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Effects of Different Edible Coatings on the Shelf Life of Fresh Black Mulberry Fruits (*Morus nigra* L.)

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Abstract: The aim of this study was to evaluate the effects of edible coatings based on gelatine, oils (sunflower and coconut) and beeswax on the physicochemical properties, bioactive compounds (total phenols and monomeric anthocyanin content), antioxidant capacity (DPPH and FRAP assays were used) and sensorial attributes of mulberry fruits during a period of storage (0, 2, 5 and 8 days) at 4 °C. The results showed that, in all samples treated with edible films, the degree of firmness was higher and the deterioration weaker compared to the control at day 8 of storage. Edible coatings significantly reduced the rate of deterioration, in terms of total phenolics and anthocyanins, in mulberry fruits over time, and the gelatine-coated mulberry samples (G_Mn) exhibited the best results. In terms of the antioxidant capacity of the coated mulberry, after 5 and 8 days of storage, only the G_Mn samples maintained significantly high DPPH radical scavenging and FRAP values compared to control. Coating improved the sensorial attributes of the mulberry during storage, and gelatine-coated fruits recorded the highest score, followed by layer-by-layer samples (O+W_G_Mn). All edible coatings used to cover black mulberry in this study extended the shelf life of the fruits, while maintaining high levels of bioactive compounds and, consequently, high antioxidant capacity, along with improved sensory qualities, during cold storage.

Keywords: mulberry; coconut oil; sunflower oil; gelatine; beeswax; total phenols; anthocyanins; antioxidant properties; sensorial attributes

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1. Introduction

The genus *Morus* is a genus of flowering plants of the *Moraceae* family. It comprises 10–16 species of trees generally known as mulberries, most commonly found in the wild and cultivated in many temperate regions of the world [1]. There are a total of 24 species of *Morus* and one subspecies, with at least 100 known varieties. Mulberry is found in temperate or subtropical regions and can grow in a wide range of climatic conditions, topographical settings and soils [2]. The most common Morus species in the world are *Morus alba* L., *Morus rubra* L. and *Morus nigra* L., which were domesticated and acclimatized in the second millennium of our era [1,2].

Due to its phytochemical composition, mulberry, especially black mulberry, has positive effects on health [3,4]. Black mulberry fruits are considered functional foods due to their high antioxidant contents, including anthocyanins, which are involved in the protection of cellular DNA [5,6]. They have tonic and depurative properties, do not contain saturated fats and help with weight loss. Ripe fruits have diuretic, laxative, depurative, alkalinizing,

sweat-inducing, tonic and refreshing properties, and unripe fruits have astringent and antidiarrheal properties [2,5,7,8].

During the growing and ripening periods of the fruit, a number of physical, biochemical and microbiological changes occur [9]. Ripe mulberries have a high moisture content, from 62.20 to 74.62%, and are highly perishable fruits [10]. For this reason, mulberry fruits are at risk of fungal attacks and undergo changes in aroma and appearance after harvest. Thus, an appropriate preservation method is required to maintain all the characteristics of mulberry fruit [11–13].

Today, there is a growing awareness among healthy food consumers about the negative effects of harsh conservation techniques on the nutritional and biological values of processed foods. Thus, consumers demand guaranteed products, as well as the preservation of nutritional and biological values and sensory characteristics in their entirety. Minimal food processing is a conservation technique that addresses these concerns, offering many advantages for both food technologists and consumers [11,14,15].

In order to prevent food degradation with a minimal preservation process, several techniques have been developed, such as packaging in a modified atmosphere [16], antimicrobial packaging [17] and the application of edible films and coatings [18]. The latter technique is particularly suitable for the preservation of fresh and minimally processed foods, as it provides a unique possibility for the incorporation of active biological substances into the film or coating, such as vitamins or natural antimicrobial agents [14,15]. The coatings serve several purposes in food systems, including improving the appearance of the exterior and reducing water loss [15,19]. Edible coatings are recommended to be made from natural compounds that are biodegradable and edible so that they can address both environmental concerns and consumer demands [18,20]. Another advantage of these coatings is the preservation of volatile flavours in the coated fruits [21,22]. Depending on the nature of the components, edible coatings can be classified into the following groups [23]: (i) polysaccharides (such as alginate, chitosan, cellulose derivates, starch); (ii) proteins (collagen, gelatine, whey proteins); and (iii) lipids (beeswax, glycerol esters). Edible protein-based coatings are good barriers against the transport of O_2 and CO_2, whilst lipid-based coatings, due to relative low polarity, block transport of moisture. Thus, a new perspective for the extension of the shelf life of perishable fruits is provided by minimum processing [14,15,20,21].

There are few studies in the literature on extending the shelf life of mulberry using the coating technique. The treatment of mulberry with chitosan-*g*-caffeic acid decreased the rotting rate compared to control samples, suggesting that components of the coating possessed antibacterial properties and could be used to extend the shelf life of postharvest mulberries during cold storage [24]. In another study, caffeic acid was used to improve the shelf life of white mulberry (*Morus alba* L.) stored for 21 days at 4 °C [25]. To the best of our knowledge, this is the first time that the effects of edible coatings, derived from easily accessible natural products and based on gelatine, oils and beeswax, have been investigated in terms of their capacities to extend the shelf life and maintain the nutritional qualities of mulberry. In this sense, the aim of this study was to treat mulberry fruits with different formulas based on gelatine, a combination of vegetable oils (sunflower and coconut) and beeswax in order to prolong the shelf life of the fruits during cool storage. Furthermore, layer-by-layer coating, combining the oils and beeswax with gelatine as the coating matrix, was performed on mulberry fruits. In addition, the outcomes for the total phenol content, anthocyanin content and antioxidant capacity were investigated in the fruits with edible coatings during the 8 days of postharvest cold storage.

2. Materials and Methods

2.1. Fruit Material

Fresh black mulberry (*Morus nigra*) fruits were hand-harvested at maturity stage from a field in Arad County, Romania (46°36′50″ N, 21°55′16″ E). Freshly picked fruits were placed in a refrigerated container and immediately transported to a laboratory. Mulberry

fruits with about 90% dark purple surfaces, uniform in size and shape and without defects or stains were selected for the edible coating treatments.

2.2. Preparation of Edible Coating Solution and Mulberry Fruit Treatments

The protocol for the preparation of the edible coatings was developed based on the study by Mladenoska 2012 with minor modifications [15]. Two types of edible coating were prepared. The first coating consisted of 10 g of gelatine dissolved in 100 mL of distilled water at 60 °C under magnetic stirring to obtain a homogenous mass (called G). The gelatine used was of animal origin (SC. GOOD PROD S.R.L., Oradea, Romania). The second coating was prepared by mixing 60 mL of virgin coconut oil (the Philippines, packaged by Adams Vision SRL, Târgu Mureș, Romania), 50 mL of cold-pressed sunflower seed oil (SanoVita company, Valcea, Romania) and 25 g of beeswax under intense stirring at 40 °C to obtain a homogeneous mass (called O+W). The oils were sourced from a local natural pharmacy, and beeswax was freshly harvested from a private producer. The edible coatings were freshly prepared and allowed to cool to room temperature before treating the mulberries.

