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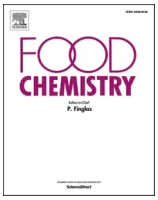

Understanding the effects of chitosan, chia mucilage, levan based composite coatings on the shelf life of sweet cherry

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ABSTRACT

Sweet cherry (*Prunus avium* L.) fruits are prone to quality and quantity loss in shelf-life conditions and cold storage due to their short post-harvest life. Until now efforts have been made to extend the shelf life of the sweet cherry. However, an efficient and commercially scalable process remains elusive. To contribute to this challenge, here in this study, biobased composite coatings consisting of chitosan, mucilage, and levan, were applied on sweet cherry fruits and tested for postharvest parameters in both market and cold storage conditions. Results demonstrated that the shelf life of sweet cherries can be extended until the 30th day while retaining important post-harvest properties like decreased weight loss, fungal deterioration, increased stem removal force, total flavonoid, L-ascorbic acid, and oxalic acid. Given the cost-effectiveness of the polymers used, the findings of this study indicate the feasibility of extending the shelf-life of sweet cherries on a larger scale.

1. Introduction

Sweet cherry is a highly perishable fruit with short postharvest life. The main postharvest problems in sweet cherries are water loss, softening, pitting, decay, and stem browning (Bernalte, Sabio, Hernandez, & Gervasini, 2003). Different techniques such as controlled atmosphere storage, modified atmosphere packaging, irradiation, and precooling are applied to extend the shelf life of fruits during postharvest. All these conventional approaches are providing limited relief to overcome these problems. The advancements in polymer technologies and coatings have attained enormous attention in the food industry. Similarly, several biobased polymers have been tested as edible coating to increase postharvest life in highly perishable fruits. Edible film coating creates a barrier between the fruit surface and the atmosphere, which decreases moisture losses, softening, pitting, decay, and stem browning (Rojas-Argudo, Pérez-Gago, & Del Río, 2005). The edible film coating improves the fruit quality by decreasing the oxygen intake from the atmosphere which limits the respiration rate and hence slows down the senescence process. The control of internal gas composition determines the success of edible film coating in fresh fruits and vegetables. Biobased polymers such as chitosan, cellulose, alginate, starch, and chia mucilage have been tested for the shelf life of different fruits. However so far, except for chitosan, no study has reported the application of chia mucilage and Levan for the extension of the shelf life of sweet cherries.

Chia (*Salvia hispanica* L.) is an annual herb that belongs to Lameacea family. According to the reports, Lameacea consists of approximately 224 genera and 5600 species worldwide (de la Paz Salgado-Cruz, Calderón-Domínguez, Chanona-Pérez, Farrera-Rebollo, Méndez-Méndez, & Díaz-Ramírez, 2013). Chia seed is a potential source for animal feed industries. Chia seeds have an excellent source of protein, fat, fatty acids, and vitamin B. It also contains vitamins and minerals, as well as natural antioxidants such as α linolenic acid (omega 3), phenolic glycoside-Q and K, chlorogenic acid, caffeic acid, quercetin, and kaempferol. These properties make it an excellent candidate for food preservative applications. Upon soaking in water chia seeds produced a transparent gel (also known as Mucilage) that is mainly composed of xylose, glucose, and methyl glucuronic acid. Chia mucilage is a branched polysaccharide with a high molecular weight (ranging from

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0.8 to 2.0 × 10 Da)., and contains about 5–6% soluble dietary fiber that can retain 27% of water in water (Muñoz, Cobos, Diaz, & Aguilera, 2012). Chia seed mucilage can be used as a foam stabilizer, suspending agent, emulsifier, adhesive, or binder in the food industry due to its water-holding capacity and viscosity.

Mucilage from chia seeds is a new source of polysaccharides and can potentially produce different polymer blends for edible films and coatings (Muñoz, Aguilera, Rodriguez-Turienzo, Cobos, & Diaz, 2012). Chitosan is the deacetylated form of chitin, that is isolated from marine waste and insects. Chemically chitosan is comprised of two sub-units as d-glucosamine and N-acetyl-d-glucosamine linked linearly with each other via 1,4-glycosidic bonds. Chitosan has successfully got the attention of the food and biomedicine industry due to its enormous properties such as biocompatibility, biodegradability non-toxicity, antioxidant, and antimicrobial activities (Mujtaba et al., 2019). Chitosan has shown excellent compatibility with other polysaccharides such as starch, cellulose, alginate, and mucilage, thanks to the functional groups across the backbone. So far chitosan has been tested with several polymers for its coating and active film-forming properties, however, no study has reported the protective edible coating ability of chitosan with mucilage and Levan.

Levan is an industrially important fructose-based homopolysaccharide. Structurally, levan is composed of β-D-fructofuranose units connected by β-(2–6) glycosidic bonds (Öner, Hernández, & Combie, 2016). Thanks to the unique properties of levan such as biocompatibility, self-assembly into spherical colloids, adhesivity, etc., it has been used in many applications such as cosmetics, pharmaceutical, textile, wastewater treatment, and food. Few plant species and a miscellaneous number of microorganisms produce levan as short, and long chains respectively (Erkorkmaz, Kırtel, Ateş Duru, & Toksoy Öner, 2018). In a recent single study by Gan et al. (2022), levan has been incorporated into pullulan/chitosan films. Despite the numerous advantages of levan, it has not been used as a fruit coating agent in combination with mucilage for sweet cherries.

To investigate the impact of these polymers, in the current study levan and mucilage were incorporated into a chitosan-based coating solution in various combinations. SEM, DSC, FT-IR, XRD, and contact angle were employed to characterize the physicochemical characteristics of composite coating films. Sweet cherries were used as model fruits to check the effects of coating solutions on post-harvest weight loss, total soluble solids, titratable acidity, taste scores, stem removal force, stem browning, pitting, fungal deterioration, fruit firmness, fruit cracking, fruit skin color (L^*, C^*, h°), antioxidant activity, total phenolic content, anthocyanin content, total flavonoid content, total chlorophyll content of stem, ascorbic acid content, organic acids, and phenolic compounds analysis were conducted at 10 days intervals for 30 days during cold storage and shelf life conditions.

2. Material methods

2.1. Materials

Sweet cherry (0900-Ziraat cultivar) was used in this study as fruit material. The cherry fruits were grown in Egirdir district in Isparta province in Turkey (37°49′55.5″N 30°51′56.3″E). The fruits were harvested at optimum harvest maturity, pre-cooled through cold water, and immediately brought to the Postharvest Physiology Laboratory, Department of Horticulture at Akdeniz University Antalya, Turkey. Fruits with any kind of defects were discarded from the experiment.

2.2. Mucilage and levan extraction

Chia mucilage was obtained with the centrifugation process. Extraction of mucilage from chia seeds was carried out using a modified method earlier reported by Dick, Costa, Gomaa, Subirade, de Oliveira Rios, and Flôres (2015). Briefly, chia seeds (*S. hispanica* L.) were rinsed in distilled water at a ratio of 1:30 and mixed mechanically at 250 rpm at 25 ± 1 °C for 2 h. The resulting mucilage solution was centrifuged at 9000 g for 30 min. Mucilage gel was separated from chia seeds. Chia gel solution was also filtered through cheesecloth to remove the remaining unwanted small particles. The obtained chia gel was stored at 4 ± 1 °C for further experiments. Levan was produced through microbial fermentation of *Halomonas smynensis* AAD6^T under controlled bioreactor conditions and purified from the cell-free culture medium as described previously (Erkorkmaz et al., 2018).

2.3. Preparation of composite film solutions

Chitosan control and chitosan composite solutions with different concentrations of mucilage and levan were prepared according to our previous study (Mujtaba, Salaberria, Andres, Kaya, Gunyakti, & Labidi, 2017). For the preparation of chitosan control (CH) solution, 1 g of chitosan (medium molecular weight and deacetylation degree ≈ 95%) was dissolved in 1% acetic acid solution. The solution was stirred (350 rpm) with a magnetic stirrer at 25 ± 1 °C for 48 h. Later 500 μL glycerol was added to the solution and stirred for 20 mins at the same conditions. Chitosan-levan (CH-LVN) composite solution was prepared by adding 30 mL levan into 1% (w/v) chitosan solution. The solution was homogenized for 20 min by using a Heidolph, SilentCrusher M homogenizer. Similarly, a chitosan-mucilage (CH-MLG) composite solution was prepared by adding 30 mL mucilage into 1% (w/v) chitosan solution. The solution was homogenized for 20 min by using a Heidolph, SilentCrusher M homogenizer. For chitosan-mucilage-levan (CH-LVN-MLG) solution preparation, 30 mL of levan and 30 mL of mucilage were supplemented to 1% chitosan solution. The solution was homogenized for 25 mins by using a Heidolph, Silent Crusher M homogenizer. To analyze the physicochemical properties of composite coatings, 25 mL of each film solution was poured into Petri dishes and incubated for 48 h at room temperature. The films were peeled-off the Petri dishes and used for further analyses.

2.4. Physicochemical analysis of coatings

The physicochemical attributes of control (CH) and composite films (chitosan (CH), Levan (CH-LVN), mucilage (CH-MLG), and Mucilage + Levan (CH-LVN-MLG)) were investigated via different analytical tools. The surface morphologies and blending patterns were determined by using Zeiss Supra40 Field Emission Scanning Electron Microscopy (FE-SEM) Gold- palladium coating was conducted before taking the pictures. The IR spectra of composite films were recorded in the wavelength range of 4000–600 cm^{-1} by using an Attenuated Total Reflectance infrared spectroscope fitted with Universal Attenuated Total Reflectance fitment containing an internal reflection diamond crystal lens. X-ray (XRD) diffraction analysis of the composite films was carried out by using Bruker AXS D8 Advance XRD instrument. Diffractograms were recorded at 2θ in the range of 10–50° (scan angle) and 40 kV/30 mA. Differential Scanning Calorimetry (DSC) (Mettler Toledo DSC822e (Schwerzenbach, Switzerland)), analysis was performed to analyze the thermal characteristics of the coating films. DSC analysis conditions were as followed; a 50 mg sample heated from − 20 to 500 °C (5 °C/min) in the hermetic aluminum pan under an N_2 atmosphere. The contact angle measurements were recorded using Bruker Theta Flex optical tensiometer.

2.5. Fruit coatings

The sweet cherries were classified into five categories for coating treatments. The first, second, third, and fourth categories of fruits were treated with chitosan (CH), levan and chitosan (CH-LVN), mucilage and chitosan (CH-MLG), and mucilage, levan and chitosan (CH-LVN-MLG), respectively. The fifth group was considered a control (CTL) with no treatment. Fruits along with stem were dipped in film solutions for 1 min. The fruits were knotted through the stem and hung on a thread for

drying purposes. The dried samples were then kept in punnets and stored for 30 days at 0 °C temperature and 90–95% relative humidity (RH). For quality analysis, the fruit samples were taken from the storage at 10 days intervals and kept at 20 °C and 60 ± 5% relative humidity for additional 3 days to simulate the shelf-life performance.

2.6. Quality analysis during cold storage and shelf-life conditions

2.6.1 Weight. loss

The sweet cherries were weighed at harvest and then after every 10 days for the determination of weight loss. The accumulative weight loss was determined as the percent of initial weight losses.

2.6.2. Taste score

The taste score in sweet cherries was determined according to Kurubaş and Erkan (2018) by using a 1–5 point hedonic scale. The fruits were coded and presented in random order. The panelists were told to take water after tasting each sample. The taste score assessment was conducted with five male and five female educated panelists. The hedonic scale was 1: very poor; 2: poor (unmarketable); 3: good (marketable); 4: very good; 5: excellent.

