Italian Journal of Food Science, 2025; 37 (1): 307–318

Characterization of chitosan films incorporating thyme oil and its effect on black olives

Pınar Çoruhlu*, Ahsen Rayman Ergün, Taner Baysal

Food Engineering Department, Engineering Faculty, Ege University, Izmir, Turkey

***Corresponding Author:** Pınar Çoruhlu, Food Engineering Department, Engineering Faculty, Ege University, Izmir, Turkey. Email: pinarcoruhlu@gmail.com

Academic Editor: Prof. Monica Rosa Loizzo – Università della Calabria, Italy

Received: 17 September 2024; Accepted: 22 November 2024; Published: 1 January 2025
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OPEN ACCESS

ORIGINAL ARTICLE

Abstract

This study purposed to determine the impacts of chitosan film (CF) (2%, w/v) coating combined with thyme oil (TO) (0.2%, 0.5%, 1%, (v/v)) on the quality properties of black olives during storage (3 months, +4 and 25°C). In the study, film properties (color, elongation at break, tensile strength, Young's modulus, and scanning electron microscope (SEM)) were analyzed. In the second stage, table olive samples were assessed for physicochemical (% moisture, pH, titratable acidity, color (L*, a*, b* and ΔE*), and microbiological (yeast–mold and total viable count) properties. The average elongation at break, tensile strength, and Young's modulus values of the obtained film coating samples were 70.02%, 16.66 MPa, 24.05 MPa for the CF; 74.69%, 14.79 MPa, 19.65 MPa for the CF + 0.2% TO; 77.21%, 10.79 MPa, 13.94 MPa for CF + 0.5% TO and 83.78%, 10.40 MPa, 12.69 MPa for CF + 1.0% TO group, respectively. Chitosan film sample was determined the highest tensile strength value and the CF+1% TO sample group was detected with the highest elongation at break. It was determined that the increase in the concentration of TO in the film increased the elongation at break and decreased the Young's modulus value. Scanning electron microscope (SEM) surface images of group CF film have a homogeneous and uniform structure but added thyme oil occured a heterogeneous appearance. The L*, a*, b*, and ΔE* values increased with the addition of thyme oil and a statistically significant difference was found between samples ($P < 0.05$). The highest moisture loss was determined in the control (uncoated) group. The highest decrease in yeast–mold and total viable count value was found 4.89 log CFU/g CF + 0.2% TO and 5.21 log CFU/g in CF + 1.0% TO sample at the end of storage period at 4°C in table olives.

Keywords: black olives; chitosan; film; microbiological; properties; quality; thyme oil

Introduction

Table olive consumption is increasing day by day in the world (International Olive Council, 2023). Table olives have an important place in the Mediterranean diet. This is due to the sensory properties and nutritional values (Lanza *et al*., 2010). Table olives, rich in phenolic compounds, have been reported to have beneficial effects on health (Boskou *et al*., 2006; Kountouri *et al*., 2007). After Spain and Egypt, Turkey ranks third in table olive production. The International Olive Council announced that 3.1 million tons of olives were produced in 2023. Today, synthetic chemicals are used to prevent spoilage in table olives. According to the Turkish Food Codex Regulation, 1000 ppm potassium sorbate and 500 ppm sodium benzoate are allowed to be used in olives and olive-based products.

Chitosan (C) was chosen for coating black olives as it is a high molecular weight cationic polysaccharide with antimicrobial effects as well as functional and film formulation properties (Adegbemiro Alimi *et al*., 2023;

Lin *et al*., 2020). Essential oils (EOs) were started to be used as alternatives to chemical preservatives in foods due to their high antimicrobial and antioxidant activities (Shi *et al*., 2021), and they are generally defined as safe (Antonioli *et al*., 2020).

Thyme oil (*Thymus vulgaris* L.) is defined as a plant of the *Lamiaceae* family, which contains thymol, carvacrol, and linalool (Leyva-Lopez *et al*., 2017; Wang, *et al*., 2021). The usage of thyme oil with chitosan can be used as an alternative to chemical preservatives due to the microbial inhibitor and oxidative prevention effects (Chouhan *et al*., 2017; Han *et al*., 2015; Hyun *et al*., 2015; Pateiro *et al*., 2021; Radünz *et al*., 2020). In recent studies, natural antimicrobial materials have been started to be used instead of chemical preservatives. Martinez *et al.* (2018) reported the effect of edible chitosan coatings incorporated with *Thymus capitatus* essential oil on the shelf-life of strawberries during cold storage. These results demonstrated a positive effect in reducing the microbial population such as aerobic mesophylls, molds, and yeasts compared with samples without treatment, and the fruits provided excellent stability against moisture loss. Tsitsos *et al.* (2023) indicated the effect of chitosan and alginate-based edible membranes with oregano essential oil and olive oil on the microbiological, physicochemical, and organoleptic characteristics of mutton. These edible coatings were reported to contribute to maintaining good quality characteristics and extending the shelf-life of mutton. Besides this, it has been found that chitosan coatings significantly reduce the total mesophilic and psychrophilic, as well as the *B. thermosphacta* and lactic acid bacteria counts in mutton. Moutsatsou *et al.* (2011) reported that the combination of edible coating application and modified atmosphere packaging (MAP) promoted weight loss, helped maintain the color and firmness, and extended shelf life of the olives. In addition, hydrophobic compounds have been reported to reduce the water vapor permeability of films and prevent food from losing water during storage (Abbaszadeh *et al*., 2014; Guerra *et al*., 2015). Although there are studies on chitosan film and essential oil, studies on the application of chitosan incorporated with thyme oil (TO) for black olives are limited in the literature. Some chemical preservatives, such as potassium sorbate and sodium benzoate, are used to prevent spoilage in table olives. However, these chemicals have been reported to pose harmful effects on human health (Cardador and Gallego, 2018). Therefore, studies have suggested that alternative natural preservatives could be used instead of potassium sorbate and sodium benzoate. In recent years, alternative natural antimicrobial substances have attracted increasing attention. As previously reported, edible coatings can effectively prevent microbial spoilage and enhance the safety of food products by inhibiting the growth of microorganisms. This study aimed to evaluate the effects of CF coating with TO on the microbial and physicochemical properties of unbrined table olives during storage. Moreover, this study will assist researchers and industries in selecting an efficient and cost-effective method for developing edible films or coatings for specific applications. Additionally, the method can be used alone or in combination with other techniques to produce edible films or coatings with higher efficiency and durability, which can help extend the shelf life and improve the commercial quality of food products.

Materials and Methods

Materials

This study used fermented black olives (Greek-style "Kalamata") supplied by UGS (Urla Food and Agriculture Products Industry and Trade Inc.). Commercial thyme oil was used as a natural antimicrobial agent (W282812, Sigma Aldrich). The water-soluble, food-grade, medium molecular weight commercial chitosan (Nanjing Lanya Chemical Co. Ltd., China) with 90–95% deacetylation degree was also used as edible film. Additionally, 0.1% mixture of potassium sorbate and sodium benzoate (Nantong Acetic Acid Chemical Co. Ltd., China) used in traditional production was used as a food-grade chemical preservative.