Four different treatments were used in this study. One set of mulberry fruits were immersed in distilled water for 10 s and represented the control (C_Mn). Other fruits were dipped into the gelatine-based edible coating for 10 s (G_Mn), and another set were immersed in the oil and wax-based edible coating for 10 s (O+W_Mn). The last treatment, O+W_G_Mn, consisted of immersing the fruits with the two films described above layer by layer. The mulberry fruits were first coated with the O+W coating for 10 s and the fruits were air dried at room temperature for 30 min, then a coating of gelatine was applied. After the treatments, all fruits were air dried at room temperature until there were no droplets on the surfaces of the mulberry fruits. All treated samples were stored at 4 °C. A total of 36 batches of 50 g (three batches per treatment) were produced, and they were maintained at 4 °C and subsequently removed after 2, 5 and 8 days of cold storage for analysis. Three biological replicates per treatment were prepared and all analyses were performed in triplicate. The experimental design of the study along with the sample identification acronyms are shown in Figure 1, and Figure S1 presents pictures of the edible coatings, their preparation and the mulberry-covered samples.

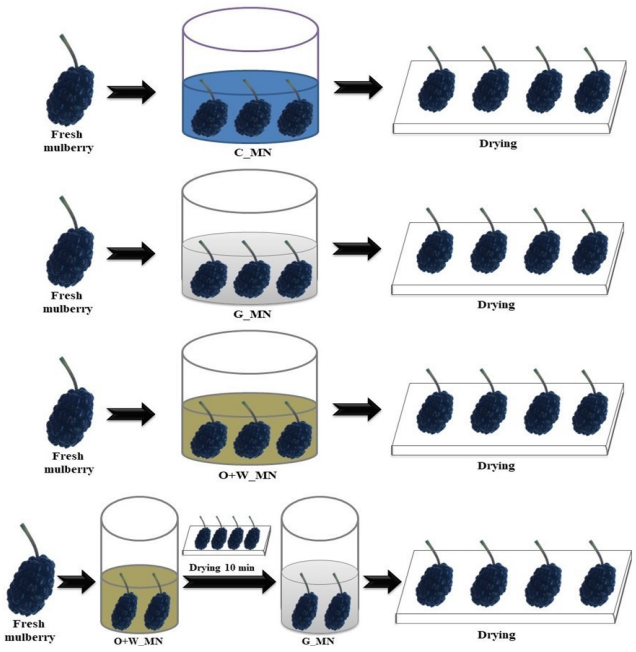


Figure 1. The experimental design for the mulberry fruits with edible coating. C_Mn—control sample, fruits immersed in distilled water; G_Mn—sample with fruits coated with gelatine; O+W_Mn—sample treated with oil and wax-based edible coating; O+W_G_Mn sample subjected to layer-by-layer treatment, the first layer being the oils with wax and the last the gelatine.

2.3. Structural Characterisation of Edible Coatings

2.3.1. Moisture Content

The moisture content of the films was determined by measuring the weight loss in films after drying them in an oven at 105 °C (Nitech Pol Eko oven, model CLN 53, Wodzisław Śląski, Poland) until a constant weight was achieved. Three replications of each film were used for the determination of the moisture content, which was calculated using Equation (1) [21]:

$$\%\text{Moisture} = \frac{Wi - Wf}{Wi} \times 100 \quad (1)$$

where *Wi* is the weight of films before drying and *Wf* is the weight of films after drying.

2.3.2. Swelling Index

The swelling index (%) was determined in terms of weight (Equation (3)) following Miteluț et al., 2021 [21]. Briefly, the coatings were pre-treated at 45 °C for 24 h and then immersed in distilled water for 24 h. Before weighing, the excess moisture was removed using sheets of filter paper. The equation used to calculate the weight was as follows:

$$\text{DS} = \frac{Ww - Wd}{wd} \times 100 \quad (2)$$

where *Ww* and *Wd* denote the weight of the swelling and the dry film, respectively.

2.3.3. FTIR Spectroscopy of Edible Films

Spectrophotometric measurements were perform using a Shimadzu FT 8400 S (Shimadzu Co., Kyoto, Japan) FTIR spectrophotometer operating in the range from 400 to 4000 cm^{-1}, following Roiu et al., 2020 [26]. Small parts of the edible coatings were placed on the surface of pure Si windows (Nicodom S.R.O., Praha, Czech Republic), and the spectral acquisition conditions were: wavelength resolution of 2.00 cm^{-1}, Happ-Genzel apodization and 3 scans/spectrum. Spectral deconvolution and second-derivative techniques was applied using Origin 8 software in order to mathematically enhance the resolution of the FTIR spectrum, taking advantage of band separation and a curve-fitting procedure [26].

2.3.4. UV-Vis Spectroscopy of Edible Coating Components and Mulberry Treated-Samples

The UV−visible spectra of gelatine, coconut oil and beeswax, dissolved in the proper solvents, were recorded using a Shimadzu PharmaSpec UV-1700 (Shimadzu Corporation, Kyoto 604-8511, Japan) spectrophotometer in the wavelength range from 200 to 300 nm. The wavelength spectrum from 300 to 600 nm was used for sunflower oil. In addition, the UV-Vis spectra of the mulberry samples treated with various edible coatings were recorded on the 8th day after the start of treatment using a Shimadzu PharmaSpec UV-1700 spectrophotometer (Shimadzu Corporation, Kyoto 604-8511, Japan) in the wavelength range from 200 to 700 nm to highlight the presence of anthocyanins and phenolic acids.

2.4. Determination of Deterioration Degree (DD) and Firmness of Mulberry Fruits

The deterioration degree refers to the loss of moisture in the samples and was calculated according to Equation (3). The weight of each fruit sample was determined before and after storage as the percentage weight loss compared to the initial weight [15].

$$DD\ (\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100 \quad (3)$$

Firmness was measured on two opposite sides of 10 fruits per treatment using a portable penetrometer (Fruit penetrometers tester, FT 327) with a 5 mm diameter probe, and the average value was expressed in Newtons (N). The firmness of the mulberries was measured on the first and last days of treatment, T0 and T8, respectively.

2.5. Extraction and Measurement of Total Phenol Content (TPh), Monomeric Anthocyanin Pigments (MAPs) and Antioxidant Capacity

The mulberry fruits (5 g) were subjected to extraction using acidified methanol (trifluoracetic acid (TFA), 0.1%) in the ratio 1:10 (g/v) in a Heidolph, Silent Crusher M homogenizer (D-91126 Schwabach, Germany) at a speed of 12,000 rpm for one minute. Then, the homogenized samples were subsequently centrifuged (NÜVE NF 200 Ankara, Turkey) at 5000 rpm for 20 min. The pellet was re-extracted until the solvent became colourless. The resulting supernatants were mixed and subsequently used for the following analysis [27].

The TPh in the mulberry fruits was determined using the Folin–Ciocalteu method with some modifications [28,29]. Briefly, the mulberry methanolic extract (100 µL) was incubated with 1700 µL of distilled water, 200 µL of Folin–Ciocalteu reagent (freshly prepared, dilution 1:10, *v/v*) and 1000 µL of 7.5% Na_2CO_3 solution for 2 h in the dark at room temperature. The absorbance was measured at 765 nm (Shimadzu miniUV-Vis spectrophotometer) and the results were expressed as mg of gallic acid equivalent (GAE) per 100 g fresh weight (fw), using gallic acid as a standard.

