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Marination of deep-water pink shrimp with rosemary extract and the determination of its shelf-life

Asli Cadun*, Duygu Kışla, Şükran Çaklı

Ege University, Faculty of Fisheries, 35100 Bornova, Izmir, Turkey

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Abstract

The effect of the antioxidant activity of rosemary extract on marinated deep-water pink shrimp (*Parapenaeus longirostris* Lucas, 1846) stored at 1 °C was investigated. Chemical, physical, instrumental, microbiological and sensory analyses were performed to investigate the quality changes and to determine the shelf-life of marinated shrimps. Chemical composition of the shrimp was determined and no significant difference (P > 0.05) was found between the control group (without rosemary extract) and the experimental group (with rosemary extract). Both groups contained 2% citric acid. There was no significant difference (P > 0.05) between the sensory analysis of control and experimental groups on storage days 0, 15, 30, 45 and 60 while rancidity was noted by the panelists in the control group on day 75. The TBA value of the control group reached the consumption limit on day 75 but it was still 'very good' for the experimental group. Although the bacterial load of both groups were lower than the consumption limits on storage day 75, TBA value limited the shelf-life of the control group but the experimental group was still of good quality for consumption after 75 days. © 2007 Elsevier Ltd. All rights reserved.

Keywords: Deep-water pink shrimp; Marination; Rosemary extract; TBA; Citric acid

1. Introduction

Deep-water pink shrimps (*Parapenaeus longirostris* Lucas, 1846) show a wide geographic distribution around the world. In 2003, annual landings of pink shrimp in the Sea of Marmara (Eastern Mediterranean) were 4059 tonnes (Anonymous, 2004), which constitutes approximately 72% of the total shrimp landings in Turkish waters (Zengin et al., 2004). The species also has a high commercial value in France, Italy, Algeria, Tunisia and Greece (Holthuis, 1980). Pink shrimps are peeled, cleaned and then stored frozen in processing plants in the Marmara and Aegean regions' of Turkey. Some quality problems peculiar to this species occur during long storage periods (Pala, Varlık, & Aran, 1988). Especially in European countries (Germany, Netherlands and Norway), deep-water pink shrimps are

E-mail address: cadun@mail.ege.edu.tr (A. Cadun).

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marinated so that new flavour and texture developments occurs.

Marinades are solutions, including sugar, spices, oil, acids (from vinegar, fruit juice and wine), that are used to improve the tenderness, juiciness, flavour and aroma, to extend the shelf-life of red meat, poultry, seafood and vegetables (Brandt, 1996; Carlos & Harrison, 1999; Chu, Huang, & Toledo, 1998; Harazak, 2000; Hull, 2001; Sheard, Nute, Richardson, & Wood, 2005; Zheng, Huang, Nelson, Bartley, & Gates, 1998). Although marination technology in the meat and poultry industry is well developed, there is less information in the literature about marinated shrimps (Cadun, Caklı, & Kışla, 2005; Chu et al., 1998; Dagal & Bazaraa, 1999; Dalgaard & Jorgensen, 1999; Huang, He, & Gates, 1995). In Turkey, there are several studies on marinated fish (Aksu et al., 1997; Bakıcı, 1987; Ersan, 1960; Kılınç & Çaklı, 2004; Kılınç & Çaklı, 2005a; Kılınç & Çaklı, 2005b), but studies on marinated shrimps are minimal (Cadun, Çaklı, & Kışla, 2005; Pala, Varlık, & Aran, 1988).

^{*} Corresponding author. Tel.: +90 232 3884000/1300; fax: +90 232 3883685.