2.6.3. Stem removal force

The stem removal force of the sweet cherries was determined with the help of Chatillon Digital Force Gauge containing notch adopters. For that purpose, the stem of thirty fruits from each replication was picked and the stem removal force was expressed in newton (N).

2.6.4. Stem browning and pitting

The sweet cherry fruits with visible symptoms of stem browning and pitting were considered browned and pitted. The stem browning and pitting were reported in percentage by using the Eq. (1).

$$\text{Stem browning/pitting (\%)} = \text{Stem browned or pitted fruits/total number of fruit} \times 100 \quad (1)$$

2.6.5. Fungal deterioration

The fungal deterioration of the sweet cherry fruits was recorded in percent. The fungal deterioration of sweet cherries was determined according to (Jan & Rab, 2012) by using the Eq. (2).

$$\text{Fungal deterioration (\%)} = \text{number of fungal deteriorated fruit/total number of fruit} \times 100 \quad (2)$$

2.6.6. Fruit firmness

The fruit firmness was recorded with Chatillon Digital Force Gauge having 8 mm tip. Thirty fruits from each replication were measured on their equatorial axis at three different locations and fruit firmness was reported in newton (N).

2.6.7. Fruit cracking

The sweet cherry fruits with visible symptoms of cracking were recorded and expressed in percentage by using the Eq. (3).

$$\text{Fruit cracking (\%)} = \text{number of cracked fruit/ total number of fruit} \times 100 \quad (3)$$

2.6.8. Fruit color

The color changes in sweet cherry fruits were measured through a chroma meter (Minolta Chroma Meter CR-400 Minolta Camera Co., Ramsey, NJ). The color meter gave numerical values for coloring as L^*, a^* and b^*. L^* value refers to lightness and varies from 0 to 100. Values of 0 represent no reflection whereas 100 represents white color i.e., perfect reflection. Positive a^* values show red whereas negative a^* values display green color. Since there are no color phenomena to be detected by the buyer and seller in markets, the numerical values of L^*, a^*, b^* were determined as chroma (C^*) and hue angle (h°) values to show real fruit colors.

2.7. Biochemical assay

2.7.1. Total soluble solids

A digital refractometer (Hanna HI 96801) was used for the determination of total soluble solids (TSS) content and expressed as percent (Selcuk & Erkan, 2015).

2.7.2. Titratable acidity

The sweet cherry fruit juice was obtained with the help of a blender for the determination of titratable acidity (TA). The juice sample of 2 mL was titrated with 38 mL distilled water along with 0.1 N NaOH to the end point of 8.1 pH. Each sample was titrated thrice, and the means were calculated. The TA was determined as g malic acid 100 mL^{-1} (Selcuk and Erkan, 2015).

2.7.3. Antioxidant activity

The sweet cherries were homogenized with the help of a blender. The puree (10 g) was extracted with 80% methanol (20 mL) for antioxidant activity, total phenolic content and total flavonoid content analysis on a fresh weight basis.

The antioxidant activity of sweet cherries extracts was analyzed according to Benvenuti, Pellati, Melegari, and Bertelli (2004) by using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The 600 μL of 1 mM DPPH was added to each tube and different concentrations of 80% methanol extracted samples (0.25, 0.50, 0.75 and 1.0 mL) were added to the tubes. The final concentration in every tube was brought to 6 mL by adding methanol (80%). The tubes were then vortexed (Heidolph Reax top) and incubated for 15 mins in dark at ambient temperature. Moreover, the control sample was prepared by the addition of 1 mM DPPH (600 μL) and 80% methanol (5.4 mL). The control sample was also vortexed and incubated for 15 mins in dark at ambient temperature. After incubation, the sample's absorbances were read at 517 nm through spectrometer (Analytik Jena AG Specord 40 ST) against blank methanol (80%) and control. Eq. (4) was used to determine the percent (%) inhibition values proportionate to each sample.

$$\%\text{Inhibition} = A_{DPPH} - A_{Extract}/A_{DPPH} * 100 \quad (4)$$

A_{DPPH}: DPPH control sample absorbance value.
$A_{Extract}$: The test sample absorbance value.

The sample volumes and inhibition values were used to obtain a graph. The linear regression model was used in the graph, and the sample curve and equation were determined. The equation was used to calculate the EC50 (effective concentration) value of the sample. The EC50 value is vital for the calculation of antioxidant activity through DPPH method. The EC50 value represents the amount of antioxidant content that inhibits half (50%) of the DPPH radical available in fruits and vegetables. The increase in EC50 value decreases the antioxidant activity (Cemeroglu, 2010). The antioxidant activity was expressed as milligrams of fresh weight (mg fw).

2.7.4. Total phenolic content, anthocyanin content, flavonoid content, stem chlorophyll content, ascorbic acid, organic acids, and phenolic compound

The detailed methodology of each assay has been provided in the supplementary section.

2.8. Statistical analysis

The study was planned according to a randomized complete block

design (CRD) with three replications. For coating treatment, a total of ninety sweet cherries from each treatment were divided into three replications. Each punnet containing thirty sweet cherries was used as a single replication. The statistical differences with $P \leq 0.05$ were considered significant. The means comparison was done according to Duncan's multiple-range test.

3. Results and discussion

3.1. Film thickness and visual appearance

The obtained chitosan film and chitosan composite films with chia mucilage/levan are presented in Fig. 1a–d and Fig. S1 (light microscopy images). The thickness of obtained films was recorded as 75.8 ± 0.21 μm for the CH, 83.6 ± 0.27 μm for CH-LVN, 76.5 ± 0.22 μm for CH-MLG and 139.7 ± 0.17 μm for CH-LVN-MLG. Considering the thickness results, the thickness of the CH-MLG film was close to the CH film. This similarity can be attributed to the excellent composite formation

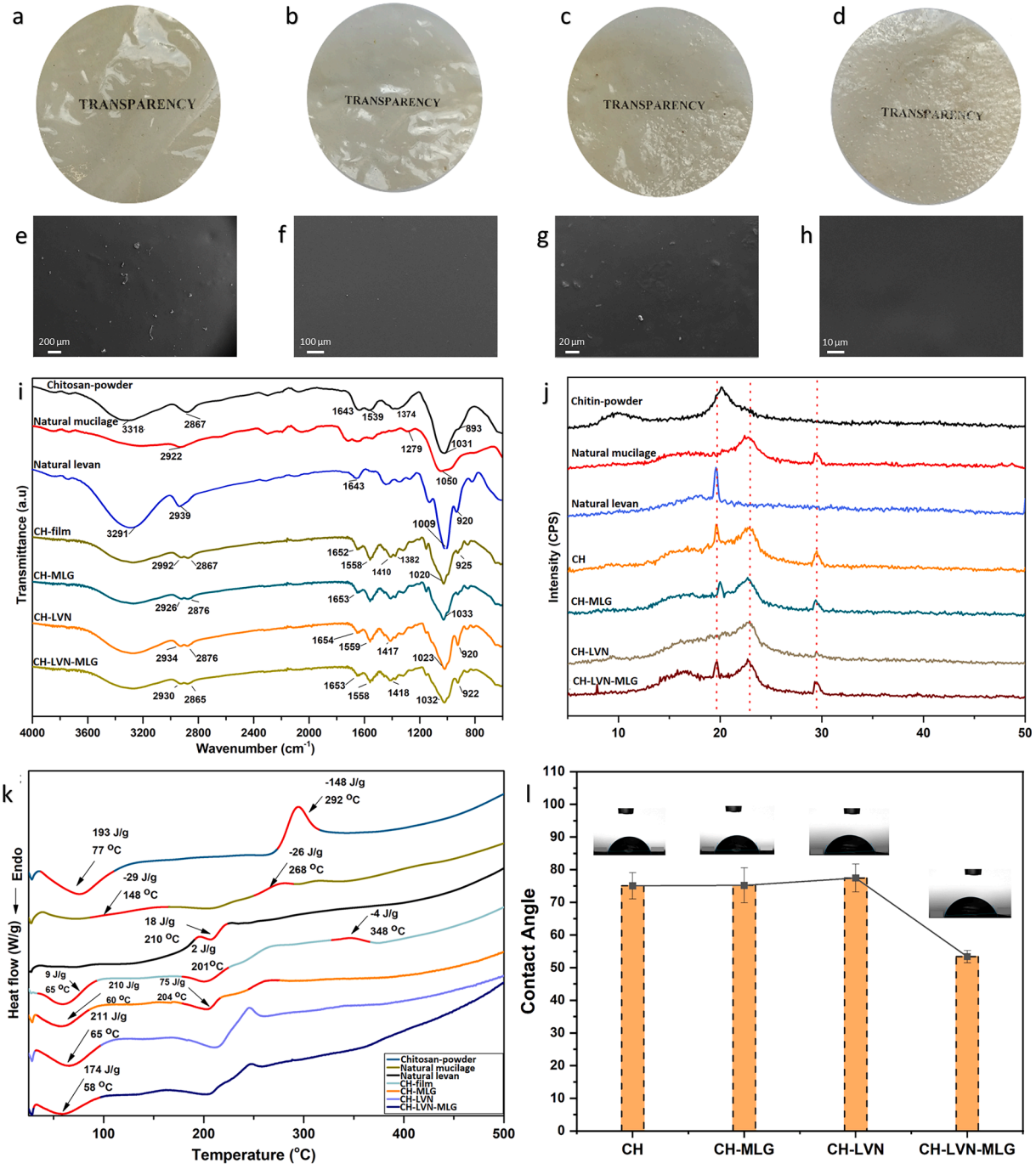

Fig. 1. Digital images of; a) CH-film, b) CH-LVN, c) CH-MLG, d) CH-LVN-MLG, SEM images of; e) CH-film, f) CH-LVN, g) CH-MLG, h) CH-LVN-MLG, i) ATR/FT-IR (Fourier-transform infrared spectroscopy) spectra of chitosan-powder, natural mucilage, natural levan, CH-film, CH-MLG, CH-LVN, CH-LVN-MLG, j) XRD diffraction curves of chitosan-powder, natural mucilage, natural levan, CH-film, CH-MLG, CH-LVN, CH-LVN-MLG, k) DSC thermograms of chitosan-powder, natural mucilage, natural levan, CH-film, CH-MLG, CH-LVN, CH-LVN-MLG, l) contact angle analysis of CH-film, CH-MLG, CH-LVN, CH-LVN-MLG.

between chia mucilage and chitosan. The thickness of the CH-LVN-MLG composite film increased significantly compared to the CH-film. It has been reported in the literature that the addition of new ingredients to the chitosan film gradually increases its thickness (Mujtaba et al., 2019; Mujtaba, Fernández-Marín, Robles, Labidi, Yilmaz, & Nefzi, 2021). In addition, thickness variation was observed according to the added material. Mujtaba et al. (2021) reported that the addition of cellulose nanofibers (CNF) and diatom to the chitosan film gradually increased its thickness.

3.2. FT-IR

FT-IR spectra of chitosan (powder), natural chia mucilage, natural levan, and corresponding composite films CH, CH-MLG, CH-LVN, and CH-LVN-MLG are presented in Fig. 1i. In the spectrum of CH, a wide O—H stretching peak was observed in the range of 3100–3500 cm^{-1}. This peak is attributed to the O—H band of water. Additionally, the incorporation of glycerol into CH film resulted in the widening of this band. Further, in the spectra of CH film, a C═O stretching at 1643 cm^{-1} in the Amide-I band, NH bending at 1536 cm^{-1} in the Amide-II band, and C—N stretching at 1374 cm^{-1} in the Amide-III band were recorded. In addition, the peaks observed at 1031 and 893 cm^{-1} are attributed to the sugar rings and the asymmetric stretching vibration of the C—O—C bonds of the glycosidic linkages. The characteristic peaks for chitosan were observed to be in-line with previous studies (Ritthidej, Phaechamud, & Koizumi, 2002).