The total MAP content in the mulberry fruits was quantified according to the pH-differential method [30]. The anthocyanin pigments were estimated from their difference in absorbance at 523 nm (λmax) and at 700 nm in 0.025 M KCl phosphate buffer at pH 1.0 and 0.4 M acetate buffer at pH 4.5 using Equation (4):

$$A = (A\ \lambda vis\text{-}max - A700)pH\ 1.0 - (A\ \lambda vis\text{-}max - A700)pH\ 4.5 \quad (4)$$

The results were expressed as mg cyanidin-3-glucoside equivalent/100 g fw based on Equation (5) [31]:

$$MAP\ (mg/100\ g) = (A \times MW \times DF \times 1000)/\varepsilon) \times 0.1 \quad (5)$$

where MW is the molecular weight of cyanidin 3-glucoside (MW = 449.2), DF is the dilution factor, 1000 is the conversion factor for grams to mg, ε is the molar absorptivity (ε = 26,900 L M^{-1} cm^{-1}) and 0.1 is the conversion factor from 1000 g to 100 g.

The antioxidant capacity of the mulberry samples was determined using two spectofophotometric assays, the ferric-reducing antioxidant power (FRAP) and the 2.2, diphenylpicril-hydrazil (DPPH) assays.

The FRAP assay tests the antioxidant power of samples and is based on an extract's ability to reduce the Fe^{3+} from the tripyridyltriazine $Fe(TPTZ)^{3+}$ complex to the blue-coloured complex $Fe(TPTZ)^{2+}$ in an acidic medium [32,33], with maximum absorption at 595 nm. Briefly, mulberry extract (100 µL) was allowed to react with 500 µL of FRAP working solution (freshly prepared by mixing 300 mM acetate buffer, pH 3.6; 20 mM $FeCl_3 \bullet 6H_2O$ solution; and 10 mM TPTZ solution in the ratio 10:1:1 (v/v/v)) and 2 mL distilled water for 1 h in the dark. The results were expressed in mmol Trolox equivalent (TE)/100 g fw.

The radical scavenging capacity of the mulberry extract was determined using the stable 2-picryl-hydrazyl-hydrate (DPPH) radical according to the method described by Brand Williams [34]. Briefly, 100 µL mulberry extract was allowed to react with 2800 µL of 80 µM DPPH methanol solution for exactly 30 min in the dark. The reduction of DPPH was monitored spectrophotometrically at 517 nm and the antioxidant capacity was expressed as radical scavenging capacity (RSC%) using Equation (6) [35].

$$RSC\ (\%) = \frac{(A_0 - A_s)}{A_0} \times 100 \quad (6)$$

where A_0 is the absorbance of the DPPH free radical solution in methanol and A_S represents the absorbance of the fruit sample.

2.6. Sensory Evaluations of Mulberry Fruits

Fresh fruits treated with edible coatings were subjected to sensory evaluation on days 0, 5 and 8 of the validity study. The sensory characteristics (appearance, aroma, taste, texture and general desirability) were evaluated by a well-trained team of six men and nine women aged 29 to 43, who were members of the Department of Food Engineering at the University of Oradea, Romania. Each characteristic was scored between 1 (unacceptable) and 10 (excellent). During the evaluation, the referees rested for 5 min between tests to minimize fatigue and transfer effects. Ballot papers were used to collect data [36–38].

2.7. Statistical Analysis

The results represent the values for the means ± the standard deviation (n = 3) of the samples and all analyses were performed in triplicate. Data were statistically processed in GraphPad Prism 3.03 software (GraphPad Software, Inc., La Jolla, CA, USA) using one-way ANOVA followed by Tukey's multiple comparison test, and statistically significant differences ($p < 0.05$, $p < 0.01$ and $p < 0.001$) were determined compared to uncoated mulberry fruits (control, C_MN). Following the analysis of DD%, firmness, TPh content, MAPs and antioxidant capacity (FRAP and DPPH), principal component analysis (PCA) was performed to evaluate the effectiveness of the coatings on the mulberries during cold storage after different periods of time (0, 2, 5 and 8 days).

3. Results and Discussion

Bioactive compounds in fruits are very susceptible to degradation, mainly due to abiotic or biotic factors. Thus, the nutritional quality of the fruit is reduced. In this context, several postharvest treatments have been developed to preserve the quality of the fruit, one of them being edible coatings [23].

3.1. The Physicochemical Characterisation of Edible Coatings

The moisture content of the gelatine and oils and wax coatings was measured in terms of weight loss (Table 1). In terms of its physicochemical properties, solubility, colour, transparency, odour and taste are the main properties that define the quality of gelatine. The water-swelling capacity of the gelatine film determined as the water-uptake capacity is shown in Table 1.

Table 1. Moisture content (%) and swelling index (%) of the gelatine coating and the combination coating using oils and beeswax.

Edible Coating Type	Moisture Content (%)	Swelling Index (%)
Gelatine [1]	91.17 ± 1.3 ***	10.69 ± 0.95
Oils and wax [2]	3.04 ± 0.05	-

[1] Gelatin-based edible coating with 10% concentration; [2] Oil and wax edible coating—coconut, sunflower oil and beeswax in the combination described in the Materials and Methods section. For the statistical significance, *** $p < 0.001$.

The UV spectrum of gelatine showed two absorptions at 249 nm and 284 nm due to its peptide bond and aromatic groups and side chains, respectively [39]. The absorption spectra of sunflower oil were obtained in the visible range from 300 to 600 nm. The sunflower oil had peaks from carotenoids at 391, 432, 457 and 485 nm, which are the pigments responsible for the oil's colour. The carotenoids detected in the sunflower oil were lutein and zeaxantin [40]. Crude sunflower seed oil, is a valuable product because it contains higher concentrations of linoleic acid, an essential fatty acid, and is an source of excellent vitamin E. Coconut oil has a high medium-chain fatty acid (caproic (C6), caprylic (C8), capric (C10) and lauric (C12) acids) content that showed weak absorption near 206 nm [41]. Similar results were obtained in [42]. Here, coconut oil from cosmetic cream had maximum UV absorption at the wavelength of 205 nm. The UV spectrum of the beeswax showed absorption at 274 nm, corresponding to the presence of conjugated

dienes and trienes. The results are in accordance with [43], where wax from sugarcane peel showed a similar pattern.

FTIR spectroscopy is a modern tool for the qualitative and quantitative analysis of oils or other measurements and aims to verify authenticity [44,45].

The main vibrational regions of gelatine are: 680–1240 cm^{-1}, corresponding to amide III; 1360–1550, amide II; 1650, amide I; and 3100–3600 cm^{-1}, amide A. Amide A arises from N-H stretching coupled with hydrogen bonding and free O-H. The most important FTIR band is amide I, due to the C=O stretching vibration, reflecting the protein secondary structure. Amide II arises from N-H bending and C-N stretching vibrations. Amide III is a more complex vibrational mode, representing the in-plane vibration of the C-N and N-H groups of amide (Figure 2a).

