COMBINE EFFECT OF SALTING AND THYME (THYMUS VULGARIS) ESSENTIAL OIL ON SHELF LIFE OF RAINBOW TROUT (ONCORHYNCHUS MYKISS) STORED AT 4°C

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Abstract

The following fish samples were examined: control salted samples (A), salted samples with 0.1% of thyme oil (B), salted samples with 0.3% of thyme oil (C), and salted samples with 0.5% of thyme oil (D). The study was based on microbiological (total viable count (TVC), Pseudomonas sp., lactic acid bacteria (LAB), Enterobacteriaceae, and H2S-producing bacteria), chemical (total volatile basic nitrogen (TVBN) and thiobarbituric acid (TBA)), and sensory (colour, odour, taste, flavor, texture, and overall acceptance) analyses of changes occurring in the product as a function of treatment and storage time. The salted samples stored at 4°C were taken as the control samples. Results showed that TVC exceeded 7 log cfu/g on day 12 of storage for control samples and day 21 for C and D samples. Populations of LAB, H2S-producing bacteria, Enterobacteriaceae, and Pseudomonas sp. reached higher final numbers in A and B samples than for C and D samples. Under B, C, and D treatments, TVBN values were lower than for A samples, whereas lipid oxidation, as judged by determination of TBA, did not occur during the refrigerated storage. Sensory scores of trout samples salted with thyme (groups B, C, D) decreased during storage time. However, at the end of the storage period, samples with thyme oil were acceptable by the panelist. The results of this study suggest that the shelf life in case of C and D samples was 21 d. The salting, thyme oil, and air packing were found to be effective, easy, and cheap methods of fish preservation.

Key words: rainbow trout, salting, thyme oil, shelf life.

Rainbow trout (Oncorhynchus mykiss) is a fish of a high commercial value and high appreciation by European consumers (11). It is sold as either whole fresh fish (stored on ice) or in fillet form (usually salted/smoked) stored under vacuum packaging.

Many fish products are perishable by nature and require protection from spoilage during their preparation, storage, and distribution to give them desired shelf life. Because fishes are now often sold in areas of the world far remote from their production sites, the need for extended safe shelf-life for these products has also expanded. Improvements in the cold distribution chain have made international trade of perishable fish possible, but refrigeration alone cannot assure the quality and safety of all perishable fish. Although the value of traditional fish preservatives has been recognised, their safety has been questioned (8).

While abundant data exists on preservation of rainbow trout using low-dose irradiation (36), ice (9, 33), ozonation (31), vacuum packaging (VP) (3), including cold smoked rainbow trout using nitrite or potassium nitrate (25), antimicrobial combinations of lactic acid and nisin (32), there is, in general, limited information in the literature on the effect of essential oils (EOs), such as oregano or thyme, used singly or in combination with other preservative technologies, i.e. salting, VP, on extension of shelf-life of freshwater fish species, including rainbow trout.

The greater consumer awareness and concern regarding synthetic chemical additives, foods preserved with natural additives have become more popular. This has led researchers and food processors to look for natural food additives with a broad spectrum of antimicrobial activity. Antimicrobial compounds present in foods can extend shelf-life of unprocessed or processed foods by reducing microbial growth rate or viability (7). Spices and herbs, originally added to change or improve taste, can also enhance shelf-life because of their antimicrobial nature. Some of these substances are also known to contribute to the self-defence of plants against infectious organisms (12, 21). Extracts from oregano, thyme, rosemary, clove, sage, and mint belong to the EOs, which are used to improve the sensory characteristics, extend the shelf-life of foods due to their natural antimicrobial and antioxidant properties (6, 15). The antimicrobial properties of EOs
are mainly attributed to their phenolic compounds, i.e. carvacrol and thymol, and to terpenes (6). A number of reports are known on the effects of EOs or their constituents (e.g. carvacrol, thymol etc.) on the shelf-life of various food products, including: meat and meat products (42, 37), vegetables (38), fresh fish, and cephalopods (4, 16, 18, 22, 23, 25). However, no information is available about the effects of the combination of salting, thyme oil, and air package process on the quality of rainbow trout stored at 4°C. The present study was not only to investigate the combine effect of salting and thyme oil on shelf-life of rainbow trout, but also to obtain good and cheap fish product.

