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MICROBIOLOGICAL QUALITY ASSESSMENT OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) MEAT TREATED WITH ROSEMARY AND THYME DRIED HERBS AND ESSENTIAL OILS

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ABSTRACT

This study investigates the effects of thyme and rosemary dried herbs, as well as thyme and rosemary essential oils (1%, w/v), on the microbiological quality of vacuum-packed rainbow trout meat stored under refrigerated conditions (4 ± 1 °C) for 7 days. The microbiological quality of meat was monitored based on total viable counts, coliform bacteria, and lactic acid bacteria counts over a designated storage period (on the 0th, 1st, 3rd, 5th and 7th day of storage). As expected, the highest total viable counts were observed on the 7th day of storage in the untreated aerobically packed control group reaching 5.723 ± 0.021 CFU.g^{-1}. The tested treatments showed potential in reducing the growth of bacteria in rainbow trout meat samples. Across all bacterial groups under investigation, rosemary essential oil appears to have the most advantageous effect in inhibiting bacterial growth compared to other tested treatments. Cultivated isolates were identified by MALDI-TOF mass spectrometry and assigned to 33 species belonging to 14 families. The most prevalent family was *Enterobacteriaceae* (18.99%), and the most abundant species found in samples was *Hafnia alvei* (8.86%). Our findings suggest the potential of using thyme and rosemary in both essential oil and dried forms as natural preservatives to enhance the microbiological quality and longevity of refrigerated rainbow trout meat.

Keywords: thyme, rosemary, essential oils, rainbow trout, vacuum packaging, microbiological quality

INTRODUCTION

Freshwater fish, including rainbow trout (*Oncorhynchus mykiss*), are a part of the diet of many people. Fish meat is in general a nutritionally valuable food source for humans **(Balami et al., 2019; Steffens, 2006)**. However, the composition and properties of fish meat make it an ideal environment for the growth of microorganisms. The flesh of healthy fish is sterile. Fish are highly susceptible to spoilage due to their elevated water activity, neutral pH, abundance of low-molecular-weight compounds, and a microbiota adapted to cold temperatures. These combined factors create ideal conditions for both biochemical and microbial deterioration. Consequently, traditional fish preservation methods (such as salting, drying, and freezing) involve significant reductions in water activity. However, modern consumers increasingly prefer minimally processed fresh food. Currently, it is estimated that 20% of fish spoil after being caught **(Hao et al., 2021)**. Three distinct mechanisms contribute to fish spoilage: autolytic spoilage (primarily involving proteolysis and lipolysis), oxidative spoilage (resulting from the oxidation of unsaturated fatty acids), and microbial spoilage (driven by the proliferation of psychrotolerant species and the production of biogenic amines). The issue of fish waste is significant in a world with a growing population and limited resources. Furthermore, fish spoilage poses food safety concerns and leads to substantial economic losses for food companies **(Hussain et al., 2021)**. Unwanted contamination with microorganisms occurs only during handling and processing. During these processes, is fish meat contaminated by two hypothetical groups of microorganisms. The first group includes microorganisms associated with the surface of the body or internal organs of fish. The second group includes microorganisms found in the environment in which fish and fish meat are processed, transported, and stored, including microbes found on surfaces, tools, or persons processing fish and fish meat. Microorganisms colonizing fish flesh cause microbial spoilage during storage through their enzymatic activity. Spoiled or decayed meat is not suitable for consumption and some microorganisms found in fish flesh can be harmful to humans **(Gram & Dalgaard, 2002)**.
Observing strict regulations throughout every step in the fish processing industry is crucial to ensure the high-quality and safe meat demanded by consumers. The principal objective of meat preservation techniques is to prevent the deterioration of meat. Meat preservation is essential for maintaining freshness, extending shelf life, and ensuring the safety of meat and meat products **(Ghaly et al., 2010)**. Traditional methods of extending the shelf life of raw foods, such as refrigeration and freezing, or even vacuum packaging, have their limitations. Therefore, new approaches for preserving food and the synergistic effect of multiple preservation techniques are currently being explored **(Dave & Ghaly, 2011)**. With increased consumer knowledge of food safety and quality, there is a strong demand for preservative (synthetic)-free food and the use of natural products as preservatives. Natural antimicrobials derived from various sources are used to keep food safe from spoilage and pathogenic microbes. Plants are the primary source of antimicrobials and include a variety of essential oils that have antimicrobial properties. Many essential oils are found in herbs and spices, such as rosemary, sage, basil, oregano, thyme, cardamom, and clove **(Mashabela et al., 2022).**
Researchers are investigating various natural ingredients, such as herbs or essential oils derived from herbs, for their potential in preserving food. Many herbs contain substances with antimicrobial and antioxidant properties, making them promising candidates for extending the shelf life of foods, including fish meat **(Calo et al., 2015; Hassoun & Çoban, 2017; Huang et al., 2021; Jackson-Davis et al., 2022)**. Several authors have recently explored the antimicrobial properties of various essential oils in freshwater fish as a strategy to reduce the use of antimicrobials in aquaculture. In their research, they assessed the inhibitory effects of 14 essential oils using disc diffusion assay (DDA) and minimum inhibitory concentration (MIC) against 20 bacteria isolated from freshwater fish. Among these essential oils, *Cinnamomum camphora* var. *Linaloolifera* EO exhibited the highest activity against most of the isolates, particularly *Aeromonas* spp. and *Enterococcus* **(Klūga et al., 2021)**.
Rosemary and thyme, commonly used in numerous cuisines to enhance the flavour and aroma of foods, are among the herbs of interest. In this study, we specifically investigated their effect in the dried form and form of essential oil on the microbiological quality of vacuum-packed rainbow trout meat during cold storage. By understanding the impact of these natural ingredients, we may contribute to the development of effective preservation methods that maintain the safety and freshness of fish meat.