Formulation of chitosan films

To create chitosan film dispersion, the mixture to be prepared with chitosan (2%, w/v), acetic acid (1%), and distilled water was obtained by using a magnetic stirrer at 25°C for 24 h. After adding thyme oil at different concentrations (0.2, 0.5, and 1% v/v) to the chitosan film, the solution was homogenized using a homogenizer at 24,000 rpm for 5 min (Ultra Turrax T25, IKA Labortechnik, Germany). The prepared film solutions were poured onto petri dishes (9 cm in diameter) in equal amounts (20–25 mL) and dried at 40°C using an incubator (Nüve ES 500, Türkiye). The resultant chitosan films are shown in Figure 1.

Application of the coating and storage

The fermented black olives were stored in the refrigerator at +4°C until further study. The black olives were sorted and their seeds were removed. The olives were then kept in water with added salt (2%) for 1 day. The excess moisture on the surface of the olives was then removed under constant airflow in the sterile cabin. The black olive samples were dipped into chitosan solutions containing different concentrations of thyme oil (25°C, 3 min) and

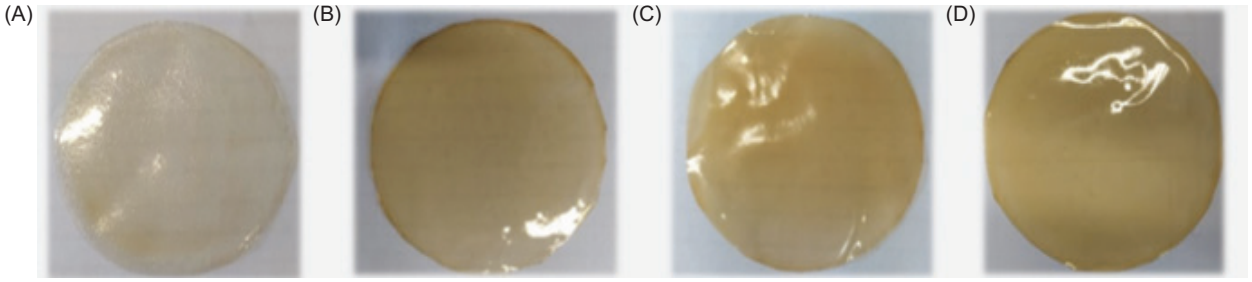

Figure 1. Images of chitosan films with TO (A) CF, (B) CF+0.2% TO, (C) CF+ 0.5% TO, (D) CF+1.0% TO.

drained to cover them with a film. The coated olives were left to air dry at ambient temperature. After that, all samples were vacuum packaged and stored at +4 and 25°C for 3 months. Samples were opened every month, and physicochemical and microbiological analyses were performed in 3 replicates and 2 parallels.

Film characterization

Color analysis

Color values of samples were estimated using the Hunter Lab Color Flex CX1633 measurement device (Managment Company, USA). Finally, the total color difference (ΔE*) value was determined from L*, a*, b* values according to Equation 1.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

While L_0^*, a_0^*, and b_0^* are defined the initial color values of the chitosan film (2% CF), L*, a*, and b* are given the color values of the chitosan films added different concentration of TO.

Mechanical analysis

Mechanical properties were measured using a texture analyzer (model TA-XT2, Stable Microsystems, Godalming, England) following ASTM D882-91. The films are prepared in strips of 20 and 50 mm length. The analyses were performed at least 5 times and recorded. The tensile strength (maximum load (N)/initial cross-sectional area (m^2) of the films), elongation at break (percentage of elongation at the breaking point of the films according to the initial length of the films), and Young's modulus (tensile strength/elongation at break) were calculated as reported by Santhosh and Sarkar (2022).

Scanning electron microscope (SEM)

Scanning electron microscopy (SEM) was used to evaluate the surface morphology of the film samples (Philips XL 30S FEG, FEI Company, Holland).

Analysis performed on coated black olives during the storage

Determination of moisture

The black olives are homogenized with a blender, and 5 g is taken. The sample was kept in the oven (105 ± 2°C) until it reached a constant weight and was cooled in a desiccator. The final weights of the samples were measured and noted (Cemeroğlu, 2010). It was calculated according to the Equation 2.

$$\text{Moisture } \% = \frac{W_0 - W_1}{W_o} \times 100 \quad (2)$$

W_0 = weight (g) of sample before drying
W_1 = weight (g) of sample after drying

pH

pH values of the black olives were calculated according to Brenes *et al.* (1995). According to the given method, 25 mL of distilled water was added to 25 g of the sample and crushed in a blender for 1 min. The pH values were determined using a pH meter (Inolab WTW, Germany) at 20°C, using the method given in Cemeroğlu (2010).

Titratable acidity

The titratable acidity was determined according to the colorimetric titration method in Cemeroğlu (2010). After the samples were homogenized with distilled water, 10 mL of the homogenate was taken in a measuring flask and made up to 100 mL with deionized water. After the filtration process, 10 mL of the filtrate was titrated against a known amount of 0.1 N NaOH until appearance of the pink turning point. Phenolphthalein was used as the indicator. The results were calculated as g/100 mL lactic acid (AOAC, 1995) according to Equation 3.

$$\text{Titratable acidity } \% = N \times V \times E \times 100/m \quad (3)$$

V = Volume of titrate (mL 0.1 NaOH)
N = Normality of titrate (0.1 N NaOH)
E = Equivalent of lactic acid
m = Volume of sample (mL)

Color analysis

Color values of samples were estimated using the Hunter Lab Color Flex CX1633 measurement device (Managment Company, USA). Finally, the total color difference (ΔE*) value was determined from L*, a*, b* values according to Equation 4.

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \qquad (4)$$

while L_0^*, a_0^*, and b_0^* are the initial color values of the uncoated black olive samples onday 0; L_0^*, a_0^*, and b_0^* are the color values of the black olives at different storage times.

Microbiological analysis

Ten grams of sample and 90 mL of 1% peptone water were added into sterile bags after homogenization using a stomacher (BagMixer 400 CC, Interscience, France). Suitable 3M Petrifilm medium plates are ready made culture media (3M Cooperation, USA). The 3M Petrifilm medium plates were incubated at 37°C for 48 h for total viable count (TVC) and at 25°C for 72 h for yeast–mold counts, which were made according to methods (990:12; 997:12) described in Anonymous (2012a and 2012b).

Statistical analysis

ANOVA and Duncan test were used to for variance analysis to evaluate the differences between treatments at a significance level of $P < 0.05$. Three replicates were made for every experiment (SPSS version 18, Chicago, IL, U.S.A).