It is known that chia mucilage is a polysaccharide containing fiber, protein, and oil in its structure (Darwish, Khalifa, & El Sohaimy, 2018). The broad peak observed around 3200–3500 cm^{-1} in the spectrum for natural chia mucilage (mucilage only) represents the − OH stretching of the hydroxyl groups in the chia mucilage's structure. Also, the sharp peak observed at 2867 cm^{-1} is attributed to the aliphatic C — H stretching vibration of aromatic rings and methyl groups representing oil content (Archana et al., 2013). Due to the protein structure of chia seed mucilage, the characteristic peaks were recorded at 1717 cm^{-1} (C═O stretching vibration of carboxylic acid), 1643 cm^{-1} (C═O bonded to an aromatic group), 1536 (–COO- stretching), and 1374 cm^{-1} (CO as ester group in a cycle). Also, a sharp peak was observed at 1031 cm^{-1} attributed to the glycosidic linkage observed. These results were observed to be in line with previous chia seed mucilage FT-IR spectra (Muñoz Hernández, 2012). The changes in peak intensities of the CH-MLG IR-spectrum demonstrated the successful composite formation between these two polymers. Chia mucilage contains several − OH groups and a small number of − COOH groups making it a suitable polysaccharide for interacting with chitosan. The sharpness of the amide-II band indicates the interaction through weak hydrogen bonding between –OH and/or –COOH groups in chia mucilage and $–NH_2$ groups in chitosan.

For natural levan, a broad peak in the range of 3500–3100 cm^{-1} was observed due to the –OH stretching of the CH_2-OH groups and the fructofuranans ring. In addition, the characteristic IR peaks for natural levan were recorded at 1009 cm^{-1} and 920 cm^{-1} due to the symmetrical bending vibration of the C—O—C bonds of the fructose ring and glycosidic linkage. These results were found to be in line with the previous IR spectra of levan (Bostan, Mutlu, Kazak, Keskin, Oner, & Eroglu, 2014). In the IR spectrum of the CH-LVN composite film, the characteristic peaks for chitosan and levan were observed (Fig. 1i). In the CH-LVN-MLG composite film, the observation of sharp peaks at 1653 cm^{-1}, 1558 cm^{-1}, 1417 $cm^{-1,}$ and 1022 cm^{-1} proved that chitosan successfully formed the intermolecular interaction with chia mucilage and levan.

3.3. XRD

XRD diffractograms of chitosan, natural chia mucilage, natural levan, CH, CH-MLG, CH-LVN, and CH-LVN-MLG composite films were recorded, and results are presented in Fig. 1j. X-Ray diffraction patterns for chitosan are known to show the characteristic diffraction peaks at approximately 10° and 20° (Al Sagheer, Al-Sughayer, Muslim, & Elsabee, 2009). In the current study, similar peaks were recorded for chitosan (powder) (Fig. 1j). For natural chia mucilage, crystalline peaks were recorded at approximately 23° and 29° (Fig. 1j). For natural levan, a typical amorphous peak was observed at $2\theta = 18°$ (Fig. 1j). These results were observed to agree with the previously reported XRD diffractograms (García-Salcedo, Torres-Vargas, del Real, Contreras-Jiménez, & Rodriguez-Garcia, 2018). For CH film, three major characteristic peaks were observed at 18°, 23° and 29° corresponding to 110 and 200 crystallographic planes (Fig. 1j). However, these crystalline characteristic peaks for chitosan at 10 and 20°, have disappeared from the diffractograms of all composite films. This can be attributed to the fact that during the production of chitosan composite films, dissolution of chitosan in weak acetic acid, the crystalline regions are distorted. The disappearance of characteristic peaks for chitosan has also been supported by previous studies (Akyuz Yilmaz, Karaduman, Cicek, Akata, & Kaya, 2021). Two characteristic peaks at 19° and 22° were recorded for CH-MLG (Fig. 1j). These peaks can be ascribed to the crystalline regions of chia mucilage and chitosan respectively. It has been reported that pure levan gives a pattern peak between 10° and 40° (Chen, Gao, & Ploehn, 2014). A single characteristic peak for levan was recorded at 18° in the diffractogram CH-LVN composite film (Fig. 1j). This result is due to the interaction between the molecules of chitosan and levan. For CH-LVN-MLG composite films, the characteristic peaks for chia mucilage and levan were observed in the diffractogram, demonstrating a successful composite formation between chitosan, chia mucilage, and levan (Fig. 1j).

3.4. DSC

The DSC thermograms are shown in Fig. 1k. Two characteristic DSC peaks were observed for chitosan powder. An endothermic peak at 77 °C can be ascribed to the loss of water from the polymeric structure of chitosan. The exothermic peak at 292 °C can be attributed to the decomposition of amine (GlcN) units (Kittur, Harish Prashanth, Udaya Sankar, & Tharanathan, 2002). Natural chia mucilage displayed both exothermic and endothermic transitions, corresponding to water leaching and degradation of the polymeric structure. The prominent endothermic peaks for transition temperatures (T_o, T_p and T_e) were recorded at 50 °C, 110 °C, and 220 °C. The broad peak at 50 °C to 110 °C can be ascribed to the loss of water. For natural levan, a peak was recorded at 210 °C. CH films displayed two endothermic peaks at 65 °C and 201 °C, which can be ascribed to the loss of water and degradation of polymeric chains respectively. For composite films, DSC thermograms displayed a shift in thermal transition temperatures. In CH-MLG, a wide endothermic peak was recorded at 69 °C corresponding to the loss of water from the polymeric structures of mucilage and chitosan. A peak at 204 °C can be ascribed to the degradation of chitosan and mucilage polymeric structure. Similarly, for CH-LVN and CH-LVN-MLG, two endothermic peaks were recorded. In both thermograms, the second endothermic peaks were wider compared to their pure form. This can be due to the composite formation with chitosan.

3.5. Contact angle

Contact angle measurements were made to evaluate the effect of mucilage and levan additions in chitosan coating films and results are presented in Fig. 1l. The contact angle of CH film was recorded as 75° ± 0.12. For CH-MLG and CH-LVN the contact angle was determined as 74° ± 0.15 and 77° ± 0.11. According to the results, mucilage and levan did not have any effect on the hydrophobicity of chitosan films when these polymers were incorporated alone. In a previous study, the contact angles of CS film, Levan/Chitosan (1:1), Levan/Chitosan (2:1) and Levan/Chitosan (5:1) films were 72.8°, 77.2°, 78.0° and was 83.1. °

respectively. It has been reported that as the levan concentration increases, the hydrogen bonding increases and the films can be made less hydrophilic (Wang, Zhou, & Han, 2022). However, a significant decrease in contact angle was observed for the CH-LVN-MLG films at 58° ± 0.22 due to their hydrophilic nature. This can be attributed to the formation of strong intermolecular interaction between hydrophilic mucilage and levan leading to the availability of free functional groups for water molecules. Current results were supported by previous literature reports regarding chitosan films containing levan and mucilage (Mujtaba et al., 2019).

3.6. SEM

The surface morphologies of chitosan, natural chia mucilage, natural

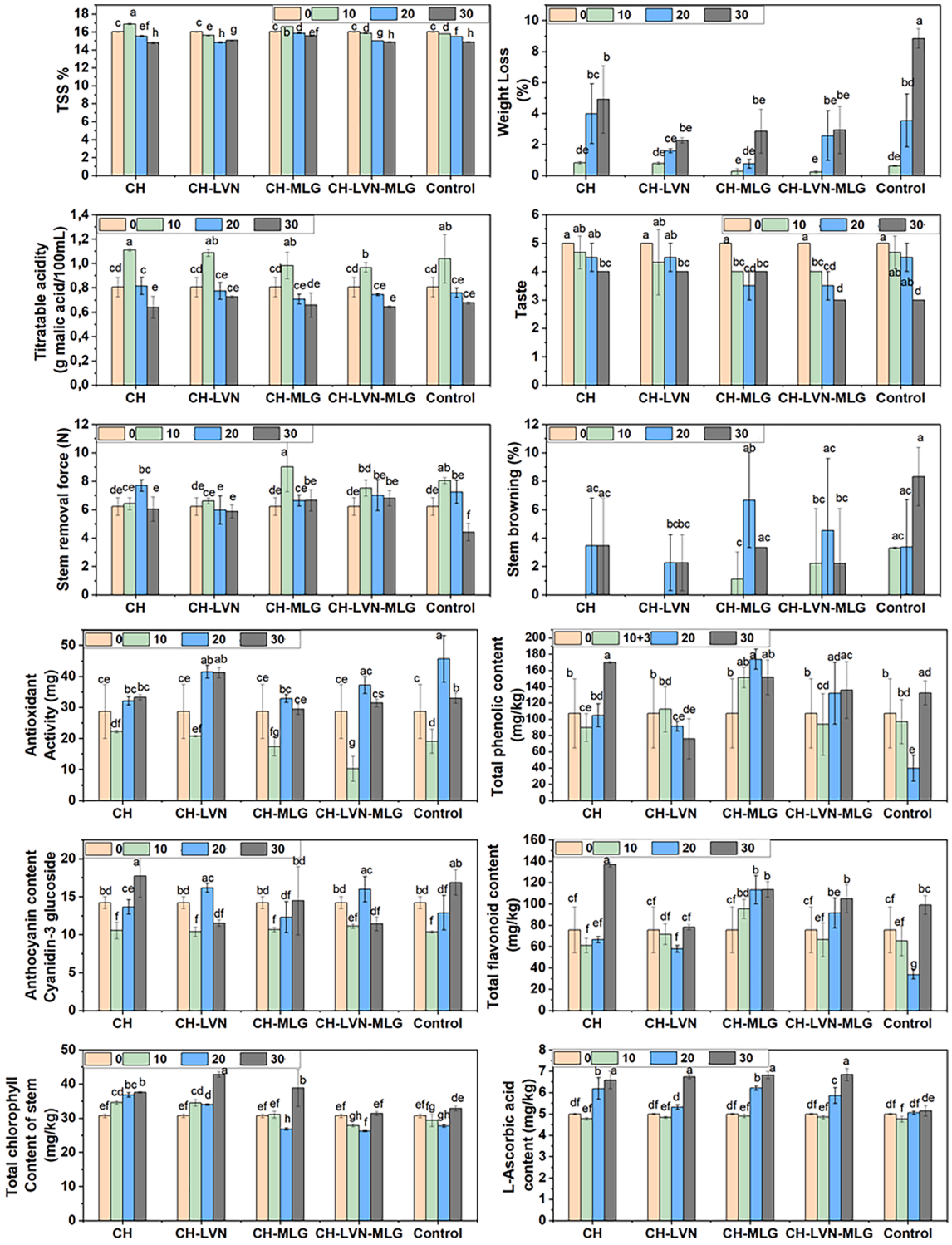

Fig. 2. Effects of chitosan, chia mucilage, levan, and their composite coatings on weight loss, TSS content, titratable acidity, taste, stem removal force, stem browning, antioxidant activity, total phenolic content, anthocyanin content, total flavonoid content, total chlorophyll content, and L-ascorbic acid content of sweet cherry during cold storage at 0 °C.

levan (Fig. S1a–c), CH, CH-MLG, CH-LVN, and CH-LVN-MLG (Fig. 1e–h) composite films were analyzed using SEM. A flat and compact surface without any fibers or pores was observed for natural levan (Fig. S1a). The SEM microscopy images of commercial chitosan revealed an uneven/rough surface with tightly bounded fibers (Fig. S2b). The mucilage displayed an irregular fibrous surface morphology with pores distributed across the surface (Fig. S2c). The rheological properties of chia mucilage are attributed to the open pores (Capitani, Ixtaina, Nolasco, & Tomás, 2013). As can be seen, the CH film has a flat surface, in contrast to the surface morphology of the natural chitosan. After dissolving the chitosan in weak acid, it formed a homogeneous structure with the addition of glycerol. Unlike chitosan in its natural form, CH film displayed a smooth and homogenous surface without any fibers and pores (Fig. 1e). This change in surface morphology is attributed to the dissolution of chitosan in acetic acid (Bilican et al., 2020). Similar surface morphology has been reported by different reports. CH-MLG composite films resulted in a smooth surface without any cracks and pores (Fig. 1f). These results were in line with our previously reported surface morphologies for chia mucilage-chitosan films where chia mucilage was incorporated at different concentrations into the chitosan matrix. Likewise, the CH-LVN composite films exhibited a flat surface morphology (Fig. 1g). The flat and smooth surface of CS-LVN films shows that levan and chitosan form composites well. This result is compatible with the literature (Wang et al., 2022). The inclusion of chia mucilage and levan (CH-LVN-MLG) in the chitosan film matrix also showed somewhat similar surface morphology i.e., smooth, and flat (Fig. 1h). The fact that the films have a flat and homogeneous matrix is indicator of their structural integrity and homogenous distribution of levan and chia mucilage inside the chitosan matrix.