Material and Methods

Fish samples. Fresh aquacultured rainbow trout (200 ±10 g and 25±2 cm) were obtained from an aquaculture farm. The fresh fish samples were packed in polystyrene boxes with crushed ice and then transferred to the laboratory. The fish were harvested, beheaded, gutted manually, and then washed.

Preparation of fish samples. The fish were filleted manually using a sterile scalp. The samples (33 gutted fishes) were immersed in a NaCl brine (10%, w/v; fish:brine ratio 1:1; brine temperature 4°C). After brining, the samples were briefly dipped in chilled tap water for 1 h. Undiluted thyme oil (Sigma Fluka, Germany) was added to the three lots of filleted samples in appropriate volumes onto the surface (two sides) of each fillet using a micropipette, to achieve final 0.1%, 0.3%, and 0.5% (v/wt) concentrations of essential oil. In all treatments (given below), the antimicrobials were massaged onto the product using gloved fingers (to avoid cross-contamination of samples and also transmission of food poisoning organisms) to obtain even distribution of the oil. The following fish samples were prepared: control salted samples (A), salted samples with 0.1% of thyme oil (B), salted samples with 0.3% of thyme oil (C), and salted samples with 0.5% of thyme oil (D). The samples were placed in plastic bags and coating stretch film. The samples were analysed microbiologically, chemically, and sensually on days 0, 3, 6, 9, 12, 15, 18, and 21 of refrigerated storage. Three experiments were carried out and two fish samples from each group were analysed.

Microbiological analysis. Twenty-five grams of trout fillet were aseptically weighed and homogenised in a stomacher (Lab Blender 400) for 2 min with 225 ml of sterile peptone water (0.1% peptone, Merck). Further decimal dilutions were made with the same diluent. For total viable counts (TVC), Plate Count Agar (Merck, 110661) was used. Plates were incubated at 30°C for 24 h. The large colonies with purple haloes were counted (30). For H2S-producing bacteria enumeration, a 1.0 ml sample was inoculated into 10 ml of molten (45°C) Sulfite Iron Agar Base (Merck, 110864). After setting, a 10 ml overlay of molten medium was added, incubated at 37°C for 2-3 d. Black colonies formed by the production of H2S were counted as H2S-producing bacteria. Three replicates of at least three appropriate dilutions, depending on the sampling day, were enumerated. Microbiological data were transformed into logarithms of the number of colony forming units (cfu/g) (20, 21).

Chemical analysis. Total volatile basic nitrogen (TVBN) content was determined according to the method of Malle and Poumeryol (28). The TBA value was determined according to the method of AOAC (2) and milligrams of malondialdehyde (MDA)/kg flesh.

Colour measurement. The colour was measured using a TMI 68-50 Brightness & Color Meter and expressed as colour L* (lightness), a* (redness), and b* (yellowness) values. The results reported (L*, a*, b*) are the mean of ten determinations.

Sensory evaluation. For sensory analysis, fish samples were cooked individually in a microwave oven at full power (1,600 W) for 5 min, including defrosting time, and immediately presented to the panelists. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature, and humidity. The samples were tested by eight panelists in small aluminum trays. The panelists were selected and trained according to ISO standards (ISO 8586-1 1993). The quality of each sample was classified using characteristics to describe the texture, taste, colour, odour, appearance, and overall acceptance. A hedonic scale from 1 to 5 was used to evaluate fish samples: 1 - very bad, 2 - bad, 3 - normal, 4 - good, and 5 - very good (23).