MATERIAL AND METHODS

Sample preparation

Rainbow trout (*Oncorhynchus mykiss*) (2000 g) were purchased from a private breeder and retailer in Nitra (Slovakia). Trout were slaughtered and promptly transported to the microbiological laboratory. The meat was processed on the day of purchase immediately after transport. Under strict antiseptic conditions, the meat was cut into 5 g pieces. All microbiological analyses were performed on raw meat samples subjected to various treatments. A total of 7 following groups of samples were analysed: (1) control untreated samples stored aerobically (C); (2) untreated vacuum sealed samples (CV); (3) untreated vacuum sealed samples treated with sunflower oil (CO); (4) vacuum sealed samples treated with thyme essential oil (TEO); (5) vacuum sealed samples treated with thyme dried herb (TDH); (6) vacuum sealed samples treated with rosemary essential oil (REO); and (7) vacuum sealed samples treated with rosemary dried herb (RDH). Vacuum sealing was performed using a vacuum sealing machine (Concept, Choceň, Czech Republic). Thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.) plant essential oils (EOs) were purchased from a private supplier and retailer (Hanus - Herbal Preparations, Ltd., Slovakia). Both plant EOs mentioned above were used at 1% (w/v) concentration (diluted with sunflower oil). Thyme and rosemary dried herbs (DH) were purchased as a commercially available culinary seasoning and used at 1% (w/v) concentration (diluted with sunflower oil). All samples were stored at 4 ± 1 °C for a designated period.

Microbiological analysis

Microbiological analyses were conducted in triplicate to ensure better accuracy and reliability. The counts of selected groups of bacteria were monitored, including the Total Viable Count (TVC), Coliform Bacteria (CB) and Lactic Acid Bacteria (LAB). Subsequently, a more accurate identification was performed using mass spectrometry MALDI TOF-MS Biotyper (Bruker Daltonics, Bremen, Germany). Microbial testing was conducted on the 1st (Day 1); 3rd (Day 3); 5th (Day 5); and 7th (Day 7) day of storage. On the day of purchase (Day 0) was analysed just untreated control group stored aerobically (C).

To determine the number of colonies forming units present in the samples (CFU.g^{-1}), a dilution plating method was employed. The initial dilution (10^{-1}) was prepared by homogenizing 5 g of the sample with 45 ml of physiological saline solution (0.9% NaCl), followed by a 30-minute homogenization process using an orbital shaker (GFL Orbital Shaker 3005) at 200 RPM. Three different types of cultivation media were used for the cultivation of microorganism. Plate Count Agar (PCA, Oxoid, Basingstoke, UK) was used for the cultivation of Total Viable Counts (TVC), Violet Red Bile Lactose Agar (VRBL, Oxoid, Basingstoke, UK) was used for the cultivation of Coliform Bacteria (CB), and De Man–Rogosa–Sharpe agar (MRS, Oxoid, Basingstoke, UK) was employed for the cultivation of Lactic Acid Bacteria (LAB). Petri dishes with the corresponding media were inoculated with samples and incubated in a thermostat (PCA at 30 °C for 72 hours; VRBL at 37 °C for 24 hours; and MRS at 37 °C for 48 hours with 5% CO_2). Plate counts (CFU.g^{-1}) were converted to log (log10) values. Pure colonies of bacteria were suspended in 75% ethanol (Ethyl Alcohol Solvanal 99.8%, Centralchem, Slovakia) and stored at −20 ± 1 °C until the subsequent stage of analysis. Before the analysis, the samples were centrifuged (12 000 RMP, 1 min) and the supernatant was discarded. The pellet was mixed with 30 µl of formic acid (Honeywell, USA) and 30 µl of 70% acetonitrile (Sigma-Aldrich, USA). The mixture was resuspended, centrifuged (12 000 RMP, 1 minute) and 1 µl of aqueous phase was utilized for further analysis.

