The investigation of the shelf life at 2 ± 1 °C of *Luciobarbus esocinus* fillets packaged with films prepared with the addition of different essential oils and chitosan

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Abstract In this study, the chemical, microbiological and sensory changes during storage at 2 ± 1 °C of *Luciobarbus esocinus* fillets coated with edible films prepared with chitosan incorporation of thyme, clove, rosemary essential oils were examined. To create the experimental samples, a total of six groups of *L. esocinus* fillets coated with different edible films (normal, vacuum-packed, chitosan, chitosan with added thyme oil, chitosan with added clove oil, and chitosan with added rosemary) were used. The food composition of the fillets and experimental samples were determined after they had been coated with edible films. The results of analysis showed that, the preservation period of fresh fillets ended on day 12th, that of vacuum-packed fillets on day 15th, that of fillets coated with chitosan incorporation of rosemary on day 27th, that of fillets coated with chitosan incorporation of thyme and chitosan incorporation of cloves on day 30th. In comparison with the control group, fish spoilage was significantly delayed in samples coated with chitosan incorporation of thyme and chitosan incorporation of cloves ($p < 0.05$). The lowest bacterial growth, values of PV, TBA and TVB-N were obtained in fish samples coated with thyme + chitosan and cloves + chitosan. In terms of the general acceptability of the fish, as determined by qualified panelists, it was determined that the highest score was given to the experimental group to which essential oil of clove had been applied.

Keywords Chitosan · Edible film · *Luciobarbus esocinus* · Essential oil · Shelf-life

Introduction

Packaging materials are one of the major aspects of our daily life because the majority of food production is supplied to consumers in packages in today's society (Cha and Chinnan 2004). There are a good deal of applications developed to inhibit spoilage and pathogen microorganisms on fish product (Gómez-Estaca et al. 2010). One of these techniques is edible film, which is commonly used in other food product as well. Edible films extend the shelf life of foods and enhance the economic efficiency of packaging material. Provided that edible films are prepared appropriately, they can exhibit all characteristics expected from a functional package (Kester and Fennema 1986; Lerdthanangkul and Krochta 1996).

Since chitosan constitutes a perfect oxygen barrier, it can be utilized as an edible film component. Furthermore, it is biologically degradable (Caner et al. 1998; Jeon et al. 2002; López-Caballero et al. 2005). Chitosan is a versatile biopolymer, having a broad range of applications in the food industry. It has been reported to have a number of functional properties that make chitosan useful in food preservation; these include its antimicrobial activity (Falowo et al. 2014) and antioxidant activity (Ojagh et al. 2010; Ruiz-Navajas et al. 2015; Jeyakumari et al. 2016) and its ability to form protective films or coatings (Santos et al. 2017).

Consumers usually prefer natural additive substances to synthetic ones (Pizzal et al. 2002). Herbs and herbal extracts which contain antioxidants and aromatic substances with antimicrobial effects are commonly used in

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foods. Since the chemical components and active ingredients of these herbs and spices are different, their relative impacts are different from each other as well (Singhal et al. 2001).

Although many studies have been carried out concerning the use of chitosan as an antioxidant and antimicrobial agent in edible films (Ojagh et al. 2010), very few have thoroughly discussed its effect on nutritional properties.

The purpose of this study was to examine the chemical, microbiological and sensory changes during storage at 2 ± 1 °C of *Luciobarbus esocinus* fillets coated with edible films prepared with various combinations of the essential oils of chitosan, thyme, clove and rosemary.

Materials and methods

Raw material

In the present thesis study, economically valuable *L. esocinus* Heckel, 1843 fish caught from Keban Dam Lake were brought to the laboratory in styrofoam boxes in ice and made into fillets the same day. The average weight and length of the fish were 12.50 ± 2.50 kg and 75.25 ± 2.75 cm. The study was conducted in three replicates and in two parallel studies.

Chitosan film preparation

The chitosan with a medium molecular weight (CAS no; 9012-76-4) used in the preparation of film solutions was obtained from the Sigma Aldrich. The essential oils of thyme (Aquaresin® Michigan, USA), clove (Aquaresin® Michigan, USA), rosemary (Aquaresin® Michigan, USA) added into the chitosan solutions in the experimental groups were supplied from the Kalsec® company. Glycerol, Tween 20 and glacial acetic acid chemical substances utilized in the formation of film were supplied by the Merck company. In the preparation of the edible films, plexiglass petri plates of 85 mm diameter (Isolab GMBH) were utilized as templates.

In the study, the films for covering of samples are prepared according to method of Ponce et al. (2008). A flow diagram for the obtaining procedure of films is given Fig. 1.

After the fillets were covered with the film sterilized under the UV (BIOBASE, Jinan, China) for 2 min, they were vacuumed into the packaging machine (Henkelman Vacuum Systems BOXER 42, Netherlands). Finally, they were organized into six experimental groups.

The experimental groups were put into storage at 2 °C and they were analyzed in terms of chemical, microbiological and sensory parameters every 3 days (on 0th, 3rd,

6th, 9th, 12th, 15th, 18th, 21st, 24th, 27th, 30th and 33rd days; food content analysis was performed only at the beginning of the storage experimental).

