



Effect of Dielectric Barrier Discharge Cold Plasma on the Bio-nanocomposite Film and its Potential to Preserve the Quality of Strawberry under Modified Atmosphere Packaging

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Received: 10 July 2023 / Accepted: 24 August 2023 / Published online: 2 September 2023
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Abstract

The present study aimed to investigate the effect of cold plasma on the developed bio-nanocomposite film. A dielectric barrier discharge system was utilized to generate the cold plasma. The films were treated under three different times (5, 10 and 15 min) and the characteristics of the films were evaluated. The cold plasma treatment influence the thermal stability and crystallization of the films. Results showed that the mechanical properties (tensile strength and elongation at break), water vapor permeability, oxygen transmission rate, moisture content and water contact angle characteristics were improved up to 69%, 31%, 34%, 3% and 28%, respectively by cold plasma treatment. In consequence, the average O₂ and CO₂ concentration of the packed strawberries decreased from 5 and 10% to 4.2% and 5.1%, respectively after 15 days. Finally, the results revealed that the mechanical properties, chemical attributes, physical characteristics and microbial activities of the samples were affected by the treated films. As a result, cold plasma modification can be applied as an effective method to maintain and preservation of fresh fruit.

Keywords Cellulose · Chitosan · Fresh fruit · Shelf life · Non-thermal treatment

Introduction

Appropriate packaging plays a key role in preserving and prolonging the shelf life of fresh fruits and vegetables. For researchers in the packaging industry, it is crucial to employ packaging materials that can effectively reduce the respiration rate, ethylene production, spoilage rate, and microbial activities associated with fresh produce. (Ceylan & Atasoy, 2023; Ranjha et al., 2023). However, overcoming the environmental problems due to the carbon dioxide released from the packaging material still is a challenge. For these reasons, bio/polymeric nanocomposites which are made up of

proteins, lipids and polysaccharides considerably have been studied and demanded by many researchers (Chaichi et al., 2023; Jafarzadeh et al., 2021; Kaur et al., 2021).

Chitosan is a natural biodegradable and antibacterial polysaccharide that has been effectively used in perishable fruit packaging (Correa-Pacheco et al., 2023; Li et al., 2023a, b). Nonetheless, the lack of enough reinforcement of chitosan films for the postharvest process was confirmed by previous studies (Behera et al., 2021; Kumar et al., 2023). Ortiz-Duarte et al. (2021) evaluated the potential of adding different component to improve biopolymer films containing Chitosan Nanocomposite. Similarly, Tavakolian et al. (2021) improved the barrier properties of nanocomposite films based on carboxymethyl cellulose to increase the efficacy of food packaging systems. The combination of chitosan and cellulose agents was suggested not only for their enhanced mechanical and thermal properties, but also for improved water vapor and gas permeability characteristics. (Ruan et al., 2022; Wardana et al., 2023). Ruan et al. (2022) and Wardana et al. (2023) reported the significant influence of cellulose nanoparticles on chitosan-based films, and their impact on the shelf life of mandarins and tomatoes,

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respectively. A significant difference in the respiration rate and weight loss of the samples among the products which were packed in chitosan film and chitosan-cellulose films were observed. Further, Chaichi et al. (2023) and Correa-Pacheco et al. (2023) improved the bionanocomposite film by adding different chemical component. Although bio nanocomposite films have potential for preservation of fresh fruit, complementary methods also affect the performance of developed bio nanocomposite films.

Cold plasma treatment is a non-thermal complementary technique that has been used to protect the functionality of bioactive compounds of different packaging film materials such as chitosan (Wang et al., 2023), chitosan/cellulose (Oberlintner et al., 2022) and chitosan/zein (Chen et al., 2019). The non-thermal decontamination method, holds significant promise as an advanced technology for the eradication of microbes and degradation of mycotoxins. This application has the potential to enhance the safety and quality of fresh fruits (Deng et al., 2020a, b). The effectiveness of the treatment is contingent upon various factors, including operational parameters, food characteristics, specific micro-organism species, and types of mycotoxins present (Deng et al., 2020a, b).