Results and Discussion

The results of film characterization

Mechanical properties

The results are shown in Table 1. The average elongation at break, tensile strength, and Young's modulus values of the obtained film samples were 70.02%, 16.66 MPa, 24.05 MPa for the CF; 74.69%, 14.79 MPa, 19.65 MPa for CF + 0.2% TO; 77.21%, 10.79 MPa, 13.94 MPa for CF + 0.5% TO; and 83.78%, 10.40 MPa, 12.69 MPa for CF+1% TO group.

Among the film samples, CF had the highest tensile strength value, and the highest elongation at break was determined in CF + 1.0% TO sample group. It was determined that the increase in the concentration of TO in the film increased the elongation at break and decreased the Young's modulus. CF samples were found to be statistically different from the samples with added TO ($P < 0.05$). The addition of TO to the film solutions can cause the film network to deteriorate and thereby decrease the tensile strength values. The addition of thyme oil increased the elongation at break values and the films became more elastic and extensible as a result. It was determined that increasing the concentration of TO increased the elongation at break values and decreased the breaking strength values. The results were found similar to that in the literature; it was found that the elongation values of the films increased and tensile strength decreased (Benavides *et al*., 2012; Shojaee *et al*., 2013). The weakening of the bonds between polymer chains reduced the mechanical strength of the films (Yan *et al*., 2012). Sanchez-Gonzalez *et al*. (2011) concluded in their study that adding EO to the hydrophilic structures of polysaccharide films caused weaker bonds to form between polarized polymers and nonpolar oil molecules in the film matrix network. It is stated that the decrease in tensile strength in films containing EOs causes a microporous structure in the film due to the volatile properties of essential oils during the drying process. Hosseini *et al*. (2009) found that EOs added to film solutions cause the film network to deteriorate and therefore reduce tensile strength values. Additionally, Jouki *et al*. (2014) reported that thyme oil increased elongation at break and decreased tensile strength.

Table 1. Analysis results of the mechanical properties of the obtained chitosan films.

Samples	% Elongation at break	Tensile strength (MPa)	Young's modulus (MPa)
CF	70.02 ± 4.53^{b}	16.66 ± 3.11^{a}	24.05 ± 6.09^{a}
CF + 0.2% TO	74.69 ± 4.63^{ab}	14.79 ± 3.84^{ab}	19.65 ± 3.86^{ab}
CF + 0.5% TO	77.21 ± 2.62^{ab}	10.79 ± 2.82^{b}	13.94 ± 3.35^{b}
CF + 1% TO	83.78 ± 10.42^{a}	10.40 ± 1.81^{b}	12.69 ± 3.49^{b}

Different letters $^{(a,b)}$ in the same column indicate a statistically significant difference ($P < 0.05$).

Color estimation

The results of color are represented in Table 2. The mean values of L*, a*, b*, ΔE* of the film samples were as follows: 8.16, −0.42, and −0.04 for the CF group; 15.39, −0.18, 4.18, and 8.38 for CF + 0.2% TO group; 14.99, 0.4, 5.34, and 8.74 for CF + 0.5% TO; and 13.48, 1.38, 8.67, and 10.38 for the CF + 1% TO group. It was determined that adding TO to the chitosan film increased the L*, a*, b*, and ΔE* values. Color values of chitosan films with added TO differed from the chitosan film sample ($P < 0.05$).

Chitosan films were smooth, transparent, and had a slightly yellow color. When TO was added, they became more opaque and darker yellow colored. It was determined that the yellowness value of the film colors

Table 2. Analysis results of the L*, a*, b*, and ΔE* of the chitosan films.

Samples	L*	a*	b*	ΔE*
CF	8.16 ± 0.07^{c}	−0.42 ± 0.11^{c}	−0.04 ± 0.19^{a}	–
CF + 0.2% TO	15.39 ± 0.43^{a}	−0.18 ± 0.25^{c}	4.18 ± 0.07^{b}	8.38 ± 0.38^{b}
CF + 0.5% TO	14.99 ± 0.18^{a}	0.40 ± 0.11^{b}	5.34 ± 0.15^{c}	8.74 ± 0.13^{b}
CF + 1% TO	13.48 ± 0.28^{b}	1.38 ± 0.13^{a}	8.67 ± 0.98^{d}	10.38 ± 0.66^{a}

Different letters $^{(a,b)}$ in the same column indicate a statistically significant difference (P < 0.05).

increased as the concentration of TO added to the chitosan films increased. The L*, a*, and b* values of the films increased on addition of TO. The color differences between a* and b* values in films containing TO is due to the phenolic compounds that contribute the yellowish colors. Additionally, the properties of the components incorporated to the film matrix and the drying conditions of the films are also believed to have an effect.

Scanning electron microscope (SEM)

Images of chitosan films are represented in Figures 2 and 3. The surface images of group CF film have a homogeneous and uniform structure. The addition of TO causes a heterogeneous appearance in the film surface areas. It was observed that the emulsion stability could not be maintained with the increase in the concentration of TO which caused some heterogeneous appearance on the film surfaces.

This resulted in a heterogeneous and rough surface due to the formation of two phases, namely, oil–polysaccharide in the film structure. Otherwise, films modified with TO showed a structure with droplets of various sizes. This rough structure caused a porous structure on the surface as a result of the movement of lipids to the film surface during drying of the films. The results were found to be similar to that in the literature. Norajit *et al.* (2010) explained that the surface of control films for SEM images was homogeneous and smooth, but with the addition of extracts, the films had heterogeneous and porous

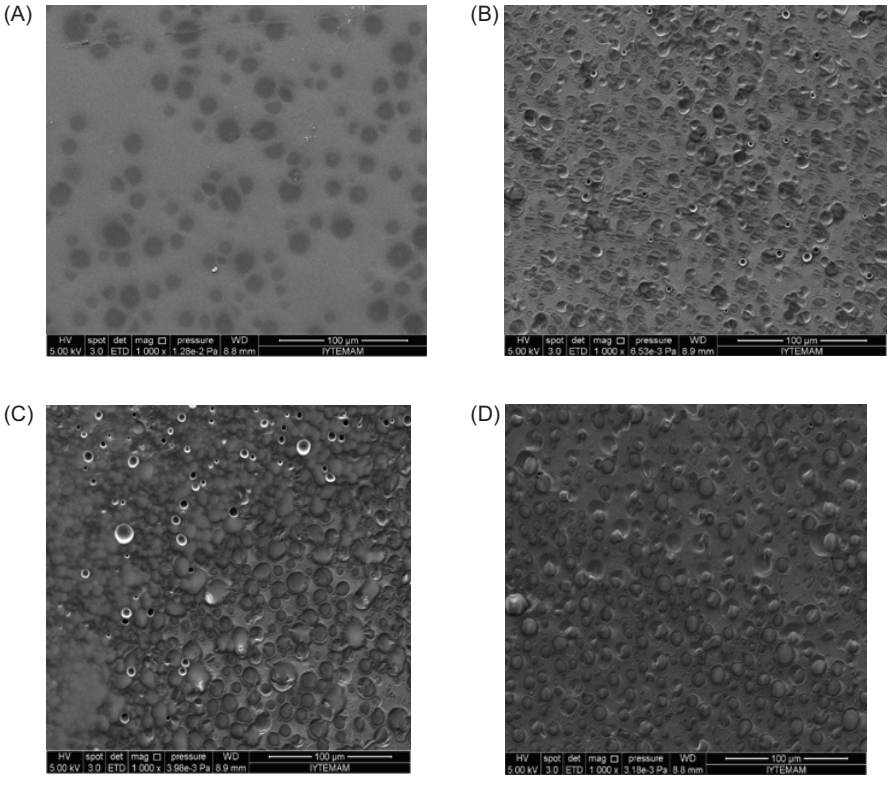

Figure 2. Scanning electron microscope images of chitosan films with TO (1000X magnification scale bar = 100 μm). (A) CF, (B) CF + 0.2% TO, (C) CF + 0.5% TO, (D) CF + 1% TO.