Statistical analysis. Analysis of the data was conducted using Statistical Analysis System (SAS) package programme. Values between groups and within group - between days were compared. Data were subjected to variance analysis in accordance with 3 x 11 x 3 x 1 factorial design and in terms of fix effects and inter-variable interactions so that “repetition number x sampling time x test groups x number of samples examined at one instance from each test group”. According to General Linear Model procedure, Fisher’s smallest squares average (LSD) test was used. Standard deviation figures of all averages were calculated (1). P<0.05 was considered as statistically significant.

Results

Results of microbiological examinations of salted samples during storage with and without thyme oil are shown in Figs 1 a-e. The initial TVC value
determined for all samples was 2.2 log cfu/g. This value exceed 8 log cfu/g in samples from group A and B by the end of storage. D and B group samples did not exceed the value by the ICMSF (20). *Pseudomonas* sp. for trout samples was 2.7 log cfu/g (Fig. 1. b) on the initial day. Pseudomonads reached counts of 7.39 log cfu/g on day 21. *Pseudomonas* sp. population was significantly (P<0.05) lower for samples from B, C, and D groups compared to A group between days 9 and 21 of storage. H$_2$S producing bacteria counts were initially 1.8 log cfu/g while A and B group samples reached 8.97 log cfu/g and 8.38 log cfu/g on day 12, respectively. These counts were significantly (P<0.05) lower in groups C and D than in A and B groups during the storage period, whereas D group showed the lowest counts, as compared to the other groups. The samples from C and D groups were characterised by a significantly (P<0.05) lower LAB and *Enterobacteriaceae* populations on final day 21 of storage period (Figs 1 d-e).

TVB-N values are shown in Fig. 2a. The initial TVB-N value was 12.56 mg/100 g. The TVB-N value of samples untreated and treated with thyme essential oil were 38.43, 30.07, 25.13, and 21.01 mg/100 g by the end of the storage period. Changes in TBA values for A, B, C, and D samples during storage are shown in Fig. 2a. As seen from the figure, changes in TBA values for samples from all groups were variable.

The initial colour was bright pink (data not shown). Colour parameters (L*, a*, and b*) in samples containing the thyme oil were not affected during storage. Changes in colour parameters were statistically insignificant (P>0.05) in all samples.

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**Fig. 1.** Changes (log cfu/g) in total viable counts (a); *Pseudomonas* sp. (b); H$_2$S-producing bacteria (c); LAB (d), and *Enterobacteriaceae* (e) in rainbow trout fillets: salted (A), salted with 0.1% of thyme oil (B), salted with 0.3% of thyme oil (C), and salted with 0.5% of thyme oil (D). Each point is the mean of three samples taken from two replicate experiments (n: 3 x 2: 6). Error bars show SD.
Fig. 2. Changes in TBA (a) and total volatile basic nitrogen (b) values of rainbow trout fillets: salted (A), salted with 0.1% of thyme oil (B), salted with 0.3% of thyme oil (C), and salted with 0.5% of thyme oil (D). Each point is the mean of three samples taken from two replicate experiments (n: 3 x 2: 6). Error bars show SD.

Table 1

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Each value is the mean of three samples taken from two replicate experiments; a, b - means within a column lacking a common superscript letter are different (P<0.05); z, y - means within a row lacking a common superscript letter are different (P<0.05). * - not analysed. A - salted, B - salted with 0.1% of thyme oil, C - salted with 0.3% of thyme oil, D - salted with 0.5% of thyme oil.

The results of the sensory evaluation (texture, taste, colour, odour, appearance, and overall acceptance) of cooked trout fillet samples are shown in Table 1. Sensory scores of group C samples in terms of all criteria slightly decreased during the storage period. As determined by sensory analysis (overall acceptance attribute), the observed shelf-life of trout fillets was the longest for group D (18 d) followed by C (9 d), B (7 d), and control (A) samples (3 d). It is noteworthy, that the presence of salt and 0.3% of thyme oil in cooked C samples produced a distinct but sensually acceptable pleasant odour. The higher concentration (0.5%) of thyme oil imparted both a bitter taste and a strong odour to the samples, resulting in the rejection of the trout.
fillets. A significant difference (P<0.05) was found between samples C and D for odour, taste, and overall acceptance.