Dielectric barrier discharge (DBD) is a frequent method of cold plasma generation by the action of two parallel electrodes (Ashtiani et al., 2023; Ebrahimi et al., 2023). The DBD attracted the attention of many researchers due to the uniformity of treatment, low energy consumption, and free of using hazardous solvents (Chen et al., 2019; Rodrigues & Fernandes, 2023; Kongboonkird et al., 2023). Chen et al. (2019) improved the functional properties of chitosan/zein film using DBD treatment. The film was treated under power 100 W for 60–90 s and increasing of the tensile strength was reported. Similarly, Dong et al. (2017) evaluate the effects of DBD Cold Plasma Treatment on physicochemical and structural properties of zein powders. Further, Wu et al. (2020), Goiana et al. (2022) and Ranjha et al. (2023) approved the application of DBD for the improvement of the bio-Nano composite films. Nevertheless, limited studies have been researched regarding the effect of dielectric barrier discharge cold plasma on bio nanocomposite based on chitosan-cellulose and its utilization for preservation of fresh and perishable fruit under actual storage condition.

Strawberries are highly perishable fruits, and their limited shelf life is primarily attributed to their physiological characteristics (Duarte et al., 2023). Modified atmosphere packaging (MAP) is a well-proven method to extend the life of fresh fruit and vegetables (Alzuabi et al., 2023; Oliveira-Bouzas et al., 2023; Zhang et al., 2022). Although several research has been carried out on the effect of MAP on the shelf life of strawberries (de Oliveira Filho et al., 2022; Li et al., 2023a, b; Pasha et al., 2023; Robles-Flores et al., 2018) and

improvement of bionanocomposite material to maintain the quality of strawberries (Luo et al., 2022; Pasha et al., 2023), no study (to the best of our knowledge) has been performed on the effect of cold plasma on the packaging film based on cellulose/chitosan nano-composite that leads to influence on the quality of strawberries. Therefore, The objectives of this study were to (i) investigate the effect of DBD treatment on the microstructure, mechanical properties, water vapor and gas permeability, moisture content and water contact angle of the developed bio nanocomposite film, (ii) evaluate the potential of the treated film on the physicochemical and morphological characterization of strawberry. Furthermore, to simulate the real storage condition, the samples were packed under modified atmosphere packaging condition.

Material and Methods

Raw Material

Chitosan powder with a molecular weight of 500–700 kDa (Molekula Company, USA) and cellulose nanoparticles with a fiber diameter of 10–30 nm were purchased (Nano Research element, USA). In addition, acetic acid and absolute grade glycerol, calcium chloride, magnesium nitrate, and sodium chloride (Merck company, Germany) were prepared. Strawberry fruits were hand-harvested during the early morning from the research garden, and fertilization was carried out using complete manure. The intact samples were transferred to the material laboratory and separated into different classes based on similarity in color and size.

Preparation of Film

The nanocomposite film was fabricated using the casting method (Olonisakin et al., 2023). For this purpose, chitosan powder based on a specified amount (1.25% w/ W) was added to 30 mm of 1% acetic acid. Then it was stirred at a temperature of 40° C with the speed of 200 rpm (FR2E model, Fusion, USA) for 100 min. In addition, a certain amount of cellulose nanoparticles (1.5% w/ chitosan W) was added to 15 ml of distilled water and stirred under the same conditions as mentioned. In the next step, the distilled water was added to the chitosan solution. Moreover, 20% v/V glycerol was added to the final solution and for uniformity the sample was stored for 24 h under temperature of 21°C. Then, the solution was homogenized by a homogenizer (IKA-T25, Ultra Turax, Germany) with a speed of 7500 rpm for 20 min and an ultrasonic device (180H, Elma, Germany) under a frequency of 40 kHz for 20 min. Also, the aeration process was performed by a vacuum oven (6258, Lab-line, USA) under a pressure of 500 mm Hg for one hour. The solutions were placed in the center of the glass plates and put into the

oven at a temperature of 30°C for 32 h. Finally, the films were separated from the cast and was placed into the oven (40°C) for 24 h to remove the remaining solvent.

Cold Plasma Treatment

The versatile pilot scale system for the generation of cold plasma based on dielectric barrier discharge (DBD) was developed. The maximum transmission power, voltage, current and frequency of the system were 50 W, 15 kV, 10 mA, and 50 kHz, respectively. The system was powered by a DC pulse type, utilizing pulse width modulation (PWM) for control. Hence, the voltage level and the timing for rapid energy release could be established according to the transmission current's needs. Furthermore, the system could amplify the voltage energy while decreasing the current through capacitor discharges (Fig. 1(a)).