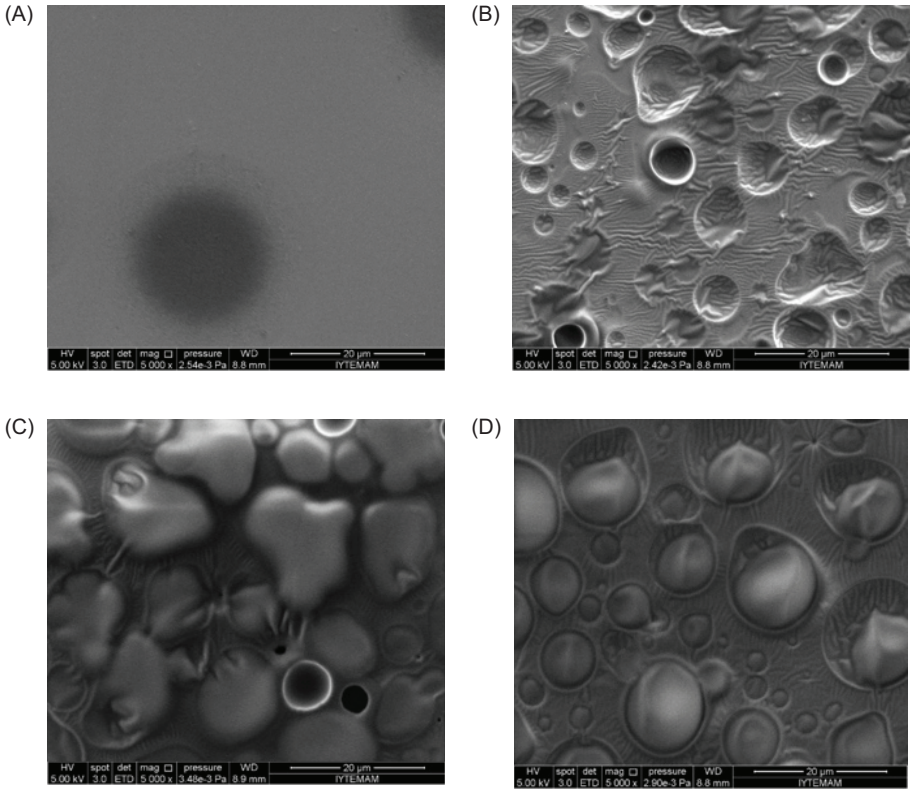

Figure 3. Scanning electron microscope images of chitosan films with TO (5000X magnification scale bar = 20 µm). (A) CF, (B) CF + 0.2% TO, (C) CF + 0.5% TO, (D) CF + 1% TO.

surfaces. Ahmad *et al*. (2012) explained that micropores are formed in the matrix of these films due to the heterogeneous distribution of lipids, which affects the tensile strength, elongation break, and Young's modulus. Kadzinska *et al*. (2019) examined the film morphologies and stated that the control film had a homogeneous structure, while apple puree + sodium alginate films had a heterogeneous structure. In this case, although it seems that the TO in the film formulation solution is important, factors affecting emulsion stability, such as reducing particle sizes, adding appropriate emulsifiers to the film formulation to reduce surface tension, or investigating temperature parameters, and drying conditions are important to improve the films.

The results of the analysis performed on black olive samples coated with chitosan films

Measurements of physicochemical properties

Black olive samples coated with chitosan films were analyzed for their moisture content, pH, and titratable acidity values (Table 3). The initial moisture content of the sample was 59.60%. Moisture content values were found to be as follows: 59.60–53.68% for the control; 59.60–54.49% for PS + SB; 59.60–55.90% for CF; 59.60–56.64% for CF + 0.2% TO; 59.60–56.38% for CF + 0.5%; and 59.60–56.82% for CF+1% TO sample at +4°C. The moisture values of the samples stored at 25°C at the beginning and end of storage were as follows: 59.60–51.22% for the control; 59.60–52.99% for P + SB; 59.60–55.60% for CF; 59.60–56.57% for CF + 0.2 % for TO; 59.60–57.25% for CF + 0.5% TO; For the CF + 1% TO group sample, it was determined as 59.60–57.28%.

A statistically significant difference was found between control, CF, CF + 0.2% TO, CF + 0.5% TO, and CF + 1% TO at the end of the storage period ($P < 0.05$). It was found that the moisture loss in the coated products was less than the control group. The minimum difference in moisture value was determined in the CF + 1% TO group. Maftoonazad *et al*. (2008) determined that the uncoated group lost more moisture than the coated groups during storage. Peach was used and was coated with sodium alginate. It was determined that the highest decrease in moisture value was in the control group due to the water retention properties of the chitosan film solution containing TO. In agreement with previous studies, chitosan coating restricted water transfer from the surface of olive samples as it formed a thin film on the top of the fruit skin. Moisture loss occurs due to the water vapor pressure difference between the inside and outside of the food. Consistent with previous studies, chitosan coating restricted water transfer from the surface of the coated

Table 3. Changes in pH, TA%, and moisture content of black olives treated by chitosan or added with TO during 90 days of storage at +4 and 25°C.