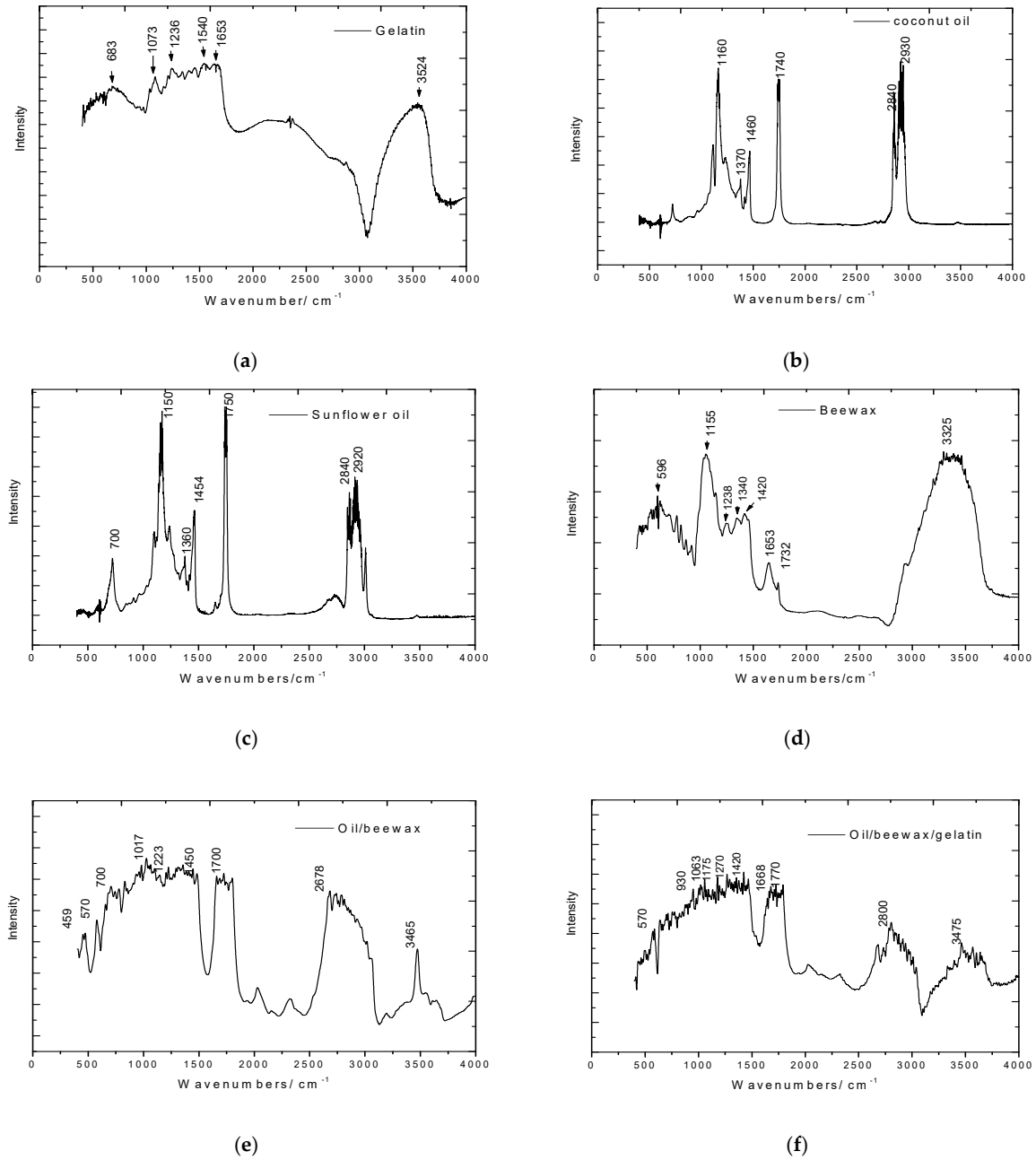


Figure 2. FTIR spectra of the edible coating components and their combinations. Spectra of (**a**) gelatine, (**b**) coconut oil, (**c**) sunflower oil, (**d**) beeswax, (**e**) combination of coconut and sunflower oil with beeswax (O+W), (**f**) combination of coconut oil, sunflower oil, beeswax and gelatine (O+W_G).

Similar results to ours were obtained in other studies at different spectral intervals using the FTIR method [44,46–50]. Cebi et al., 2019 applied the ATR-FTIR spectroscopic method to classify gelatine candies according to the gelatine source. In this case, the spectral characteristics were obtained from the FTIR spectra that resulted from the presence of gelatine, this being one of the major components of gum candies, and the most significant band related to gelatine was observed in the spectral range from 1700 to 1600 cm^{-1}. This information was also confirmed by Cebi in another study conducted in 2016 [46,47].

The marker bands in the FTIR spectrum of coconut oil (Figure 2b) are: 1160 cm^{-1}, due to the stretching vibration of C-O in the group O=C-OR-; 1370 cm^{-1}, due to the bending vibration of C-O in CH_2 groups; 1460 cm^{-1}, due to the stretching vibration of C-H in CH_2 and CH_3 groups; and 1740 cm^{-1}, assigned to the stretching vibration of C=O bonds. The absorption bands in the higher region of the wavenumbers are 2840 cm^{-1} and 2930 cm^{-1} (symmetric and asymmetric vibrations of C-H in CH_2 and CH_3 groups). Regarding the spectral range between 1100 and 3000, similar results were obtained by Neves et al., 2020 in a study on the authentication and identification of adulterants in virgin coconut oil, where it was observed that the peaks are mainly related to the stretching and bending of C–H, C–C, C–O and C=O bonds [51]. We also found a similarity between the peaks of coconut oil detected here and those from the study by Srivastava et al., 2017 [44].

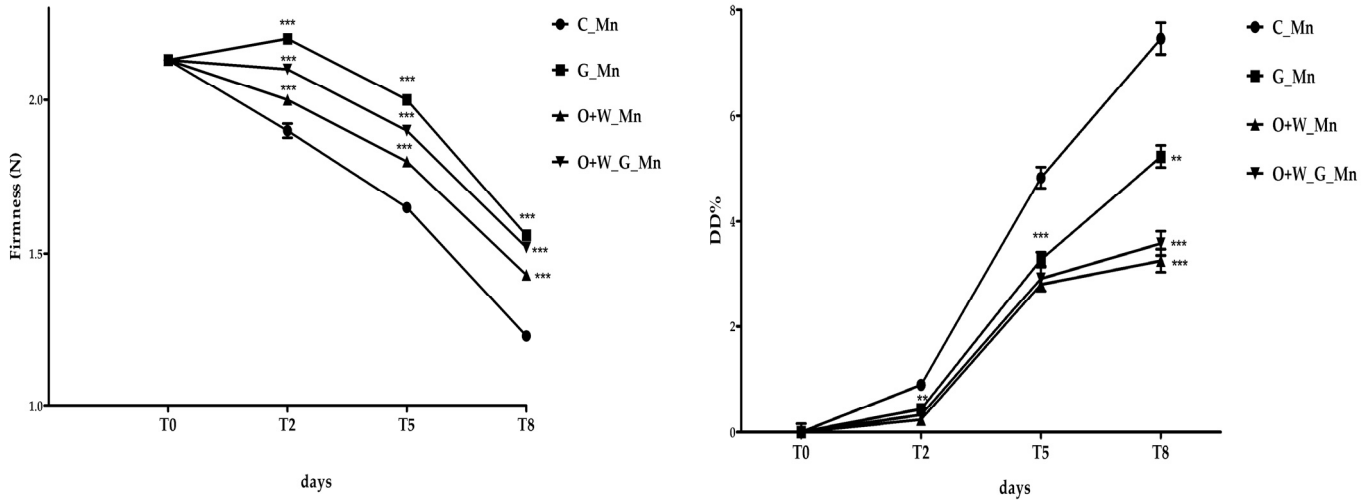
The FTIR fingerprints of sunflower oil (Figure 2c) are: 700 cm^{-1}, corresponding to the rocking vibration of CH_2 groups; 1150 cm^{-1}, due to the stretching vibration of C-O; 1360 cm^{-1}, due to CH_3 symmetric vibrations; 1454 cm^{-1}, due to CH_2 scissoring vibrations; and 1750 cm^{-1} (the major band), arising from C=O stretching vibrations of aldehydes, ketones and carboxylic acids. In the high region of the wavenumbers, the peaks at 2840 and 2920 cm^{-1} are due to symmetrical and asymmetrical CH_2 vibrations, respectively. The FTIR fingerprints of the beeswax samples were observed as a strong band at around 1155 cm^{-1}, corresponding to C-O asymmetric stretching of esters, and at 1653 and 1732 cm^{-1}, corresponding to C=O stretching in esters and fatty acids, while a large and strong band around 3320 cm^{-1} can be attributed to O-H stretching vibrations in aliphatic group (symmetric and asymmetric). The hydrocarbon –CH_2 in-plane vibration can be observed at around 1420 cm^{-1} (Figure 2d). Our results are consistent with other studies, both for sunflower oil [52,53] and beeswax [50,54].