In order to ensure a uniform dispersion of cold plasma across the packaging film, a 1-L water container was utilized. Consequently, the electrode was immersed within the water, and the effectiveness was determined by adjusting the separation between the water container (not the electrode) and the film. From the pre-tests, a distance of 3–8 cm was considered to control the uniformity of plasma distribution, and the best performance was observed at a distance of 5 cm (Fig. 2(b)). Additionally, the duration of treatment is critical parameters impacting system’s performance (Ranjha et al., 2023). As a result, three different treatment times, namely 5, 10, and 15 min were taken into account.

Film Characterization

Mechanical Properties

Mechanical tests were conducted using a texture analyzer (TA.XT Plus, Stable Micro Systems UK) according to the

standard method (ASTM D882). The films were cut (15×55 mm) and placed between the upper and lower of the device's clamp. The grip separation, the movement speed of the clamp, and utilized load cell were 30 mm, 5 mm.s⁻¹ and 50 N. Then, tensile strength (TS) and elongation at break (EAB) of the film were calculated by Eqs. (1) and (2), respectively:

$$TS = F.A^{-1} \tag{1}$$

$$EAB = \Delta l.l^{-1} \tag{2}$$

where *F*, *A*, Δl and *l* are the maximum force applied to film (N), the cross-sectional area of initial film (m²), elongation at break (m) and initial film length (m), respectively (Pasha et al., 2023).

Water Vapor Permeability (WVP) and Oxygen Transmission Rate (OTR)

The WVP was measured using the water method gravimetrically based on the ASTM-E96/E96-05 Standard. For this purpose, the film was placed on a plate (diameter: 100 mm) and 25 ml of distilled water was added. It is noticeable, a specific distance (almost 9 mm) between the water surface and the film was considered. Then, the plate was placed in a controlled chamber (temperature: 21 ± 0.5 °C and relative humidity: 50 ± 5%) and the weight was measured on each test day using a digital balance with an accuracy of 0.01 g (PFB-6000-I, Kern, Germany). Finally, the WVP can be calculated by Eq. 3:

$$WVP = P.(A.\Delta C)^{-1} \tag{3}$$

where *P*, *A* and ΔC present mass flow rate of the gas (kg.s⁻¹), area of the film (m²) and specific molar fraction difference.

The OTR was measured by flask method based on ASTM D3985. For this purpose, a specific amount of nitrogen was

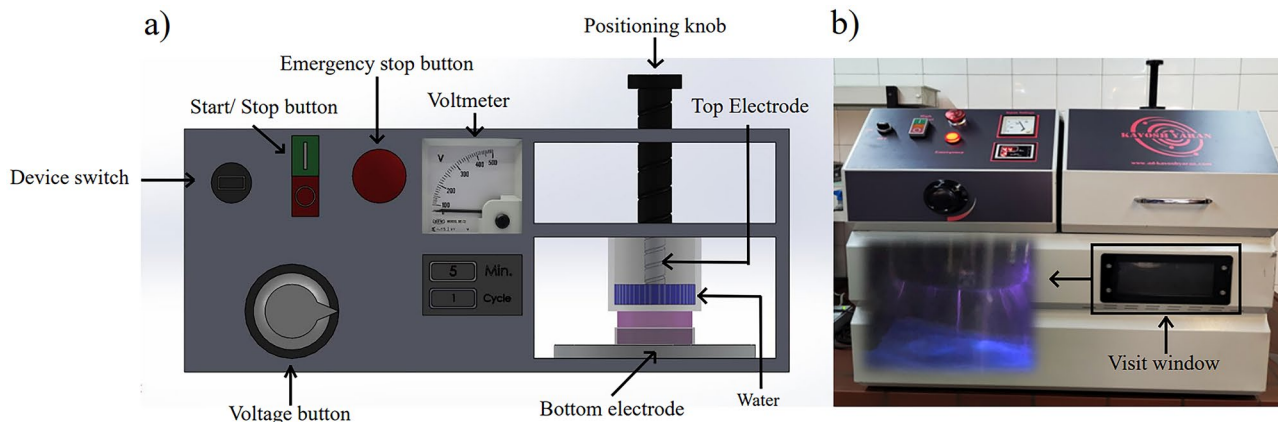


Fig. 1 a Schematic of the DBD cold plasma system b treatment view of the bionanocomposite film