Parameter	Storage period						
Moisture % **Samples**	**0 days**	**30 days**		**60 days**		**90 days**	
		4°C	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**
Control	59.60 ± 0.51^{aA1}	55.64 ± 1.29^{bB1}	55.54 ± 1.16^{cB1}	53.70 ± 1.16^{bC1}	53.67 ± 0.73^{bC1}	53.68 ± 0.73^{bC1}	51.22 ± 0.76^{dD2}
PS + SB	59.60 ± 0.51^{aA1}	55.64 ± 1.29^{bB1}	56.75 ± 2.08^{abB1}	54.76 ± 0.51^{bBC1}	54.49 ± 0.57^{bC1}	54.49 ± 0.57^{bC1}	52.99 ± 0.56^{cC2}
CF	59.60 ± 0.51^{aA1}	57.74 ± 0.98^{aB1}	58.34 ± 1.86^{aAB1}	55.96 ± 0.85^{aBC1}	57.32 ± 0.63^{aBC1}	55.99 ± 0.65^{aC1}	55.60 ± 0.13^{bC1}
CF + 0.2% TO	59.60 ± 0.51^{aA1}	58.46 ± 0.66^{aAB1}	57.97 ± 0.86^{abB1}	57.24 ± 1.05^{aBC1}	57.64 ± 1.07^{aB1}	56.64 ± 0.42^{aC1}	56.57 ± 0.49^{abB1}
CF + 0.5% TO	59.60 ± 0.51^{aA1}	58.80 ± 0.70^{aAB1}	57.68 ± 0.59^{abB1}	57.58 ± 1.24^{aBC1}	57.38 ± 0.65^{aB1}	56.38 ± 0.48^{aC1}	57.25 ± 0.88^{aB1}
CF + 1% TO	59.60 ± 0.51^{aA1}	58.96 ± 0.07^{aB1}	58.78 ± 0.22^{aA1}	57.95 ± 0.11^{aC1}	57.48 ± 0.42^{aB1}	56.82 ± 0.16^{aD1}	57.28 ± 1.09^{aB1}
pH **Samples**	**0 days**	**30 days**		**60 days**		**90 days**	
		4°C	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**
Control	4.06 ± 0.01^{aA1}	3.91 ± 0.01^{abB1}	4.02 ± 0.02^{aB2}	3.92 ± 0.00^{dB1}	3.98 ± 0.00^{dC2}	3.84 ± 0.08^{bB1}	3.83 ± 0.01^{aBC2}
PS + SB	4.06 ± 0.01^{aA1}	3.87 ± 0.01^{bB1}	3.94 ± 0.06^{bcdC1}	3.86 ± 0.01^{eB1}	4.00 ± 0.00^{bcAB2}	3.87 ± 0.01^{bB1}	3.95 ± 0.01^{bC2}
CF	4.06 ± 0.01^{aA1}	3.97 ± 0.01^{aAB1}	3.88 ± 0.02^{eC2}	3.97 ± 0.01^{bCB1}	3.97 ± 0.00^{eB1}	3.70 ± 0.09^{cC1}	3.90 ± 0.01^{cC2}
CF + 0.2% TO	4.06 ± 0.01^{aA1}	3.86 ± 0.06^{bC1}	3.96 ± 0.00^{abcC1}	3.96 ± 0.00^{cB1}	3.99 ± 0.00^{cdB2}	4.01 ± 0.01^{aAB1}	3.83 ± 0.01^{dD2}
CF + 0.5% TO	4.06 ± 0.01^{aA1}	3.85 ± 0.02^{bC1}	4.00 ± 0.00^{abB2}	3.98 ± 0.00^{bB1}	4.01 ± 0.00^{abB2}	4.00 ± 0.01^{aAB1}	3.89 ± 0.01^{cC2}
CF + 1% TO	4.06 ± 0.01^{aA1}	3.88 ± 0.05^{bC1}	3.92 ± 0.05^{cdB1}	4.00 ± 0.02^{aAB1}	4.02 ± 0.00^{aA1}	4.00 ± 0.01^{aB1}	3.89 ± 0.01^{cB2}
TA% **(*Equivalent of lactic acid*)** **Samples**	**0 days**	**30 days**		**60 days**		**90 days**	
		4°C	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**
Control	0.52 ± 0.03^{aA1}	0.48 ± 0.00^{bB1}	0.54 ± 0.00^{bA1}	0.56 ± 0.03^{aA1}	0.53 ± 0.02^{aA1}	0.54 ± 0.00^{bc,A,1}	0.54 ± 0.00^{cA1}
PS + SB	0.52 ± 0.03^{aA1}	0.58 ± 0.07^{aA1}	0.48 ± 0.00^{cB1}	0.55 ± 0.04^{aA1}	0.45 ± 0.00^{bcB2}	0.56 ± 0.03^{bA1}	0.56 ± 0.03^{bcA1}
CF	0.52 ± 0.03^{aA1}	0.57 ± 0.05^{aB1}	0.60 ± 0.03^{aA1}	0.49 ± 0.02^{bCB1}	0.50 ± 0.03^{abB1}	0.70 ± 0.06^{aA1}	0.64 ± 0.03^{aA1}
CF + 0.2% TO	0.52 ± 0.03^{aA1}	0.58 ± 0.03^{aB1}	0.54 ± 0.00^{bB1}	0.49 ± 0.02^{bCB1}	0.44 ± 0.03^{bcC1}	0.52 ± 0.03^{bcB1}	0.62 ± 0.03^{abA1}
CF + 0.5% TO	0.52 ± 0.03^{aA1}	0.60 ± 0.06^{aA1}	0.56 ± 0.03^{abA1}	0.46 ± 0.03^{cB1}	0.52 ± 0.03^{cB1}	0.48 ± 0.05^{dB1}	0.56 ± 0.03^{bcA1}
CF + 1% TO	0.52 ± 0.03^{aA1}	0.54 ± 0.03^{abA1}	0.60 ± 0.06^{aA1}	0.53 ± 0.02^{aBA1}	0.46 ± 0.03^{bcB2}	0.54 ± 0.00^{bcA1}	0.62 ± 0.03^{abA2}

Different letters $^{(a,e)}$ represent a statistically significant different in the same column ($P < 0.05$). Different letters $^{(A,D)}$ represent a statistically significant different in the same row ($P < 0.05$). Different numbers $^{(1,2)}$ represent a statistically significant different between storage temperature +4 and 25°C in the same column ($P < 0.05$).

olive samples as it formed a thin film on the olive surface (Campus *et al*., 2018; Ozilgen and Bucak, 2018; Bonilla *et al*., 2014; Sheikh *et al*., 2013; Pranoto and Rakshit, 2012; Maftoonazad *et al*., 2008).

In olive processing, pH and titratable acidity are important regarding technology and health. According to Türkiye Food Codex Communique on table olives, the pH value of table olives was expected to be less than <4.5. The initial pH value of the samples is 4.06. The pH values of the sample groups were found to be between 4.06 and 3.84 stored at +4°C and 4.06 and 3.83 when stored at 25°C, respectively. pH values of control, CF, and CF+ 1% TO samples were determined as the statistically significant difference at the end of storage at +4°C ($P < 0.05$). pH values of control, PS + SB, CF + 0.2% TO, and CF + 1% TO samples were determined as the statistically significant difference at the end of storage at +25°C ($P < 0.05$). The results indicated that the film coating application protected the pH value of the product during storage. The initial % TA value of the sample groups was 0.52. The % TA values of the sample groups were between 0.48 and 0.52 stored at +4°C and 0.52 and 0.64 when stored at 25°C, respectively. A statistically significant difference was determined between control, CF, and CF + 0.2% TO groups in TA values at +4°C at the end of the storage period ($P < 0.05$). According to the results, it was seen that the changes in titratable acidity values were parallel to the changes in pH values.

