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EFFECT OF THYME AND ROSEMARY ESSENTIAL OILS ON THE SHELF LIFE OF MARINATED RAINBOW TROUT

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ABSTRACT

The present study aimed to investigate the effect of essential oils (EO) (thyme and rosemary) on the quality of marinated rainbow trout during storage at 4ºC. Three different treatment were tested: C1; (control samples), C2; (marinating with 1% [v/w] rosemary EO added) and C3 (marinating with 1% [v/w] thyme EO added). Fillets were subjected to microbiological (total aerobic mesophilic bacteria, psychrotrophic bacteria, lactic acid bacteria, yeast and mould), chemical (pH, thiobarbituric acid reactive substances-TBARS, total volatile base nitrogen-TVB-N) analyses and sensory quality testing throughout the storage period. Difference in microbiological and chemical changes between samples was found to be significant ($p<0.05$) during storage period. In conclusion, essential oils treatment to marinated had positive effect on shelf life rainbow trout, especially thyme oil showed more positive effect when compared with rosemary oil.

Key words: Rainbow trout, marinating, essential oil, shelf life, antimicrobial activity.

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is the widespread cultured fish species all over the world and has major economic importance in Turkey. Rainbow trout is one of the most widely farmed species with a total production in Turkey of 114.569 tons in 2012 (Australian Fisheries Statistics 2012; TUIK 2013). In Turkey, they are generally marketed fresh, chilled, frozen or smoked. Dressing or splitting the fish, and processing by curing, burger, döner production and/or marinating will enable the fish industry to put a number of desirable, new products on the market (Erkan 2003; Ozden and Erkan 2006). Microorganisms can grow in the muscle tissue of aquatic products due to their high nutrient content. So, the conservation and handling of aquatic products are very important. One of the processesing method of aquatic products is marinades technology (Serdaroğlu and Felekoğlu 2005; Duman *et al.* 2012). Marinated fish are typically inspired as ready-to-eat products with no heat treatment (Ibrahim Sallam *et al.* 2007; Duyar and Eke 2009). Marinades are solutions, including sugar, spices, oil, acids (from vinegar, fruit juice, wine) and they are used to improve tenderness, juiciness, flavour and aroma and to extend shelf life of meat, poultry, seafood and vegetables (Duman *et al.* 2012; Cadun *et al.* 2008). Marinades are semipreserves; acid, usually acetic acid and salt are added to the fish to retard the action of bacteria and enzymes. The preserving principal is the combination of acetic acid and salt. The aim is not only to prevent microorganism growth. Marination is also used to tenderise or to change taste, textural and structural properties of raw material (Gokoglu *et al.* 2004; Ibrahim Sallam *et al.* 2007). Some additives can be used during marination in order to improve taste and aroma. If these additives are antibacterial and antioxidant, the organoleptic characteristics of the product will be improved and its preservation period will be extended. With this purpose usage of natural essence oils are being widely used today (Can 2011; Can and Ersan 2013). Essential oils has proven to be an effective preservation method for the extension of shel-life of fresh (Harpaz *et al.* 2003; Giatrakou *et al.* 2008; Quitral *et al.* 2009; Emir Coban *et al.* 2012; Erkan 2012). Essential oils (EOs) are aromatic, oily liquids that are obtained from plant material. Extracts from oregano, thyme, rosemary, clove, sage and mint are among the EOs used to improve the sensory characteristics and extend the shelf life of foods. A number of EOs and some of their components have been reported to have antimicrobial activity against a wide range of spoilage and pathogenic bacteria (Lambert *et al*. 2001; Burt 2004). Very little information is available in the literature on the effect of essential oils in fish marination. The aim of this study was to determine the effects of thyme and rosemary essential oils on the shelf life of refrigerated (4ºC) marinated rainbow trout fillets by evaluating microbiological, chemical and sensory parameters.

MATERIALS AND METHODS

Preparing Samples: Marinated fish was prepared from rainbow trout (*O. mykiss*). Rainbow trout (250±25 g) were obtained from the Ataturk University Agricultural College Fisheries Department's rainbow trout breeding and research center. The fishes were individually beheaded, gutted, filleted and washed with tap water (Robb *et al.* 2002). The treatments included the

following: C1 (control samples), C2 (marinating with 1% [v/w] rosemary EO added) and C3 (marinating with 1% [v/w] thyme EO added).