When the coatings containing wax and oils (respectively, wax, gelatine and oils) were formed, the marker bands were superposed in some spectral regions and, for this reason, they were difficult to identify. However, when gelatine was present in the film composition, the spectral region from 1660 to 1780 cm^{-1} became larger and more structured due to amide I vibration concomitantly with modifications in the vibrational features of the band at 3475 cm^{-1} (Figure 2e,f).

3.2. Effects of Edible Coating Treatments on Deterioration Degree (DD) and Firmness of Mulberry Fruits

The incorporation of vegetable oils and waxes into protein matrices to form edible composite films and coatings results in improvements to the film moisture barrier [55].

Firmness is an important feature of fresh berry fruits because softening is a major factor in quality deterioration. Figure 3a shows that, after eight days of cold storage, all mulberries became softer. In the case of the C_Mn (control sample), the firmness decreased by 26.83, 23.58 and 16.26% compared to G_Mn, O+W_G_Mn and O+W_Mn, respectively. The change in the firmness of the fruit tissues was a consequence of the biodegradation of pectic substances (Burzo et al., 1999), substances that in mulberry fruits are not found in overly large quantities [56]. For example, due to the lower pectin content compared to other fruits used for processing, Baston et al., 2021 implemented various processes to obtain jams from black, white or red mulberry fruit by using gelling with the addition of pectin [57].



(**a**) (**b**)

Figure 3. The firmness (N) (**a**) and deterioration degree (%) (**b**) of mulberry fruits treated with edible coatings over 8 days at cold storage. All values are expressed as the means ± SD (n = 3). The statistical significance (** $p < 0.01$; *** $p < 0.001$) was determined compared with the control group at the corresponding times.

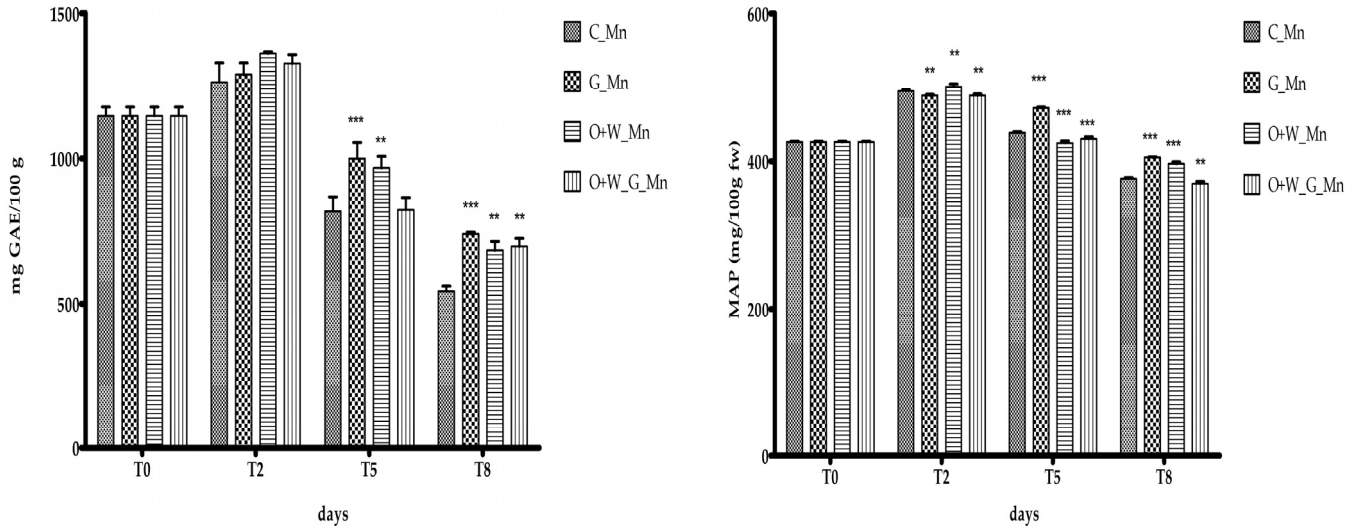
In our study, at 8 days of cold storage (4 °C), the firmness of the coated mulberries was significantly higher than the control ($p < 0.001$). No significant difference was found between the G_Mn treatment and other treatments (O+W_Mn and O+W_G_Mn) during postharvest storage ($p > 0.05$). The results suggested that the coatings had a positive effect on maintaining the firmness of the mulberries.

Weight losses in fresh fruit and vegetables, which can be expressed as the deterioration degree (%), are primarily due to the loss of water caused by transpiration and respiration processes [24]. Figure 3b shows the weight losses in mulberry fruits without (C_Mn) and with coatings. After 8 days of cold storage, the deterioration degree in the treated mulberry fruits (G_Mn, O+W_Mn and O+W_G_Mn) reached 5.23, 3.25 and 3.58%, respectively. A significantly lower weight loss was recorded in the case of the control (C_Mn) compared to the mulberry fruits with edible coatings. Edible coatings form semi-transparent layers on the surfaces of mulberry fruits and function as protective barriers that reduce the respiration rate and the transpiration through the fruit surfaces [24,58,59]. The mulberry fruit with the gelatine coating recorded the lowest weight loss compared to the control, followed by the layer-by-layer and edible oil coatings. Our results are consistent with other studies that have shown that gelatine is the best option to avoid weight loss in fruits after harvest and thus extend their shelf life [58,60].

3.3. Effects of Edible Coating Treatments of Mulberry Fruits on Bioactive Compounds and Antioxidant Capacity

In our study, edible coatings based on gelatine, coconut and sunflower oils and beeswax, as well as combinations of these, were used to preserve the mulberry fruits. Figure 4a,b show the effects of edible coatings on the TPh and MAP content in the mulberries during the storage at 4 °C for 2, 5 and 8 days (T2, T5 and T8). The total phenol content tended to decrease during storage (Figure 4a) in both the control (uncoated) and coated mulberry fruits, which was due to the degradation of anthocyanins and breakdown of the cellular structure during the senescence period [58,61]. The TPh content in mulberry fruits varied both with different coatings and across storage days. A slight increase in phenols after 2 days of storage was observed in all samples, followed by differing trends between samples at 5 and 8 days of cold storage. In the G_Mn and O+W_Mn samples, TPh exhibited a significant increase compared to the control with the same storage time. After 8 days

of cold storage (T8), the TPh content was significantly lower compared to the first day of treatment (T0), but all treated samples showed significantly higher amounts of polyphenols compared to the control kept under the same conditions (Figure 4a). A similar trend was obtained in the case of anthocyanins: after 8 days of storage, the amount of MAP in all samples was significantly higher compared to the control (Figure 4b).



(**a**) (**b**)

Figure 4. The effects of edible coatings on total phenol content (mg GAE/100 g fw) (**a**) and monomeric anthocyanin pigments (mg/L) (**b**) in mulberry fruits over 8 days of cold storage. All values are expressed as the means ± SD (n = 3). The statistical significance (** $p < 0.01$; *** $p < 0.001$) was determined compared to the control group at the corresponding time.