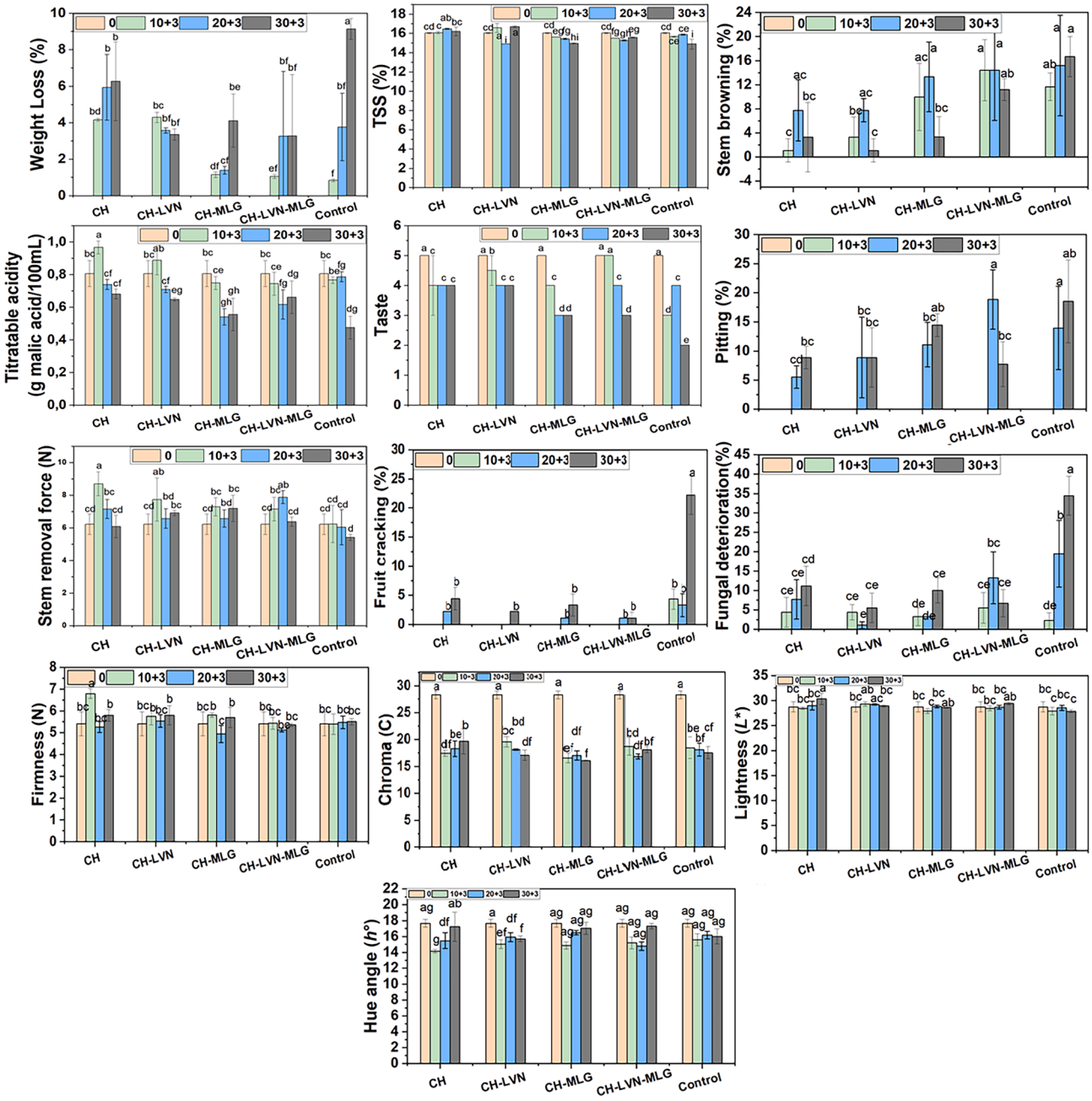

Fig. 3. Effect of chitosan, chia mucilage, levan and their composite coatings on the weight loss, TSS content, titratable acidity, taste, stem removal force, pitting, fungal deterioration, firmness, fruit cracking, L^*, C^*, and h° values of sweet cherry during shelf-life conditions at 20 °C.

3.7. Quality analysis of coated cherries

3.7.1 Weight. loss

As it is known that the fruits of sweet cherry display weight losses with an extension in storage time under both cold storage and shelf-life period. In the current study, coating sweet cherry fruits have resulted in a significant reduction in total weight loss during cold storage and shelf-life conditions. At the end of cold storage, the maximum weight loss (8.85%) was recorded for CTL, while the minimum weight loss (2.27%) was recorded for samples coated with CH-LVN film solutions. No statistically significant differences were observed between CH, CH-LVN, CH-MLG, and CH-LVN-MLG treatments (Fig. 2). CH-LVN treatment resulted initially in high weight losses on 10 + 3 days, however later showed slightly lower weight loss with extension in shelf-life conditions. At the end of the shelf-life period, the highest weight loss (9.14%) was recorded in CTL, however, the lowest weight loss (3.28%) was recorded for the CH-LVN-MLG treatment. No, statistically significant differences were observed among CH, CH-LVN, CH-MLG, and CH-LVN-MLG treatments (Fig. 3).

Considering the results, the coating treatments have significantly reduced the overall weight loss in sweet cherries during shelf life and cold storage conditions compared to the control treatments (CTL). These outcomes were observed in agreement with the results of Nourozi and Sayyari (2020), who mentioned a reduction in weight loss of apricot fruits after the application of pure basil seed chia mucilage film (natural chia mucilage) or in combination with *Aloe vera* gel. This decrease in weight loss of coated fruits samples at the end of cold storage and shelf-life period can be ascribed to the effects of coating on the reduction of transpiration rate and CO_2 production as explained by Alonso and Alique (2004); Del-Valle, Hernández-Muñoz, Guarda, and Galotto (2005) in strawberry and sweet cherry respectively.

3.7.2. Total soluble solids

The interaction between storage time and edible coatings treatments exhibited statistically significant effects on the TSS content of sweet cherry fruits in cold storage and shelf-life period. The TSS content at harvest was recorded as 16.03%. CH and CH-MLG treatment increased TSS content on day 10 and later decreased throughout the remaining duration of storage. The CH-LVN-MLG and CTL treatments displayed a decrease in TSS content during the storage whereas the CH-LVN treatment resulted in a decrease until day 20 and then an increase in TSS content on day 30. At the end of cold storage, the highest TSS content (15.57%) was recorded for CH-MLG treatment while the lowest TSS content (14.80%) was determined for CH treatments. However statistically no significant differences were observed between CH, CH-LVN-MLG, and CTL treatments on day 30 of the cold storage (Fig. 2).

The CH treatment increased the TSS content up to 20 + 3 days and then decreased on 30 + 3 days. CH-LVN and CTL treatments displayed fluctuation in TSS contents. The CH-MLG application resulted in the decline of TSS content in sweet cherry fruits. The CH-LVN-MLG treatment indicated a decline in TSS content for 20 + 3 days and later increased on 30 + 3 days. At the end of the shelf-life period, the maximum TSS content (16.70%) was in CH-LVN with the minimum TSS content (14.90%) in CTL treatment. There were no statistically significant differences between CH-MLG and CTL treatments on 30 + 3 days of the shelf-life period (Fig. 3).

The highest TSS contents in CH-MLG and CH-LVN treated sweet cherry at the end of cold storage and shelf-life period respectively can be due to the modification in the inner environment of the fruit, with alleviated levels of O_2, enhancement in the amount of CO_2 production and inhibition of ethylene production triggered by coating treatments as described by Petriccione et al. (2015) in sweet cherry.

3.7.3. Titratable acidity

In the current study, it was demonstrated that interaction between storage time and edible coatings treatments had statistically significant effects on the titratable acidity (TA) in cold storage and shelf-life period. The TA value determined at the harvest was 0.806 g malic acid 100 mL^{-1}. The TA of sweet cherry fruits increased initially on day 10 and later decreased until day 30. At the end of cold storage, the highest TA (0.726 g malic acid 100 mL^{-1}) was recorded for CH-LVN with the lowest TA (0.640 g malic acid 100 mL^{-1}) for CH treatment. There were no statistically significant differences between CH with CH-LVN, CH-MLG, CH-LVN-MLG, CTL; and CH-LVN with CH, CH-LVN-MLG, and CTL (Fig. 4). The CH and CH-LVN treatments showed a similar trend in cold storage. On the other hand, CH-MLG, and CH-LVN-MLG resulted in a decreased TA at day 20 + 3 and later increased on day 30 + 3. The CTL treatment displayed fluctuation in TA values with an extension in shelf-life conditions. At the end of the shelf-life period, the maximum TA (0.681 g malic acid 100 mL^{-1}) was recorded for CH while the minimum TA (0.475 g malic acid 100 mL^{-1}) for CTL. However, no statistically significant differences were observed between CTL and CH-MLG treatments (Fig. 3).

The highest TA with CH treatment in the shelf-life period showed similarity with the results obtained by Tokatlı and Demirdöven (2020) who mentioned that CH-treated sweet cherry had higher TA as compared to the control. The possible reason for this outcome can be the effect of CH coating on slowing down the utilization of organic acids in the respiration process by acting as a barrier (Nabifarkhani, Sharifani, Daraei Garmakhany, Ganji Moghadam, & Shakeri, 2015).

3.7.4. Taste score

The taste scores of cherries in all treatments were under marketable limits during both cold storage and shelf-life conditions. The interaction between storage time and edible coating treatments had statistically significant effects on the taste score of sweet cherry fruits in cold storage and shelf-life period. The taste score at harvest was 5.00. The taste scores showed a decrease in all treatments during both cold storage and shelf-life conditions. At the end of cold storage, the CH, CH-LVN, and CH-MLG treatments showed the highest taste score (4.00) while CTL and CH-LVN-MLG exhibited the lowest taste score (3.00) (Fig. 4). Similar trend was observed in all treatments during shelf-life conditions which showed a decrease in taste scores with extension in storage time. At the end of the shelf-life period, the maximum taste score (4.00) was determined in CH, and CH-LVN with a minimum taste score (2.00) was found in CTL (Fig. 3).

The decrease in taste scores of sweet cherries with extending storage time was also reported by Kurubaş, Özalp, and Erkan (2018). The edible coating treatments had the highest taste values as compared to the CTL which agreed with the outcomes of Ergin, Yaman, and Dilek (2018). These researchers explained that the coated strawberries and loquat had better sensory points that the uncoated ones. However, their findings contradicted our outcome in cold storage where CH-LVN-MLG had recorded similar taste scores with CTL. The highest taste scores in edible coating treatments can be because of their effects on the improvement of sensorial and nutritional attributes as reported by Bouaziz et al. (2016) in almond gum-coated potato chips.

