and degradation of active essential oil components increases (Burt, 2004). Another study showed that oregano essential oil has an antimicrobial effect only for a short period of time, and high concentrations of essential oils can provide protection against microorganisms. In this study, a significant antibacterial effect was observed during the first two days of storage, and a higher concentration of oregano had a significant antibacterial effect in the first few days. Eventually, the growth of bacteria increased, and this may be caused by the presence of low sub-lethal concentrations of carvacrol and thymol, which are the major antibacterial components of oregano to induce the expression of stress-response proteins in the microorganisms for adapting and overcoming adverse environments to facilitate cell survival and growth (Burt *et al.*, 2007) and this determines the repair of the damaged membrane (Lambert *et al.*, 2001). However, increased concentrations of essential oils or other natural antimicrobial substances provide cell death, especially for Gram-positive bacteria. Meanwhile, Gram-negative bacteria are more resistant to the activity of essential oils and their components (Burt & Reinders, 2003).

Conclusions

Our study has shown that oregano essential oil affects sensory attributes positively. In the first four days, the main volatile components in the oregano essential oil are well-known for their antimicrobial activity, which significantly inhibits the proliferation of microorganisms. However, after four days of storage, microorganisms adapted to the stress, and oregano essential oil-treated samples spoiled rapidly. This is because most of the antimicrobial compounds are evaporated, and the remnants of organic substances in oregano essential oil can provide adequate nutrients and improve the growth of microorganisms. For this reason, samples with oregano essential oil had reduced the shelf life by two days compared with the untreated group. The effects of essential oils in food preservation, using combinations with other antimicrobial compounds and their interaction with food composition components and different packaging systems, need to be further investigated.

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Raudonėlių eterinio aliejaus poveikis Atlanto menkių nugarinių galiojimo laikui ir juslinėms savybėms

(Gauta 2024 m. vasario mėn.; atiduota spaudai 2024 m. kovo mėn.; prieiga internete nuo 2024 m. gegužės 10 d.)

Santrauka

Šis tyrimas atliktas žuvies perdirbimo įmonės užsakymu, siekiant pailginti žuvies produktų galiojimo laiką. Nuspręsta išnagrinėti raudonėlių eterinio aliejaus poveikį atšildytų Atlanto menkės (*Gadus Morhua*) nugarinės, laikomų šaldytuve, jusliniams požymiams ir galiojimo laikui.

Tyrimui buvo naudojamos trys grupės. Kontrolinė grupė – atšildytos menkės nugarinės be raudonėlių eterinio aliejaus. Antroji grupė – nugarinė su 0,5 % raudonėlių eterinio aliejaus, trečioji – 5 % raudonėlių eterinio aliejaus. Po

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apdorojimo juslinis įvertinimas ir mikrobiologinis tyrimas buvo atlikti iš karto po apdorojimo ir praėjus 2, 4, 6 ir 8 dienoms.

Su raudonėlių aliejumi apdorotos nugarinės šeštąją laikymo dieną jusliškai vertinant tapo nepriimtinomis, todėl vertinimas buvo nutrauktas, o kontrolinė grupė tik po aštuonių dienų jusliškai buvo įvertinta neigiamai. Bendrieji bakterijų kiekiai abiejose apdorotose grupėse su raudonėlių eteriniu aliejumi viršijo 7 log10 KSV/g po šešių dienų, o kontrolinė grupė pasiekė tą patį rodiklį tik po aštuonių dienų. Tyrimas parodė, kad Atlanto menkių nugarėlės su raudonėlių eteriniu aliejumi galiojimo laikas sumažėjo dviem dienomis, palyginti su kontroline grupe. Remiantis tyrimo rezultatais galima teigti, kad raudonėlių eterinis aliejus pasižymi antibakterinėmis savybėmis, tačiau tik tam tikromis sąlygomis.

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