Isolates identification

The matrix used for MALDI-TOF MS was α-cyano-4-hydroxycinnamic acid (HCCA) diluted in an organic solvent (10 mg.ml^{-1}) (Sigma-Aldrich, USA). The organic solvent composed of 50% acetonitrile (Sigma-Aldrich, USA), 47.5% ultrapure distilled water, and 25% trifluoroacetic acid (Sigma-Aldrich, USA). A 1 µl of aqueous sample solution of the selected bacterial isolate was transferred to the target metal plate designed for MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and cowered with 1 µl of matrix mixture. The matrix coated sample was allowed to air dry and analysed using a MALDI-TOF MS Biotyper instrument (Bruker Daltonics, Bremen, Germany) with MALDI Biotyper 3.0 software (Bruker Daltonics, Bremen, Germany).

Statistical analysis

Basic descriptive statistical methods, including mean and standard deviation, were used to evaluate the results. Additionally, the statistical techniques of paired t-test, Analysis of Variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) test were employed to compare the results and determine statistically significant differences between the data groups. Because the data from the CB and LAB samples do not follow a normal distribution, a non-parametric Kruskal-Wallis ANOVA was conducted, followed by a post-hoc Dunn's test. Statistical analyses were conducted using XLSTAT software (Addinsoft, Pris, France). Significance was determined at a level of $p \leq 0.05$.

RESULTS

Trout meat samples treated in different ways showed different microbial loads for all the investigated microbial groups. The results of Total Viable Counts (TVCs), Coliform Bacteria (CB) and Lactic Acid Bacteria (LAB) are displayed in Table 1, Tables 2, and Table 3, respectively. Statistically significant differences ($p \leq 0.05$) were observed among the different treated samples and in all tested microbial groups.

According to the paired t-test, the application of EOs (TEO, REO) and DHs (TDH, RDH) significantly reduced the number of TVCs on the 1st of storage compared with the 0th. Samples without the addition of EO or DH (C, CV, CO) displayed significantly higher numbers of microorganisms. As expected TVCs exhibited the highest microbial loads among all the groups of microorganisms investigated. Overall TVCs ranged between 2.407 ± 0.015 log CFU.g^{-1} in the REO group on 1st day of storage to 5.723 ± 0.02 log CFU.g^{-1} in the C group on the 7th day of storage. The highest counts overall were observed in the C group. Throughout the entire period, there were no statistically significant differences observed between the CV and CO groups. Similarly, on the 1st day of storage, no statistically significant differences were found between TDH and RDH. Furthermore, on the 3rd day, there were no statistically significant differences observed between TDH and REO. It can be concluded that nearly all of the treatments tested demonstrated the potential to impact bacterial growth.

Moreover, CB were detected in more than half of the samples with bacterial loads ranging from 1.020 ± 0.020 log CFU.g^{-1} in the C group on the 0th day to 2.637 ± 0.040 020 log CFU.g^{-1} in the C group on the 7th day of storage. The highest counts overall were observed in the C group. CB were not detected (≤ 1 log CFU.g^{-1}, initial ten-fold serial dilution (10^{-1}) used for plate counts) in three types of samples, namely TEO, REO and RDH group. Obviously, throughout the entire period, there were no statistically significant differences observed between the TEO, REO and RDH groups. Similarly, on the 1st day of storage, no statistically significant differences were found between TEO, TDH, REO and RDH.

However, LAB were observed in less than half of the samples with bacterial loads ranging from 1.200 ± 0.010 log CFU.g^{-1} in the RDH group on the 5th day of storage to 1.983 ± 0.021 log CFU.g^{-1} in the TEO group on the 7th day of storage. The highest counts overall were observed in the TEO group. LAB were not detected (≤ 1 log CFU.g^{-1}, initial ten-fold serial dilution (10^{-1}) used for plate counts) in three types of samples, namely C, CO, TDH and REO group. As is evident, throughout the entire period, there were no statistically significant differences observed between the TDH and REO groups. Similarly, on the 1st day of storage, no statistically significant differences were found between any of the groups; on the 3rd day only the TEO group showed significant differences compared to the other groups; and on the 5th and 7th day of storage C, CO, TDH and REO showed no significant differences. Overall, rosemary EO seems to have a more positive effect compared to thyme EO and a more positive effect compared to rosemary DH in all monitored groups of bacteria.