Measurement of film thickness

Film thickness measurements were made on the experimental groups by means of a digital micrometer (Asimeto, Weißbach, Germany) with 1 µm sensitivity. It was made calibrating of the micrometer with a parallel block master set, before measurement of film thickness was performed, and then film thicknesses were measured. Each film samples was measured from three different points. Then, the averages of these measurements were taken into consideration to determine the ultimate thickness.

Chemical analysis

The proximate composition (moisture, crude protein, crude oil and crude ash) of the fish samples was determined according to standard methods (AOAC 2002). The pH values of fillets were determined with a pH meter (Thermo Scientific Orion 3-Star). The sample was homogenized in distilled water in the ratio 1:10 (wt/vol), and the measurement was made using a pH meter (Thermo Scientific Orion 3-Star Benchtop, Cambridge, UK) (AOAC 2002). The total volatile basic nitrogen (TVB-N) content was determined according to the method of Conell and Shewan (1979). Peroxide value (PV), expressed in milliequivalents of peroxide oxygen per kilogram of fat, was analyzed according to Mattissek et al. (1992). Thiobarbituric acid reactive substances (TBARs) was analyzed according to method of Tarladgis et al. (1960) to evaluate the oxidation changes in the fillets during storage, and the results were expressed as the TBARs value in milligrams of malondialdehyde per kilogram (mg MA kg^{-1}) of fish flesh.

Microbiological analysis

Fish meat (10 g) was mixed with 90 ml of sterile Ringer's solution (one-quarter strength) and then stomached (Stomacher 400) for 3 min. Moreover, decimal dilutions were made and then 0.1 ml of each dilution was pipetted onto the surface of plate count agar (Merck 1.05463) plates for determination of aerobic bacteria (AB) and psychrophile bacteria (PB), incubated at 30 ± 1 °C for 3 days and at 5 ± 1 °C for 7 days. Anaerobic bacteria (AnB) were determined in Brewer Anaerob Agar (Merck 1.05410) plates incubated at 30 ± 1 °C for 3 days and an anaerobic atmosphere kit (Anaerogenk, Oxoid) was placed together with the plates inside the anaerobiosis jar. Coliform count (CB) was determined in Violet Red Bile agar (VRB agar,

Fig. 1 Process flow diagram for the preparation of edible films

Merck) plates incubated at 35 °C for 1 day. For lactic acid bacteria (LAB), 0.1 ml of each dilution was pipetted onto the surface of de Man Rogosa Shape agar (MRS; Merck 1.10660) and the plates covered with an overlay of MRS medium (2–3 mm). To count of lipolytic microorganisms (LB) Tributyrin Agar (Merck 1.01957) was used. After plates were incubated at 30 ± 1 °C for 2 days, a clear zone was evaluated around the colonies. Potato Dextrose Agar medium (Merck 1.10130) was used to count the number of yeast and fungi colonies after the cultivated plaques were incubated at 25 ± 1 °C for 4–5 days (Harrigan 1998).

Sensorial analysis

Ten experienced panellists, all of whom were academic staff trained in sensorial evaluated for the samples, were used to evaluate the quality of fillets during storage. The samples were cooked for 10 min at 180 °C in a cooking bag before the samples were presented to the panellists. Panelists evaluated the general acceptability of the samples on a 10-point hedonic scale ranging from inconsumable (1) to excellent (10). All samples were stored at 2 °C until sensorial analysis was performed (Amerine et al. 1965).

Statistical analysis

The chemical, microbiological and sensorial values obtained during storage of the experimentally prepared fillets were statistically analyzed. For statistical analysis, the IBM SPSS®22 (SPSS Inc., Chicago, IL, USA) statistical software package was used. The statistical significance of differences between groups and storage days was determined using analysis of variance (ANOVA).

Results and discussion

Film thickness changes

The thickness of the edible film made up of chitosan solution was measured as 0.034 ± 0.01 µm. It was observed that the addition of thyme, clove and rosemary essential oils after Tween 20 into the film solutions increased the film thickness significantly ($p < 0.05$) (Table 1).

Chemical composition changes

Changes observed in the food composition of the sample experimental groups investigated in the study are presented

Table 1 Thickness of produced films in the study

The type of film	Film thickness (μm) $\bar{X} \pm S \bar{x}$
Chitosan	0.034 ± 0.010^x
Thyme + chitosan	0.058 ± 0.011^y
Clove + chitosan	0.061 ± 0.014^y
Rosemary + chitosan	0.059 ± 0.013^y

^{x, y}Different letters on the same column display differences among groups

in Table 2. The average moisture content of the *L. esocinus* fillets used in the experiment was determined as $71.27 \pm 1.20\%$. It was determined that this rate was inclined to decrease as edible film was applied to the experimental samples (B, C, D, E and F groups). Accordingly, it was considered that this was a result of edible films absorbing the moisture content of the fillets. According to the statistical analysis in the present study, the difference among the groups was significant in terms of moisture content ($p < 0.05$). Significant differences were determined between the fillet and processed fish in terms of the amount of crude protein ($p < 0.05$). On the other hand, the crude fat rate of the fish meat was determined as $7.75 \pm 1.20\%$. Crude fat amounts determined in the B, C, D, E and F groups were found to be 7.75 ± 1.20 , 10.22 ± 0.49 , 10.32 ± 0.91 , 9.11 ± 0.77 and $9.43 \pm 0.44\%$, respectively. In studies the nutritional values of *L. esocinus* fillets was measures and, the crude fat content was observed as $7.75 \pm 0.71\%$ Gürel İnanlı et al. (2006). Our results were in agreement with the values reported by other groups. When the crude fat contents of the experimental groups were considered, a significant difference was observed between groups ($p < 0.05$). The ash content of fillets was determined as $1.19 \pm 0.28\%$.