Marination process: The fillets were dipped in 4 % acetic acid, 10 % salt marination solutions at a ratio of 1: 1.5 (fish: marination solution, w/w) for 48 h. This marination process was performed at 4±1°C. After marination, the fish were drained at ambient temperature (18±1°C) for 15 min (Ozden and Erkan 2006). The marinated fishes were divided into three groups. Two hundred grams of marinated rainbow trout fillets were packed into glass jars. Thyme and rosemary essential oils were added to two lots of the filleted samples in appropriate volumes to cover both sides of each fillet using a micropipette to achieve final concentrations of 1% (v/wt) EO. After the application of essential oils and the marinated treatments were complete, the rainbow trout fillets were stored under refrigeration (4±1ºC) and were subjected to microbiological (total aerobic mesophilic bacteria, psychrotrophic bacteria, lactic acid bacteria, yeast and mould), chemical (pH, thiobarbituric acid reactive substances-TBARS and total volatile base nitrogen-TVB-N) and sensory analysis (colour, odour, taste and general acceptance). The microbiological, chemical and sensory analysis were performed at 0, 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 days of storage.

Microbiological analysis: A sample (25 g) was taken from each fillet, transferred aseptically to a stomacher bag containing 225 mL of 0.1% peptone water and was homogenized for 60 s using a Stomacher blender (Lab Stomacher Blender 400-BA 7021 Seward Medical, England) at room temperature. For microbial analyses, 0.1 mL samples of serial dilutions (1:10, diluent: 0.1% peptone water) were inoculated on agar plates. The total mesophilic aerobic bacteria (TMAB) and total psychrotrophic aerobic bacteria (TPAB) numbers were determined using plate count agar (PCA Merck 1.05463.0500) plates that were incubated at 30ºC for 2 days or at 10ºC for 7 days, respectively. The number of lactic acid bacteria (LAB) was determined using Man Rogosa Sharpe agar (MRS, de Man, Rogosa Sharpe Agar Oxoid CM0361) plates that were incubated at 30°C for 2 days. The number of yeast and mould was determined using RBC (Rose Bengal Chloramphenicol) agar (Merck 1.00467.0500) plates that were incubated at 25°C for 5 days.

Chemical analysis: The total volatile base nitrogen (TVB-N) content was determined using the method reported by Malle and Tao (1987). The TVB-N contents were expressed as mg 100 g^{-1} of fish muscle. The thiobarbituric acid-reactive substance (TBARS) content was determined as reported by Lemon (1975) and Kilic and Richards (2003). The TBARS content was expressed as µmol of malondialdehyde (MDA) kg^{-1} of fish muscle. The pH was determined according to the method of Gokalp *et al.* (2001).

Sensory Evaluation: Sensory analysis of rainbow trout marinades was performed by a panel of five experienced panelists (Fernández-Fernández *et al.* 2002). The quality of the rainbow trout fillets was assessed based on the colour, odour, taste and general acceptance characteristics using a five-point descriptive scale. The scores 5, 4, 3, 2 & 1 indicate “very good”, “good”, “normal”, “bad” and “very bad” quality, respectively. All of the samples were stored at 4 °C until the sensory analysis were performed.

Statistical analysis: The experimental design consisted of completely randomised design in a factorial arrangement: three treatments of rainbow trout (C1, C2 and C3), eleven storage time (0, 15, 30, 45, 60, 75, 90, 105, 120, 135 and 150 days) and two replicates. All statistical calculations were performed using SAS Statistical Software (SPSS 17.0; Chicago, IL, USA) (1998). Duncan’s multiple range tests and variance analysis were used to evaluate the significance level ($p<0.05$) for statistical differences.