A total of 33 species belonging to 14 families were identified. Table 4 summarizes the bacteria reliably identified by mass spectrometry. In Table 5 they are classified according to their families. Namely, the families are *Aeromonadaceae* (11.39%), *Bacillaceae* (2.53%), *Enterobacteriaceae* (18.99%), *Erwiniaceae* (2.53%), *Hafniaceaea* (8.86%), *Lactobacillaceae* (5.06%), *Listeriaceae* (2.53%), *Lysobacteraceae* (1.27%), *Microbacteriaceae* (7.59%), *Moraxellaceae* (2.53%), *Pseudomonadaceae* (17.72%), *Sphingobacteriaceae* (3.80%), *Staphylococcaceae* (2.53%), *Yersiniaceae* (12.66%). *Enterobacteriaceae* (18.99%) family was the most abundant family and *Lysobacteraceae* (1.27%) was the least abundant. The most abundant species was *Hafnia alvei* (8.86%), which was present in all samples, and the least (1.27%) abundant species were *Bacillus altitudinis*, *Stenotrophomonas rhizophila*, *Microbacterium liquefaciens*, *Pseudomonas proteolytica*, *Pseudomonas libanensis*, *Pseudomonas rhodesiae* (found solely in C samples); *Staphylococcus warneri*, *Bacillus cereus*, *Microbacterium phyllosphaerae* (found solely in CV samples); *Aeromonas media*, *Aeromonas sobria* (found solely in TEO samples); *Macrococcus caseolyticus*, *Aeromonas eucrenophila* (found solely in TDH samples); and *Serratia grimesii* (found solely in REO samples). Figure 1 displays the representation of the bacterial composition found in the samples.

Table 1 Total Viable plate Counts (TVC)

Treatment	Count ± SD (log CFU.g-1)				
	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**
C	2.777 ± 0.032	3.107 ± 0.021 a	4.010 ± 0.020 a	4.903 ± 0.015 a	5.723 ± 0.021 a
CV	-	2.890 ± 0.010 b	3.467 ± 0.031 b	4.020 ± 0.030 b	4.447 ± 0.015 b
CO	-	2.890 ± 0.020 b	3.423 ± 0.021 b	4.013 ± 0.015 b	4.410 ± 0.026 b
TEO	-	2.630 ± 0.030 c	2.720 ± 0.020 c	3.023 ± 0.023 c	3.410 ± 0.010 c
TDH	-	2.530 ± 0.026 d	2.650 ± 0.010 d	2.840 ± 0.010 d	3.033 ± 0.015 d
REO	-	2.407 ± 0.015 e	2.600 ± 0.010 d	2.900 ± 0.010 e	2.977 ± 0.021 e
RDH	-	2.530 ± 0.020 d	2.793 ± 0.021 e	3.090 ± 0.010 f	3.697 ± 0.015 f

Note: The results of the count show the mean values and standard deviations (SD) of plate counts, each conducted in triplicate. Data within the same column followed by different letters are considered significantly different according to Tukey's Honestly Significant Difference (HSD) test ($p \leq 0.05$). (C) untreated samples stored aerobically; (CV) untreated vacuum sealed samples; (CO) untreated vacuum sealed samples treated with sunflower oil; (TEO) vacuum sealed samples treated with 1% thyme EO; (TDH) vacuum sealed samples treated with 1% thyme DH; (REO) vacuum sealed samples treated with 1% rosemary EO; (RDH) vacuum sealed samples treated with 1% rosemary DH.

Table 2 Coliform bacteria (CB) plate counts

Treatment	Count ± SD (log CFU.g-1)				
	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**
C	1.020 ± 0.020	1.407 ± 0.021 a	1.870 ± 0.020 a	2.020 ± 0.017 a	2.637 ± 0.040 a
CV	-	1.120 ± 0.026 b	1.347 ± 0.006 b	1.600 ± 0.010 b	1.857 ± 0.021 b
CO	-	1.207 ± 0.021 c	1.410 ± 0.010 c	1.683 ± 0.015 c	1.803 ± 0.015 c
TEO	-	≤ 1 d	≤ 1 d	≤ 1 d	≤ 1 d
TDH	-	≤ 1 d	1.100 ± 0.020 e	1.187 ± 0.015 e	1.313 ± 0.015 e
REO	-	≤ 1 d	≤ 1 d	≤ 1 d	≤ 1 d
RDH	-	≤ 1 d	≤ 1 d	≤ 1 d	≤ 1 d