3.7.5. Stem removal force

The stem removal force of sweet cherry fruit increased initially and later declined in both cold storage and shelf-life period. The interaction between storage time and edible coating treatments displayed statistically significant effects on the stem removal force of sweet cherry fruits in cold storage and shelf-life period. The stem removal force determined at the harvest was 6.22 N. CH treatment resulted in an increase in stem removal force for 20 days and later decreased on day 30. CH-LVN, CH-LVN-MLG, and CTL coating treatments showed an increase in stem removal force on day 10 and later decreased throughout the storage. CH-MLG treatment displayed fluctuation in stem removal force throughout the cold storage. At the end of cold storage, the highest stem removal force (6.81 N) was recorded for CH-LVN-MLG coated samples with lowest stem removal force (4.42 N) was determined for CTL. There were

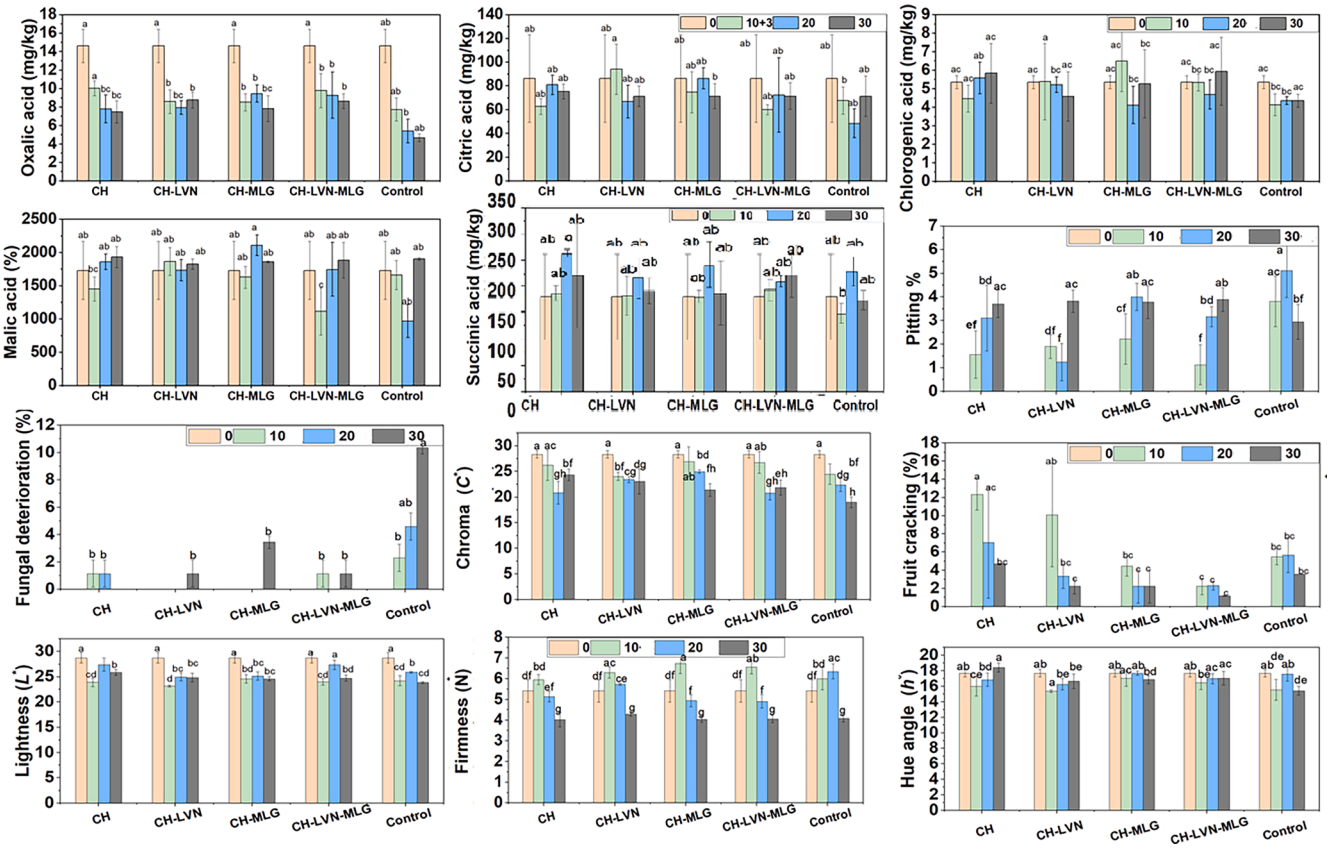

Fig. 4. Effect of chitosan, chia mucilage, levan and their composite coatings on the oxalic acid, citric acid, malic acid, succinic acid, and chlorogenic acid contents, pitting, fungal deterioration, firmness, fruit cracking, L^*, C^* and h° values of sweet cherry during cold storage at 0 °C.

no statistically significant differences between CH-MLG, CH, CH-LVN, and CH-LVN-MLG on day 30th of the cold storage (Fig. 4). The CH treatment displayed an increase in stem removal force on day 10 + 3 and later decreased throughout the shelf-life period. The CH-LVN and CH-MLG treatments exhibited fluctuation in stem removal force with extension in shelf-life conditions. The CH-LVN-MLG treatment displayed an increase in stem removal force for 20 + 3 days and then decreased on day 30 + 3. The CTL treatment recorded no significant changes in stem removal force with extension in shelf-life conditions. At the end of the shelf-life period, the highest stem removal force (7.19 N) was recorded in CH-MLG-coated samples with the lowest stem removal force (5.42 N) in CTL. No statistically significant differences were observed between CH-MLG and CH-LVN on day 30 + 3 of the shelf-life period (Fig. 3). The finding of the current study in both cold storage and shelf-life period agreed with the results of Martínez-Romero et al. (2006), who mentioned low stem removal force in controlled samples (only water) as compared to *Aloe Vera* gel coated cherries. The highest stem removal force in coated fruit samples can be ascribed to the constant maintenance of fruit freshness due to coating.

3.7.6. Stem browning

Stem browning is a serious problem in sweet cherries, influencing marketability. In the current study, biobased coatings showed less stem browning as compared to control in sweet cherry fruits (in both cold storage and shelf-life period conditions). The interaction between storage time and coating treatments showed statistically significant effects on the stem browning of sweet cherry fruits in cold storage and shelf-life period. There was no stem browning at harvest. CH and CH-LVN treatments resulted in increased stem browning until the 20th day and then remained constant until the 30th day. The CH-MLG and CH-LVN-MLG coating treatments exhibited an increase in stem browning until the 20th day and then displayed a decreasing pattern until the 30th day. The CTL treatment displayed an increase in stem browning throughout the cold storage. At the end of cold storage, the highest stem browning (8.33%) was recorded for CTL, while the lowest stem browning (2.22%) was recorded for CH-LVN-MLG. There were no statistically significant differences between CH-LVN-MLG and CH-LVN on day 30 of the cold storage (Fig. 4).

CH, CH-LVN, and CH-MLG treatments displayed an increase in stem browning for 20 + 3 days and then a decrease on day 30 + 3. CH-LVN-MLG treatment showed an increase in stem browning on day 10 + 3, remained constant on day 20 + 3, and then decrease on day 30 + 3. CTL treatment exhibited a rapid increase in stem browning with the advancement in shelf-life conditions. On the contrary, a slower increase rate was witnessed in stem browning for coated fruit samples. At the end of the shelf-life period, the maximum stem browning (16.67%) was recorded for CTL samples, while the minimum stem browning (1.11%) was observed for CH-LVN treatment. There were no statistically significant differences between CTL with CH-LVN-MLG and CH-LVN with CH and CH-MLG on day 30 + 3 days of the shelf-life period (Fig. 3). The delay in stem browning triggered by coating treatments can be attributed to the decrease in dehydration preventing a complete stem dryness (Martínez-Romero et al., 2006). Lim, Stathopoulos, and Golding (2011) reported a significant reduction in stem browning after coating sweet cherry with gelatin, carboxy-methylcellulose, and soy protein isolate. These results are also consistent with the findings of the current study.

3.7.7. Pitting

The interaction between storage time and edible coating treatments displayed statistically significant effects on the pitting percentage of sweet cherry fruits in cold storage and shelf-life conditions. CH and CH-LVN-MLG treatments displayed an increase in pitting percentage with advancement in storage time. CH-MLG and CTL treatments increased the pitting percentage for 20 days and then decrease on day 30. CH-LVN

treated samples showed fluctuation in pitting percentage throughout the storage. At the end of cold storage, the highest pitting (3.89%) was recorded for CH-LVN-MLG treated samples with the lowest pitting (2.95%) for CTL (Fig. 4). CH, CH-LVN, CH-MLG, and CTL treatments exhibited an increase in pitting percentage throughout shelf-life conditions. CH-LVN-MLG treatment enhanced the pitting percentage for 20 + 3 days and then decreased it to 30 + 3 days. At the end of the shelf-life period, maximum pitting (18.54%) was observed in CTL with minimum pitting (7.78%) in CH-LVN-MLG treated samples. There were no statistically significant differences between CH-LVN-MLG, CH, and CH-LVN treatments on 30 + 3 days of shelf-life conditions (Fig. 3).

Park and Zhao (2006) reported that edible coatings (calcium caseinate and chitosan) did not reduce pitting in 'Bing' sweet cherries which agreed with our current findings obtained at the end of cold storage. These researchers further mentioned that the enhancement in pitting may be due to the physical damage during harvesting and handling or can be because of stress from adjoining stems in transportation and storage. The lower pitting percentage obtained with edible coatings treatments in shelf life can be attributed to their effects on decreasing the moisture loss and alteration of the internal environment.

3.7.8. Fungal deterioration

The coating treatments significantly reduced the fungal deterioration of sweet cherry fruits in both cold storage and shelf-life period. The interaction between storage time and edible coatings treatments had statistically significant effects on the fungal deterioration of sweet cherries in cold storage and shelf-life condition. Fungal deterioration increased until day 10 in fruit samples coated with CH. After the 10th day, the rate of deterioration displayed no statistically significant difference throughout the storage. CH-LVN-MLG treatment indicated fluctuation in fungal deterioration with advancement in storage time. At the end of cold storage, the greatest fungal deterioration (10.34%) was recorded for CTL, while no (0.00%) fungal deterioration was recorded in CH-coated fruit samples. There were no statistically significant differences among coating treatments (Fig. 4). CH-LVN treatment showed fluctuation in fungal deterioration with advancement in storage. CH-LVN-MLG treatment displayed an increase in fungal deterioration for 20 + 3 days and then a decrease for 30 + 3 days. At the end of the shelf-life period, the highest fungal deterioration (34.44%) was in CTL with the lowest fungal deterioration (5.59%) in CH-LVN. There were no statistically significant differences between CH-LVN, CH-MLG, and CH-LVN-MLG on 30 + 3 days of shelf-life conditions (Fig. 3).

The edible coating treatments reduced the fungal deterioration due to their antifungal effects on grey mold and blue mold in sweet cherries as mentioned by Romanazzi, Nigro, and Ippolito (2003) in the case of CH treatment also reported the antifungal characteristics of an edible coating comprising *Aloe vera* against *Penicillium expansum* and *Botrytis cinerea*. The minimum fungal deterioration recorded in edible coating treatments during our study may be due to their antifungal properties against blue mold, grey mold, and brown rot.