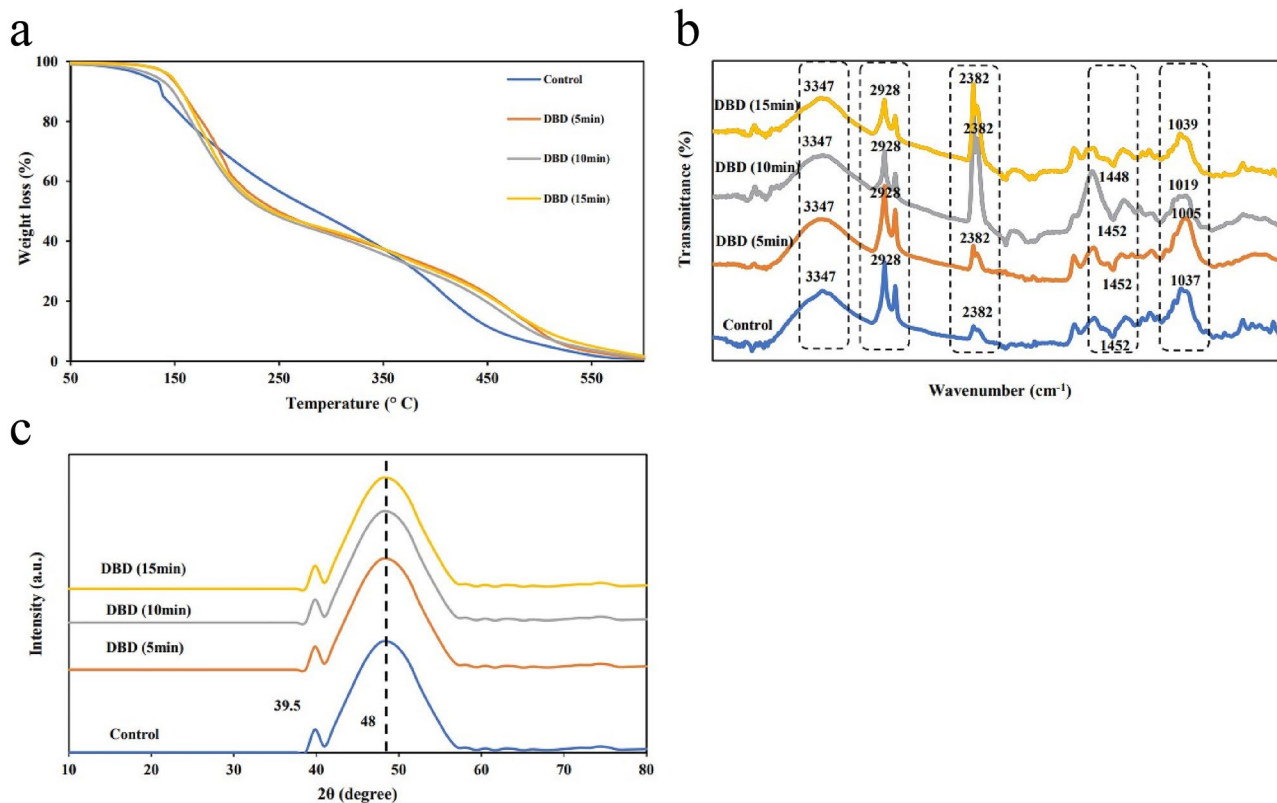


Fig. 2 a TGA thermograms b FTIR spectra and c XRD patterns of control and treated films

injected into a flask (volume = 2L) to diminish the molar fraction of O₂. The molar fraction of O₂ was measured by a tunable Diode Laser (G pro 500, Switzerland) and the oxygen permeability was calculated by Eq. 4:

$$OTR = C \cdot e^{-\frac{pA}{\rho VL}t} \quad (4)$$

where C , p , ρ , V , L and t are oxygen molar fraction in the flask at selected time (%), permeability coefficient (kg.m.m⁻².s⁻¹), density of O₂ (kg.m⁻³), volume of the flask (m³) and thickness of the film (m), respectively.

Moisture Content (MC) and Water Contact angle (WCA)

To determine the MC of the film, the samples (40×40mm) were cut and dried in hot air oven at 100 °C for 24 h. The weight loss of the samples was measured before and after the drying process and the MC Moisture content (%) was defined using the weight loss divided by the weight before drying (Kang et al., 2021).

To measure the WCA on the film a CA analyzer (Theta Flex model, Biolin scientific, Sweden) was used. For this purpose, the film samples were cut (50×50 mm) and placed on the horizontal plate which equipped with WCA analyzer. A specific amount of water (20 μl) was dropped on the film surface and the CA was measured.