Note: The results of the count show the mean values and standard deviations (SD) of three plate counts, each conducted in triplicate. Data within the same column followed by different letters are considered significantly different according to Dunn's test ($p \leq 0.05$). (C) untreated samples stored aerobically; (CV) untreated vacuum sealed samples; (CO) untreated vacuum sealed samples treated with sunflower oil; (TEO) vacuum sealed samples treated with 1% thyme EO; (TDH) vacuum sealed samples treated with 1% thyme DH; (REO) vacuum sealed samples treated with 1% rosemary EO; (RDH) vacuum sealed samples treated with 1% rosemary DH.

Table 3 Lactic acid bacteria (LAB) plate counts

Treatment	Count ± SD (log CFU.g-1)				
	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**
C	≤ 1	≤ 1 a	≤ 1 a	≤ 1 a	≤ 1 a
CV	-	≤ 1 a	≤ 1 a	1.290 ± 0.026 b	1.630 ± 0.026 b
CO	-	≤ 1 a	≤ 1 a	≤ 1 a	≤ 1 a
TEO	-	≤ 1 a	1.420 ± 0.026 b	1.703 ± 0.021 c	1.983 ± 0.021 c
TDH	-	≤ 1 a	≤ 1 a	≤ 1 a	≤ 1 a
REO	-	≤ 1 a	≤ 1 a	≤ 1 a	≤ 1 a
RDH	-	≤ 1 a	≤ 1 a	1.200 ± 0.010 d	1.577 ± 0.031 d

Note: The results of the count show the mean values and standard deviations (SD) of three plate counts, each conducted in triplicate. Data within the same column followed by different letters are considered significantly different according to Dunn's test ($p \leq 0.05$). (C) untreated samples stored aerobically; (CV) untreated vacuum sealed samples; (CO) untreated vacuum sealed samples treated with sunflower oil; (TEO) vacuum sealed samples treated with 1% thyme EO; (TDH) vacuum sealed samples treated with 1% thyme DH; (REO) vacuum sealed samples treated with 1% rosemary EO; (RDH) vacuum sealed samples treated with 1% rosemary DH.

Table 4 Families and species of isolated microorganisms

Family	Species
Aeromonadaceae	*Aeromonas salmonicida, Aeromonas bestiarum, Aeromonas media, Aeromonas sobria, Aeromonas eucrenophila*
Bacillaceae	*Bacillus altitudinis, Bacillus cereus*
Enterobacteriaceae	*Buttiauxella gaviniae, Buttiauxella warmboldiae, Buttiauxella agrestis, Lelliottia amnigena*
Erwiniaceae	*Pantoea agglomerans*
Hafniaceaea	*Hafnia alvei*
Lactobacillaceae	*Latilactobacillus sakei*
Listeriaceae	*Brochothrix thermosphacta*
Microbacteriaceae	*Microbacterium liquefaciens, Microbacterium maritypicum, Microbacterium phyllosphaerae*
Moraxellaceae	*Acinetobacter johnsonii*
Pseudomonadaceae	*Pseudomonas fragi, Pseudomonas lundensis, Pseudomonas extremorientalis, Pseudomonas proteolytica, Pseudomonas libanensis, Pseudomonas rhodesiae*
Sphingobacteriaceae	*Sphingobacterium faecium*
Staphylococcaceae	*Macrococcus caseolyticus, Staphylococcus warneri*
Lysobacteraceae	*Stenotrophomonas rhizophila*
Yersiniaceae	*Ewingella americana, Serratia fonticola, Serratia liquefaciens, Serratia grimesii*

Table 5 Bacteria isolated from rainbow trout meat subjected to various treatments