Chemical changes

The changes in pH values of *L. esocinus* fillets prepared during storage are displayed in Table 3. In regard to these measurements of experimental fillets, the highest pH value was observed for group B as 6.63 ± 0.14 on 3rd day; the lowest value was measured declared group C as 6.02 ± 0.07 on 30th day. The results indicated that pH value for significantly decrease in all experimental groups according to the storage length ($p < 0.01$). However, no significant difference was observed among groups in terms of pH value ($p > 0.05$). Again, when all groups were considered within the storage period, no significant difference was evident in terms of pH value ($p > 0.05$). These decreases in pH values could be associated with the increasing population of lactic acid bacteria toward the end of the storage period. Gürel İnanlı et al. (2006) reported the pH value as 6.57 ± 0.18 . This was lower than our findings.

The average TVB-N amount in the *L. esocinus* fillets used in our study was determined as 9.33 ± 1.84 mg/100 g (Table 3). When TVB-N values were examined alongside the storage life, an increase was observed in all groups in this period; but the TVB-N increase rate in the groups to which edible film had been applied was limited with respect to other groups. In terms of TVB-N amounts, significant differences were determined among the groups on the 3rd, 6th, 9th, 12th and 15th days of storage ($p < 0.05$). However, no significant difference was determined between groups on some of the measurement days (0th, 18th, 21st, 24th, 27th, 30th and 33rd days) ($p > 0.05$). It has been reported by many researchers that TVB-N values of fish meat increase with respect to the storage remained limited in fish meat coated with chitosan-based edible film (Gómez-Estaca et al. 2010; Jeon et al. 2002; López-Caballero et al. 2005). Whereas Gürel İnanlı et al. (2006) reported TVB-N value in fresh fish meat as 7.06 ± 1.07 mg/100 g. Our results were in agreement with

Table 2 Average chemical composition of experimental fillets prepared in the study

Groups	Food composition (%)			
	Moisture (%) $\bar{X} \pm S \bar{x}$	Crude protein (%) $\bar{X} \pm S \bar{x}$	Crude fat (%) $\bar{X} \pm S \bar{x}$	Crude ash (%) $\bar{X} \pm S \bar{x}$
A	71.27 ± 1.20^a	19.21 ± 1.78^{ab}	7.75 ± 1.20^a	1.19 ± 0.28
B	71.27 ± 1.20^a	19.21 ± 1.78^{ab}	7.75 ± 1.20^a	1.19 ± 0.28
C	66.91 ± 1.35^{bc}	21.13 ± 2.26^{ab}	10.22 ± 0.49^b	0.97 ± 0.27
D	67.56 ± 2.55^b	19.26 ± 0.49^a	10.32 ± 0.91^b	0.98 ± 0.52
E	67.64 ± 0.47^{bc}	19.30 ± 0.42^a	9.11 ± 0.77^{ab}	0.83 ± 0.16
F	66.27 ± 0.78^c	22.24 ± 0.45^b	9.43 ± 0.44^{ab}	0.82 ± 0.17

^{a, b, c}The difference between average values with different letters on the same column