RESULTS AND DISCUSSION

Microbiological changes: The changes in the TMAB content of the refrigerated marinade samples during the storage period are shown in Fig. 1a. Microbiological counts in fillets were found to be significant ($p<0.05$) during storage time. On the initial day (day 0), the TMAB content (Fig. 1a) of the marinade samples was 2.0 log CFU g^{-1}. The TMAB contents of C1 rainbow trout fillets exceeded 7 log CFU g^{-1}, which is considered the upper limit for acceptable TMAB levels in fresh marine species (ICMSF 1992) on days 150 of storage. This limit was not exceeded throughout storage in C2 and C3. At the end of the 150 day storage period, the TMAB levels in C1, C2 and C3 had reached up to 10.36, 7.04 and 6.10 log CFU g^{-1}, respectively. Initially (day 0), psychrotrophic bacteria content (Fig. 1b) of the marinated rainbow trout was 2.0 log CFU g^{-1}. The psychrotrophic bacteria levels in C1 rainbow trout fillets exceeded 7 log CFU g^{-1}, which is considered the upper limit of acceptability of psychrotrophic bacteria in fresh marine species (ICMSF 1992), on days 150 of storage. This limit was not exceeded throughout storage in C2 and C3. At the end of the storage period, the levels of psychrotrophic bacteria in C1, C2 and C3 had reached 10.63, 7.21 and 6.19 log CFU g^{-1}, respectively. Because the control group developed higher levels of total aerobic mesophilic bacteria and psychrotrophic bacteria during storage, thyme and rosemary EO’s were observed to have an inhibitory effect on microbe growth. Microbial growth and lipid oxidation are factors important to shelf-life and consequently to consumer acceptance of fresh and processsed meat (Erkan *et al.* 2011). Control group

showed the higher bacteria count than essential oils applied group. These results showed thyme and rosemary EO having the greatest antimicrobial activity. A number of EOs and some of their components have been reported to have antimicrobial activity against a wide range of spoilage and pathogenic bacteria (Lambert *et al.* 2001; Burt 2004). Thyme contains high concentrations of phenolic compounds including carvacrol, thymol, p-cymene and γ-terpinene (Komaki *et al.* 2015). The thyme and rosemary oils can be considered effectively inhibitory on the total aerobic flora. Similar results were observed by several researchers (Cadun *et al.* 2008; Duman *et al.* 2012; Can and Ersan 2013). Cadun *et al.* (2008), reported that they detected lower TMAB in rosemary group compared to the control group in marination of rosemary-added pink deep water shrimps. Duman *et al.* (2012) found that thyme and rosemary essential oils reduced the growth of total aerobic and psychrotrophic bacteria.

The initial LAB content (Fig. 1c) on day 0 was 2.0 log CFU g^{-1}. At the end of the storage period, the LAB contents of C1, C2 and C3 were 8.12, 6.47 and 5.54 log CFU g^{-1}, respectively. In this study the number of LAB was found lower in experimental groups compared to the control group. Lower LAB count was found for experimental groups compared to control group. Similar LAB were reported for marinated fish by Kilinc and Cakli (2004), Cadun *et al.* (2005). Viuda-Martos *et al.* (2008), stated that the most effective one on LAB was rosemary oil. Can and Ersan (2013) observed that rosemary oil treatment weren't reduced the growth of LAB.

The yeast and mould levels (Fig. 1d) were 2.0 log CFU g^{-1} on day 0. At the end of the storage period, of C1, C2 and C3 were 6.19, 4.94 and 3.95 log CFU g^{-1}, yeast and mould, respectively. The highest yeast and mould count control groups, as compared with essential oil-treated groups. These results showed the antifungal activity of essential oils. Several researchers have observed similar results (Kilinc and Cakli 2004; Duman *et al.* 2012; Can and Ersan 2013). Cadun *et al.* (2008), reported that rosemary oil was effective on yeast and mould. Duman *et al.* (2012) observed that thyme and rosemary treatments reduced yeast-mould counts. Their findings are similar to the findings of this paper.