Various synthetic and natural antioxidants are used to prevent oxidation of lipids in foods subjected to long-term storage. In recent year, there has been increasing concern about the appropriateness of synthetic food additives (Vareltzis, Koufidis, Gavriilidou, & Vasiliadou, 1997). Svnthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tert-butylhydroquinone (TBHQ), are widely used in the food industry, but BHA and BHT have been suspected of being responsible for liver damage and carcinogenecity in laboratory animals (Wang, Weng, & Cheng, 2000). Therefore, there has been an interest in the use of compounds obtained from natural sources, such as grains, oilseeds, spices, fruits and vegetables, to stabilize fat-containing foods (Chen, Muramoto, Yamauchi, & Huang, 1996). Rosemary (Rosmarinus officinalis L.) has been reported to contain certain compounds including, rosmanol, rosmariquinone, rosmaridiphenol and carnosol, which may be up to four times as effective as butylated hydroxyanisole (BHA) and equal to butylated hydroxytoluene (BHT) as antioxidants (Houlihan, Ho, & Chang, 1984; Houlihan, Ho, & Chang, 1985; Nakatani & Inatani, 1984). Several authors reported that some compounds in rosemary extracts could have antibacterial activity (Cuvelier, Richard, & Berset, 1996; Del Campo, Amiot, & Nguyen-The, 2000).

The application of plant extracts to prevent fish oxidative rancidity has been studied in ceratin fish products like fillets and mince gels (Aubourg et al., 2004; Perez-Mateos, Gomez-Guillen, Hurtado, Solas, & Montero, 2002; Serdaroğlu & Felekoglu, 2005; Weilmeier & Regenstein, 2004). Quercetin and rosemary extracts are among the most used natural antioxidants.

This study was conducted to evaluate the antioxidative effect of rosemary extract on marinated shrimps. Chemical, physical, instrumental, microbiological and sensory analyses were done to investigate the quality changes and to determine the shelf-life of marinated shrimp during storage at 1 $^{\circ}$ C.

2. Materials and methods

2.1. Material

Shrimps were caught in November 2006 in the Sea of Marmara by a commercial fishing trawler. Shrimp were put in boxes including ice just after being caught. Average weight of the shrimp was 10 ± 0.7 g and the average temperature of the sea at the time of the catch was 14.7 °C. Shrimp were brought to the processing plant in boxes including ice immediately and they were immersed in a dilute (1.25% by weight) sodium metabisulphite (Na₂S₂O₅) solution for 1 min (maximum uptake limit 150 mg/kg shrimp, Turkish Food Codex, 1997) before they were peeled. Head, tail, legs and shell were removed by hand. Then they were kept at -40 °C in a blast freezer for 12 h and then stored at -18 °C until use. In this study, 2-month frozen shrimp were used.

2.2. Method

2.2.1. Processing method

Frozen shrimp were put into plastic bags and boiled in a water bath for 10 min. Then they were divided into two groups (control and experimental). Marinade solution was prepared by adding 2% citric acid (JT Baker, Deventer, Holland), 4% NaCl (Merck KGaA, Darmstadt, Germany) and preservatives, 0.1% sorbic acid (JT Baker), 0.1% benzoic acid (JT Baker), cited in the Turkish Food Codex (1997) to the tap water. This marinade solution was used for both groups except that 300 ppm of rosemary extract (Dragoco, Holzminden, Germany) was added to the marinade solution mentioned above for the experimental group. Shrimp and marinade solution were filled into plastic containers at a ratio of 1:1 (shrimp/marinade solution; w/v), sealed with their lids and stored at 1 ± 0.5 °C during for 75 days.

2.3. Analytical methods

2.3.1. Proximate composition

Moisture (Ludorff & Meyer, 1973), crude fat (Bligh & Dyer, 1959), crude protein (AOAC, 1984) and ash contents (AOAC, 1984) were determined for raw and marinated shrimp.

2.3.2. Physical and chemical quality analysis

Thiobarbituric acid, TBA as mg malonaldehyde equiv./ kg (Tarladgis, Watts, Younathan, & Dugan, 1960), total volatile base-nitrogen, TVB-N, mg N/100 g (Antonacopoulos & Vyncke, 1989) and pH values were measured in raw and marinated shrimp (Amtliche, 1980). The pH value was recorded using a Hanna 211 model pH meter (Cluj-Napoca, Romania). The pH of the marinade solution (brine) during storage was also measured. The gas electrode was dipped directly into the minced shrimp meat and brine.

