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EFFECTS OF ROSEMARY AND THYME OILS ON SHELF LİFE OF MARINATED SAUCE CRAYFISH

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ABSTRACT

The natural preservative effect of rosemary and thyme essential oils on marinated crayfish (*Astacus leptodactylus* Esch., 1823) stored at 4 ºC was investigated. Microbiological, chemical and sensory analyses were performed to monitor the changes in quality and to determine the shelf life of marinated crayfish. Microbiological results showed that natural compounds from rosemary and thyme essential oils resulted in lower bacterial growth in marinated crayfish during the chilled storage period. The total volatile basic nitrogen (TVB-N) and the thiobarbituric acid (TBA) values were observed to increase in all groups during the storage period. As for the TBA value, the difference between Group C (Control, without essential oils), Group R (with rosemary oil), and Group T (with thyme oil) was significant ($p < 0.05$). The shelf life of marinated crayfish was found to be 42 days for Group C, 56 days for Group R, and 70 days for Group T according to the results of sensory and microbiological assessments.

Keywords: *Astacus leptodactylus*, marination, rosemary oil, thyme oil, shelf life

INTRODUCTION

Astacus leptodactylus is naturally and widely distributed in rivers lakes and ponds throughout of Turkey. Its distribution area has considerably expanded in Turkey after 1985 because of its commercial importance and declined catches from traditional fisheries. The consumption of *A. leptodactylus* has always been very low in Turkey and it had been exported to Western Europe until 1986. However, due to over-fishing, pollution, and disease (crayfish plague), the total production dramatically decreased from 5.000 to 200 tons per year (Harlioğlu, 2004). In recent years, there has been a gradual increase in the production of crayfish in Turkey. Total catch in 2010 was reported to be 1.030 tons (TUIK, 2010).

Marinades are semi-preserves; the preservation is based on acetic acid and salt, whose combined effects inhibit the reproduction of bacteria and activities of enzymes. The aim of marination is not only to prevent microbial growth, but also to tenderize or change the taste, texture, and structural properties of the fish or meat, resulting in a product with a characteristic flavor and an extended but still limited shelf life (McLay, 1972). Although the marination technology is well developed for meat, poultry, and fish industries, no information is yet available in the literature about preservative effects of rosemary and thyme essential oils (EOs) on marinated crayfish (Sallam *et al*., 2007; Cadun *et al*., 2008; Gökoğlu *et al*., 2009).

Increasingly, consumers are demanding more natural, minimally processed products. To satisfy these requirements, one of the major challenges in the food industry consists of reducing conventional chemical additives in food formulation (Sanchez-Gonzalez *et al*., 2011). The increased demand for safe and natural food without chemical preservatives motivates many researchers to investigate the antimicrobial effects of natural compounds. Numerous investigations have confirmed the antimicrobial action of essential oils (EOs) in modeled as well as actual food systems (Harpaz *et al*., 2003; Serdaroğlu and Felekoğlu, 2005). Recently, rosemary (*Rosmarinus officinalis*) and thyme (*Thymus vulgaris L*.) are used as natural preservatives in foods, but no information is yet available in the literature about the preservative rosemary and thyme essential oils on marinated sauced crayfish Rosemary and thyme plant species native to the Mediterranean area are now widely cultivated as spices in temperate climes. According to Wang *et al*. (2008), rosemary contains a number of important compounds such as 1, 8-cineole (27.23%), α-pinene (19.43%), camphor (14.26%), camphene (11.52%) and β-pinene (6.71%). On the other hand, thyme contains a number of critical compounds such as the phenols: thymol (44–60%) and carvacrol (2.2–4.2%) (Solomakos *et al*., 2008).

In this study we evaluated the preservative effects of rosemary and thyme essential oils on marinated sauced crayfish. Microbiological, chemical, and sensory analyses were conducted to investigate the changes in quality and to determine the shelf life of marinated crayfish in sauce during storage at 4ºC.