Chemical changes

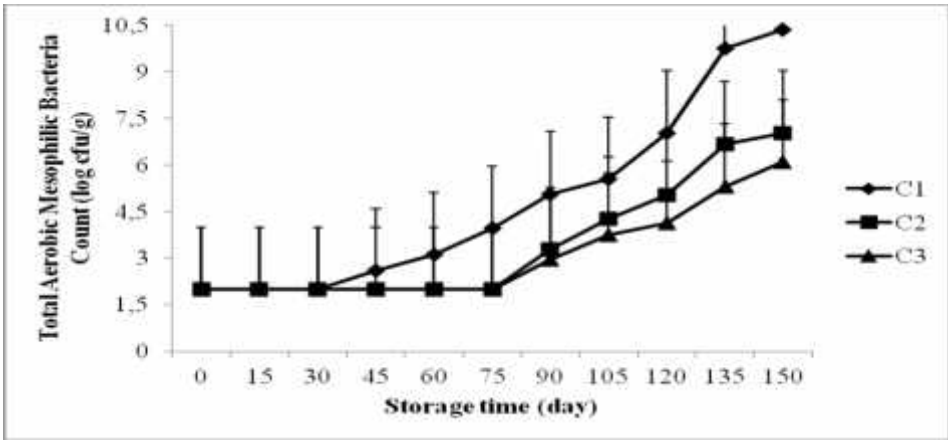
TVB-Nitrogen: The amount of TVB-N is an important criterion for determining the freshness of fish and fish products (Kose and Koral 2005). Total volatile basic nitrogen (TVB-N) is a product of bacterial spoilage, and the activity of endogenous enzymes and TVB-N levels are often used as an index to assess the quality and shelf life of products (Ruiz-Capıllas and Moral 2005; Ozogul *et al.* 2006; Ucak *et al.* 2011). TVB-N values of the marinated trout (Fig. 2a) were 6.35, 5.58 and 4.11 mg 100 g^{-1} initially and were 20.67, 18.65 and 17.08 mg 100 g^{-1} at the end of the storage period for C1, C2 and C3, respectively. All samples were not exceeded the limit (25 mg 100 g^{-1}) for rainbow trout (Robb *et al.* 2002) at the end of the storage period. The TVB-N values of all of the groups increased during the storage period. The highest TVB-N values were determined in control groups, while the lowest in essential-treated groups. These increases in the TVB-N value can be explained by proteolysis driven by the enzymatic and microbial activity of the samples. Values similar to our TVB-N data have been reported for marinated fish (Ozden and Baygar 2003; Gokoglu *et al.* 2004; Kilinc and Cakli 2005; Can and Ersan 2013; Topuz *et al.* 2014).

Lipid oxidation: Thiobarbituric acid (TBA) is a widely used indicator for the assessment of degree of lipid oxidation (Jeon *et al.* 2002; Ibrahim Sallam 2007; Ojagh *et al.* 2010). The consumability limit value of the TBA content was between 7 and 8 mg MDA kg^{-1} In food suitable for consumption, the TBA values might reach the upper limit of 7 to 8 mg of MDA kg^{-1} (Emir Coban and Ozpolat 2013); in "perfect material," the TBA value should be less than 3 mg of MDA/kg, and in "good material," the TBA value should be no more than 5 mg of MDA kg^{-1}. The TBA values indicate the degree of rancidity of products, and values greater than 3-4 mg of MDA kg^{-1} indicate a loss of product quality (Frangos *et al.* 2010). The TBARS values of the rainbow trout fillets (Fig. 2b) were 2.8, 2.0 and 1.86 µmol MDA kg^{-1} initially and were 13.93, 11.97 and 10.88 µmol kg^{-1} at the end of the storage period for C1, C2 and C3, respectively. TBARS values increased in the duration of storage time in all groups. The lowest TBARS values were obtained from the group treated with essential oils. These results showed the antioxidant characteristics of essential oils. A similar pattern of the increase in TBARS has been reported in marinated sardine (Kilinc and Cakli 2004); pink shrimp (Cadun *et al.* 2005); anchovy (Gokoglu *et al.* 2009) and rainbow trout (Can ve Ersan 2013).

pH: The pH of fresh fish flesh is approximately neutral. In the post-mortem period, the decomposition of nitrogenous compounds leads to an increase in the pH of the fish flesh, which indicates a loss of quality (Can 2011). The pH values of the samples (Fig. 2c) were 4.47, 4.35 and 4.23 initially and 4.08, 4.02 and 4.00 at the end of the storage period for C1, C2 and C3, respectively. pH values of marinated samples decrease after marination process. Marinades have a low pH due to acetic acid content. During the storage of marinades, heterofermentative lactic acid bacteria can grow and cause the amino acids to degrade. Thus, the formation of carbon dioxide and other decarboxylation products is observed. Due to these degradation products the pH of marinade increases (Topuz *et al.* 2014). Similar findings were reported by other researchers (Ozden and Erkan

2006; Gokoglu *et al.* 2009; Duman *et al.* 2012; Topuz *et al.* 2014).