2.3.3. Instrumental analysis

Colour measurements were carried out on homogenized samples of raw and marinated shrimp by using a Dr. Lange Spectro Pen[®]. This is a colourimeter operating on the spectral method described in DIN 5033 (Deutsches Institut für Normung, CIE 95, 2000) using the 45/0 °C circular viewing geometry, the sample is illuminated with polychromatic light encircling it at an angle of 45°, with the optical unit observing the reflected light from a horizontal angle (0°) towards the sample surface. The colour was measured on homogenates prepared from shrimp by using a Kitchen Aid KPM5 Professional meat grinder (St. Joseph, MI, USA). The homogenate was placed in glass petri dishes (12 cm diameter) and the colour measurement was repeated 10 times using different parts of the surface. In the CIE $L^*a^*b^*$ system, L^* denotes lightness on a scale from 0 to 100 from black to white; a^* denotes (+) red or (-) green; b^* denotes (+) yellow or (-) blue (Schubring, Meyer, Schlüter, Boguslawski, & Knorr, 2003).

2.3.4. Sensory analysis

Sensory evaluation of marinated shrimp was performed by five previously trained panelists, who were asked to evaluate appearance, flavour, odour and texture by using a previously developed form (modified) (Ludorff & Meyer, 1973). According to the scoring table, a total score of 30 points (maximum score of the test) in overall quality of shrimp means 'first quality', a score from 29.9 to 26 indicates 'second quality', a score from 25.9 to 22 indicates 'third quality' and a score from 21.8 to 12 indicates 'fourth quality'.

2.3.5. Microbiological analysis

For all microbial counts, 10 g of shrimp were weighed and transferred into 90 ml of 0.1% peptone water (Oxoid, Basingstoke, UK), and samples were homogenized in a Stomacher (IUL Instruments, Barcelona, Spain) for 1 min. From the prepared dilutions, total aerobic plate (AP) count, psychrotrohic bacteria (PB) count, yeast-mold (YM) count and lactic acid bacteria (LAB) count were carried out. Plate Count Agar (PCA, Oxoid CM 325) was used for AP and PB counts with incubation periods of 30 °C/24-48 h and 7 °C/10 days, respectively, while Potato Dextrose Agar (PDA, Oxoid CM 139) and de Man Rogosa Sharp Agar (MRSA, Oxoid CM 361) were used for YM and LAB counts, respectively, with incubation period of 30 °C/3–5 days (Ariyapitun, Mustafa, & Clarke, 1999; Dalgaard & Jorgensen, 1999; Harrigan & McCance, 1976).

2.3.6. Statistical analysis

Data were subjected to analysis of variance (STATISTI-CA StatSoft Inc. (1996), Tulsa, OK, USA) to determine the significant differences (P < 0.05) between mean values.

3. Results and discussion

3.1. Proximate composition

Proximate compositions of raw and marinated shrimp are given in Table 1. Moisture content of raw shrimp was determined as $84.7 \pm 1.4\%$ but moisture content in the control and experimental groups decreased to $77.3 \pm 0.6\%$ and $82.3 \pm 3.4\%$, respectively, due to the water loss during the boiling process (P > 0.05). Fat content of raw shrimp was $0.9 \pm 0.1\%$. After the marination process, fat content in

Table 1

Proximate composition								
Material	Moisture (%)	Fat (%)	Protein (%)	Ash (%)				
Raw shrimp	$84.7^{\rm a}\pm1.4^{\rm 1}$	0.9 ± 0.1^1	12.3 ± 0.3^{1}	2.3 ± 0.6^1				
Control ^b	81.30 ± 0.6^1	1.4 ± 0.2^1	19.2 ± 0.4^2	2.5 ± 0.2^1				
Experimental ^c	82.3 ± 3.4^{1}	1.4 ± 1.4^{1}	18.0 ± 0.5^2	$2.4\pm0.5^{\rm 1}$				

^{1,2}Means in the same column with the same number do not differ significantly at the level of 0.05 significance.

^a *n*:3 (arithmetic mean \pm SD).

. . .

^b Marinated shrimp without rosemary extract.

^c Marinated shrimp with rosemary extract

the control and experimental groups increased to $1.4 \pm 0.2\%$ and $1.4 \pm 1.4\%$, respectively. Protein contents of marinated shrimp also increased after marination as compared to the protein content of raw shrimp (Table 1). No significant difference was found between the ash contents of raw and marinated shrimp.