MATERIALS AND METHODS

Raw material: We studied crayfish (*Astacus leptodactylus*) which were caught in June 2010 in Cemisgezek Region of Keban Dam Lake, Turkey. Crayfish samples were placed in ice boxes and transferred within 1 h to the laboratory of the Faculty of Fisheries at Firat University. The average weight and length of the crayfish were 59.36 ± 18.84 g and 12.04 ± 0.85 cm, respectively. Approximately 50 kg of crayfish were used for this study. Crayfish were washed with clean water, boiled for 10 min at 100° C, and separated from their shells.

Rosemary and thyme essential oils: Water-soluble Rosemary (Herbalox® Seasoning) and thyme (Aquaresin® Thyme Code:35-06-39) essential oils were purchased (Kalsec, Inc., Kalamazoo, MI).

Marination and sauce process: The preparation process was carried out in a cold room at a temperature of 4 °C. Crayfish were then divided into three batches (~3.5 kg each) and prepared for marination. The first group (C) was marinated by immersing the crayfish in a pre-chilled (4 °C) solution containing a combination of 4% acetic acid (v/v) and 5% NaCl (w/v); the second group (R) was marinated in a pre-chilled solution containing 4% (v/v) acetic acid, 5% (w/v) NaCl and 300 mL/L rosemary. The third group was marinated in a pre-chilled solution containing 4% (v/v) acetic acid, 5% (w/v) NaCl and 300 mL/L thyme. Crayfish and marinade solution were filled into glass containers at a ratio of 1:1.5 (crayfish/ marinade solution; w / v), and the containers were sealed with their lids. The maturation and brining processes were completed after seven days at 4 ± 1 º C.

The marinated crayfish were removed from the solution and put into glass jars. Three groups of marinated crayfish were placed in carrot sauce. Carrot sauce was prepared using olive oil, carrots, tomato paste, onion, salt, bay leaf, and water. The selected ratio of crayfish to carrot sauce was 120 g : 50 g. This ratio was determined in preliminary trials. Packed samples were stored at 4 ±1ºC and analyzed on the 0^{th}, 7^{th}, 14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th}, and 84^{th} days to determine the changes in product quality.

Sauce preparation: 20 ml olive oil, 120 g grated carrots, 30 g tomato paste, 60 g grated onion, 3 g salt, 2 g garlic, 1 bay leaf were used as ingredients. Chopped onions, garlic, and tomato paste were cooked in olive oil until tender. Then grated carrots, salt, bay leaf, and water were added and the sauce was cooked for approximately 15 minutes.

Analyses

Proximate composition: The moisture content of crayfish was determined by drying the meat in an oven at 105 ºC until a constant weight was obtained (AOAC, 1990). Crude protein content was calculated by converting the nitrogen content determined by Kjeldahl's method (6.2 x N). Fat content was determined as described by the AOAC (1990) using the Soxhlet system. Ash content was determined by dry ashing in a furnace at 550 ^{0}C for 4 h (AOAC, 1990).

Microbiological analysis: For all microbiological counts, 10 g of sample was taken and placed into 90 ml 0.1% peptone water (Difco, 0118-17-0) and homogenized. Other decimal dilutions were prepared from the 10^{-1} dilution. Appropriate dilutions of samples were prepared in sterile 0.1% peptone water and plated, in duplicate, on the growth media by using the pour plate method. Plate Count Agar (PCA): 30 ± 2°C for 3 days for total viable count (TVC) and 4 ± 1 °C for 10–14 days for psychrotrophic viable count (PVC). Dichloran Rose Bengal Chloramphenicol Agar (DRBC): 25 ± 2°C for 5 days for yeast and mold count (YM). Man Rogosa Sharpe Agar (MRS): 37 ± 2°C for 2 days for lactic acid bacteria count (LAB). The microbial counts were expressed as log cfu g^{-1} (ICMSF, 1986).