Family	Species
C	*Hafnia alvei, Serratia fonticola, Bacillus altitudinis, Pseudomonas fragi, Stenotrophomonas rhizophila, Pseudomonas lundensis, Microbacterium liquefaciens, Microbacterium maritypicum, Pseudomonas extremorientalis, Aeromonas salmonicida, Acinetobacter johnsonii, Pseudomonas proteolytica, Pseudomonas libanensis, Pseudomonas rhodesiae, Latilactobacillus sakei*
CV	*Hafnia alvei, Serratia fonticola, Buttiauxella gaviniae, Pseudomonas fragi, Pseudomonas lundensis, Staphylococcus warneri, Bacillus cereus, Microbacterium phyllosphaerae, Aeromonas bestiarum, Aeromonas salmonicida, Latilactobacillus sakei*
CO	*Hafnia alvei, Buttiauxella gaviniae, Buttiauxella warmboldiae, Pseudomonas fragi, Brochothrix thermosphacta, Pseudomonas lundensis, Microbacterium maritypicum, Pseudomonas extremorientalis, Serratia liquefaciens, Ewingella americana, Serratia fonticola, Pantoea agglomerans*
TEO	*Hafnia alvei, Serratia fonticola, Buttiauxella gaviniae, Buttiauxella warmboldiae, Latilactobacillus sakei, Acinetobacter johnsonii, Aeromonas bestiarum, Aeromonas media, Aeromonas sobria, Sphingobacterium faecium, Serratia liquefaciens*
TDH	*Hafnia alvei, Buttiauxella gaviniae, Buttiauxella agrestis, Lelliottia amnigena, Brochothrix thermosphacta, Pseudomonas lundensis, Pseudomonas extremorientalis, Sphingobacterium faecium, Microbacterium maritypicum, Macrococcus caseolyticus, Aeromonas bestiarum, Aeromonas salmonicida, Aeromonas eucrenophila*
REO	*Hafnia alvei, Serratia fonticola, Buttiauxella gaviniae, Buttiauxella agrestis, Pseudomonas lundensis, Sphingobacterium faecium, Microbacterium maritypicum, Serratia grimesii, Ewingella americana*
RDH	*Hafnia alvei, Serratia fonticola, Pantoea agglomerans, Buttiauxella gaviniae, Buttiauxella agrestis, Lelliottia amnigena, Latilactobacillus sakei, Ewingella americana*

Note: (C) untreated samples stored aerobically; (CV) untreated vacuum sealed samples; (CO) untreated vacuum sealed samples treated with sunflower oil; (TEO) vacuum sealed samples treated with 1% thyme EO; (TDH) vacuum sealed samples treated with 1% thyme DH; (REO) vacuum sealed samples treated with 1% rosemary EO; (RDH) vacuum sealed samples treated with 1% rosemary DH.