3.2. Physical and chemical quality analysis

The pH value of raw shrimp was measured as 7.61 (data not shown). Changes in pH values of the shrimp and brine in the control and experimental groups during storage are given in Table 2. No significant difference was observed between the pH values of shrimp during storage in both groups (P > 0.05). In marinated products, the pH value should not be more than 4.8 (Rehbein & Oehlenschlager, 1996). Almost all food poisoning and most of the growth of spoilage bacteria may be prevented by a pH below 4.8 (McLay, 1972). In this study, the pH of the shrimp and brine in both groups during storage remained below 4.8.

TVB-N and TBA values were determined to investigate the chemical quality changes in marinated shrimp (Table 3). The TVB-N value of raw shrimp was 13.3 mg N/100 g(data not shown) but in the marinated groups, TVB-N values were found to be lower than this value during storage. It is probable that the acid and salt combination decreased TVB-N values of the shrimp. TVB-N is well documented as an index of the quality of fresh or frozen fish because its increase is related to spoilage by bacteria and the activity of endogenous enzymes (Vareltzis, Koufidis, Gavriilidou, & Vasiliadou, 1997). Crustaceans may have unusually high TVB-N values (Cabellero, Gancalves, & Nunes, 2002; Lou, 1998; Oehlenschlager, 1997; Shamshad, Nisa, Riaz, Zuberi, & Qadri, 1990). In this study, the control and experimental groups started with TVB-N value of 7.5 mg N/100 g and finished with the values of 7.0 ± 2.8 and 5.6 ± 0.00 mg N/100 g, respectively (Table 3). No significant difference was observed between TVB-N values for the control group during storage (P > 0.05). However, the initial TVB-N value of the experimental group decreased significantly (P < 0.05) at day 15 and continued to decrease significantly during storage. No work in the literature was found regarding the influence of rosemary extract on TVB-N values but this study showed that addition of rosemary extract decreased TVB-N values significantly during storage at 1 °C (P < 0.05). Both groups were rated 'good quality' with respect to their TVB-N values.

TBA (mg malonaldehyde equiv./kg) analysis, an important quality index indicating lipid oxidation, was carried out with the marinated shrimp (Table 3). The TBA value of raw shrimp was 0.26 mg malonaldehyde equiv./kg (data not shown). At the beginning of the storage period TBA values of the control and experimental groups were 0.9 ± 0.04 and 0.4 ± 0.1 mg malonaldehyde equiv./kg, respectively, whereas at the end of the storage period they reached 6.6 ± 0.4 and 2.4 ± 0.7 mg malonaldehyde equiv./ kg, respectively. However, this increase in the experimental

pН	Storage period (da	Storage period (days)							
	0	15	30	45	60	75			
Shrimp ^A	4.2 ± 0.03^{a1B}	4.6 ± 0.06^{a1}	4.2 ± 0.02^{a1}	4.5 ± 0.05^{a1}	4.1 ± 0.01^{a1}	4.2 ± 0.01^{a1}			
Shrimp ^C	4.2 ± 0.01^{a1}	4.5 ± 0.04^{a1}	4.2 ± 0.03^{a1}	4.4 ± 0.04^{a1}	$4.3\pm0.03^{\mathrm{a}2}$	$4.2\pm0.01^{\rm a1}$			
Brine	$4.4\pm0.07^{\mathrm{a1}}$	4.7 ± 0.03^{a1}	4.4 ± 0.11^{a1}	4.7 ± 0.02^{a1}	$4.1 \pm 0.02^{\mathrm{a1}}$	$4.3\pm0.01^{\mathrm{a1}}$			
Brine ^C	4.3 ± 0.01^{a1}	$4.6\pm0.02^{\mathrm{a}2}$	4.3 ± 0.02^{a1}	4.5 ± 0.9^{a2}	$4.3\pm0.03^{\mathrm{a}2}$	4.3 ± 0.01^{a1}			

Table 2 Changes in pH values of shrimp and brine during storage

^aMeans in the same row with the same letter do not differ significantly at the level of 0.05 significance.