Edible coatings function as a barrier inhibiting the oxygen and moisture supply for the enzymatic oxidation of phenolic compounds [62].

Among the three treatments, the gelatine coating treatment (G_Mn) maintained significantly higher levels of total phenol content and anthocyanins in mulberry after 8 days of storage compared to control. Our results are in accordance with the study by Aitboulahsen et al., 2018, who used gelatine alone or gelatine with mentha essential oil as coatings, the latter being the most effective treatment in reducing losses in total phenol content after 10 days' storage of strawberry [58].

There are few studies on how to extend the shelf life of mulberries by applying edible coatings. In one study, edible coatings utilizing two aqueous extracts of medicinal plants (*Parkia biglobosa* and *Lonicera japonica*) were applied to mulberry fruit but, as protective materials, the effects they provided were minimal for the postharvest treatment of mulberry fruit [63]. Kahramanoğlu et al., 2020 investigated the effects of different bio-materials on the postharvest quality of *M. nigra* fruits [64]. The bio-materials consisted of black seed oil (*Nigella sativa* L.), eggshell extract and Mediterranean wild thyme oil (*Thymus capitatus* L.). The mulberry fruits' quality parameters, such as weight loss, soluble solids concentration and fruit rotting rate, were measured for 15 days at intervals of 3 days. The results [64] showed that all the bio-materials investigated had a significant influence on the prolongation of the shelf life of the mulberry fruits, with the *Nigella sativa oil* and eggshell extract treatments being noted in particular.

After the mulberries covered with various edible films had been stored for 8 days, the UV-Vis absorption spectra were recorded between 200 and 800 nm using a UV-Vis spectrophotometer (Figure 5). The anthocyanin pigments in the mulberries showed two absorption peaks, one at λ_{max} = 280 nm in the UV region and another at λmax = 523 nm in the visible region. Additionally, a peak was recorded at λmax = 317 nm. Saha et al., 2019

suggested that the presence of the peak range between 310 and 340 nm indicates that the sugar fragment is acylated. The ratio of the acylation maximum to the visible maximum was 0.61 in our mulberry samples, suggesting the presence of one acylated group [65]. Qin et al., 2009 identified cyanidin 3-O-rutinoside as the main mulberry pigment, followed by cyanidin 3-O-glucoside. Pelargonidine 3-O-glucoside and pelargonidine 3-O-rutinoside have also been identified as minor pigments in mulberry [66].

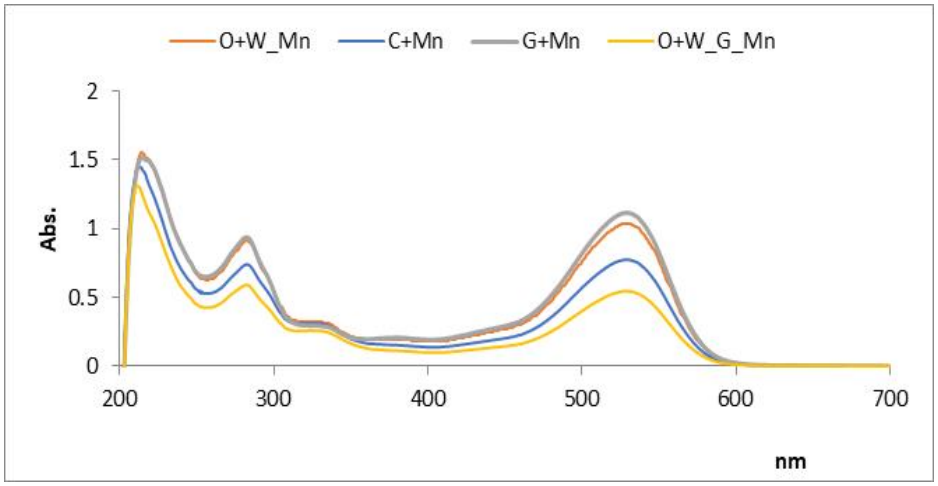
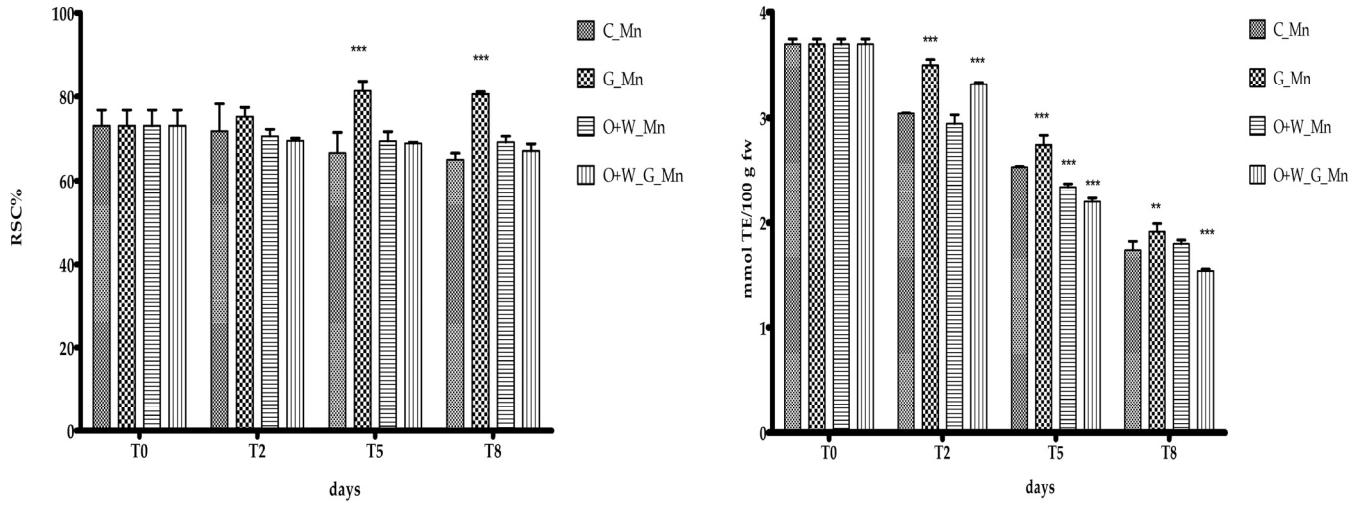


Figure 5. The UV-Vis absorption spectra for anthocyanins in mulberry extracts with different coating treatments after 8 days of storage.

The FRAP and DPPH methods are the most commonly used to determine the antioxidant capacity of fruits [67–69]. The antioxidant capacities of mulberry fruits treated with different coatings and stored for 8 days at 4 °C are represented in Figure 6a,b.



(**a**) (**b**)

Figure 6. The effects of edible coatings on the antioxidant capacities of mulberry fruits after 8 days of cold storage. (**a**) RSC% as determined by DPPH assay; (**b**) FRAP assay. All values are expressed as the means ± SD (n = 3). The statistical significance (** $p < 0.01$; *** $p < 0.001$) was determined compared to the control group at the corresponding time.

The mulberry fruits treated with edible coatings exhibited similar changes in DPPH radical scavenging activities during storage, except for the samples coated with gelatine (G_Mn). After 5 and 8 days of storage, G_Mn samples recorded significantly higher DPPH

radical scavenging capacities ($p < 0.001$) compared to the other treatments. Using the FRAP method, which measures the ferric-reducing antioxidant power, it was found that, after 2 days of storage, the G_Mn and O_W+G samples recorded significantly higher values compared to the control. In contrast, after 5 and 8 days of storage, only the gelatine-treated mulberry fruits maintained significantly higher FRAP values compared to control.