Table 4. Changes in color of black olives coated by chitosan added with TO during 90 days storage at +4 and 25°C.

Parameter	Storage period							
L*	**0 days**		**30 days**		**60 days**		**90 days**	
Samples	**4°C**	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**
Control	17.84 ± 0.11^{bA1}	17.84 ± 0.11^{bA1}	14.89 ± 0.09^{dB1}	14.81 ± 0.45^{dC1}	12.63 ± 0.43^{dD1}	15.99 ± 0.37^{bB2}	13.96 ± 0.07^{bC1}	13.89 ± 1.06^{cD1}
PS+ SB	17.07 ± 0.03^{cA1}	17.07 ± 0.03^{cA1}	12.20 ± 0.11^{bD1}	14.87 ± 0.15^{dB2}	15.23 ± 0.11^{cB1}	17.17 ± 0.27^{aA2}	13.19 ± 0.04^{dC1}	13.46 ± 0.15^{cC2}
CF	16.42 ± 0.05^{dA1}	16.42 ± 0.05^{dB1}	15.22 ± 0.11^{cB1}	16.90 ± 0.04^{bA2}	10.97 ± 0.10^{eD1}	12.36 ± 0.08^{eD2}	12.94 ± 0.03^{eC1}	15.03 ± 0.08^{bC2}
CF + 0.2% TO	15.89 ± 0.09^{eB1}	15.89 ± 0.09^{eB1}	13.50 ± 0.05^{eC1}	15.38 ± 0.14^{cC2}	16.34 ± 0.41^{bA1}	14.84 ± 0.19^{dD2}	13.74 ± 0.03^{cC1}	16.89 ± 0.07^{aA2}
CF + 0.5% TO	18.21 ± 0.03^{aB1}	18.21 ± 0.03^{aA1}	21.14 ± 0.12^{aA1}	17.80 ± 0.04^{aB2}	12.60 ± 0.64^{dC1}	15.52 ± 0.13^{cC2}	13.21 ± 0.15^{dC1}	14.09 ± 0.07^{cD2}
CF + 1.0% TO	18.19 ± 0.15^{aA1}	18.19 ± 0.15^{aA1}	16.59 ± 0.01^{bC1}	15.14 ± 0.04^{cC2}	17.20 ± 0.05^{aB1}	14.09 ± 0.07^{cD2}	15.92 ± 0.06^{aD1}	15.75 ± 0.13^{bB1}
a*	**0 days**		**30 days**		**60 days**		**90 days**	
Samples	**4°C**	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**
Control	5.30 ± 0.09^{bB1}	5.30 ± 0.09^{bC1}	7.93 ± 0.13^{dA1}	14.81 ± 0.45^{dC1}	2.81 ± 0.18^{aC1}	15.99 ± 0.37^{bB2}	2.57 ± 0.04^{dC1}	13.89 ± 1.06^{cD1}
PS + SB	3.12 ± 0.04^{dD1}	3.12 ± 0.04^{dD1}	6.73 ± 0.17^{eA1}	14.87 ± 0.15^{dB2}	4.45 ± 0.04^{eC1}	17.17 ± 0.27^{aA2}	4.90 ± 0.05^{aB1}	13.46 ± 0.15^{cC2}
CF	4.68 ± 0.15^{cC1}	4.68 ± 0.15^{cD1}	10.69 ± 0.11^{bA1}	16.90 ± 0.04^{bA2}	7.21 ± 0.22^{cB1}	12.36 ± 0.08^{eD2}	3.02 ± 0.12^{cD1}	15.03 ± 0.08^{bC2}
CF + 0.2% TO	3.08 ± 0.05^{dC1}	3.08 ± 0.05^{dD1}	6.57 ± 0.21^{eA1}	15.38 ± 0.14^{cC2}	5.42 ± 0.22^{dB1}	14.84 ± 0.19^{dD2}	2.56 ± 0.13^{dC1}	16.89 ± 0.07^{aA2}
CF + 0.5% TO	6.60 ± 0.06^{aC1}	6.60 ± 0.06^{aC1}	11.28 ± 0.16^{aA1}	17.80 ± 0.04^{aB2}	7.66 ± 0.48^{bB1}	15.52 ± 0.13^{cC2}	3.28 ± 0.09^{bD1}	14.09 ± 0.07^{cD2}
CF + 1% TO	3.12± 0.09^{dB1}	3.12 ± 0.09^{dD1}	9.96 ± 0.15^{cA1}	15.14 ± 0.04^{cC2}	10.11 ± 0.09^{aA1}	14.09 ± 0.07^{cD2}	3.02 ± 0.04^{cB1}	15.75 ± 0.13^{bB1}
b*	**0 days**		**30 days**		**60 days**		**90 days**	
Samples	**4°C**	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**
Control	3.44 ± 0.04^{bB1}	3.44 ± 0.04^{bC1}	7.43 ± 0.11^{dA1}	7.57 ± 0.24^{aA1}	0.93 ± 0.08^{fC1}	6.83 ± 0.22^{aB2}	1.02 ± 0.05^{fC1}	2.27 ± 0.34^{dD2}
PS + SB	1.83 ± 0.07^{dC1}	1.83 ± 0.07^{dC1}	4.79 ± 0.11^{eA1}	6.80 ± 0.10^{bA2}	2.54 ± 0.05^{eB1}	5.09 ± 0.17^{bB2}	2.56 ± 0.10^{aB1}	2.12 ± 0.04^{dC1}
CF	2.22 ± 0.21^{cC1}	2.22 ± 0.21^{cC1}	8.96 ± 0.19^{cA1}	6.87 ± 0.20^{bA2}	4.83 ± 0.15^{cB1}	4.43 ± 0.15^{cB2}	1.62 ± 0.04^{eC1}	1.82 ± 0.14^{eC2}
CF + 0.2% TO	2.30 ± 0.12^{cC1}	2.30 ± 0.12^{cD1}	4.59 ± 0.24^{eA1}	6.76 ± 0.09^{bA2}	3.93 ± 0.09^{dB1}	3.66 ± 0.09^{dB2}	2.08 ± 0.09^{cD1}	3.19 ± 0.02^{bC2}
CF + 0.5% TO	4.92 ± 0.11^{aC1}	4.92 ± 0.11^{aC1}	11.96 ± 0.11^{aA1}	6.89 ± 0.06^{bB2}	7.90 ± 0.49^{bB1}	7.11 ± 0.19^{aA1}	2.28 ± 0.04^{bD1}	2.90 ± 0.03^{cD2}
CF + 1% TO	2.27± 0.16^{cC1}	2.27 ± 0.16^{cD1}	10.03 ± 0.22^{bA1}	6.63 ± 0.03^{bA2}	9.13 ± 0.15^{aB1}	3.41 ± 0.19^{dC2}	1.88 ± 0.07^{dC1}	3.86 ± 0.06^{aB2}
ΔE*	**0 days**		**30 days**		**60 days**		**90 days**	
Samples	**4°C**	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**	**4°C**	**25°C**
Control	0.00 ± 0.00^{eC1}	0.00 ± 0.00^{eC1}	5.62 ± 0.30^{cB2}	6.77± 0.35^{aA1}	6.30 ± 0.30^{bA1}	4.61 ± 0.35^{cB2}	5.33 ± 0.06^{bB1}	4.20 ± 0.16^{bB2}
PS + SB	2.82 ± 0.10^{bC1}	2.82 ± 0.10^{bC1}	5.97 ± 0.12^{cA1}	5.04 ± 0.13^{bA2}	2.89 ± 0.12^{cC1}	2.61 ± 0.13^{eC2}	4.75 ± 0.02^{dB1}	4.70 ± 0.02^{aB1}
CF	1.97 ± 0.17^{dD1}	1.97 ± 0.17^{dD1}	8.15 ± 0.16^{bA1}	5.21 ± 0.11^{bB2}	7.26 ± 0.16^{aB1}	5.57 ± 0.11^{bA2}	5.70 ± 0.07^{aC1}	3.32 ± 0.14^{cC2}
CF + 0.2% TO	3.17 ± 0.06^{aB1}	3.17 ± 0.06^{aB1}	4.67 ± 0.42^{dA1}	4.62 ± 0.19^{cA1}	1.58 ± 0.42^{dC1}	3.01 ± 0.19^{dB2}	5.12 ± 0.09^{cA1}	1.14 ± 0.08^{eC2}
CF + 0.5% TO	2.00 ± 0.19^{dD1}	2.00 ± 0.19^{dC1}	10.02 ± 0.89^{aA1}	4.16 ± 0.14^{dB2}	7.27 ± 0.89^{aB1}	5.64 ± 0.14^{bA2}	5.18 ± 0.22^{bC1}	4.35 ± 0.09^{bB2}
CF + 1% TO	2.50 ± 0.09^{cD1}	2.50 ± 0.09^{cC1}	8. 17 ± 0.09^{bA1}	4.48 ± 0.07^{cB2}	7.48 ± 0.09^{aB2}	8.91 ± 0.07^{aA1}	3.36 ± 0.12^{eC1}	2.41 ± 0.32^{dC2}