^{1,2}Means in the same column with the same number in the same kind of sample do not differ significantly at the level of 0.05 significance.

^A Control (without rosemary extract).

^B *n*:3 (arithmetic mean \pm SD).

^C Experimental (with rosemary extract).

Table 3		
Chemical quality change	ges of marinated	shrimps during storage

Analysis	Storage period (days)							
	0	15	30	45	60	75		
<i>TVB-N^A</i> Control Experimental	$\begin{array}{c} 7.5 \pm 2.14^{a1B} \\ 7.5 \pm 0.81^{a1} \end{array}$	$\begin{array}{c} 6.1 \pm 0.8^{a1} \\ 5.6 \pm 1.4^{b1} \end{array}$	$\begin{array}{c} 6.5\pm 0.8^{a1} \\ 6.1\pm 0.8^{b1} \end{array}$	$\begin{array}{c} 6.3 \pm 1.0^{a1} \\ 6.3 \pm 1.0^{b1} \end{array}$	$\begin{array}{c} 7.0 \pm 0.0^{a1} \\ 6.1 \pm 0.8^{b1} \end{array}$	$\begin{array}{c} 7.0 \pm 2.8^{a1} \\ 5.6 \pm 0.00^{b1} \end{array}$		
<i>TBA^C</i> Control Experimental	$\begin{array}{c} 0.9 \pm 0.04^{a1} \\ 0.4 \pm 0.1^{a2} \end{array}$	$\begin{array}{c} 1.5 \pm 0.4^{b1} \\ 0.4 \pm 0.1^{a2} \end{array}$	$\begin{array}{c} 1.8 \pm 0.1^{b1} \\ 0.7 \pm 0.1^{a2} \end{array}$	$\begin{array}{c} 2.9 \pm 0.5^{c1} \\ 1.0 \pm 0.1^{a2} \end{array}$	$\begin{array}{c} 3.1 \pm 0.6^{c1} \\ 1.7 \pm 1.1^{b2} \end{array}$	$\begin{array}{c} 6.6 \pm 0.4^{a1} \\ 2.4 \pm 0.7^{c2} \end{array}$		

^{a,b,c}Means in the same row with the same letter do not differ significantly at the level of 0.05 significance.

^{1,2}Means in the same column with the same number in the same analysis do not differ significantly at the level of 0.05 significance.

^A mg/100 g.

^B n:3 (arithmetic mean \pm SD).

^C mg malonaldehyde/kg.

group increased slowly (mainly after the 60th day) and addition of rosemary extract led to a final TBA value of marinated shrimp that was 2.7 times lower than the control group. There are several studies concerning the use of rosemary extracts to prevent oxidative rancidity in fish products (Frankel, Huang, & Aeschbach 1996; Montero, Gimenez, Perez-Mateos, & Gomez-Guillen, 2005; Perez-Mateos et al., 2002; Serdaroğlu & Felekoglu, 2005; Wada & Fang, 1992). In these studies, it was shown that use of rosemary extracts at different concentrations prevented lipid oxidation in fish minces, gels and emulsions. In high quality materials the TBA value should be less than 3 mg malonaldehyde equiv./kg and, in good quality material, TBA values should not be more than 5 mg malonaldehyde equiv./kg. The consumption limit is 7–8 mg malonaldehyde equiv./kg (Schormüller, 1968; Schormüller, 1969). As shown in Table 3, TBA values of marinated shrimp in the control group were still high quality until the 60th day, whereas this value reached the consumption limit at day 75. In the experimental group, TBA values of marinated shrimp stayed at high quality to the end of storage.