Sensory changes: The results of the sensory evaluation (colour, odour, taste and general acceptance) of the marinated rainbow trout samples are presented in Fig. 3a-d. A significant difference ($p<0.05$) was found to be between the samples for colour, odour, taste and general acceptance during storage time. In terms of sensorial evaluation, panellists gave the highest scores especially to C3 group. These results showed that samples with a high level of thyme oil had acceptable overall scores due to the effect of thyme on limiting microbiological activity and decreasing the TVB-N value as well as the positive attributes of its flavor. These conclusions were supported by the results from microbiological and chemical quality analyses. Similar results have been reported in other recent studies (Ozpolat *et al.* 2010; Duman *et al.* 2012).



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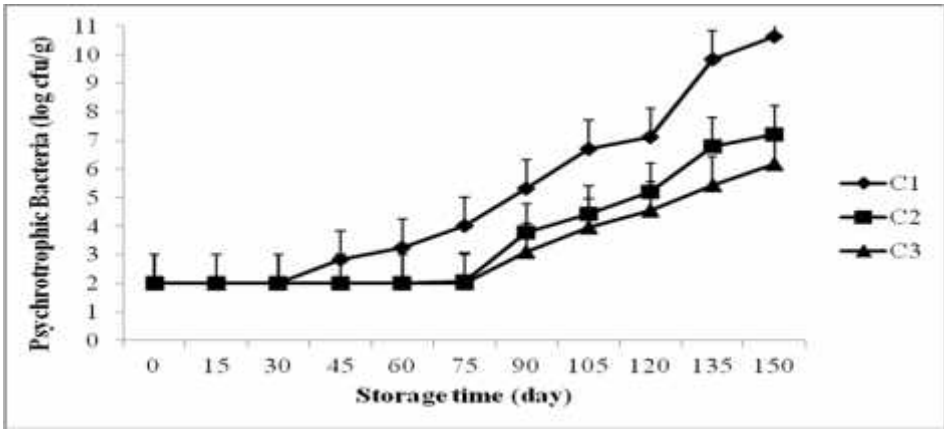

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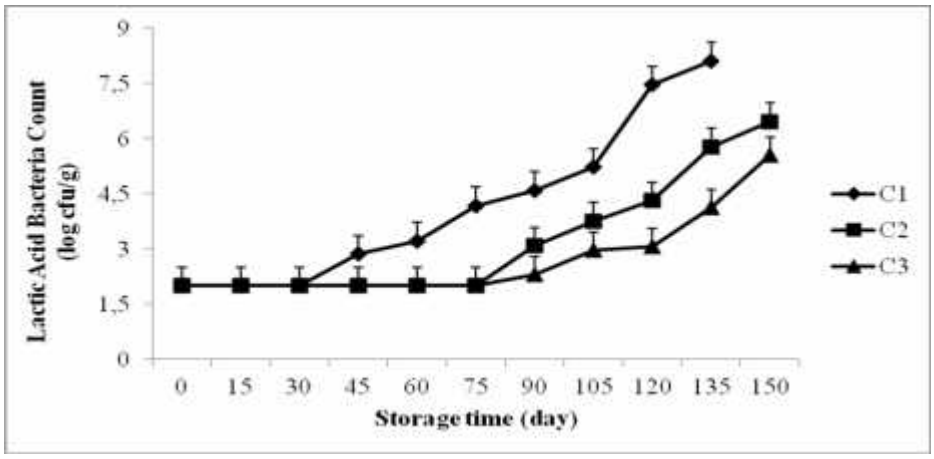