Physical and chemical quality analysis: The pH value was measured using a pH-meter (Thermo Scientific Orion 3-Star Plus). The pH-meter was calibrated using standard pH solutions before each measurement *(*AOAC, 1990). Total Volatile Basic Nitrogen (TVB-N) was determined according to the method of Varlik *et al.* (1993). Thiobarbituric acid value (TBA, mg malonaldehyde/kg) was determined using the spectrophotometric method (Tarladgis *et al*., 1960).

Sensory analysis: The sensory quality of the marinated crayfish was evaluated by five experienced panelists. The samples were heated individually in a microwave oven at full power, for 2 min and immediately presented to the panellists. Panelists scored the products for sensory characteristics such as color, odor, flavor, and general acceptability using a five-point hedonic scale. A score of 5 indicated ''very good'' quality, a score of 4 ''good'' quality, a score of 3 "acceptable", a score of 1-2 denoted as ''spoiled''. (Kurtcan and Gonul, 1987).

Statistical analysis: All data were studied with one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test. Statistical significance level was considered to be α = 0.05. All statistical analyses were carried out using SPSS Version 12.0 (SPSS, IL, USA).

RESULTS AND DISCUSSION

Proximate composition: Proximate compositions of raw and marinated crayfish are given in Table 1. The compositions of the raw crayfish are similar to the findings of other researchers (Gurel and Patir, 2001;

Erkan *et al*., 2009). Moisture content of raw crayfish was determined as 79.15 ± 0.07% but after the marination process, it decreased to 77.70 ± 0.59%, 78.38 ± 1.00%, and 77.91 ± 1.70% in the control, rosemary, and thyme groups, respectively ($p > 0.05$). Fat content of raw crayfish was 2.97 ± 0.23%. After the marination process, fat content increased to 7.98 ± 1.52%, 8.01 ± 0.81%, and 8.03 ± 0.20% in the control, rosemary, and thyme groups, respectively. Protein contents of marinated crayfish also decreased after marination as compared to the protein content of the raw crayfish (Table 1). Significant difference was found between the protein, fat, and ash contents of raw and marinated crayfish ($p < 0.05$). This result can be explained by the effects of sauce.

Microbiological analysis: The results of microbiological analyses are illustrated in Fig 1. Initial contamination of the fresh samples in terms of total viable count (TVC) of bacteria was low (3.75 log cfu/g). After marination, the control showed values of 3.78 log cfu/g on the 1st and 6.70 log cfu/g on the 56th days respectively, and these levels were statistically significantly higher than the other groups (Fig. 1). The TVC in rosemary and thyme samples were still low even after 70 days at 4 ºC. This result can be explained by the effects of rosemary and thyme on total viable counts (Elgayyar *et al*., 2001; Özkan *et al*., 2004; Cadun *et al*., 2008). Significant statistical differences were found between the samples ($p < 0.05$). The maximum microbiological growth had already been reached in the control group by the 56th day. While R and T groups were still found acceptable by the panelists, the control samples were scored as unacceptable for all sensory criteria. Similar maximum TVC were also observed in other studies (Cadun *et al*., 2008; Gutierrez and Barry-Ryan 2009). Ahn *et al*. (2007) and Fan *et al*. (2008) reported that mesophilic aerobic counts in fish treated with plant extracts were lower than those in non-treatment samples during cold storage (-5 ºC ~ 10 ºC). Bacteria in the control group grew more quickly than treated groups, indicating antibacterial effects of rosemary and thyme oils on marinated crayfish. Thyme was less effective than rosemary extract in reducing microbial growth.