3.3. Instrumental analysis

Colour measurements of marinated shrimp during the storage period are shown in Table 4. L^* values of shrimp

in the control group were 48.9 ± 0.3 and 72.9 ± 0.5 at the beginning and at the end of the storage period, respectively. Significant differences (P < 0.05) were determined between the first three periods and last three periods. a^* values of the same group were determined as 1.9 ± 0.5 and 0.9 ± 0.1 at the beginning and at the end of the storage period, respectively, and these differences were found to be not significant ($P \ge 0.05$). There was no significant difference (P > 0.05) between b^* values of the control group at the beginning and at the end of the storage. L^* values of shrimp in the experimental group were 50.4 ± 0.4 and 70.4 ± 1.1 at the beginning and at the end of the storage period, respectively. Significant differences (P < 0.05) were observed between L^* values from the first three periods and last three periods with the control group. No significant difference $(P \ge 0.05)$ was determined between a^* values of the experimental group at the beginning and at the end of storage. For b^* values of the same group, significant differences (P < 0.05) were determined between the first three periods and last three periods. Significant differences were determined between L^* values of the control and experimental group at the beginning and at the end of the storage period. The experimental group had significantly higher a^* values than the control group at day 45 and 60. The experimental group had significantly lower b^* values than the control group at day 0, 60 and 70.

Table 4	
Changes in colour parameters of marinated shrimps during storage at 1 °C	

Colour parameters	Storage period (days)						
	0	15	30	45	60	75	
Control							
L^*	48.9 ± 0.3^{a1A}	$49.7\pm0.4^{\rm a1}$	49.8 ± 0.5^{a1}	$71.0 \pm 1.1^{\mathrm{b1}}$	$72.5\pm0.6^{\rm b1}$	$72.9\pm0.5^{\rm b1}$	
a*	$1.9\pm0.5^{\rm a1}$	$1.9\pm0.6^{\rm a1}$	$1.8\pm0.2^{\rm a1}$	$0.8\pm0.5^{\rm a1}$	$0.8\pm0.1^{\mathrm{a1}}$	$0.9\pm0.1^{\mathrm{a1}}$	
b^*	9.8 ± 0.3^{a1}	9.0 ± 0.4^{a1}	9.8 ± 0.4^{a1}	$6.5\pm1.1^{\rm b1}$	$7.5\pm0.3^{\rm b1}$	10.0 ± 0.3^{a1}	
Experimental							
L^{*}	$50.4\pm0.4^{\rm a2}$	50.5 ± 0.3^{a1}	$50.9\pm0.7^{\mathrm{a1}}$	$70.4 \pm 0.9^{\mathrm{b1}}$	$71.3\pm0.9^{\rm b1}$	70.4 ± 1.1^{b2}	
a*	$1.9\pm0.5^{\rm a1}$	$1.9\pm0.49^{\mathrm{a1}}$	$1.9\pm0.3^{\rm a1}$	$2.4\pm0.4^{\mathrm{b}2}$	$2.6\pm0.2^{\mathrm{b}2}$	$2.0\pm0.7^{\rm a1}$	
b^*	$9.0\pm0.2^{\mathrm{a}2}$	8.9 ± 0.45^{a1}	9.0 ± 0.4^{a1}	6.3 ± 0.7^{b1}	$6.8\pm0.1^{\rm b2}$	$7.5\pm0.5^{\rm b2}$	

^{a,b}Means in the same row with the same letter do not differ significantly at the level of 0.05 significance.

^{1,2}Means in the same column with the same number in the same attribute do not differ significantly at the level of 0.05 significance.

^A *n*:3 (arithmetic mean \pm SD).

Table 5	
Sensory evaluations of marinated shrimps during period	

Group	Storage period (days)							
	0	15	30	45	60	75		
Control Experimental	$\begin{array}{c} 28.2\pm0.3^{a1} \\ 28.1\pm0.2^{a1} \end{array}$	$\begin{array}{c} 27.3 \pm 0.2^{a1} \\ 28 \pm 0.4^{a1} \end{array}$	$\begin{array}{c} 27.4 \pm 0.3^{a1} \\ 27.7 \pm 02^{a1} \end{array}$	$\begin{array}{c} 25.4 \pm 0.8^{b1} \\ 26.8 \pm 0.4^{a1} \end{array}$	$\begin{array}{c} 24.4 \pm 0.7^{b1} \\ 26.5 \pm 0.5^{a2} \end{array}$	$\begin{array}{c} 21.9 \pm 0.5^{c1} \\ 26 \pm 0.6^{a2} \end{array}$		

n:5.