Figure 1 Visualization of the bacterial composition of the samples

DISCUSSION

Many studies have focused on the addition of EO as a natural additive to extend the shelf life of foods. However, most have focused on either other EOs than thyme or rosemary, or other fish and seafood. In recent years numerous reviews have focused on conducting a thorough comparison of the existing studies **(Hao et al., 2021; Hassoun & Çoban, 2017; Mei et al., 2019; Olatunde & Benjakul, 2018)**. It is well known that vacuum packaging itself improves the microbial quality of food. Additionally, plant herbs and derived EOs have antimicrobial activity. The combination of these two techniques appears to be a compelling strategy for prolonging the shelf life of food. Several studies have explored the use of vacuum packaging and/or EOs derived from herbs and their application in preserving the quality of rainbow trout meat during cold storage. For example, **Çoban et al. (2016)** reported that sage EO (2%; 4%, w/v), when used in combination with vacuum packaging, significantly improved the quality and extended the shelf life of the meat during storage at 4 °C. **Kunová et al. (2021)** demonstrated *Citrus limon* and *Cinnamomum camphora* EOs (0.5%; 1%, w/v) with conjunction with vacuum packaging during storage at 4 °C reduced spoilage of the rainbow trout meat. **Pyrgotou et al. (2010)** found that oregano EO (0.2%; 0.4%, w/v) reduced bacterial growth and extended the shelf life of rainbow trout fillets stored at 4 °C, especially when combined with modified atmosphere packaging with reduced oxygen levels (5%). Similarly, **Arashisar et al. (2004)** demonstrated on trout fillets that modified atmosphere packaging is more efficient than vacuum packaging on its own. Furthermore, **Mexis et al. (2009)** also studied the antibacterial effects of oregano plant EO (0.4%, w/v), but in combination with an O_2 absorber on the shelf life of rainbow trout fillets stored at 4 °C. The most effective inhibition effect was achieved when combining an O_2 absorber with oregano EO. **Ozogul et al. (2017)** developed nanoemulsions based on thyme, rosemary, sage and laurel EOs (4%, w/v). Thyme and rosemary nanoemulsions were the most effective and were recommended as fish preservatives for prolonged shelf life due to their potential to reduce bacterial growth. As well as in our study, rosemary EO was even more effective than thyme EO. The shelf life of rainbow trout treated with thyme and rosemary EO's nanoemulsion was found to be extended by 3 days compared to the untreated control group (stored at 2 ± 2 °C). The initial TVCs (3.45 log $CFU.g^{-1}$) were higher compared to our study. However, TVCs of both treated and untreated groups were comparable or slightly lower to our observations (4.0-6.0 log $CFU.g^{-1}$) on the 7th day of storage. The effect of nanoemulsion with the addition of thyme plant EO on the microbiological quality of rainbow trout meat during storage (9 days, 4 °C) was also investigated by **Meral et al. (2019)**. The study reported an almost 30% reduction in the bacterial growth of nanoemulsion treated samples compared with untreated samples. A study by **Linhartová et al. (2019)** showed that commercially sold rosemary extract (0.5%; 1.0% and 2%, w/v) could effectively retard microbial deterioration and extend the shelf life of vacuum-packed rainbow trout fillets stored under refrigerated conditions (4.3 ± 0.6 °C). Samples treated with 0.5% extract showed the lowest numbers of TVCs. As in our case, the highest TVCs were observed in the control untreated samples. The initial bacterial load was slightly lower, ranging from 1.93 to 2.29 log $CFU.g^{-1}$, and showed a rapid increase to 5.10–5.27 $CFU.g^{-1}$ after 6 days of storage. Thyme and rosemary EOs and their antimicrobial potential were proven to be effective when applied directly on the surface of trout flesh, as well as in coatings, films, and glazes **(Çoban, 2013; Dehghani et al., 2018; Gómez-Estaca et al., 2010; Jouki et al., 2014; Tokur et al., 2016)**. Thyme and rosemary EOs have been studied not only on fresh trout's meat but also on smoked trout's meat. The addition of rosemary and thyme EOs (1%, w/v) in vacuum-packed and modified atmosphere packed (no O_2 levels) hot smoked rainbow trout under storage at 4°C also resulted in a longer shelf life. According to **Yıldız (2015a)** study, thyme oil was more effective in reducing bacteria than rosemary oil, which contrasts with our study. In contrast, there was also a lower initial load of TVCs (2.0 log $CFU.g^{-1}$) and a higher initial load of LAB counts (2.0 log $CFU.g^{-1}$). Nevertheless, the observed average TVCs after 10 days were lower (did not exceed 3 log $CFU.g^{-1}$) than our observations after 7 days. **Yıldız (2015b)** also compared thyme and clove EOs (1%, w/v) treatments and packaging treatments (vacuum and modified atmosphere with no O_2 levels) on the storage of hot smoked rainbow trout at 4°C. Both EOs had the potential to retard bacterial growth, but clove EO was more effective than thyme EO especially when speaking about the combination of vacuum packaging and modified atmosphere packaging. Yıldız also stated that the bacterial growth rate in the modified atmosphere packaging samples was found to be lower compared to the vacuum-packaged samples. As well as in the previous Yıldız study, the initial load of TVCs and LAB were in contrast with our study. TVCs were lower (2.0 log $CFU.g^{-1}$) and LAB higher (2.0 log $CFU.g^{-1}$). However, the average TVCs observed after 10 days were lower (not exceeding 3 log $CFU.g^{-1}$) compared to our findings after 7 days. Furthermore, the addition of rosemary plant extract (1%, w/v) in conjunction with hot smoking and vacuum packing has been shown to extend the shelf life of products stored at 2°C by two weeks, compared to the untreated vacuum-packed control samples **(Erkan et al., 2011)**. Comparable results were also reported by **Erkan (2012)** using thyme EO (1%, w/v). The incorporation of EO, similar to the aforementioned application of plant extracts, prolonged the shelf life of vacuum-packed (2°C) hot smoked trout meat by an additional two weeks. This extension was attributed mainly to the suppression of bacterial spoilage. **Yıldız (2016)** also focused on the combined effect of two other preservation techniques, namely marinating and the addition of a natural preservative in the form of thyme and rosemary EO (1%, w/v). The utilization of these EOs in marinated trout fillets has demonstrated a beneficial impact on microbial characteristics during storage (4 °C). Also in this Yıldız study, thyme EO was shown to be more effective than rosemary EO in suppressing bacterial growth, which is again in contrast to our study.

The antimicrobial potential of spices and DHs has been known for a long time and has also been investigated by many studies. Several studies compared the observations of many researchers **(Bor et al., 2016; Ceylan & Fung, 2004; Tajkarimi et al., 2010)**. Numerous spices harbor noteworthy antimicrobial agents, presenting a potential source of inhibitory substances against food spoilage and foodborne pathogens **(Sulieman et al., 2023)**. As mentioned above, the antibacterial properties of rosemary and thyme have been quite extensively documented. However, their preservative properties in the direct form of dried culinary herbs in fish meat products, have not been thoroughly studied. Existing studies focus primarily on EOs and plant extracts made out of herbs. Among others, analysing the results of many existing studies is challenging mainly due to variations in composition, concentration, application methods, and storage conditions, including air exposure and temperature.