Psychotropic pathogens can grow in refrigerated foods and may cause some foodborne diseases. Psychrotrophic bacteria count was 1.65 log cfu/g in the raw crayfish and it decreased to 1.61 log cfu/g in group R and increased to1.94 log cfu/g in group T. On the 28th day, psychrotrophic bacteria count increased to 3.76 log cfu/g in the control, 3.34 log cfu/g in group R, and 3.72 log cfu/g in group T (Fig. 1). These figures are comparable to Schulze (1985). In control group samples psychrotrophic bacteria counts exceeded the value of 6 log cfu/g, considered as the upper acceptability limit for marine species (Erkan *et al*.,, 2007) on 56th storage day. On the other hand, in R and T group samples psychrotrophic bacteria counts exhibited a growth under the 6 log cfu/g on the 70th storage day. Statistically significant differences were found between the samples with respect to the storage duration ($p < 0.05$).

The initial LAB count of the raw crayfish was determined to be 4.05 log cfu/g. After processing, the LAB count was decreased to 3.16 log cfu/g, 2.98 log cfu/g and 2.16 log cfu/g in the control, rosemary, and thyme groups, respectively. Statistically significant differences were found between the samples ($p < 0.05$). In terms of the LAB count of marinated crayfish, thyme essential oil was determined to be the most effective during storage (Fig. 1). On the 56th day of storage, 5.98 log cfu/g, 4.89 log cfu/g, and 3.88 log cfu/g levels were observed for the C, R, and T samples, respectively. These differences may explain the effects of thyme essential oil on the marinated crayfish in sauce. Zaika *et al*. (2006) found that rosemary, marjoram, sage, and thyme had an inhibitory effect on the LAB count.

Yeast-mold count (YM) in the raw crayfish was 3.66 log cfu/g. The initial YM count was significantly ($p < 0.05$) reduced after the marination process. YM count increased with increasing storage time (Fig. 1). However, YM count of marinated crayfish did not exceed the acceptable limit during storage period for any of the groups (YM count < 6 log cfu/g). Significant differences were observed: the control group contained high level of YM compared to treatment groups. Antibacterial and antifungal activities of rosemary and thyme have been reported in previous studies (Hammer *et al*. 1999; Elgayyar *et al*. 2001; Burt, 2004).

Some authors have suggested that rosemary and thyme have significant bacteriostatic/inhibition properties for pathogenic and spoilage microorganisms (Tajkarimi *et al*., 2010), the present study showed that the used thyme oil was more effective as an antibacterial agent for the preservation of marinated crayfish, probably due to the presence of carvacrol and thymol in the thyme essential oil.

Chemical analysis and pH: The changes in pH, TVB-N, and TBA values of raw materials and marinated crayfish in sauce are given in Fig. 2.

The initial pH of the raw crayfish was 6.01 ± 0.02. After processing, the pH values were 4.42, 4.39, and 4.40, for C, R, and T, respectively (Fig. 2). No statistically significant difference was found between the samples ($p > 0.05$). Generally, bacteria that cause food poisoning and spoilage may only grow above a pH of 4.8 (McLay, 1972), therefore the pH value of marinated products should not be more than 4.8 (Rehbein and Oehlenschlager, 1996). In compliance with the literature, pH values of our samples were below 4.8. This was supported by other chemical and sensory analyses (Ludorff and Meyer, 1973 Varlik *et al*., 1993). This can be explained by acetic acid concentration used in marination which causes a decrease in pH value.

TVB-N could be used as a quality indicator for fish products (Jay, 1992) and is associated with the amino acid decarboxylase activity of microorganisms during storage. Changes in TVB-N value during storage are shown in Fig. 2. The initial TVB-N in raw crayfish was 18.5 ± 0.74 mg/100 g which is noticeably higher than the results reported by Mol and Turkmen (2008). It is suggested that TVB-N value is affected by the species, catching season, and region as well as the age and sex of seafood. A level of 35 mg/100 g has been considered the upper limit above which fishery-products are regarded unacceptable (Sikorski *et al*., 1990). The TVB-N counts of all samples increased with storage time. Significant differences were observed between C, R, and T groups ($p < 0.05$). Samples treated with thyme oil had the lowest levels of TVB-N compared to the other samples. This is attributed to the effect of thyme extract on microbial population and the growth of bacteria as an antimicrobial agent (Yasin and Abou-Taleb, 2007; Kenar *et al*., 2010; Tajkarimi *et al*., 2010).