Table 3 Chemical values during storage of the samples prepared

	Groups	Storage days					
		0	3	6	9	12	15
pH*	A	6.49 ± 0.25 ^{bc}	6.59 ± 0.14 ^c	6.62 ± 0.22 ^c	6.57 ± 0.12 ^c	6.35 ± 0.49 ^c	6.30 ± 0.42 ^b
	B	6.49 ± 0.25 ^{bc}	6.63 ± 0.14 ^c	6.53 ± 0.09 ^c	6.53 ± 0.08 ^c	6.55 ± 0.06 ^c	6.22 ± 0.24 ^b
	C	6.49 ± 0.18 ^{bc}	6.53 ± 0.11 ^c	6.61 ± 0.16 ^c	6.61 ± 0.15 ^c	6.62 ± 0.14 ^c	6.33 ± 0.06 ^b
	D	6.53 ± 0.09 ^{bc}	6.50 ± 0.13 ^c	6.48 ± 0.16 ^c	6.49 ± 0.14 ^c	6.55 ± 0.07 ^c	6.43 ± 0.07 ^b
	E	6.53 ± 0.09 ^{bc}	6.50 ± 0.13 ^c	6.48 ± 0.16 ^c	6.49 ± 0.14 ^c	6.55 ± 0.07 ^c	6.43 ± 0.07 ^b
	F	6.48 ± 0.09 ^{bc}	6.44 ± 0.14 ^c	6.51 ± 0.15 ^c	6.52 ± 0.13 ^c	6.54 ± 0.13 ^c	6.34 ± 0.01 ^b
TVB-N (mg/100 g ^{***})	A	9.33 ± 1.84 ^a	14.72 ± 0.22 ^{b3}	19.68 ± 0.94 ^{c3}	25.73 ± 0.83 ^{d2}	32.44 ± 1.13 ^{e1}	39.53 ± 1.39 ^{f3}
	B	9.33 ± 1.84 ^a	13.00 ± 1.01 ^{b23}	15.49 ± 0.43 ^{c2}	19.90 ± 0.77 ^{d1}	22.02 ± 1.48 ^{e1}	27.74 ± 1.72 ^{f2}
	C	8.57 ± 1.16 ^a	11.32 ± 1.30 ^{b12}	13.32 ± 1.41 ^{e1}	18.06 ± 0.84 ^{d1}	20.90 ± 1.69 ^{e1}	23.06 ± 0.85 ^{f1}
	D	8.24 ± 1.01 ^a	10.44 ± 1.37 ^{b12}	13.62 ± 0.87 ^{e12}	18.42 ± 0.46 ^{d1}	19.90 ± 2.25 ^{e1}	20.87 ± 2.21 ^{f1}
	E	8.48 ± 1.11 ^{a1}	10.35 ± 1.24 ^{b1}	13.54 ± 0.47 ^{e12}	18.03 ± 0.28 ^{d1}	19.89 ± 0.83 ^{e1}	22.68 ± 0.87 ^{f1}
	F	8.92 ± 0.52 ^{a12}	11.05 ± 1.02 ^{b12}	14.07 ± 0.83 ^{e12}	17.73 ± 0.81 ^{d1}	21.75 ± 1.35 ^{e1}	23.95 ± 0.74 ^{f1}
Peroxide value (meq/kg) ^{***}	A	3.46 ± 0.81 ^{a12}	5.13 ± 1.42 ^{a3}	7.66 ± 1.24 ^{ab3}	9.00 ± 2.05 ^{b3}	14.33 ± 1.22 ^{c3}	17.66 ± 1.24 ^{de3}
	B	3.46 ± 0.81 ^{a12}	4.00 ± 0.40 ^{a23}	5.50 ± 0.40 ^{ab23}	6.63 ± 0.96 ^{b2}	10.00 ± 0.81 ^{c2}	13.33 ± 1.88 ^{de2}
	C	3.06 ± 0.24 ^{a12}	2.63 ± 0.53 ^{a12}	3.33 ± 0.82 ^{ab12}	4.36 ± 0.44 ^{b12}	6.30 ± 1.39 ^{c1}	6.53 ± 1.03 ^{de1}
	D	3.20 ± 0.43 ^{a12}	2.63 ± 0.47 ^{a12}	2.13 ± 1.20 ^{ab1}	4.86 ± 0.26 ^{b12}	5.50 ± 1.79 ^{c1}	7.33 ± 1.47 ^{de1}
	E	2.13 ± 0.24 ^{a1}	1.83 ± 0.23 ^{a1}	2.26 ± 1.26 ^{ab1}	3.76 ± 0.63 ^{b1}	4.96 ± 1.58 ^{c1}	7.16 ± 0.84 ^{de1}
	F	3.96 ± 1.44 ^{a2}	2.26 ± 0.37 ^{a1}	2.93 ± 1.56 ^{ab12}	5.13 ± 0.63 ^{b12}	6.13 ± 1.21 ^{c1}	7.50 ± 1.71 ^{de1}
TBARs (mg MA kg ⁻¹) ^{****}	A	0.45 ± 0.24 ^{ax1}	1.20 ± 1.07 ^{bx2}	3.04 ± 0.79 ^{cx2}	3.01 ± 0.05 ^{cdx4}	5.12 ± 0.27 ^{dex4}	6.03 ± 0.22 ^{efgmx5}
	B	0.45 ± 0.24 ^{ay1}	1.20 ± 0.13 ^{by2}	1.85 ± 0.08 ^{bey1}	2.17 ± 0.13 ^{cdy3}	2.98 ± 0.24 ^{dey3}	3.64 ± 0.24 ^{efghy4}
	C	0.22 ± 0.07 ^{ay2}	0.40 ± 0.03 ^{by1}	1.28 ± 0.11 ^{bey1}	1.40 ± 0.16 ^{cdy1}	1.45 ± 0.01 ^{dey1}	1.86 ± 0.09 ^{efghy2}
	D	0.19 ± 0.03 ^{ay2}	1.28 ± 0.02 ^{by2}	1.37 ± 0.05 ^{bey1}	1.56 ± 0.07 ^{cdy12}	1.91 ± 0.08 ^{dey2}	2.63 ± 0.04 ^{efghy3}
	E	0.22 ± 0.05 ^{ay2}	1.21 ± 0.02 ^{by2}	1.54 ± 0.25 ^{bey1}	1.72 ± 0.20 ^{cdy2}	1.71 ± 0.06 ^{dey12}	1.21 ± 0.29 ^{efghy1}
	F	0.17 ± 0.07 ^{ay12}	1.41 ± 0.34 ^{by2}	1.35 ± 0.03 ^{bey1}	1.40 ± 0.01 ^{cdy1}	1.39 ± 0.01 ^{dey1}	2.20 ± 0.3 ^{efghy23}
	Groups	Storage days					
		18	21	24	27	30	33
pH*	A						
	B	6.15 ± 0.47 ^{bc}					
	C	6.45 ± 0.12 ^{bc}	6.45 ± 0.06 ^{bc}	6.43 ± 0.03 ^{bc}	6.20 ± 0.16 ^b	6.02 ± 0.07 ^a	
	D	6.51 ± 0.06 ^{bc}	6.29 ± 0.21 ^{bc}	6.40 ± 0.08 ^{bc}	6.39 ± 0.01 ^b	6.26 ± 0.11 ^a	6.14 ± 0.10 ^a
	E	6.51 ± 0.06 ^{bc}	6.29 ± 0.21 ^{bc}	6.40 ± 0.08 ^{bc}	6.39 ± 0.01 ^b	6.26 ± 0.11 ^a	6.14 ± 0.10 ^a
	F	6.46 ± 0.01 ^{bc}	6.47 ± 0.04 ^{bc}	6.47 ± 0.07 ^{bc}	6.43 ± 0.02 ^b	6.17 ± 0.12 ^a	