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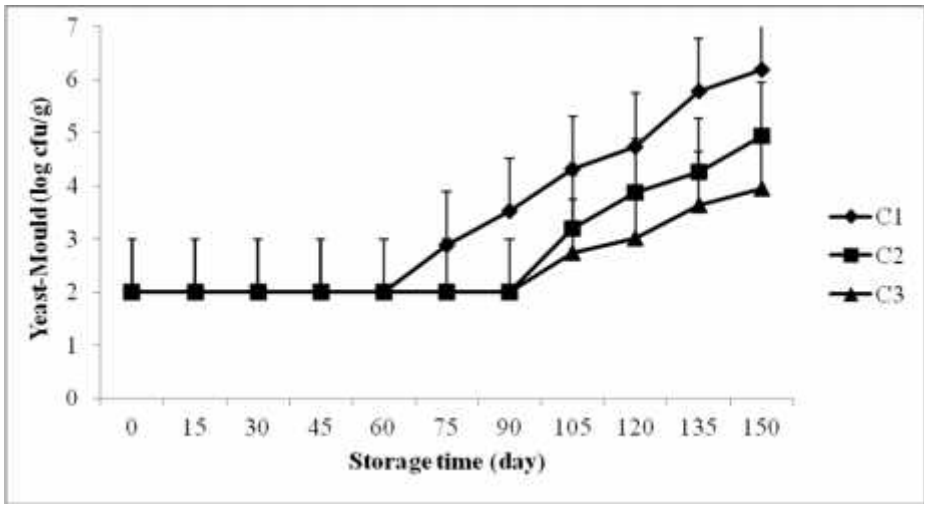
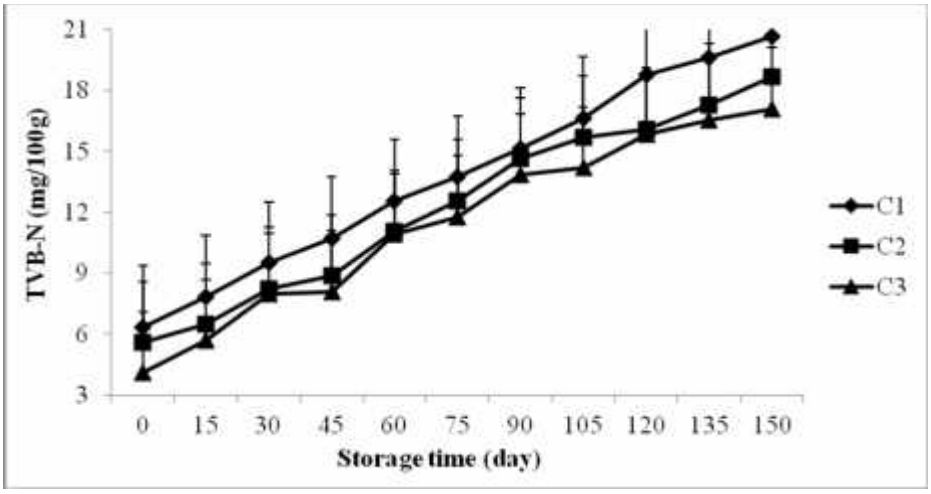


d

Figure-1. Total aerobic mesophilic bacteria (TMAB) counts (a), psychrotrophic bacteria (b), lactic acid bacteria counts (c), and yeast and mould (d) changes of treatment with rosemary and thyme essential oils (1 v/w) marinated rainbow trout fillets during cold storage.



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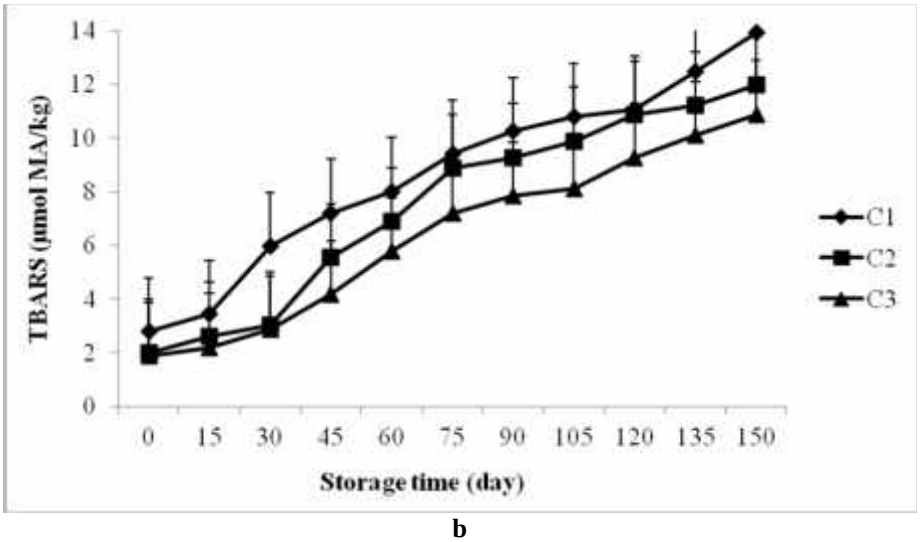