Highly unsaturated lipids in fat-rich fish are susceptible to oxidation that results in a rancid smell and taste as well as alterations in texture, color, and nutritional value (Olafsdottir *et al*., 1997). TBA (thiobarbituric acid) value is a widely used indicator for the assessment of the degree of lipid oxidation. It has been suggested that a maximum TBA value, indicating the good quality of the fish, is 5 mg malonaldehyde/kg while fish may be consumed up to a TBA value of 8 mg malonaldehyde (MA) kg^{-1} (Schormuller, 1969).

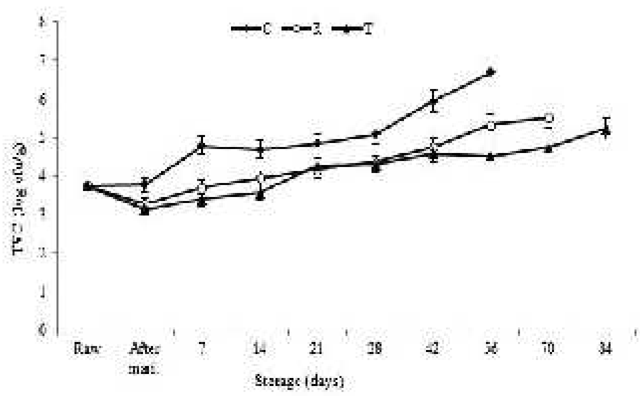
TBA values of the raw crayfish were 0.31 ± 0.43 mg MA kg^{-1}. Initial TBA values of C, R, and T groups were found to be 0.61, 0.37, and 0.32 mg MA kg^{-1}, respectively (Fig. 2). All samples showed an increased TBA value with extended storage period. Significant differences were observed among C, R, and T groups ($p < 0.05$). Similarly, these findings are in agreement with the antioxidative effect of rosemary oil reported by Cadun *et al*. (2008). They reported that application of rosemary and thyme oils at doses of 300 ml/L also led to significantly lower final TBA values compared to the control for marinated crayfish at 56 days. In addition, similar TBA values have been reported for meat treated with oregano and sage essential oils (Fasseas *et al*., 2007). In the present study, the highest TBA values were observed in the control group during the storage period.

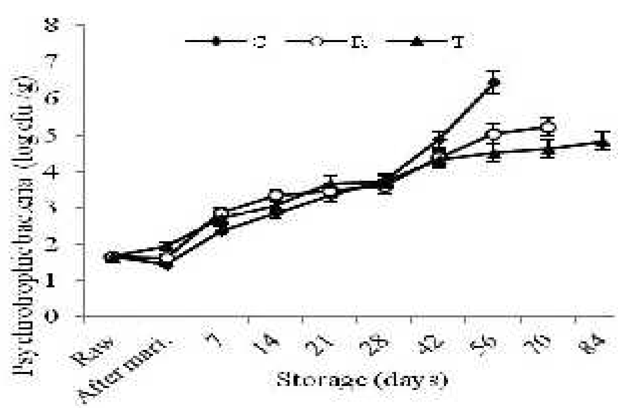
Sensory analysis: Fig 3 shows the results of the sensory evaluation (overall acceptability, odor, color, taste) of marinated sauce crayfish. Scores of all groups decreased at the end of the storage period. The limit of acceptability of taste was reached after 56 days for the control group, after 70 days for T and 84 days for R groups. The storage life of fish is affected by the initial microbial load of the fish and the storage temperature (Erkan and Bilen, 2010). The shelf life of Asian sea bass (*Lates calcarifer*) treated with 0.05% oregano and / or thyme was observed to be 33 days at 0 – 2 ^{0}C (Harpaz *et al*., 2003). Similar findings were reported by other researchers as well (Gimenez *et al*., 2005).