Table 3 continued

	Groups	Storage days					
		18	21	24	27	30	33
TVB-N (mg/100 g ^{**})	A						
	B	37.46 ± 2.56 ^{fg}					
	C	25.06 ± 0.81 ^{fg}	27.12 ± 0.89 ^{fg}	29.56 ± 1.97 ^g	34.68 ± 0.51 ^h	39.06 ± 0.82 ⁱ	43.04 ± 0.86 ^j
	D	21.32 ± 2.18 ^{fg}	26.84 ± 1.31 ^{fg}	28.60 ± 1.47 ^g	32.47 ± 1.81 ^h	35.40 ± 1.77 ⁱ	42.06 ± 0.73 ^j
	E	23.76 ± 0.82 ^{fg}	25.77 ± 1.36 ^{fg}	27.80 ± 1.27 ^g	31.66 ± 1.37 ^h	34.04 ± 0.85 ⁱ	
	F	26.78 ± 0.17 ^{fg}	28.52 ± 0.73 ^{fg}	32.08 ± 1.53 ^g	34.94 ± 0.04 ^h	39.40 ± 0.63 ⁱ	
Peroxide Value (meq/kg) ^{***}	A						
	B	16.00 ± 1.63 ^{de}					
	C	7.90 ± 0.53 ^{de}	9.06 ± 0.04 ^{cd}	9.40 ± 0.37 ^{cde}	10.33 ± 0.47 ^{de}	12.33 ± 0.47 ^{ef}	
	D	8.83 ± 0.23 ^{de}	8.66 ± 0.47 ^{cd}	9.16 ± 0.09 ^{cde}	9.50 ± 0.41 ^{de}	9.56 ± 0.32 ^{ef}	12.33 ± 0.47 ^f
	E	9.36 ± 0.38 ^{de}	9.43 ± 0.31 ^{cd}	9.60 ± 0.08 ^{cde}	9.90 ± 0.24 ^{de}	11.66 ± 0.47 ^{ef}	12.20 ± 1.63 ^f
	F	9.03 ± 0.77 ^{de}	8.70 ± 0.92 ^{cd}	8.76 ± 1.11 ^{cde}	8.66 ± 0.47 ^{de}	11.00 ± 1.41 ^{ef}	
TBARs (mg MA kg ⁻¹) ^{****}	A						
	B	4.13 ± 0.32 ^{efghy}					
	C	2.12 ± 0.17 ^{efghy}	2.89 ± 0.07 ^{efy}	3.32 ± 0.24 ^{efghy}	3.80 ± 0.08 ^{ghy}	3.78 ± 0.47 ^{hy}	
	D	2.77 ± 0.06 ^{efghy}	2.82 ± 0.05 ^{efy}	2.91 ± 0.07 ^{efghy}	3.64 ± 0.31 ^{ghy}	3.61 ± 0.28 ^{hy}	3.47 ± 0.31 ^{ghy}
	E	1.91 ± 0.14 ^{efghy}	1.99 ± 0.08 ^{efy}	2.24 ± 0.12 ^{efghy}	2.72 ± 0.30 ^{ghy}	3.19 ± 0.21 ^{hy}	3.17 ± 0.17 ^{ghy}
	F	2.38 ± 0.08 ^{efghy}	2.73 ± 0.01 ^{efy}	2.22 ± 0.39 ^{efghy}	3.36 ± 0.63 ^{ghy}	3.87 ± 0.63 ^{hy}	

^a, ^b, ^cDifferences among averages with the same letters on the same row indicate statistical differences among storage days ($p < 0.01$)

^{**a}, ^b, ^c, ^d, ^e, ^f, ^g, ^h, ⁱ, ^jDifferences among averages with the same letters on the same row indicate statistical differences among storage days ($p < 0.01$); ¹, ², ³Differences among averages with different numbers on the same column indicate statistical differences among days of each specific group ($p < 0.05$)

^{**a}, ^b, ^c, ^d, ^e, ^fDifferences among averages with the same letters on the same row indicate statistical differences among storage days ($p < 0.01$); ¹, ², ³Differences among averages with different numbers on the same column indicate statistical differences among days of each specific group ($p < 0.05$)

^{***a}, ^b, ^c, ^d, ^e, ^f, ^g, ^hDifferences among averages with the same letters on the same row indicate statistical differences among storage days ($p < 0.01$); ¹, ², ³, ⁴, ⁵Differences among averages with different numbers on the same column indicate statistical differences among days of each specific group ($p < 0.05$); ^x, ^yDifferences among averages with different numbers on the same column indicate statistical differences among days of each specific group ($p < 0.05$)

the values reported by other groups (Gürel İnanlı et al. 2006).