Thermogravimetric Analysis (TGA)

To determine the thermal stability of the sample, a TGA analyzer (HZ2329, Huazheng, China) was utilized. The samples (20mg) were analyzed for different temperature ranges (50–600 °C) and at rate of 10 °C/min in a nitrogen atmosphere (Behera et al., 2021).

Fourier-transform Infrared Spectroscopy (FTIR)

To detect the interactions between the components of the samples, a FT-IR spectrometer (Alpha model, Ettlingen, Germany). In addition, a horizontal ATR trough plate crystal cell was used (Madison, WI, USA). Infrared spectra of the samples were recorded in the 4000- 400 cm⁻¹ wavenumber range with a spectral resolution of 4 cm⁻¹ (Ruan et al., 2022).

X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM)

To investigate the crystallinity of the film, X-ray diffractometer (GNR APD 2000 PRO, Vericheck, USA) was used where the 10×10 mm of the sample was cut and placed into the device. The Cu Kα radiation, voltage, and current were 0.15 nm, 40 kV, 40 mA, respectively. Moreover, the XRD

analysis was performed in a range of $2\theta = 10^\circ - 80^\circ$ with a step of $10^\circ/\text{min}$ intervals at ambient temperature (Behera et al., 2021).

The surface morphology of the film was visualized using a SEM apparatus (WEGA-II TESCAN, the Czech Republic). In this case the film was fixed on brass support with double-sided conductive platinum. The morphology analysis was conducted at 10 kV acceleration voltage and $1000\times$ magnification.

Modified Atmosphere Packaging (MAP) Process

Considering the literature (Barikloo & Ahmadi, 2018; Matar et al., 2018; Pasha et al., 2023) and preliminary tests, the amount of 10% CO_2 , 5% O_2 and 85% N_2 was selected for packaging by modified atmosphere apparatus. Four strawberries were placed in each package and then the interior air of the packages was evacuated by the apparatus and the selected gas concentration was injected into the package. The packed samples were stored in a controlled room (10°C and $85 \pm 5\%$ RH) for 15 days and physical, mechanical and chemical analyses were performed every five days. In addition, O_2 and CO_2 concentration of the package has been measured using a Checkmate 3 instrument (PBI Dansensor, Ringsted, Denmark) in each test day. For gas analysis, a specific amount of gas (5ml) was acquired from the package (with three repetitions).

Characterization of Packaged Strawberries

Weight Loss (WL)

The weight loss of the samples was calculated by digital balance with 0.01 g accuracy (PFB-6000-I, Kern, Germany). The WL were evaluated at each test time. The WL was defined as the ratio of percentage of weight loss to the initial weight at each test time.

Color

The color of the samples was determined by means of Commission Internationale de l'Eclairage (CIE). The L^* (measure the range from white to black), a^* (measure the degree of greenness and redness) and b^* (measure the degree of blueness and yellowness) indexes were evaluated using a colorimeter (Minolta CR 400 ChromaMeter, Minolta Corp, Tokyo, Japan). The colorimeter was calibrated with a white reference plate and measurements were performed on three different points of each sample. Then, the results were converted to hue angle degrees ($H^\circ = \arctan(-1.b^*.a^*-1)$) and chroma ($C^* = [a^{*2} + b^{*2}]^{0.5}$) which define the CIE $L^*a^*b^*$ color space.

Firmness

Mechanical properties of the samples were measured by penetration test using a texture analyzer (TA.XT Plus, Stable Micro Systems UK). In this case, the utilized load cell, diameter of the probe, penetration depth and movement speed were 500N, 5.2 mm, 2.5 mm and 5 mm.s^{-1} (Barikloo & Ahmadi, 2018). The firmness was calculated by the force–deformation curve. In addition, stress (Eq. 5) and strain (Eq. 6) were obtained to calculate the elasticity modulus by the Bosonic theory (Eq. 7).

$$\sigma = \frac{F}{\pi r^2} \quad (5)$$

$$\epsilon = \frac{L_0 - L_1}{L_0} \quad (6)$$

$$E = \left[\frac{0.338F(1 - \mu^2)}{D^{\frac{9}{2}}} \right] . K \left(\frac{1}{R} \right)^{\frac{1}{2}} \quad (7)$$

where F , r , L_0 , L_1 , μ , D , K , and R were force (N), radius of the probe (mm), initial length (mm), final length (mm), poisson ratio, deformation (mm), constant based on the standard and radius of curvature in contact points (mm), respectively.