In this study, we evaluated edible coatings based on gelatine, oils and wax applied in a thin layer on the surface of mulberry fruits in order to maintain their freshness. The coatings prevent the exchange of gases with the air, which is essential to maintain the quality of the product [70].

3.4. Principal Component Analysis (PCA)

Principal component analysis is a widely used statistical method employed to discover the relationships between variables. A PCA plot was applied for the following variables: DD, firmness, TPh, MAP, DPPH and FRAP. The uncoated mulberry (C_Mn) showed different behaviour with respect to storage time compared to the coated samples. Differences between the uncoated and coated fruits with respect to the cold storage period and the different coatings used are shown in a 3D plot (Figure 7). The eigenvalues of the covariance matrix showed that the set of the three principal components (PC1, PC2 and PC3) accounted for 96.16% of the total variance with respect to cold storage in the dataset. PC1 explained 72.326% of the variance, PC 2 explained an additional 14.624% and PC3 explained 9.210%. Different behaviours in the samples were observed at different times during cold storage (0, 2, 5 and 8 days).

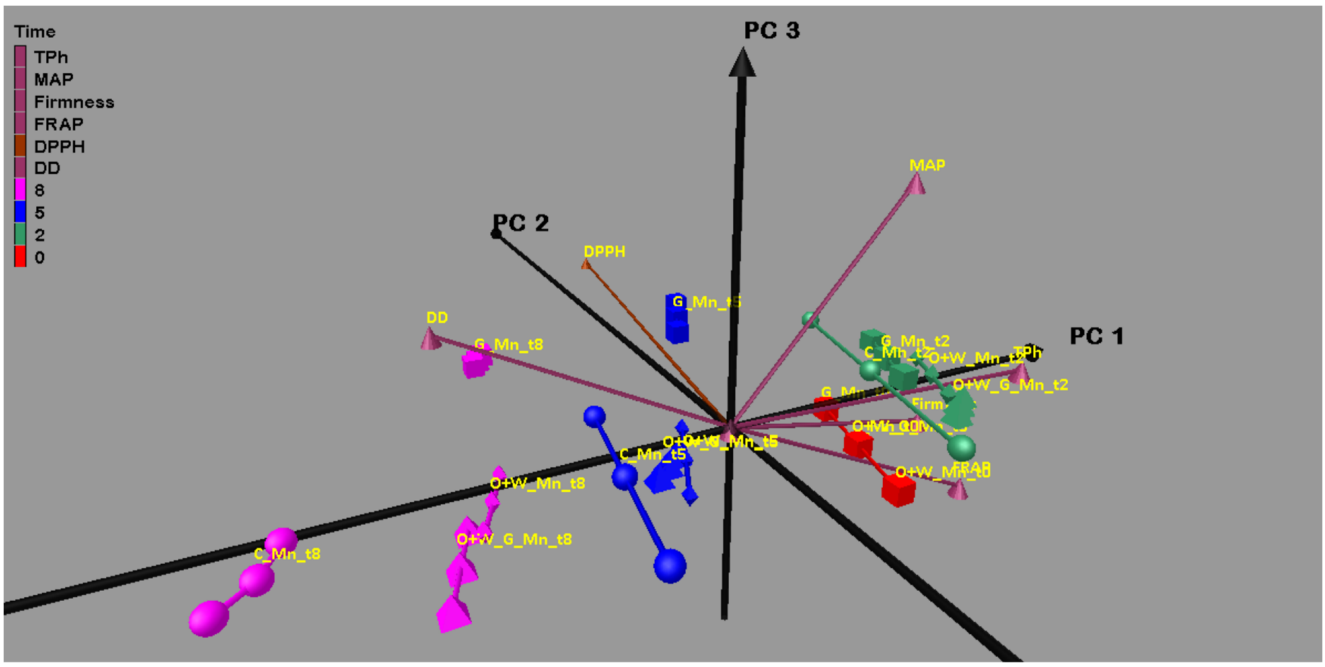


Figure 7. Three-dimensional principal component analysis of the variables (DD, firmness, TPh, MAP, FRAP and DPPH) during cold storage on the first day (t0, red colour) and after two days (t2, green colour), five days (t5, blue colour) and eight days (t8, pink colour) for uncoated (C_Mn) and coated mulberry samples (G_Mn, O+W_Mn, O+W_G_Mn).

The gelatine-coated fruit exhibited a higher rate of deterioration after 8 days of storage but other variables showed a higher shift in different PCs, suggesting that the TPh, MAP, DPPH, FRAP and firmness were maintained at high levels compared to the control sample, thus demonstrating that, for the mulberry treatment, this was the best preservative coating.

3.5. Sensory Characterization of Mulberry Coating Samples

The importance of sensory analysis for the development of new products is increasing, especially in terms of smell and taste, because, in addition to the nutritional value and the beneficial effects that accumulate in the body through the consumption of certain foods, products attract consumers primarily due to their appearance and taste. Sensory analysis is also important in terms of the acceptance or rejection of the food product [38,62]. The results of the sensory analysis of the mulberry fruits treated with different edible coatings are presented in Figure 8. The method for the sensory evaluation of the coated mulberry samples and the control (the mulberry without edible coatings) took into account the visual attributes (colour), three mouth-feel attributes (texture, taste and flavour) and the harmony of the overall characteristics. Fruits' colour is important in its acceptance or rejection by consumers [62]. The gelatine-coated mulberry showed higher scores for all sensory attributes compared to the other treatments and the control.