**Discussion**

Low initial (day 0) TVC (Fig. 1a) value (2.22 log cfu/g) is in agreement with the results by Özoğul and Özoğul (33) and Pyrgotou et al. (34) for rainbow trout, whole uncut rainbow trout (9), wild brown and farmed rainbow trout (17), and rainbow trout fillets (3, 13). TVC is an important criterion for quality evaluation. Maximum recommended bacterial count for acceptable quality products is 7 log cfu/g (20). The trout samples from A and B group exceeded the value of 7 log cfu/g for TVC on days 12 and 15 of storage, respectively, while C and D group samples did not reach this value throughout the 21 d storage period. C and D samples had significantly lower TVC count compared to A and B samples between days 7 and 12 of storage. The combination of salt and thyme oil resulted in extension of the microbiological shelf-life to 21 d (D group). This was attributed to the antimicrobial effects of both compounds, especially phenolic components, carvacrol and thymol, showing antimicrobial activity (6). Mahmoud et al. (26) found that dipping carp fillets in carvacrol/thymol solution (1%) not only reduced the initial TVC counts but also extended the shelf-life from 4 d to at least 12 d at 5°C.

Giatrakou et al. (16) found that the combined use of MAP and oregano oil (0.1%) prolonged the storage period of sword fish fillets by 8 d, as established by sensory and microbiological analysis. Pyrgotou et al. (34) reported that MAP, salt, and oregano oil resulted in extension of the microbiological shelf-life of fish samples. In another study, the combination of VP, salt, and oregano oil resulted in a microbiological shelf-life extension to 18 d (13). In the presented study, thyme oil can be considered as an effective inhibitor of the total aerobic flora. *Pseudomonas* sp. was 2.7 log cfu/g (Fig. 1b) on day 0 of storage. Frangos et al. (13) found that initial count of *Pseudomonas* sp was 2.5 log cfu/g. Chouliara et al. (10) reported that *Pseudomonas* sp. count for salted and irradiated sea bream fillets stored under VP for 32 d at 4°C was 7 log cfu/g. B, C, and D treatments examined in this study significantly affected growth of *Pseudomonas* sp., producing final counts of ca 6 logs, which was also reported by Skandamis and Nychas (37) for minced beef treated with thyme oil and stored under MAP. Skandamis et al. (38) found that the pseudomonads were the most resistant spoilage flora to oregano oil.

Counts of H₂S producing bacteria have been used as spoilage indicators of seafood products (39). In our study, the samples from groups A and B reached 8.97 and 8.38 log cfu/g on day 12, respectively. Similar initial counts of H₂S producing bacteria have been reported for trout fillets with oregano oil (34). Frangos et al. (13), reported that in salted and aerobically packed trout samples, H₂S producing bacteria (about 8-9 log) were detected on day 9 of storage. In the presented study, H₂S producing bacteria (about 9 log) were detected on day 12 of storage.

According to the literature, LAB and *Enterobacteriaceae*, both being facultative anaerobic bacteria species, were found to be a significant part of the microbial flora of trout fillets. The combined effect of thyme oil and salt did not inhibit the growth of LAB. Tassou et al. (40) observed that the addition of olive oil/lemon juice/oregano oil on cold fresh fish fillets, under MAP, reduced the final LAB counts, only by 0.5 log cfu/g, as compared to the control. In another study, a low number of LAB was found in the microbial flora of refrigerated fish, because of the storage under various MAP conditions (39). Recently, Kykkidou et al. (24) observed that the combined use of thyme oil and MAP did not significantly affect LAB population in swordfish fillets. The limited action of essential oils is attributed to the high tolerance of LAB to the action of the oils, due to their ability to generate ATP and to deal with conditions of osmotic stress (6). It is also possible that the greater resistance of the LAB is related to their better ability to deal with conditions of osmotic stress and respond more effectively to K⁺ efflux caused by many of these antimicrobials. Pygotou et al. (34), reported lower LAB counts for group with oregano oil, as compared to salted group on storage day 21 (salted, 0.2% and 0.4% oregano oil were approximately 8.2, 5.7, and 5.6 log CFU/g, respectively).