Different letters$^{(a,f)}$ represent a statistically significant different in the same column ($P < 0.05$). Different letters $^{(A,D)}$ represent a statistically significant different in the same row ($P < 0.05$). Different numbers $^{(1,2)}$ represent a statistically significant different between storage temperature +4 and 25°C in the same column ($P < 0.05$).

Table olive colors may depend on the type of olive, the geographical, and climatic conditions and the processing methods. L*, a*, b*, and ΔE* values of black olive samples coated with chitosan films are given in Table 4. L* values were found to be as follows: 17.84–13.96 for control; 17.07–13.19 for P + SB; 16.42–12.94 for CF; 15.89–13.74 for CF + 0.2% TO; 18.21–13.21 for CF + 0.5%TO; and 18.19–15.92 for CF + 1% TO group at the beginning and end of the third month at +4°C.

The L* values of the samples stored at 25°C at the beginning and end of the 3rd month were as follows: 17.84–13.89 for control; 17.07–13.46 for P + SB; 16.42–15.03 for CF; 15.89–16.89 for the CF + 0.2% TO; 18.21–14.09 for CF + 0.5% TO; 18.19–15.75 for CF + 1% TO group sample. The least change between the initial L* values at the end of the storage period was determined as CF + 1.0% TO, CF + 0.2% TO, and CF, respectively. It was determined that the coated products gave better color results and the change in L* value was less compared to the L* values of the olives PS + SB treated with the traditional method. A statistically significant difference was found between all groups at the end of the third month at +4°C ($P < 0.05$). At the end of the third month at 25°C, a statistically significant difference was observed between the control, CF + 0.2% TO, and CF + 1% TO groups ($P < 0.05$). a* and b* values of black olives were found to be different from each other due to the range in color from brown to black. The least ΔE* values at the end of the 3rd month were determined in CF + 1% TO groups at +4 and 25°C. ΔE* values of the sample groups changed in parallel with L*, a*, and b* values.

Microbiological results

The microbiological changes in the TVC and yeast-mold counts for uncoated (control) and coated black olives during storage at +4 and 25°C are presented in Tables 5 and 6. The initial TVC count of olive samples was 5.21 log CFU/g. At the end of the third month of storage at +4°C, 2.10 log CFU/g for the PS + SB; 2.91 log CFU/g for CF, 2.93 log CFU/g for CF + 0.2% TO; 0.87 log CFU/g for CF + 0.5% TO; and not detected in the CF + 1%TO.

At the end of the third month of storage at 25°C, the following values were observed: 2.14 log CFU/g for PS + SB; 3.53 log CFU/g for CF; 2.57 log CFU/g for CF + 0.2% TO; 2.35 log CFU/g for CF + 0.5% TO; and 0.86 in CF + 1% TO. In black olives, at the end of the third month, the TVC value decreased by 5.21 $\log_{10}$ CFU/g in the CF + 1%TO group at +4°C, and by 4.35 log CFU/g in the CF + 1.0% TO group at 25°C.

The initial yeast–mold count of olive samples was found to be as 5.22 log CFU/g. At end of the third month storage at +4°C, the following values were observed: 0.67 log CFU/g for PS + SB; 0.68 log CFU/g for CF; 0.33 log CFU/g for CF + 0.2% TO; 3.61 log CFU/g for CF + 0.5% TO; and 1.43 log CFU/g for CF + 1% TO. Whereas, at the end of the third month at 25°C, the following values were observed: 3.88 log CFU/g for PS + SB; 4.58 log CFU/g for CF; 4.01 log CFU/g for CF + 0.2% TO; 3.62 log CFU/g for CF + 0.5% TO; and 4.19 log CFU/g for CF + 1% TO. The highest decrease in mold–yeast value in black olives at the end of storage was in the CF + 0.2%TO sample stored at +4°C, and a reduction of 4.89 log CFU/g was observed.

It would be useful to investigate the effect of coatings combined with the strong antimicrobial effect of TO on inhibiting target microorganisms that may cause spoilage in olives. Additionally, the effectiveness of different coating materials on different olive varieties should be evaluated. The edible film and coating applied on olives are important for the applicability of this technology, as it does not contain synthetic preservatives. One of the research topics emphasized is preventing microbial growth by combining edible films with EO. The effect of

Table 5. TVC of black olive samples during 90 days storage at +4 and 25°C.