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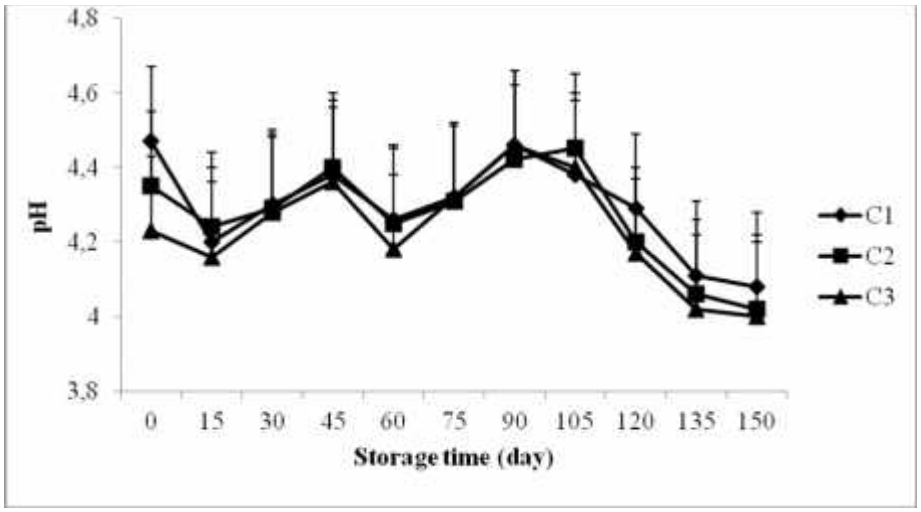
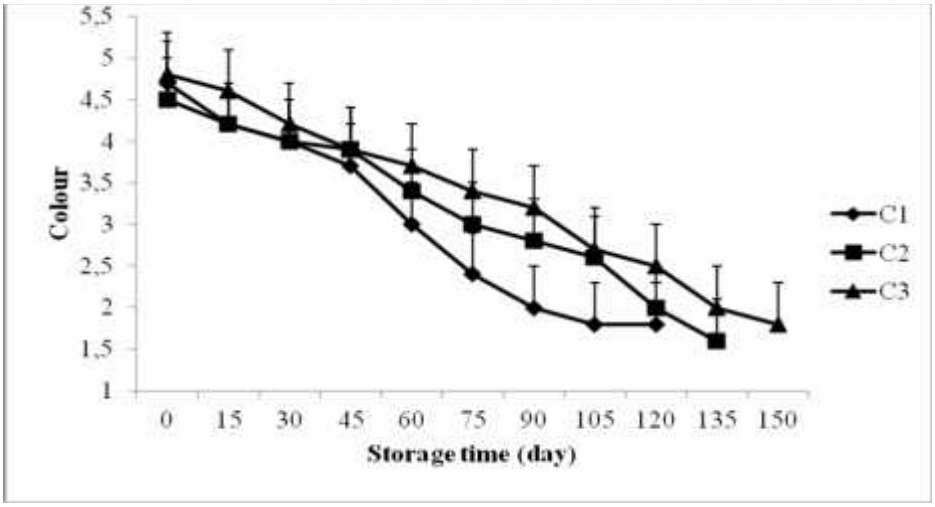


c

Figure-2. TVB-N (a), TBARS (b), pH (c) changes of treatment with rosemary and thyme essential oils (1 v/w) marinated rainbow trout fillets during cold storage.



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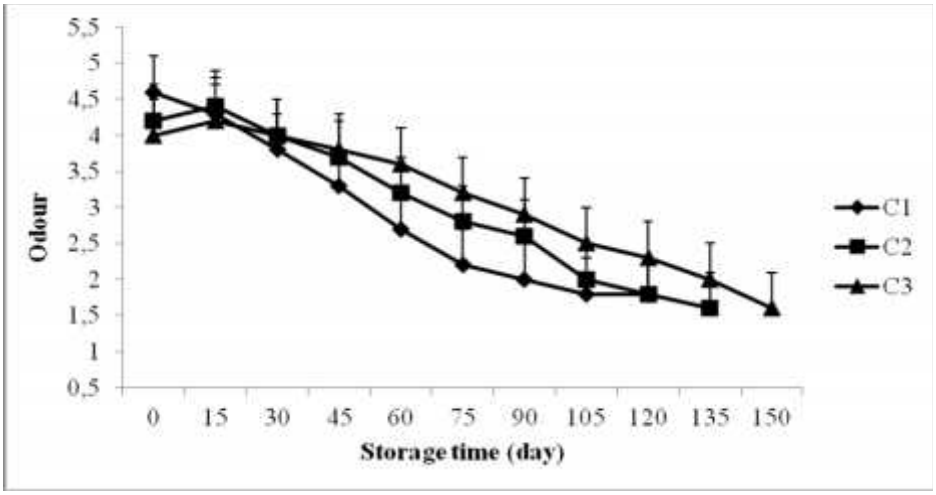