3.7.9. Fruit firmness

The interaction between storage time and edible coating treatments displayed statistically significant effects on the fruit firmness in cold storage and shelf-life condition. All the coatings treatments showed an increase in fruit firmness on day 10 and later decreased throughout the storage. CTL treatment exhibited an increase in fruit firmness for 20 days and then a decline on day 30. At the end of cold storage, there were no statistically significant differences between edible coatings treatments and CTL (Fig. 4). CH, CH-LVN, and CH-MLG treatments displayed fluctuation in fruit firmness values throughout the shelf-life period. CH-LVN-MLG and CTL treatment exhibited no statistically significant differences with extension in shelf-life conditions. At the end of the shelf-life period, there were no statistically significant differences between the edible coating treatments and CTL (Fig. 3). These outcomes were observed to be in line with a previous study by Park et al. (2006). As per the literature report, the initial increase in fruit firmness with extension in storage time can be ascribed to the enhanced ratio of solid to liquid as the integrity of cherry skin is preserved with the availability of moisture in the surrounding during storage.

3.7.10. Fruit cracking

The interaction between storage time and edible coating treatments displayed statistically significant effects on the fruit cracking both in cold storage and shelf-life conditions. CH, CH-LVN, and CH-MLG treatments showed an increase in fruit cracking until day 10 and then decreased throughout the storage. CH-LVN-MLG and CTL treatments exhibited an increase in fruit cracking on day 10 and later displayed no statistically significant differences throughout the cold storage (Fig. 4). CH, CH-MLG, and CH-LVN-MLG treatments showed an increase in fruit cracking on day 20 + 3 and later displayed no statistically significant differences on day 30 + 3. CH-LVN treatment exhibited fruit cracking on day 30 + 3 of shelf-life conditions. CTL treatment indicated fluctuation in fruit cracking throughout shelf-life conditions. At the end of the shelf-life period, the highest fruit cracking (22.22%) was in CTL with the lowest fruit cracking (1.11%) in CH-LVN-MLG. There were no statistically significant differences among coated fruit samples (Fig. 3).

Our results in the shelf life period were consistent with the outcomes of Kaiser, Fallahi, Meland, Long, and Christensen (2014) who mentioned that fruit cracking of the sweet cherry was reduced by the application of biofilm. The minimum cracking percentage in edible coating treatments can be because of their waterproof action on the surface of cherries which fills the microcracks in the cuticle.

3.7.11. Fruit color (L^*, C^*, h°)

The color of sweet cherry fruits was significantly affected by coatings in both cold storage and shelf-life condition. The lightness (L^*) value at harvest was recorded as 28.76. All treatments showed fluctuations in L^* values with extension in storage and recorded lower L^* values as compared to the harvest. At the end of cold storage, the highest L^* value (25.87) was observed in CH-treated fruit samples. The lowest L^* value i.e., 23.89 was recorded in CTL. No statistically significant differences were observed among edible coating treatments on day 30 of cold storage (Fig. 4). CH and CH-LVN-MLG treatments showed no statistically significant differences in L^* values for 20 + 3 days followed by an increase on day 30 + 3. CH-LVN treatment displayed an increase in L^* values on day 10 + 3 and later decreased throughout shelf-life conditions. CH-MLG and CTL treatments indicated no statistically significant differences with advancement in shelf-life conditions. At the end of the shelf-life period, the maximum L^* value (30.38) was observed in CH with the minimum L^* value (27.88) was recorded in CTL. There were no statistically significant differences between CH with CH-LVN, CH– LVN-MLG, and CTL with and CH-LVN on day 30 + 3 of the shelf-life period (Fig. 3).

Petriccione et al. (2015) also reported the highest L^* values with the application of CH. The lower L^* values recorded at the end of cold storage in our study may be due to the surface darkening of sweet cherry fruits (Yousuf, Qadri, & Srivastava, 2018).

The C^* value at harvest was recorded as 28.34. CH and CH-LVN-MLG treatments displayed a decrease in C^* values for 20 days and later increase on day 30. CH-LVN, CH-MLG, and CTL treatments indicated a decline in C^* values throughout the cold storage. At the end of cold storage, the highest C^* value (24.34) was recorded for CH while the lowest C^* value (18.98) was recorded in CTL. There were no statistically significant differences between CTL, CH-MLG, and CH-LVN-MLG on day 30 of the cold storage (Fig. 4). CH treatment exhibited a decline in C^* values on day 10 + 3 and later increase throughout the shelf life. CH-LVN and CTL treatments indicated a decrease in C^* values with the advancement in shelf-life conditions. CH-MLG treatment displayed fluctuation throughout the shelf-life period. CH-LVN-MLG treatment exhibited a decrease for 20 + 3 days and later increase on day 30 + 3. At

the end of the shelf-life period, the greatest C^* value (19.70) was in CH with the lowest C^* value (16.12) in CH-MLG. There were no statistically significant differences between CH with CH-LVN-MLG and CH-MLG with CH-LVN, CH-LVN-MLG, and CTL on day 30 + 3 of shelf-life conditions (Fig. 3).

The maintenance of the C^* value by CH coating was also described by (Gol, Patel, & Rao, 2013) which is also in line with current findings in cold storage. The highest C^* values in CH coatings showed that the vividness of the color was maintained by the treatments as reported by (Mozetič, Trebše, Simčič, & Hribar, 2004).

The interaction between storage time and edible coating treatments displayed statistically significant effects on the hue (h°) values of sweet cherries in cold storage and shelf-life conditions. The h° value at harvest was 17.68°. CH, CH-LVN, and CH-LVN-MLG treatments exhibited a decrease in h° on day 10 and later increase throughout the cold storage. CH-MLG and CTL treatments indicated fluctuation in h° values with an extension in storage time. At the end of cold storage, the highest h° value (18.42°) was recorded in CH with the lowest h° value (15.44°) was observed in CTL. There were no statistically significant differences between CH and CH-LVN-MLG on day 30 of cold storage (Fig. 4). CH and CH-MLG treatments displayed a decrease on day 10 + 3 and later increase for 30 + 3 days. CH-LVN and CTL treatments exhibited fluctuation in h° values with the advancement in shelf-life conditions. CH-LVN-MLG treatment indicated a decrease in h° values for 20 + 3 days and later an increase for 30 + 3 days. At the end of the shelf-life period, the maximum h° value (17.32°) was in CH-LVN-MLG with the minimum h° value (15.73°) in CH-LVN. There were no statistically significant differences between CH-LVN-MLG and CH (Fig. 3).

Petriccione et al. (2015) mentioned that h° values were maintained by the application of edible coatings which showed similarity with findings obtained in this study. The color changes in edible coating treatments were much slower as compared to the CTL treatment because of their effects on the modification of the internal atmosphere by acting as an obstacle to gas exchange and decreasing the oxygen intake which limits the physiological process like respiration rate hence delayed the ripening and senescence process as expressed by Guerreiro, Gago, Faleiro, Miguel, and Antunes (2015).

3.7.12. *Antioxidant activity*

The interaction between storage time and edible coating treatments showed statistically significant effects on the antioxidant activity of sweet cherry fruits in cold storage and shelf-life period. The antioxidant

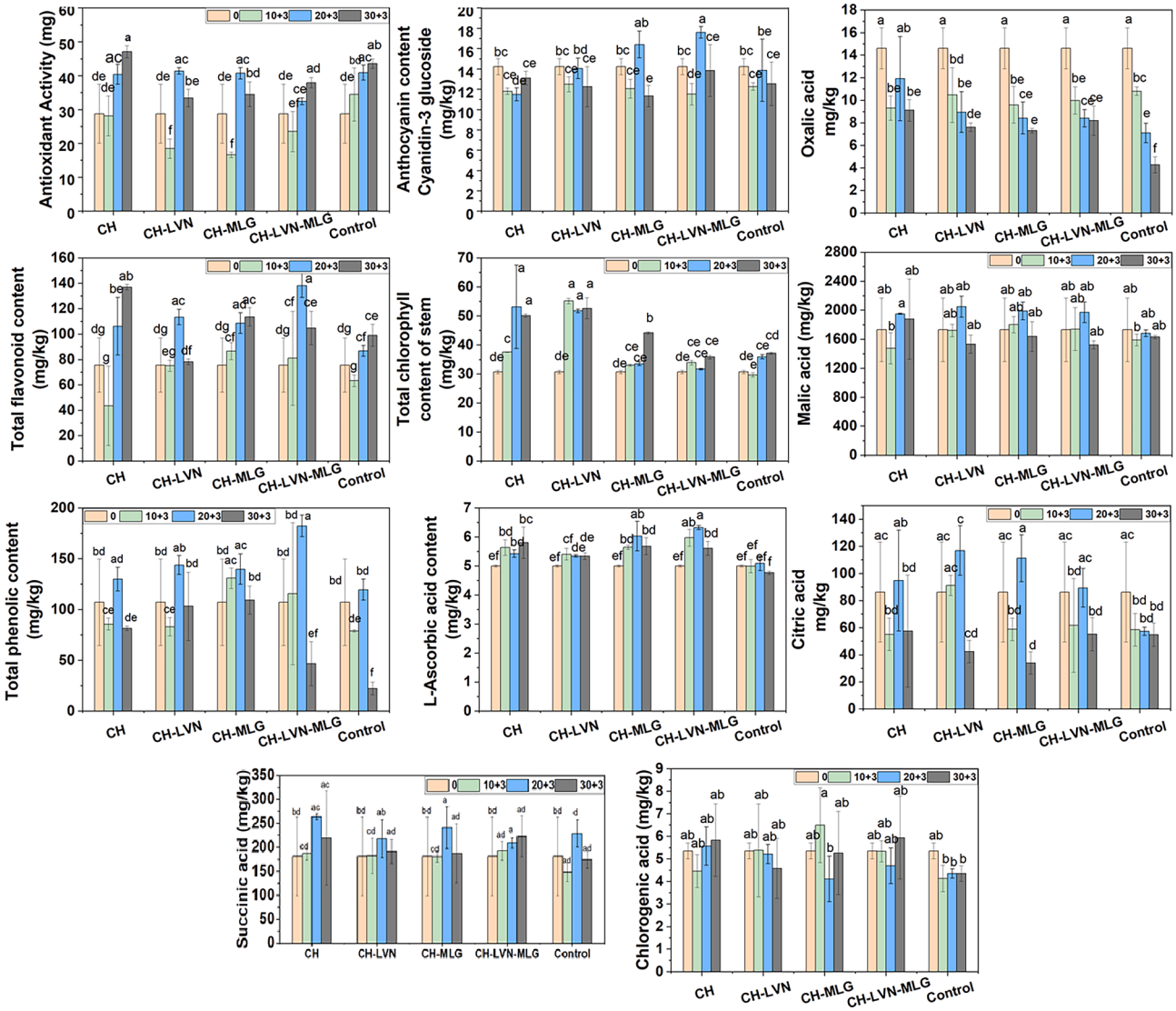

Fig. 5. Effect of chitosan, chia mucilage, levan and their composite coatings on the antioxidant activity, anthocyanin content, total flavonoid content, total chlorophyll content, total phenolic content, and L-ascorbic acid content, organic acids (oxalic acid, citric acid, malic acid, and succinic acid) and phenolic compounds (chlorogenic acid) contents of sweet cherry during shelf-life conditions at 20 °C.

activity at harvest was recorded as 28.75 mg fw. CH and CH-LVN treatments exhibited an increase in antioxidant activity on day 10 and later a decrease throughout storage. CH-MLG, CH-LVN-MLG, and CTL treatments displayed fluctuation in antioxidant activity with the advancement in storage duration. At the end of cold storage, the maximum antioxidant activity (29.44 mg fw) was observed in CH-MLG with minimum antioxidant activity (41.20 mg fw) in CH-LVN (Fig. 2). CH treatment showed no statistically significant differences in antioxidant activity on 10 + 3 days and later decrease throughout the storage. CH-LVN and CH-MLG treatment indicated fluctuation in antioxidant activity with extension in shelf-life condition. CH-LVN-MLG treatment displayed an increase in antioxidant activity on day 10 + 3 and later decrease throughout the shelf life. CTL treatment exhibited a decline in antioxidant activity with the advancement in the shelf-life period. At the end of the shelf-life period, the highest antioxidant activity (33.43 mg fw) was in CH-LVN with the lowest antioxidant activity (47.07 mg fw) in CH. There were no statistically significant differences between CH, CH-LVN-MLG, and CTL on day 30 + 3 of the shelf-life period (Fig. 5).