In the present research, the peroxide value in fillets was determined as 3.46 ± 0.81 (meq O_2 /kg), as displayed in Table 3. After the film-coating process, the peroxide value did not change in all groups and remained in the range of 2.13 ± 0.24 – 3.96 ± 1.44 meq O_2 /kg, respectively. When the effect of the storage period on the peroxide value was considered, significant differences were determined ($p < 0.01$). In terms of peroxide value, it was determined that the effect of the storage period was statistically significant in all experiment groups ($p < 0.05$). In the study of Jeon et al. (2002), investigating the preservative effect of chitosan as an edible film, while the peroxide value of herring fish from the experimental group did not exceed 10 meq O_2 /kg until 8th day of storage, the control group in the experiment exceeded this value (10 meq O_2 /kg) on the 4th day of storage.

In terms of the fluctuation of measured TBARs data, whereas the lowest value was measured in the F group as 0.17 ± 0.07 mg MA kg^{-1} on day 0, the highest value was measured in group A as 6.03 ± 0.22 mg MA kg^{-1} on the 15th day (Table 3). During the storage period, significant increases were observed in the TBARs values ($p < 0.01$), and, additionally, the differences among groups in terms of the TBARs values were found to be statistically significant as can be observed in Table 3 ($p < 0.05$). Additionally, when TBARs values during the storage period were compared among all groups, significant differences were determined ($p < 0.05$). Gürel İnanlı et al. (2006) determined the TBARs value of fresh fish meat as 0.20 ± 0.00 , which corresponds to our findings.

Microbiological changes

The population of aerobic bacteria (AB) determined during the storage period of experimental groups examined in this study is given in Fig. 2a. As the population of AB in the fillets used in the study was determined as 2.65 ± 0.81 log CFU g^{-1} , it was determined to be in the range of 2.65 ± 0.81 – 3.32 ± 0.43 log CFU g^{-1} in all film coated groups (B, C, D, E, and F). According to the statistical analysis data collected during the storage period, it was observed that there was a significant increase in population of microorganisms over time ($p < 0.01$). Furthermore, it was determined that the storage period was statistically important in all groups ($p < 0.05$). The total AB population determined in the present study exceeded the acceptable limits on the 12th day in the A and B groups, on the 30th day in the C and F groups and on the 33rd day in the D and E groups. Jeon et al. (2002) reported 3 log decrease in microbiological populations in both herring and cod fish coated with chitosan during their storage period.

Additionally, similar results were reported by a number of studies investigating the effectiveness of chitosan-coated films in the prevention or reduction of microbiological degradation (Fan et al. 2009; Gómez-Estaca et al. 2010; Ojagh et al. 2010; Vasconez et al. 2009). Gomez-Estaca et al. (2007) reported that gelatin-chitosan films were highly efficient in the prevention of microbiological development in cold-smoked sardine. In another study which investigated effect of chitosan in various concentrations (1, 1.5, 2, 2.5 and 3%) on the shelf life of *Ctenopharyngodon idellus* freshly vacuum-packaged and stored at 4 °C, it was reported that the chitosan solution had an effect on the reduction of bacteria development and that a 2% chitosan concentration was ideal for the extension of the shelf life of product (Runfeng and Li 2011).

The counts of anaerobic bacteria measured within the experimental groups during the storage period are given in Fig. 2b. When the groups were compared with each other in the storage period, significant differences were determined with the AnB population on the 0th, 6th, 9th, 12th and 15th days ($p < 0.05$). Ye et al. (2008) covered cold-fumed salmon with chitosan. At the end of the 8th week, the bacteria population of the chitosan-coated fillets did not exceed 6.4 log CFU g^{-1} . This finding confirms to our results.

In the experimental groups, it was determined that total population of the psychrophilic aerobic bacteria (PB) only exceeded the limit value in group B on the 18th day of the storage period. It is seen this counts in Fig. 2c. Furthermore, it was determined that the variation in population of PB during the storage period was significant ($p < 0.01$) and those differences among groups were statistically significant ($p < 0.05$). Furthermore, it was determined that in terms of the PB population that the storage period had a significantly statistical effect on all groups ($p < 0.05$). The difference between the reported results and our findings could be due to the fish species studied or our practice using edible film. In the available studies in the literature, it was stated that thyme and sage essential oils reduced psychrophilic bacteria activities (Emir Çoban et al. 2016). This finding also confirms to our results.

The lactic acid bacteria population was determined for fresh *L. esocinus* fillets as 2.94 ± 0.24 log CFU g^{-1} (Fig. 2d). This value increased rather high values at the end of the shelf life of all groups in the experiment and this increase was considered as statistically significant ($p < 0.01$). A significant difference was determined among groups in terms of bacteria population ($p < 0.05$). Additionally, it was determined that the effect of the storage period was statistically significant on all groups ($p < 0.05$). Gómez-Estaca et al. (2010) reported that the LAB population decreased by 4.00 log CFU g^{-1} in the experimental groups to which film had been applied with respect to the

AB: Aerobic bacteria, AnB: Anaerobic bacteria, PB: Psychrophile bacteria,
LAB: Lactic acid bacteria, LB: Lipolytic bacteria, CB: Coliform bacteria

Fig. 2 Microbiological Changes during storage at 2 ± 1 °C. *AB* aerobic bacteria, *AnB* anaerobic bacteria; *PB* psychrophile bacteria, *LAB* lactic acid bacteria, *LB* lipolytic bacteria, *CB* coliform bacteria

control group. The fact that this value was less than our finding could be associated with the fish species and the chitosan–gelatin practice. Ouattara et al. (2000) and López-Caballero et al. (2005) determined in their studies that it was effective in preventing the growth of the LAB bacteria.