Table 1. Proximate compositions of raw and marinated crayfish.

Sample componet	Raw Crayfish	C	R	T
Moisture (%)	79.15±0.07	77.70±0.59	78.38±1.0	77.91±1.7
Protein (%)	16.27±0.03a	11.19±0.46b	11.52±0.21b	11.48±0.2b
Lipid (%)	2.97±0.23a	7.98±1.52b	8.01±0.81b	8.03±0.2b
Ash(%)	1.51±0.25a	2.02±0.03b	2.04±0.04b	2.08±0.37b

C: control group, without added rosemary and thyme oils; R : plus 300 mL/L rosemary oil; T: plus 300 mL/L thyme oil. Each value is a mean (n=3). Values in the same line followed by different letter are significantly different ($p < 0.05$).





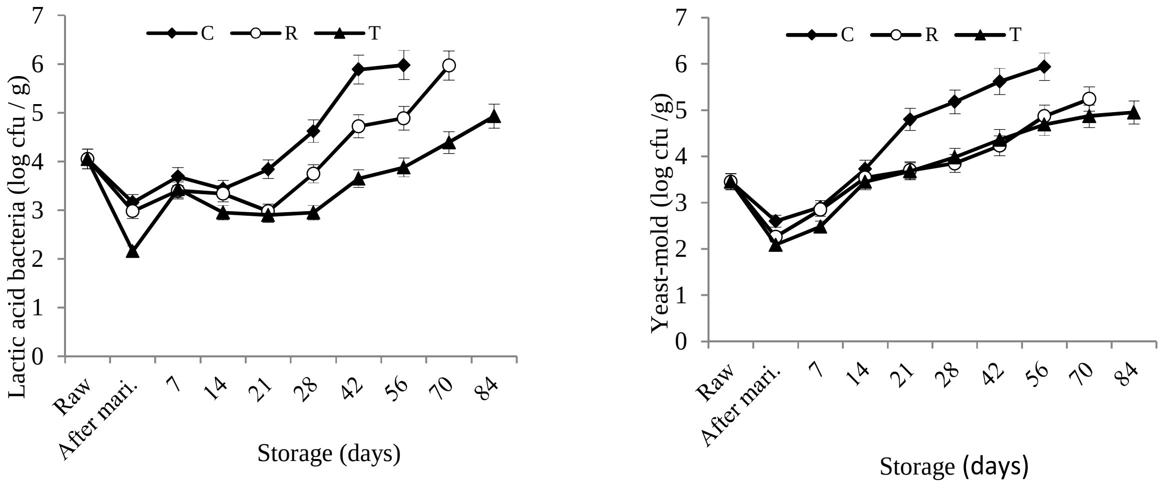


Fig.1. Changes microbial counts (log cuf /g) in production phases/ marinated sauced crayfish during storage at 4°C. After mari: After marination. C: Control R: plus 300 mL/L Rosemary oil T: plus 300 mL/L Thyme oil .Each value is a mean (n=3).