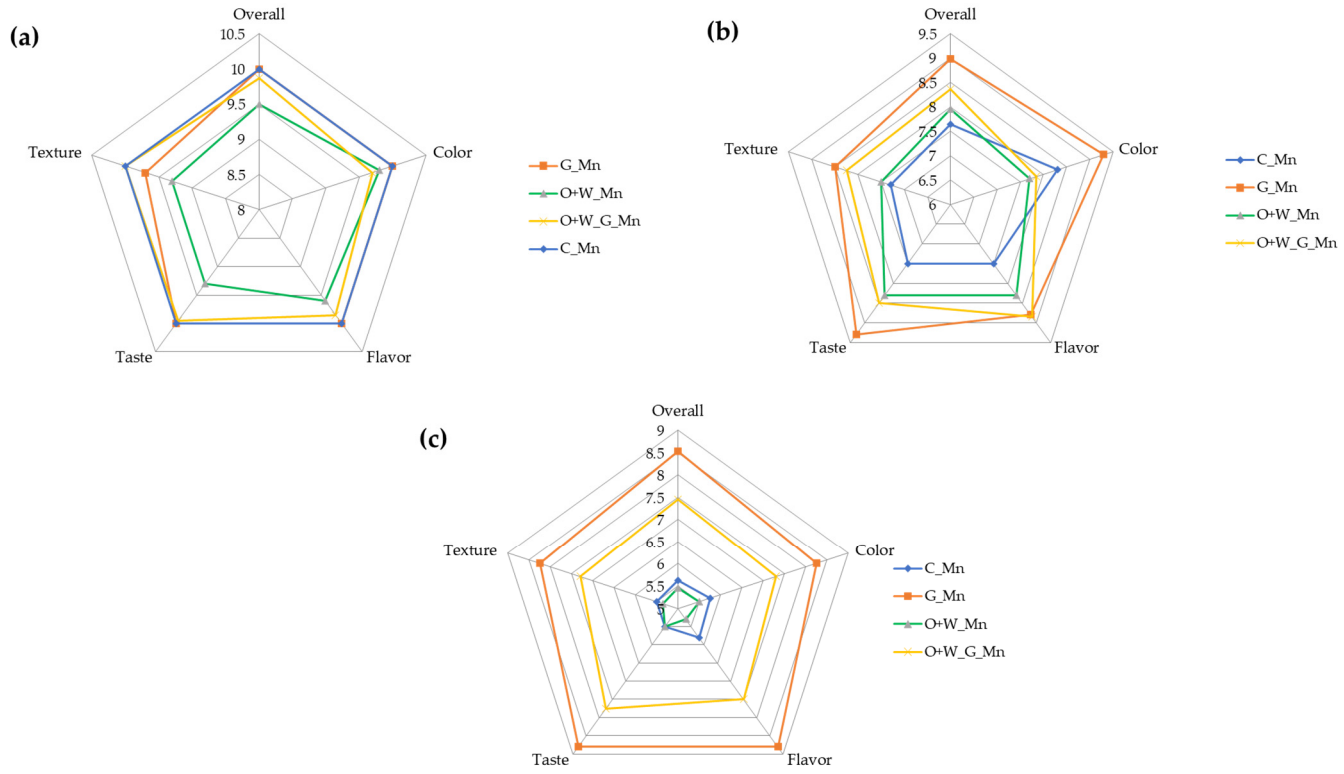


Figure 8. The effects of different treatments on the sensory evaluation of mulberry on T0 (**a**), T5 (**b**) and T8 (**c**) of storage at refrigeration temperature (4 °C).

The most appreciated were the fruits covered with gelatine, their taste being compared to that of jellies but with much fresher attributes. The mulberry fruits treated with edible coatings had much better results compared to the untreated fruits (C_Mn), providing a better texture and outer gloss in addition to the other evaluated attributes. The appearances of untreated mulberry fruits and those treated with edible coatings at different points in the period of storage at 4 °C are shown in Figure 9.

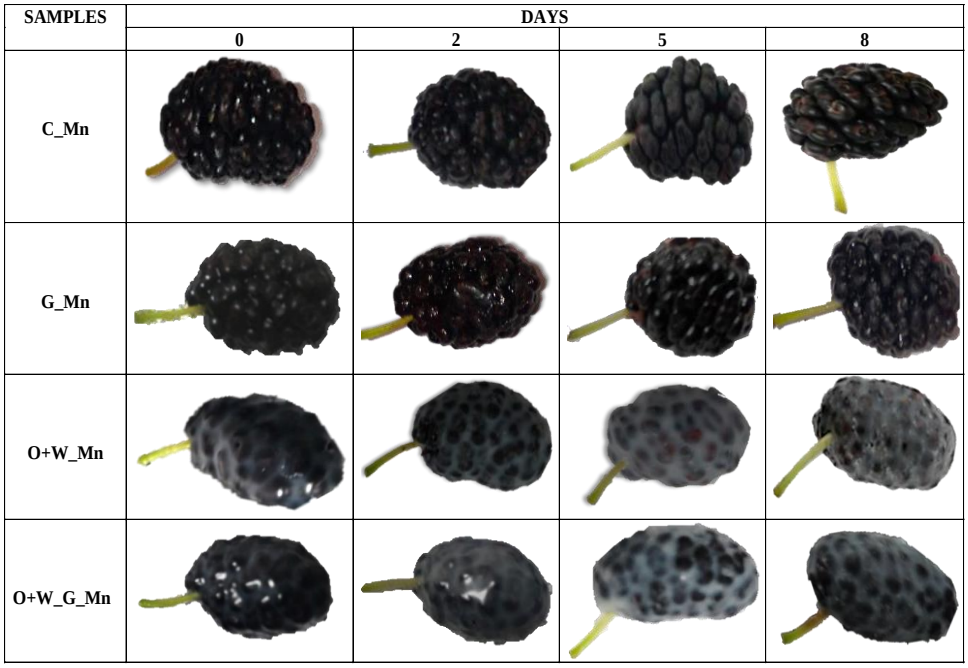


Figure 9. Appearances of the treated (G_Mn; O+W_Mn; O+W_G_Mn) and untreated (C_Mn) mulberry fruits throughout the storage period (8 days) at 4 °C.

There is currently interest in developing edible coatings or films, known as intelligent and active packaging, for use during the storage of perishable fruits or vegetables in order to extend their shelf life and maintain the nutritional and sensory qualities at a high level [71]. Thus, Jancikova et al., 2021 developed edible films based on carrageenan and chitosan in which they included various extracts (red cabbage, sweet potato and blue tea) in order to improve the shelf life of fresh-cut apple pieces [71].

Edible coatings were found to extend shelf life by reducing weight loss in mango, apple, peach, blueberry and strawberry fruits, decreasing breathing and oxidative reaction rates and reducing or even avoiding the occurrence of physiological disorders [20,22,72].

When Vargas et al., 2006 [73] applied edible protein coatings, such as gelatine and beeswax, to fruits, they observed good preservation of colour and mechanical properties, while Amal et al., 2010 [74] identified lower moisture loss in coated strawberries.

It has been shown in several studies [75,76] that the use of beeswax as a coating not only prevented moisture loss but also improved the texture and overall appearance of fruit during long periods of storage. It was suggested that the edible beeswax coatings decrease the breathing rate of the fruit, reducing weight loss and thus increasing the shelf life of the minimally processed perishable fruit [75–78]. Cold-pressed coconut oil used as a coating reduces the rates of breathing and perspiration, as well as contributing to binding in the ethylene biosynthesis process [75,79]. It has antimicrobial, antiviral and antifungal properties due to its high lauric acid content, which has the ability to turn endogenous into monolaurin, thus attracting increasing interest in its properties [15,75,80]. Likewise, sunflower oil is rich in fatty acids, such as oleic acid, linoleic acid, palmitic acid and stearic acid, and has lower concentrations of palmitoleic acid, arachidic acid, eicosenoic acid, behenic acid and lignoceric acid [81].

4. Conclusions

Application of various postharvest coatings to perishable fruit is intended to prevent deterioration during storage, maintain sensory characteristics and improve or maintain bioactive compounds in terms of antioxidant activity, firmness and degree of damage. In this study, a new approach was applied involving the use of protein, oil and beeswax coatings for black mulberry fruit in order to extend the shelf life of fresh fruit and, at the

same time, maintain compounds of interest at high levels. The edible coatings with oils, beeswax and gelatine positively influenced the physiological changes in the mulberry fruit during the 8 days of storage. Edible coatings slowed the rate of deterioration, thus improving postharvest quality. Minimal losses in firmness and appearance and good results in sensory evaluations (texture, taste and aroma) were obtained in mulberry fruits covered with gelatine. The gelatine treatment also maintained high phenol and monomeric anthocyanin contents and high antioxidant capacity. Future studies on the metabolomics of the main phenolic and anthocyanin constituents of black mulberry fruit are needed to elucidate the metabolites generated and how they are involved in the rapid deterioration of fruits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12071068/s1, Figure S1: The edible coatings (gelatine, coconut and sunflower oils, beeswax) and their preparation.

Author Contributions: Conceptualization, S.I.V. and A.R.M.; methodology, A.C.T., A.R.M. and A.V.T.; formal analysis, A.C.T.; investigation, A.C.T. and O.S.M.; writing—original draft preparation, S.I.V., S.C. and A.R.M.; writing—review and editing, S.I.V. and S.C.; visualization, S.I.V.; supervision, S.I.V.; funding acquisition, A.N.V., A.V.T. and O.S.M. All authors have read and agreed to the published version of the manuscript.

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