Overall, several studies provide strong evidence that MALDI-TOF MS fingerprinting is efficient method for identifying bacterial strains obtained from both fresh and processed seafood and freshwater fish (**Böhme et al., 2010; Böhme, Fernández-No, Barros-Velázquez, et al., 2011; Böhme, Fernández-No, Gallardo, et al., 2011; Fernández-No et al., 2010; Tütmez et al., 2023**). The bacterial composition observed in our samples was similar to previous studies performed on aerobically stored rainbow trout meat, indicating a high level of agreement. *Enterobacteriaceae* was the most abundant family in our case (18.99%), closely followed by *Pseudomonadaceae* (17.72%) and *Aeromonadaceae* (11.39%). **According to Chytiri et al. (2004), pseudomonads were the most prevalent bacteria in the spoilage microflora of whole ungutted and filleted trout during storage in ice. Lower levels of *Enterobacteriaceae* were also detected**. In general, *Pseudomonas* species are recognized as one of the primary bacteria responsible for spoiling fresh fish stored on ice, irrespective of the fish's source (**Gram & Huss, 1996).** The predominant intestinal microflora (both intestinal epithelial mucosa and digesta) of rainbow trout has been identified as bacteria belonging to the genera *Pseudomonas* and *Aeromonas*, as well as the *Enterobacteriaceae* family (**Huber et al., 2004**; **Kim et al., 2007**; **Merrifield et al., 2009**). The high prevalence of these species in our samples is likely due to contamination of meat by bacteria from the gut microbiome. Furthermore, the study conducted by **Salgado-Miranda et al. (2010)** illustrates that aeromonads are in general prevalent among rainbow trout. Their research found that aeromonads were the most frequently isolated bacteria from various organs of farmed rainbow trout, including the gills, liver, spleen, intestine, and kidney, followed by pseudomonads and *Escherichia coli* belonging to the *Enterobacteriaceae* family. However, in contrast to earlier findings and presumptions, a low abundance of LAB (5.06%) was detected. Several studies conducted by **Lyhs et al. (1998, 1999, 2001, 2002**) have successfully identified the spoilage bacteria present in processed vacuum-packed rainbow trout. Among these bacteria, LAB were found to be the most prevalent, with species such as *Latilactobacillus sakei* commonly detected (**Lyhs et al., 2002**). The presence of these bacteria has been directly linked to the spoilage of vacuum-packed rainbow trout, resulting in significant changes in both microbiological quality and sensory characteristics. The low abundance of LAB could be attributed to the antimicrobial activity of plant essential oils. Numerous studies have indicated that specific essential oils, such as oregano, rosemary, cinnamon, and clove, may inhibit the growth of LAB in fish and different meat products (**Badia et al., 2020; Gómez-Estaca et al., 2010; Zhang et al., 2017**). However, conflicting research suggests that certain essential oils may have minimal or negligible impact on LAB in meat (**Emiroğlu et al., 2010; Michalczyk et al., 2012**). Therefore, further research is necessary to determine the specific impact of essential oils and vacuum packaging on the microbial community of rainbow trout meat.

CONCLUSION

Based on the results, it is evident that the tested treatments demonstrated the potential to impact bacterial growth in the rainbow trout meat samples. The differences in TVCs among different treatments and storage periods provided insights into the impact of the various preservation methods on bacterial growth in the rainbow trout meat samples. Samples without the addition of EO or DH (C, CV, CO) displayed significantly higher numbers of bacteria. No statistically significant differences between the CV and CO groups suggest that the presence of sunflower oil, which was used as a diluent, does not affect bacterial growth. The presence of specific bacteria, such as CB and LAB, varied across different treatment groups during cold storage periods. The presence of CB was detected in more than half of the samples, with varying bacterial loads observed in different treatment groups throughout the storage period. The highest counts were consistently observed in the C group, while the TEO, REO, and RDH treated groups demonstrated the absence of CB. LAB were observed in less than half of the samples, with the highest counts found in the TEO group, whilst the C, CO, TDH and REO treated groups demonstrated the absence of LAB. In general,

rosemary EO appears to have a more beneficial impact when compared to thyme EO and rosemary DH across all bacterial groups under observation (TVC, CB, LAB). The abundance and distribution of 33 species belonging to 14 families across different treatment groups and storage periods further contribute to our comprehensive understanding of the microbial composition in rainbow trout meat samples and changes as a result of different treatment methods and periods of storage. These findings underscore the significance of vacuum packaging, EOs, and DHs application in reducing bacterial growth and preserving the quality of fish meat during cold storage.

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