TVC (log CFU/g) Samples	0 days	30 days		60 days		90 days	
		4°C	25°C	4°C	25°C	4°C	25°C
Control	5.21 ± 0.23^{aA1}	4.69 ± 0.08^{aB1}	4.98 ± 0.14^{aA2}	4.52 ± 0.37^{aB1}	4.87 ± 0.15^{aA1}	4.28 ± 0.38^{aB1}	4.79 ± 0.29^{aA1}
PS + SB	5.21 ± 0.23^{aA1}	2.83 ± 0.19^{bC1}	2.86 ± 0.12^{cB1}	4.18 ± 0.56^{aB1}	2.81 ± 0.32^{bB2}	2.10 ± 0.89^{bC1}	2.14 ± 0.99^{bB1}
CF	5.21 ± 0.23^{aA1}	4.57 ± 0.48^{aB1}	3.77 ± 0.31^{bB1}	2.31 ± 0.22^{bB1}	1.19 ± 0.32^{dC2}	2.91 ± 0.59^{bB1}	3.53 ± 0.80^{bB1}
CF + 0.2% TO	5.21 ± 0.23^{aA1}	2.96 ± 0.05^{bB1}	2.30 ± 0.27^{dB2}	1.01 ± 0.19^{cC1}	2.17 ± 0.12^{cB2}	2.93 ± 0.54^{bB1}	2.57 ± 0.38^{bB1}
CF + 0.5% TO	5.21 ± 0.23^{aA1}	2.65 ± 0.09^{bB1}	2.32 ± 0.25^{dB1}	2.35 ± 0.11^{bB1}	2.42 ± 0.29^{cB1}	0.87 ± 0.50^{cC2}	2.35 ± 0.19^{bB1}
CF + 1% TO	5.21 ± 0.23^{aA1}	2.77 ± 0.03^{bB1}	2.19 ± 0.21^{dB1}	2.17 ± 0.06^{bC1}	2.12 ± 0.16^{cB1}	0.00 ± 0.00^{dD2}	0.86 ± 0.50^{cC1}

Different letters $^{(a,d)}$ represent a statistically significant different in the same column ($P < 0.05$). Different letters $^{(A,D)}$ represent a statistically significant different in the same row ($P < 0.05$). Different numbers $^{(1,2)}$ represent a statistically significant different between storage temperature +4 and 25°C in the same row ($P < 0.05$).

Table 6. Yeast–mold count of black olive samples during 90 days storage at +4 and 25°C.

Yeast–Mold count (log CFU/g) Samples	0 days	30 days		60 days		90 days	
		4°C	25°C	4°C	25°C	4°C	25°C
Control	5.22 ± 0.35^{aA1}	4.61 ± 0.27^{aA1}	4.79 ± 0.16^{aA1}	5.04 ± 0.08^{aA1}	5.04 ± 0.33^{aA1}	4.92 ± 0.10^{aA1}	4.83 ± 0.46^{abA1}
PS + SB	5.22 ± 0.35^{aA1}	2.14 ± 0.04^{cC2}	3.32 ± 0.34^{bC1}	2.26 ± 0.05^{bB2}	4.29 ± 0.29^{bB1}	0.67 ± 0.15^{cD2}	3.88 ± 0.22^{abBC1}
CF	5.22 ± 0.35^{aA1}	0.00 ± 0.00^{dD2}	2.77 ± 0.21^{cC1}	1.00 ± 0.10^{dB2}	4.33 ± 0.05^{bB1}	0.68 ± 0.18^{cC2}	4.58 ± 0.50^{aB1}
CF + 0.2% TO	5.22 ± 0.35^{aA1}	0.00 ± 0.00^{dC2}	3.81 ± 0.13^{bC1}	0.00 ± 0.00^{eC2}	3.95 ± 0.04^{cC1}	0.33 ± 0.08^{dB2}	4.01 ± 0.01^{abB1}
CF + 0.5% TO	5.22 ± 0.35^{aA1}	0.00 ± 0.00^{dD2}	2.37 ± 0.25^{cC1}	1.00 ± 0.10^{dC2}	3.96 ± 0.04^{cB1}	3.61 ± 0.43^{bB1}	3.62 ± 0.63^{bB1}
CF + 1% TO	5.22 ± 0.35^{aA1}	2.36 ± 0.12^{bB1}	2.69 ± 0.44^{cC1}	1.94 ± 0.06^{cB2}	4.05 ± 0.06^{bcB1}	1.43 ± 0.68^{cB2}	4.19 ± 0.47^{abB1}

Different letters $^{(a,e)}$ represent a statistically significant different in the same column ($P < 0.05$). Different letters $^{(A,D)}$ represent a statistically significant different in the same row ($P < 0.05$). Different numbers $^{(1,2)}$ represent a statistically significant different between storage temperature +4 and 25°C in the same row ($P < 0.05$).

EOs added to edible film formulations on microorganisms is important. Microbiological development is important for the shelf life of olives and consumer acceptance. Jouki *et al*. (2014) reported the antibacterial effect of films (edible films were prepared by adding thyme oil at concentrations of 1, 1.5, and 2% v/v). The film containing 1% thyme oil indicating an efficient inhibitory effect against all test microorganisms. The values obtained in this study were similar to those conducted in the literature. There are no previous studies in the literature on unbrined black olives. Therefore, the current study sheds light on future research. Several studies have been conducted on different food products. This study was initially carried out to measure the antimicrobial effect of chitosan film and thyme oil on olives and to investigate their effects on the physicochemical properties. Due to the positive results, future studies will focus on conducting sensory tests and the rheological properties of the coated olives.

Conclusion

Among the film samples obtained, the CF+1% TO group was the one with the highest elongation at break. Increasing the concentration of TO increased the elongation at break values. While L* value of the films increased with the addition of TO, and b* values increased, leading to the film colors approaching yellow. The SEM images of group CF films have a homogeneous and uniform structure. The addition of TO caused a heterogeneous appearance in the film surface areas. It has been observed that chitosan films with TO added in coated products prevent moisture loss. It was concluded that film-coated samples with added TO had inhibitory effects on TVC and yeast–mold values. The results demonstrated the potential for the use of these coating formulations in extending the shelf life and improving the quality of olives during storage. The results concluded that edible film applications can be an alternative to synthetic preservatives (potassium sorbate–sodium benzoate).

Based on the results of this study, the application of edible film coatings may provide valuable insights for future studies on the analysis of their antimicrobial effects and extension of the shelf life of foods. Additionally, it will serve as a guide for testing edible films and essential oils on different food products and as an alternative to chemical preservatives. In addition, it is thought that this study, which has not yet been industrially implemented in our country, will be beneficial for our country's olive exports by increasing the competitiveness rate by developing an industrially adaptable production method.

Authors Contribution

Pınar Çoruhlu contributed to the conceptualization, investigation, formal analysis, data curation, and writing. Ahsen Rayman Ergün was involved in data curation, writing, and validation. Taner Baysal contributed to the writing of the "Methodology" section and prepared the original draft.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

No funding was obtained for this study.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

Data Availability

Data will be made available on request.

Acknowledgments

The authors thank the Ege University, Engineering Faculty, Food Engineering Department, Izmir, Turkey.

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