In counts of the lipolytic microorganisms included in the prepared experimental groups, as given in Fig. 2e, whereas the lowest value was counted in the F group as 2.54 ± 0.21 log CFU g⁻¹ on the day 0, the highest value was counted in the A group as 5.80 ± 0.41 log CFU g⁻¹ on the 15th day. A significant difference was determined among all fillet groups ($p < 0.05$), and, additionally, it was observed that there were increases in the LB population during the storage period ($p < 0.01$). In terms of the bacteria population, it was found that effect of the storage period on all groups was statistically significant ($p < 0.05$). Ahmed and Abd El-Rahman (2002), who investigated the population of lipolytic microorganisms in grounded meat, added essential oils in their study, reported that the storage period was not statistically significant.

The counts of yeast-mold determined in samples during storage period are shown in Fig. 2f. Whereas the amount of yeast-mold determined in fillets was 2.30 ± 0.16 log₁₀ kob/g, it was determined for the A, B, C, E, D and F groups at the end of the storage periods as 6.33 ± 0.44 ; 6.68 ± 0.36 ; 6.93 ± 0.32 ; 6.39 ± 0.07 ; 5.96 ± 0.54 and 6.92 ± 0.31 log CFU g⁻¹, respectively. There was a significant difference among the groups in terms of yeast-mold amount found ($p < 0.05$). Furthermore, the storage period caused a significant increase in amount of yeast-mold ($p < 0.01$). In terms of yeast-mold amount, it was determined that the storage period had statistically significant effect on all groups ($p < 0.05$). Inhibited effect of thyme on the amount of yeast-mold has been reported by researchers (Rasooli and Owlia 2005).

The amount of coliform-group bacteria determined in *L. esocinus* fillets covered with chitosan edible films was 3.21 ± 0.58 log CFU g⁻¹ (Fig. 2g). At the end of the storage period, whereas the highest count of bacteria was determined in the B group on the 18th day (6.61 ± 0.26 log CFU g⁻¹), the lowest count was determined in the D group on the 33rd day (4.14 ± 0.73 log CFU g⁻¹). The storage period was found to have a significant effect on the amount of CB in the fillet experimental groups ($p < 0.01$). Additionally, the storage period was found statistically significant on all groups in terms of CB ($p < 0.05$). In the study of Kenar (2009) on investigating the CB amount, whereas this value was determined as 10^3

Fig. 3 Sensorial changes during storage at 2 ± 1 °C

log CFU g⁻¹ at the beginning of the storage period, it was reported at the end of the 20th day of storage period 8.08, 6.23 and 6.80 log CFU g⁻¹ in the control, rosemary and sage experiment groups respectively. The researcher observed that CB development retarded in sardine fillets processed with rosemary and sage, and the highest antibacterial effect was measured with rosemary.

Sensorial evaluations

The E group earned the highest preference score (9.72 ± 0.20 on the 3rd day) (Fig. 3) and the lowest value was measured in A group on 12th day (3.66 ± 0.47), in B group on 15th day (3.33 ± 0.42), in C and F groups on 27th (3.13 ± 0.27 – 2.33 ± 0.27) and in D and E groups on 30th day (3.61 ± 0.28 – 4.55 ± 1.03). In terms of General Acceptability as determined by panelists between the groups, it was determined that the highest score was given to the experimental group, which clove essential oil had been applied on the third day. The significant differences were determined among General Acceptability scores measured during the storage period ($p < 0.01$). Furthermore, it was determined that effective of the storage period was statistically significant on all experimental groups ($p < 0.05$).

Conclusion

According to the findings of the present study, it was determined the edible films prepared with chitosan had a positive effect on the shelf life of the experimental samples prepared in the study. Additionally it was determined chitosan and chitosan incorporated with essential oils of thyme, clove and rosemary had a positive effect on chemical, microbiological and sensorial characteristics of *L. esocinus* fillets. It is concluded that chitosan based films

incorporated with essential oils of thyme, clove and rosemary had been more effective in fish fillets preservation.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest reading the publication of this paper.