The higher antioxidant activity with edible coatings was reported by Petriccione et al. (2015) which showed similarity with our outcomes except for CH-LVN in cold storage, CH and CH-LVN-MLG in shelf life. The higher antioxidant activity in CH-MLG and CH-LVN edible coatings in our study may be due to their effects on the maintenance of sweet cherry quality traits, decrease in fungal deterioration, and retarding the enzymatic activities responsible for the breakdown of antioxidant compounds as expressed by Nourozi and Sayyari (2020) in apricots through the application of basil seed chia mucilage.

3.7.13. Total phenolic content

The interaction between storage time and edible coating treatments displayed statistically significant effects on the total phenolic content of sweet cherry in both cold storage and shelf life period. The total phenolic content at harvest was 107.15 mg GAE kg^{-1} fw. CH and CH-LVN-MLG treatments displayed a decrease in total phenolic content on day 10 and then an increase throughout the cold storage. CH-LVN treatment showed an increase in total phenolic content on day 10 and later declined throughout the storage. CH-MLG treatment indicated an increase in total phenolic content for 20 days and a later decrease on day 30. CTL treatment manifested a decrease in total phenolic content for 20 days and then an increase on day 30. At the end of cold storage, the greatest total phenolic content (170.07 mg GAE kg^{-1} fw) was in CH with the lowest total phenolic content (76.07 mg GAE kg^{-1} fw) in CH-LVN. There were no statistically significant differences between CH, CH-MLG, CH-LVN-MLG, and CTL on day 30 of cold storage (Fig. 2). CH, CH-LVN, and CTL treatments displayed fluctuation in total phenolic content with the advancement in shelf-life conditions. CH-MLG and CH-LVN-MLG treatments exhibited an increase in total phenolic contents for 20 + 3 days and then decline on day 30 + 3. At the end of the shelf-life period, the maximum total phenolic content (109.18 mg GAE kg^{-1} fw) was in CH-MLG with minimum total phenolic content (22.22 mg GAE kg^{-1} fw) in CTL. There were no statistically significant differences between CH-MLG with CH-LVN and CTL with CH-LVN-MLG on day 30 + 3 of the shelf-life period (Fig. 5).

The higher total phenolic content with the application of edible coatings in apricot was reported by Petriccione et al. (2015) which was in confirmation with our outcomes regarding maximum total phenolic contents in CH and CH-MLG treatments in cold storage and shelf life respectively. These researchers further mentioned that this might be described by the development in the synthesis of phenolic compounds through the application of *Aloe vera* gel and basil seed chia mucilage edible coatings which successively decrease the fungal deterioration and enhance the shelf life.

3.7.14. Anthocyanin content

The interaction between storage time and edible coating treatment had statistically significant effects on the anthocyanin content of sweet cherry fruits in both cold storage and shelf-life period. The anthocyanin content at harvest was 14.23 mg Cya 3 kg^{-1} fw. CH, CH-MLG, and CTL treatments displayed a decline in anthocyanin content on day 10 and later increase throughout the cold storage. CH-LVN and CH-LVN-MLG treatments showed fluctuation in anthocyanin content during storage. At the end of cold storage, the highest anthocyanin content (17.72 mg Cya 3 kg^{-1} fw) was in CH with the lowest anthocyanin content (11.44 mg Cya 3 kg^{-1} fw) in CH-LVN-MLG. There were no statistically significant differences between CH with CTL and CH-LVN-MLG with CH-LVN on day 30 of cold storage (Fig. 2). CH treatment showed a decrease in anthocyanin content for 20 + 3 days and later increased on day 30 + 3. CH-LVN, CH-MLG, CH-LVN-MLG, and CTL treatments displayed fluctuation in anthocyanin content with advancement in shelf-life conditions. At the end of the shelf-life period, there were no statistically significant differences between edible coating treatments and CTL (Fig. 5).

The lower anthocyanin contents in CH-LVN-MLG and CH-LVN edible coatings during cold storage in this study may be described by its inhibitory effects on the ripening of fruit as reported by EL Ghaouth, Arul, Ponnampalam, and Boulet (1991) in strawberries. The non-significant effects of edible coatings on sweet cherry fruit as compared to the uncoated fruits were also reported by Petriccione et al. (2015) which showed similarity with our outcomes at the end of the shelf life period.

3.7.15. Total flavonoid content

The interaction between storage time and edible coating treatments had statistically significant effects on the total flavonoid contents of cherries in both cold storage and shelf-life period. The total flavonoid content at harvest was 75.61 mg catechin kg^{-1} fw. CH and CH-LVN-MLG treatments showed a decrease in total flavonoid content on day 10 and later increase throughout the cold storage. CH-LVN and CTL treatments exhibited a decline in total flavonoid content for 20 days and later increase on day 30. CH-MLG treatment indicated an increase in total flavonoid content throughout the storage. At the end of cold storage, the highest total flavonoid content (136.98 mg catechin kg^{-1} fw) was in CH with the lowest total flavonoid content (78.20 mg catechin kg^{-1} fw) in CH-LVN (Fig. 2). CH and CTL treatments displayed a decline in total flavonoid content on day 10 + 3 and later increase throughout shelf-life conditions. CH-LVN treatment indicated an increase in total flavonoid content on day 20 + 3 and later decrease on 30 + 3 days. CH-MLG treatment exhibited an increase in total flavonoid content with the advancement in the shelf-life period. CH-LVN-MLG treatment manifested an increase in total flavonoid content for 20 + 3 days and later decline on day 30 + 3. At the end of the shelf-life period, the maximum total flavonoid content (136.98 mg catechin kg^{-1} fw) was in CH with minimum total flavonoid content (78.20 mg catechin kg^{-1} fw) in CH-LVN treatment (Fig. 5).

The highest total flavonoid contents recorded with CH may be due to the enhanced levels of total phenolic contents in CH treatment. Wang and Gao (2013) mentioned that CH edible film application maintained the nutraceutical characteristics of strawberries resulting in a high quantity of phenols, anthocyanins, and flavonoids which agreed with the outcomes of this study.

3.7.16. Total chlorophyll content of stem

The total chlorophyll content of the stem determines the freshness of sweet cherry fruit. The interaction between storage time and edible coating treatments exhibited statistically significant effects on the total chlorophyll content of the stem of cherries in both cold storage and shelf-life conditions. The total chlorophyll content of the stem at harvest was 30.69 mg kg^{-1} fw. CH treatment showed an increase in the total chlorophyll content of the stem throughout the cold storage. CH-LVN and CH-MLG treatments displayed fluctuation in the total chlorophyll content of the stem during storage. CH-LVN-MLG and CTL treatments exhibited a decline in total chlorophyll content of the stem for 20 days

and later increase on day 30. At the end of cold storage, the highest total chlorophyll content of stem (42.80 mg kg^{-1} fw) was in CH-LVN with the lowest total chlorophyll content (31.40 mg kg^{-1} fw) in CH-LVN-MLG (Fig. 2). CH treatment exhibited an increase in total chlorophyll content of stem for 20 + 3 days and later decrease on day 30 + 3. CH-LVN and CH-LVN-MLG treatments showed fluctuation in the total chlorophyll content of the stem throughout shelf-life conditions. CH-MLG treatment displayed an increase in the total chlorophyll content of the stem with the advancement in the shelf-life period. CTL treatment indicated a decline in total chlorophyll content of stem on day 10 + 3 and later increase throughout the shelf-life period. At the end of the shelf-life period, the highest total chlorophyll content of stem (52.63 mg kg^{-1} fw) was in CH-LVN with the lowest total chlorophyll content (35.84 mg kg^{-1} fw) in CH-LVN-MLG treatment. There were no statistically significant differences between CH-LVN and CH on day 30 + 3 of the shelf-life period (Fig. 5).

The CH-LVN edible coating treatment in cold storage and CH-LVN along with CH treatment in shelf life showed higher total chlorophyll contents in the stem of sweet cherry fruit which was consistent with the findings of Rehman, Asi, Hameed, and Bourquin (2020) who mentioned that the application of *Aloe vera* gel coating in guava fruits reduced the ripening, senescence, color change and resulted in higher total chlorophyll content. The possible reason for the reduction in total chlorophyll content loss with CH-LVN treatment in cold storage, CH-LVN and CH treatment in shelf life could be their effects on the decrease in the ethylene production and respiration rates as reported by Hong, Xie, Zhang, Sun, and Gong (2012) with the application of CH edible coating in guava.

3.7.17. L-ascorbic acid content

The interaction between storage time and edible coating treatments had statistically significant effects on the L-ascorbic acid content of sweet cherry fruits in both cold storage and shelf-life period. The L-ascorbic acid content at harvest was 5.00 (mg kg^{-1} fw). All treatments exhibited a decline in ascorbic acid content on day 10 and later increase throughout the cold storage. At the end of cold storage, the highest L-ascorbic acid content (6.84 mg kg^{-1} fw) was in CH-LVN-MLG with the lowest L-ascorbic acid content (5.15 mg kg^{-1} fw) was in CTL. There were no statistically significant differences among edible coating treatments on day 30 of cold storage (Fig. 2). CH and CTL treatments showed fluctuation in L-ascorbic acid content with advancement in shelf-life conditions. CH-LVN treatment indicated an increase in L-ascorbic acid content on day 10 + 3 and later decline throughout the shelf-life period. CH-MLG and CH-LVN-MLG treatments displayed an increase in L-ascorbic acid content for 20 + 3 days and then a decline on day 30 + 3. At the end of the shelf-life period, the greatest L-ascorbic acid content (5.80 mg kg^{-1} fw) was in CH with the lowest ascorbic acid content (4.76 mg kg^{-1} fw) was in CTL (Fig. 5).

The higher ascorbic acid content with the application of edible coatings was reported by (Petriccione et al., 2015) who mentioned that the ascorbic acid loss was reduced through CH coating as compared to the uncoated sweet cherry. It is thought that the maximum ascorbic acid content in edible coatings treatments could be because of their effects on reduction in ascorbic acid oxidation by restricting the availability of oxygen for oxidative breakdown, inhibition of the fruit degradation and senescence as reported by Rehman et al. (2020) with the application of *Aloe vera* gel in guava.

3.7.18. Organic acids

The results showed that oxalic acid, citric acid, malic acid, and succinic acid were identified in cherries. The interaction between storage time and edible coatings treatments displayed statistically significant effects on the oxalic acid content of sweet cherry fruits in both cold storage and shelf-life conditions. The oxalic acid content at harvest was 14.61 mg kg^{-1} fw. CH and CTL treatments showed a decrease in oxalic acid content throughout the cold storage. CHLVN, CH-MLG, and CH-LVN-MLG exhibited a decline in oxalic acid content on day 10 and later showed no statistically significant differences throughout the cold storage. At the end of cold storage, the highest oxalic acid content (8.74 mg kg^{-1} fw) was in CH-LVN with the lowest oxalic acid content (4.66 mg kg^{-1} fw) was found in CTL. There were no statistically significant differences among edible coating treatments on day 30 of cold storage (Fig. 4). CH treatment exhibited fluctuation in oxalic acid content with advancement in shelf-life conditions. CH-LVN, CH-MLG, CH-LVN-MLG, and CTL treatments displayed a decline in oxalic acid content throughout the shelf-life period. At the end of the shelf-life period, the highest oxalic acid content (9.11 mg kg^{-1} fw) was in CH with the lowest oxalic acid content (4.29 mg kg^{-1} fw) in CTL. There were no statistically significant differences between CH and CH-MLG on day 30 + 3 of the shelf-life period (Fig. 5).