^{a,b,c}Means in the same row with the same letter do not differ significantly at the level of 0.05 significance.

^{1,2}Means in the same column with the same number do not differ significantly at the level of 0.05 significance.

3.4. Sensory analysis

Sensory evaluations of marinated shrimp during the storage period are shown in Table 5. Significant differences were determined for the control group during storage (P < 0.05). The control group was 'fourth quality' and the panelists recognized 'rancidity' at day 75. However, no significant differences (P > 0.05) in the experimental group were determined during the storage period. The experimental group was 'first quality' during the entire per-

iod. Sensory evaluations were parallel to the results of the TBA analysis.

4. Microbiological analysis

The initial AP, PB, YM and LAB counts of raw shrimp were determined as 5.76, 5.25, 1.74 and 4.04 log CFU/g, respectively (data not shown). Microbial load decreased just after the shrimps were marinated (Table 6). AP counts of control and experimental groups were 1.75 and

Changes in microbial f	flora of marinated	shrimps during	storage at 1 °C
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Analysis	Storage period (days)						
	0	15	30	45	60	75	
Control							
log AP/g	1.75 ± 0.65^{a1A}	$1.53 \pm 0.40^{\rm a1}$	$1.69 \pm 0.27^{\mathrm{a1}}$	$1.60 \pm 0.42^{\mathrm{a1}}$	$1.48 \pm 0.00^{\mathrm{a1}}$	$1.24 \pm 0.34^{\mathrm{a1}}$	
log PB/g	_ ^B	_	_	_	_	_	
log YM/g	_	_	1.15 ± 0.21	_	_	_	
log LAB/g	_	1.26 ± 0.24	_	_	_	-	
Experimental							
log AP/g	$1.82\pm0.30^{\mathrm{a}1}$	$1.65\pm0.30^{\mathrm{a}1}$	$1.71\pm0.40^{\mathrm{a}1}$	1.46 ± 0.15^{a1}	1.48 ± 0.67^{a1}	$1.43\pm0.60^{\mathrm{a1}}$	
log PB/g	_	-	-	-	_	_	
log YM/g	-	_	-	-	-	_	
log LAB/g	_	_	_	_	_	_	

^aMeans in the same row with the same letter do not differ significantly at the level of 0.05 significance.

¹Means in the same column with the same number in the same analysis do not differ significantly at the level of 0.05 significance.

^A *n*:3 (arithmetic mean \pm SD).

^B Not detected.

1.82 log CFU/g, respectively, at day 0 and AP counts of both groups stayed close to each other until 75th day, ending with counts of 1.24 and 1.43 log CFU/g, respectively. There were no significant differences $(P \ge 0.05)$ in both control and experimental groups between AP counts during storage at 1 °C and no significant difference (P > 0.05) was found in AP counts between the two groups for each storage period. LAB and YM were detected at day 15 and 30, respectively, in the control group. It was thought that this case resulted from the contamination because no YM and LAB nor PB were detected again during the storage period. In the experimental group, no microbial load other than AP was detected. Sorbates and benzoates are primarily effective against yeasts and moulds, but it has been reported that they are effective against a wide range of bacteria (Jay, 1991). Einarsson and Lauzon (1995) reported that shrimp, subjected to a marination treatment with NaCl, citric acid and glucose, was found at 10 days to have some bacterial counts (log AP and PB counts ≥ 6) but a sorbate-benzoate solution (0.1% for each) preserved the brined shrimp for 59 days at 4.5 °C. According to the findings from a previous study (Cadun, Cakli, & Kışla, 2005), addition of sorbic and benzoic acids to the formulation for marinated shrimp inhibited bacterial growth during 40 days of storage at 1 °C as compared to the control treatment (including no sorbic and benzoic acids in the formulation). In the present study this inhibition of bacterial growth in both groups was observed to the end of day 75 at 1 °C (Table 6). Thus, the addition of rosemary extract did not render any additional antibacterial activity on the microbial load of the experimental group in the present study (Table 6).

In conclusion, according to the sensory and TBA analyses, rosemary extract at the level used helped to prolong the shelf-life of marinated shrimp.

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