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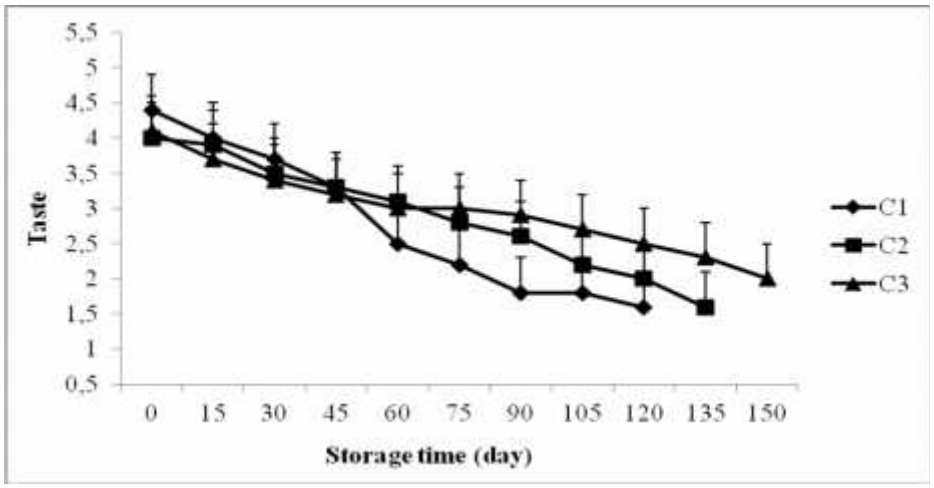

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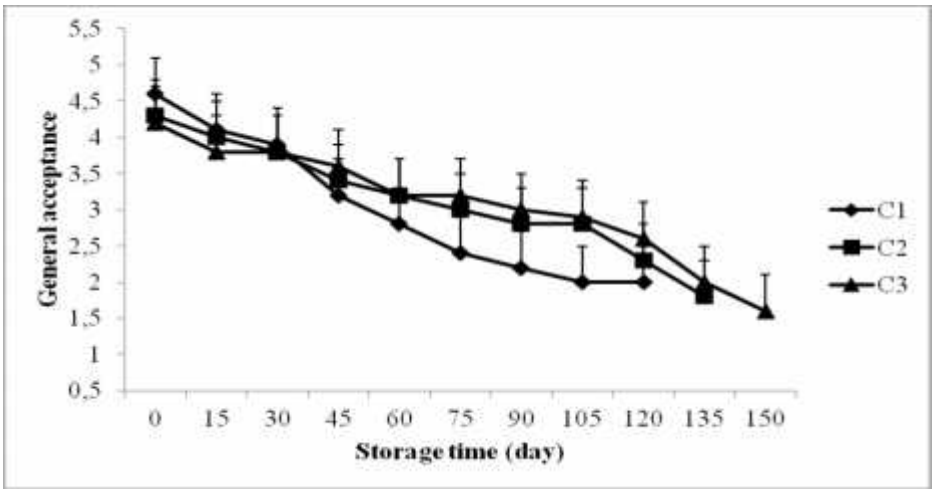

d

Figure-3. Colour (a), odour (b), taste (c), general aacceptance (d) changes of treatment with rosemary and thyme essential oils (1 v/w) marinated rainbow trout fillets during cold storage.

Conclusion: In conclusion, essential oils treatment to marinated rainbow trout were showed positive effect on the shelf life of, especially clove oil showed more positive effect when compared with thyme oil. In the present study, usage of these EOs in marinated fish showed that EOs have a positive effect on the product's chemical, microbial and sensory attributes. Overall, the use of thyme EO (1%, v/w) showed a synergistic effect, extending the shelf life of rainbow trout fillets. According to the results of TMAB analyses, shelf life of rainbow trout fillets was estimated as 120 and 150 days for C1 and C2 respectively. C3 sample did not exceed 7 log CFU/g after 150 days of storage.

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