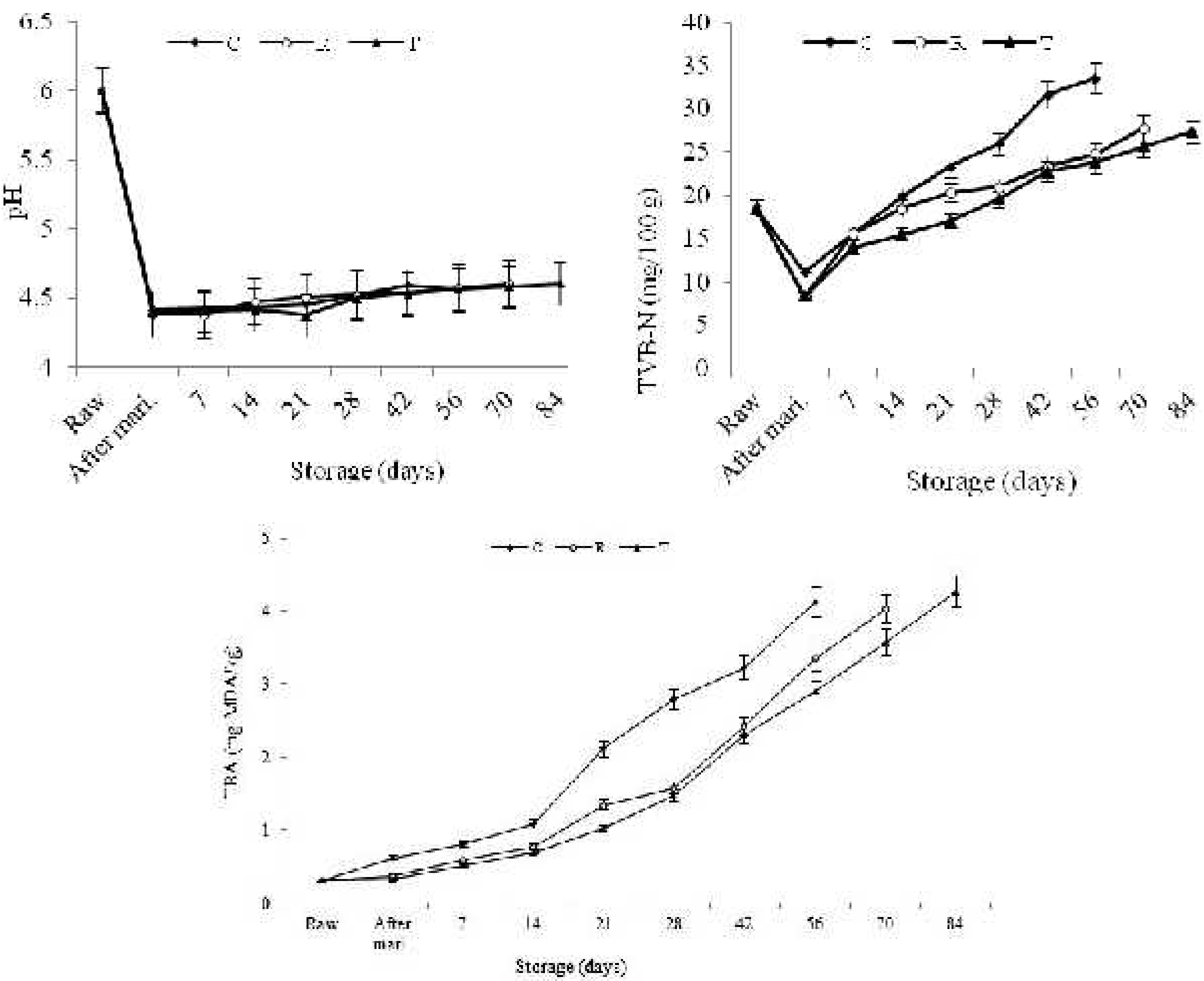


Fig.2. Chemical changes in production phases/ marinated sauced crayfish during storage at 4°C. After mari: After marination. C: Control R: plus 300 mL/L Rosemary oil T: plus 300 ppm Thyme oil. Each value is a mean (n=3).

In conclusion, the results of the study showed that addition of rosemary oil and thyme oil in marination solution resulted in longer shelf life compared to the control. Sauce marinades were preferred by the panelists. Rosemary and thyme essential oils were effective in controlling the growth of bacteria and biochemical indices.