References

- Ahmed AM, Abd El-Rahman HA (2002) Effect of ground sage on some microbial characteristics of refrigerated minced beef. *J King Saud Univ Agric Sci* 15:47–52
- Amerine MA, Pangborne RM, Roessler EB (1965) Principles of sensory evaluation of food. Academic Press, New York, p 602
- AOAC (2002) Official methods of analysis of the Association of Official Analytical Chemists, 17th edn. Association of Official Analytical Chemists, Gaithersburg
- Caner C, Vergano PJ, Wiles JL (1998) Chitosan film mechanical and permeation properties as affected by acid. *Plast Storage J Food Sci* 63:6
- Cha DS, Chinnan MS (2004) Biopolymer-based antimicrobial packaging: a review. *Crit Rev Food Sci Nutr* 44:223–237
- Conell JJ, Shewan JM (1979) Past, present and future fish science. In: *Advances in fish science and technology*. Fishing News Books Ltd., England
- Emir Çoban Ö, Patır B, Özpolat E, Karaton Kuzgun N (2016) Improving the quality of fresh rainbow trout by sage essential oil and packing treatments. *J Food Saf* 36:299–307
- Falowo AB, Fayemi PO, Muchenje V (2014) Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: a review. *Food Res Int* 64:171–181 (**View at Publisher View at Google Scholar View at Scopus**)
- Fan W, Sun J, Chen Y, Qiu J, Zhang Y, Chi Y (2009) Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. *Food Chem* 115:66–70
- Gomez-Estaca J, Montero P, Gime'nez B, Gómez-Guillén MC (2007) Effect of functional edible films and high pressure processing on microbial and oxidative spoilage in cold-smoked sardine (*Sardina pilchardus*). *Food Chem* 105:511–520
- Gómez-Estaca J, López De Lacey A, López-Caballero ME, Gómez-Guillén MC, Montero P (2010) Biodegradable gelatine-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiol* 27:889–896
- Gürel İnanlı A, Emir Çoban Ö, Özpolat E, Dartay M (2006) Determination of the shelf-life of fish croquettes during cold storage (*Barbus esocinus*, Heckel, 1843). *Sünder* 25:26, 40–44
- Harrigan WF (1998) Laboratory methods in food microbiology, 3rd edn. Academic Press, London
- Jeon YJ, Kamil J, Shahidi F (2002) Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. *J Agric Food* 20(50):5167–5178
- Jeyakumari A, George Ninan Joshy CG, Parvathy U, Zynudheen AA, Lalitha KV (2016) Effect of chitosan on shelf life of restructured fish products from pangasius (*Pangasianodon hypophthalmus*) surimi during chilled storage. *J Food Sci Technol*. <https://doi.org/10.1007/s13197-016-2174-3>
- Kenar M (2009) Investigation of sensory, chemical and microbiological effects of natural antioxidant obtained from aromatic plants on fish fillets. MSc Thesis, Çukurova University
- Kester JJ, Fennema OR (1986) Edible films and coatings: a review. *Food Technol* 40(12):47–58
- Lerdthanangkul S, Krochta JM (1996) Edible coating effects on postharvest quality of green bell peppers. *J Food Sci* 61(1):97–103
- López-Caballero ME, Gómez-Guillén MC, Pérez-Mateos M, Montero P (2005) A chitosan-gelatin blend as a coating for fish patties. *Food Hydrocolloids* 19:303–311
- Mattisek R, Schnepel MF, Steiner G (1992) *Lebensmittelanalytik, Grundzüge, Methoden, Anwendungen*. Springer, Zweite korrigierte Auflage, Berlin
- Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH (2010) Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chem* 120(1):193–198
- Ouattara B, Simard RE, Piette G, Bégin A, Holley RA (2000) Inhibition of surface spoilage bacteria in processed meats by application of antimicrobial films prepared with chitosan. *Int J Food Microbiol* 62:139–148. [https://doi.org/10.1016/S0168-1605\(00\)00407-4](https://doi.org/10.1016/S0168-1605(00)00407-4)
- Pizzal L, Bortolomeazzi R, Vichi S, Überegger E, Conte LS (2002) Antioxidant activity of sage (*Salvia officinalis* and *S. fruticosa*) oregano (*Origanum onites* and *O. onites*) extracts related to their phenolic compound content. *J Sci Food Agric* 82:1645–1651
- Ponce AG, Roura S, Del Valle CE, Moreira MR (2008) Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: in vitro and in vivo studies. *Postharvest Biol Technol* 49:294–300
- Rasooli I, Owlia P (2005) Chemoprevention by thyme oils of *Aspergillus paraciticus* growth and aflatoxin production. *Phytochemistry* 66:2851–2856
- Ruiz-Navajas Y, Viuda-Martos M, Barber X, Sendra E, Perez-Alvarez JA, Fernández-López J (2015) Effect of chitosan edible films added with *Thymus moroderi* and *Thymus piperella* essential oil on shelf-life of cooked cured ham. *J Food Sci Technol*. <https://doi.org/10.1007/s13197-015-1733-3>
- Runfeng W, Li Z (2011) Effect of chitosan on fresh-keeping refrigerated fillets of fresh *Ctenopharyngodon idellus*. In: *New technology of agricultural engineering (ICAE)*. IEEE, pp 1061–1064
- Santos FMS, Silva AIM, Vieira CB, Araujo MH, Silva ALC, Cunha MGC, Souza BWS, Bezerra RS (2017) Use of chitosan coating in increasing the shelf life of liquid smoked Nile tilapia (*Oreochromis niloticus*) fillet. *J Food Sci Technol*. <https://doi.org/10.1007/s13197-017-2570-3>
- Singhal RS, Kulkarni PR, Rege DV (2001) University of mumbai handbook of herbs and spices, Volume 1 (K. V. Peter (ed.)). Woodhead Publishing Limited, Cambridge
- Tarladgis BG, Watts BM, Younathan MT, Dugan JR (1960) A distillation method for the quantitative determination of malonaldehyde in rancid foods. *J Am Oil Chem Soc* 3:44–48
- Vasconez MB, Flores SK, Campos CA, Alvarado J, Gerschenson LN (2009) Antimicrobial activity and physical properties of chitosan-tapioca starch based edible films and coatings. *Food Res Int* 42(7):762–769
- Ye M, Neetoo H, Chen H (2008) Effectiveness of chitosan-coated plastic films incorporating antimicrobials in inhibition of *Listeria monocytogenes* on cold-smoked salmon. *Int J Food Microbiol* 127:235–240