It was reported that essential oil inhibited the growth of bacteria (6). Lower counts of *Enterobacteriaceae* for A, B, C, and D trout samples (5.7, 5.4, 4.9, and 3.7 log cfu/g, respectively), were observed probably due to the action of salt and antimicrobial effect of the thyme oil (Fig. 1e). The combined use of salt and thyme oil (0.5%) inhibited the growth of *Enterobacteriaceae* for the entire storage period, as their population remained below 4 log cfu/g. It was also reported that the treatment of fresh sea bream fillets with a mixture of olive oil, lemon, and oregano oil decreased *Enterobacteriaceae* counts by approximately 2.5 log cfu/g by the end of the storage period, as compared to the control (40).

The TVBN as an index for determination of degree of spoilage by bacteria and endogenous enzymes, has been used for the quality control of many fish species (14). At the beginning of storage, the TVB-N value of 12.56 mg/100 g is in quite good agreement with findings of Chytri et al. (9) and Neratzaki et al. (31) for rainbow trout samples. The TVBN values for the samples from groups A, B, C, and D were 38.43, 30.07, 25.13 and 21.01 mg/100 g, respectively, at the end of the storage period. An upper acceptable limit of TVBN of 30-35 mg N/100 for fresh fish was suggested by Huss (19). On the basis of this limit, for the initiation of fresh rainbow trout spoilage, samples A exceeded the proposed limit by days 15, 18, and 21. Among the treatments applied in the presented study, C and D produced significantly lower TVB-N values as compared to the A and B samples after day 7 and until the end of the storage period. Pyrgotau et al. (34)
reported lower TVBN values for trout treated with oregano oil, as compared to control on final day. Similarly, Frangos et al. (13) reported lower TVBN values, ca 40 mg N/100 g, for essential oil groups as compared to control in refrigerated trout fillets. Mahmoud et al. (26) reported a TVBN value of 30 mg N/100 g after 12 d of storage at 5°C, after dipping carp fillets in a solution of 0.5% carvacrol and thymol, whereas the control reached this value after 4 d. Furthermore, Goulas and Kontominas (18) reported that the combination of light salting, MAP, and oregano essential oil (0.4%-0.8%) extended the shelf-life of fresh sea bream by ca 11–18 d.

TBA values are commonly used to measure the level of rancidity and are mainly related to development of secondary oxidation products (18). The lipid oxidation is evidenced by measuring malondialdehyde (MDA), which are the initial reaction products of polyunsaturated fatty acids with oxygen (5, 35). The initial TBA index value for the trout fillets was 1.34 mg MDA/kg and was in agreement with Yanar (43) and Cakli et al. (11), who reported a TBA index value for hot smoked tilapia and rainbow trouts of 0.84 and 1.4 mg MDA/kg. This result is caused by the use of a different method. The TBA values for samples from all groups were variable. The use of thyme oil to protect meat against oxidation has been reported in the literature. Mariutti et al. (29) and Erkan and Bilen (15) observed that garlic and thyme oils were effective means of controlling lipid oxidation in chicken and fish meat, as reflected in thiobarbituric acid reactive substance values. Similar results have been obtained in the literature on the effective antioxidant activity of essential oils in mackerel (41), carp (27), and sea bream (18).

In conclusion, the combination of salting, thyme oil, and air packaging led to production of a good quality fresh fish product, which could be alternative, reliable, easy, and cheap food in Turkey. Salting process of trout fillets cannot provide storage for a long time. Additional use of thyme oil inhibits growth of bacteria and improves the taste and flavour. On the basis of sensory, chemical, and microbiological analyses, shelf-life of the fish product was 21 d.

Reference

17. Gonzalez C.J.: Bacterial microflora of wild brown trout (Salmo trutta), wild pike (Esox lucius) and aquacultured rainbow trout (Oncorhynchus mykiss). J Food Protect 1999, 62, 1270–1277.