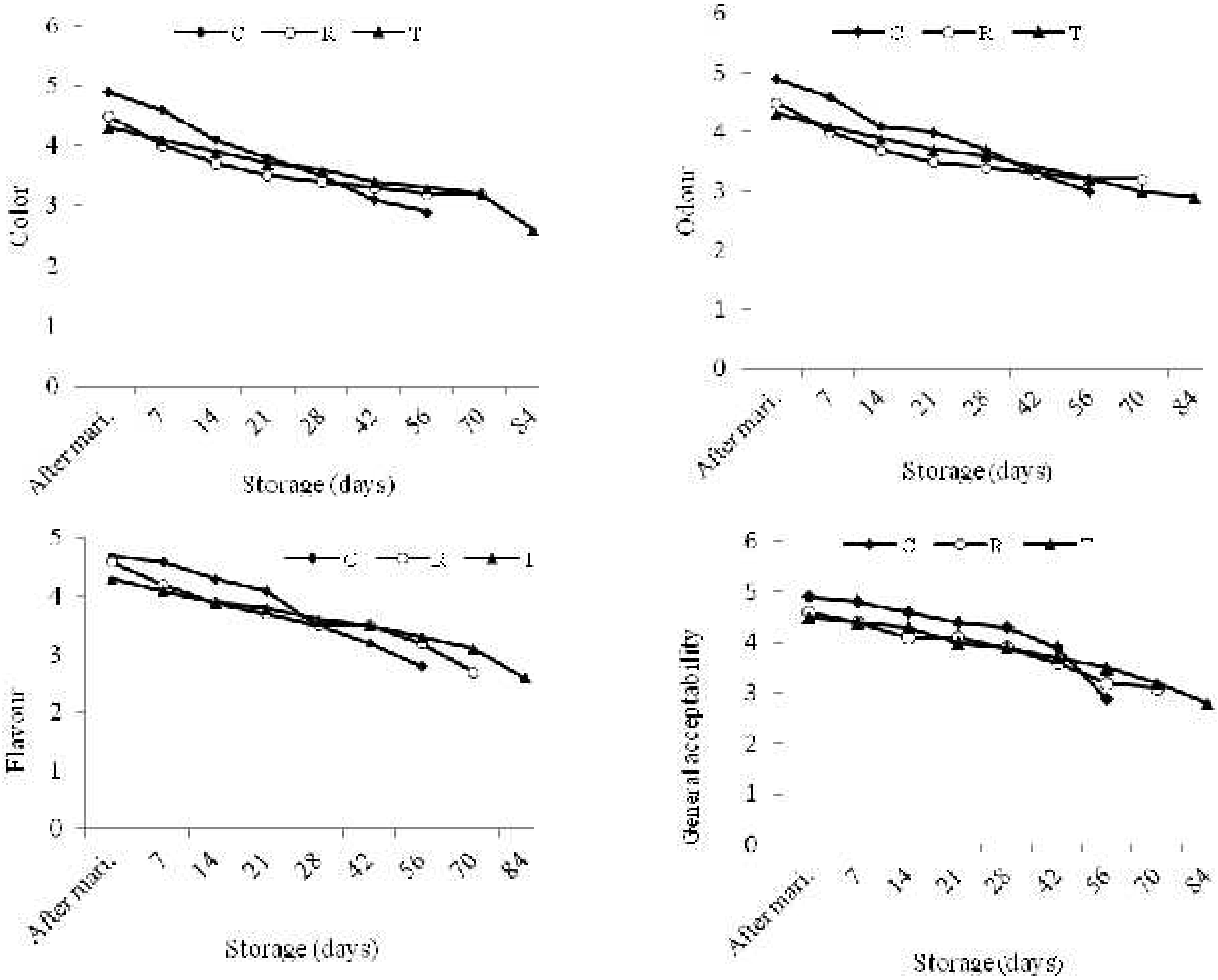


Fig. 3. Sensory scores of marinated sauced crayfish during storage at 4°C. After mari: After marination. C: Control R: plus 300 mL/L Rosemary oil T: plus 300 mL/L Thyme oil Each value is a mean (n=3). Color, Odour, Flavor and General acceptability, n =5 panelists.

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