The interaction between storage time and edible coating treatments was statistically non-significant in citric acid content in cold storage. The citric acid content at harvest was 86.23 mg kg^{-1} fw. All treatments showed statistically non-significant effects on the citric acid content of sweet cherries in cold storage (Fig. 4). The interaction between storage time and edible coating treatments was statistically significant on the citric acid content of sweet cherries in shelf life. CH, CH-MLG, and CH-LVN-MLG treatments displayed fluctuation in citric acid content with advancement in the shelf-life period. CH-LVN treatment indicated an increase in citric acid content for 20 + 3 days and later decline on day 30 + 3. CTL treatment exhibited a decrease in citric acid content on day 10 + 3 and later showed no statistically significant differences throughout the shelf-life condition. At the end of the shelf-life period, the maximum citric acid content (57.49 mg kg^{-1} fw) was in CH with the minimum citric acid content (33.94 mg kg^{-1} fw) in CH-MLG. There were no statistically significant differences between CH with CH-LVN-MLG, CTL, and CH-MLG with CH-LVN on day 30 + 3 of the shelf-life period (Fig. 5).

The interaction between storage time and edible coating treatments displayed statistically significant effects on the malic acid content of sweet cherry fruits in cold storage. The malic acid content recorded at the harvest was 1729.53 mg kg^{-1} fw. CH and CH-LVN-MLG treatments showed a decrease in malic acid content on day 10 and later increase throughout the cold storage. CH-LVN and CH-MLG treatments displayed no statistically significant differences throughout the storage. CTL treatment exhibited a decline in malic acid content on day 20 and later increase on day 30. At the end of cold storage, there were no statistically significant differences between edible coatings treatments and CTL (Fig. 4). The interaction between storage time and edible coatings treatments were statistically non-significant throughout the shelf-life period. At the end of the shelf-life period, there were no statistically significant differences between edible coatings treatments and CTL (Fig. 5).

The interaction between storage time and edible coating treatments showed statistically significant effects on the succinic acid content of sweet cherry fruit. The succinic acid at harvest was 181.28 mg kg^{-1} fw. CH, CH-LVN, CH-MLG, and CTL treatments showed fluctuation in succinic acid content throughout cold storage. CH-LVN-MLG treatment displayed an increase in succinic acid content for 20 days and later decline on day 30. At the end of cold storage, the highest succinic acid content (252.78 mg kg^{-1} fw) was in CH with the lowest succinic acid content (195.45 mg kg^{-1} fw) in CH-LVN-MLG. There were no statistically significant differences between CH-LVN-MLG, CH-LVN, CH-MLG, and CTL on day 30 of cold storage (Fig. 4). The interaction between storage time and edible coatings treatments was statistically non-significant during the shelf-life condition. At the end of the shelf-life period, there were no statistically significant differences between edible coating treatments and CTL (Fig. 5).

The major organic acid was malic acid which was followed by succinic, citric, and oxalic acid in the current study, which was also in line with the results of (Öztürk, Ağlar, Karakaya, Saracoğlu, & Sefa, 2019) who mentioned that the dominant organic acid was malic acid in sweet

cherry. Application of the edible film treatments maintained the oxalic acid in both cold storage and shelf-life conditions. The possible reason for these outcomes can be the effect of coating on the creation of a semi-permeable membrane which reduces the respiration rate and hence maintained the organic acids contents used as a substrate as reported by Yaman and Bayoındırlı (2002) in sweet cherry coated with Semperfresh.

3.7.19. Phenolic compound

The phenolic compounds enhance the antioxidant potential of fruits. In this study, the phenolic compound recorded was chlorogenic acid. The interaction between storage time and edible coating treatments was statistically significant on the phenolic compound of sweet cherries in both cold storage and shelf-life period. The chlorogenic acid content at harvest was 5.35 mg kg^{-1} fw. CH treatment showed an increase in chlorogenic acid content for 20 days and then a decline on day 30. CH-LVN treatment displayed fluctuation in chlorogenic acid content throughout cold storage. CH-MLG treatment exhibited an increase in chlorogenic acid content on day 10 and later declined throughout storage. CH-LVN-MLG treatment indicated a decrease in chlorogenic acid content for 20 days and later increased on day 30. CTL treatment manifested a decline in chlorogenic acid content on day 10 and later increased throughout the storage. At the end of cold storage, the highest chlorogenic acid content (6.54 mg kg^{-1} fw) was in CH-LVN-MLG with the lowest chlorogenic acid content (4.85 mg kg^{-1} fw) in CH-MLG. There were no statistically significant differences between CH-LVN-MLG, CTL, CH-LVN, and CH on day 30 of cold storage (Fig. 4). CH, CH-LVN, and CH-LVN-MLG treatments displayed no statistically significant differences with the advancement in shelf-life condition. CH-MLG treatment showed fluctuation in chlorogenic acid content with extension in the shelf-life period. CTL treatment indicated a decrease in chlorogenic acid content on day 10 + 3 and later manifested no statistically significant differences throughout the shelf-life period. At the end of the shelf-life period, there were no statistically significant differences between edible coatings treatments and CTL (Fig. 5).

The major phenolic compound was chlorogenic acid in sweet cherry as reported by Kelebek and Selli (2011) which agreed with our outcome as the phenolic compound recorded in our study was chlorogenic acid. Our results regarding the maintenance of chlorogenic acid content by application of edible coatings in cold storage agreed with the findings of Nair, Saxena, and Kaur (2018) who mentioned that CH and alginate-coated samples incorporated with olive oil extracts maintained the phenolic compounds in sweet cherry. However, it contradicted our findings in the shelf-life period where no significant effects between edible coatings treatment and CTL were observed on day 30 + 3 of shelf life.

3.7.20. Visual appearance of the fruits

The coated fruits including the control (CTL) were photographed at 10 days intervals until 30 days in both shelf life and cold storage conditions and the photos are provided in Fig. 6. As can be seen from the images, the control group displayed pitting and significant fungal deterioration.

Besides, fungal deterioration continued in CH-coated fruits. From the physical appearance, it is evident that CH-LVN, CH-MLG, and CH-LVN-

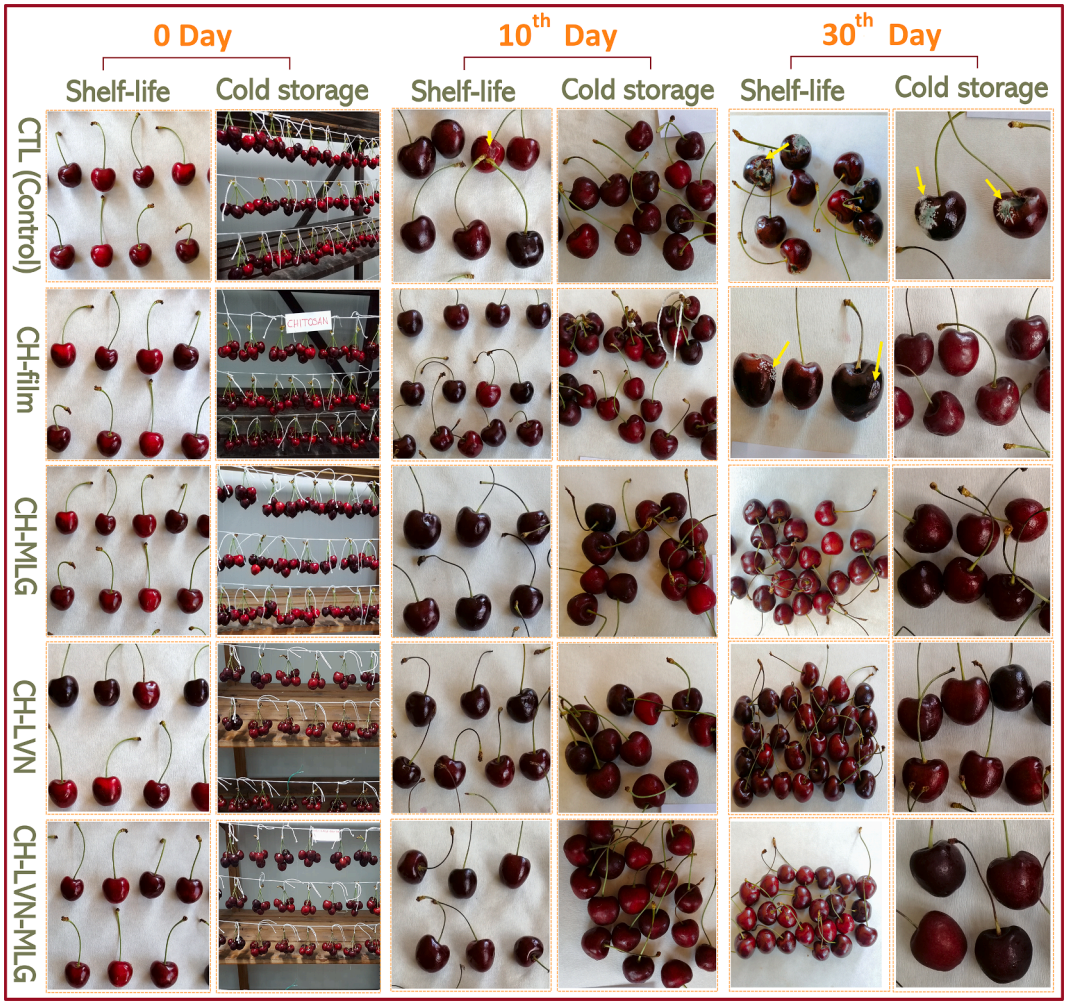

Fig. 6. The physical appearance of coated and uncoated sweet cherries at 0-day, 10th-day, and 30th-day under shelf life and cold storage conditions.

MLG coated fruit samples retained their physical properties up to the maximum acceptable level until the 30th day in both test environments.

4. Conclusions

The edible coating treatments preserved the postharvest quality of sweet cherries by minimizing weight loss and fungal deterioration and preserving stem removal force, total flavonoid content, L-ascorbic acid content, and oxalic acid content. Edible coating treatments showed non-significant effects on fruit firmness and malic acid content. The major organic acid and phenolic compounds recorded were malic acids and chlorogenic acid respectively. Among the edible coating treatments, CH application showed maximum total flavonoid content with CH-LVN-MLG exhibiting minimum total chlorophyll content of stem. CH and CH-LVN-MLG treatments displayed higher h° values. CTL treatment recorded an increase in weight loss, and fungal deterioration with a decrease in stem removal force, L-ascorbic acid content, and oxalic acid content. There have been numerous studies concentrating on small-scale materials development to extend the shelf life of various fruits, however many of these approaches have been relegated to the literature with no market application practicality. We demonstrated here that the new edible coating treatments can increase the shelf life of sweet cherries on a commercial scale.

CRediT authorship contribution statement

Muhammad Mujtaba: Conceptualization, Methodology, Writing – original draft, Visualization. **Qasid Ali:** Formal analysis, Investigation, Writing – original draft. **Bahar Akyuz Yilmaz:** Investigation, Writing – original draft. **Mehmet Seckin Kurubas:** Formal analysis, Investigation. **Hayri Ustun:** Investigation. **Mustafa Erkan:** Validation, Resources. **Murat Kaya:** Resources, Visualization, Writing – review & editing. **Mehmet Cicek:** Formal analysis. **Ebru Toksoy Oner:** Resources, Writing – review & editing.

Declaration of Competing Interest

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 paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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