Chemical Attributes

The strawberry juice was extracted by a blender machine from five samples and the pH of the juice was measured using a pH meter (PHS-550, Lohand, China). In addition, The soluble solid content (SSC) was measured by a digital refractometer (rx-5000a, Atago, Japan) and expressed as % (sugar equivalents in g.100.g^{-1}). Total ascorbic acid (AA) was obtained using Hitachi LaChromUltra UHPLC system with a diode array detector and a LaChromUltra C18 $2 \mu\text{m}$ column (Hitachi, Ltd., Tokyo, Japan) based on Avalos-Llano et al. (2020) method. The AA was expressed in g.kg^{-1} on a dry weight basis.

Microbial Analysis

To microbial count estimations, four strawberries (30–40 g) were placed in each bag, including 15 ml of sterile recovery diluter for 5 min. Bacteria and yeasts/moulds count were determined by surface plating of proper aliquots on plate count agar (PCA, APHA, Germany) and potato dextrose agar (PDA, BD Difco, USA) respectively. PCA plates were placed at 40°C for 48 h. The PDA plates were placed. All experiments were conducted in triplicate and, the microbial

parameters were expressed in $\text{Log}_{10}\text{CFU g}^{-1}$. Also, the strawberries of each package were selected for visual assessment of the initial appearance and severity of mold lesions or splitting on the surface of the strawberries. Finally, the ratio of infected strawberries in package to total samples in package was applied to calculate decay rate.

Morphological Analysis

Scanning electron microscope (SEM) was applied to observe the microstructure changes of the samples during storage time. To perform the SEM process, the sample were placed in 40% ethanol solution to prevent them from drying and plasticizing during preparation. Afterward, the samples were merged using different concentrations of ethyl alcohol, including 40%, 60%, 80%, 95% and 100% for 15 min. The samples were picked up from ethanol and immersed in 100% butyl alcohol for 40 min. The dry process of the container was taken 3 h and immediately stored in a desiccator on a water-resistant calcium sulfate substance (Robles-Flores et al., 2018). The samples were covered with gold at 15 nm to avoid electric charge during experiments. The experiments were conducted using SEM apparatus (WEGA-II TESCAN, the Czech Republic) with a voltage of 10 kV.

Statistical Analysis

Minitab 17 software was utilized for data analysis. Following an assessment of the normality of each parameter's dataset, a parametric approach was employed to compare the samples. Consequently, a two-way ANOVA was conducted to assess the impact of DBD-time treatment, storage duration, and their interaction on all measured parameters. Additionally, a multiple pairwise comparison using Tukey's honestly significant difference test was applied to ascertain significant mean differences at a 95% confidence level. Principal Component Analysis (PCA) was used to assess inter-parameter correlations and patterns within the samples. For each examined DBD treatment, the Pythagorean distance between the sample at day 0 and subsequent samplings was computed to determine the treatment that most comprehensively contributed to maintaining quality attributes during storage.

Results and Discussion

Thermogravimetric analysis, Fourier-transform Infrared Spectroscopy and X-ray Diffraction

Thermogravimetric Analysis (TGA) was employed to assess the thermal stability of plasma-treated films, involving the measurement of weight loss attributed to the generation of volatile compounds during thermal degradation.

The TGA curve of the control film can be divided into three stages (Fig. 2(a)). In the first stage, the initial mass loss was observed from 50° C to almost 150° C. Based on the Mohammadi Sadati et al. (2022) findings the reason for this trend was the evaporation of water from the cellulosic samples. The second stage, which revealed the major weight loss started at 150° C and continues until 400° C. In this stage, dehydration and depolymerization of glycerol have occurred which leads to weight loss (Wildan & Lubis, 2021). The trend of weight loss in the third stage (400° C—600° C) was sharply decreased that it was mainly due to the oxidation of chitosan and cellulose molecules (Mohammadi Sadati et al., 2022).

In comparison to the control film, the treated films displayed a modest downward trend during the initial phase. This suggests that the application of cold plasma intensified the hydrogen bonding between chitosan and nanoparticles, leading to enhanced water continuity (Oberlintner et al., 2022). On the contrary, in the second stage, the weight loss happened dramatically which corresponds to the rapid volatilization of chitosan chains due to the division of glycosidic connections (Oberlintner et al., 2022; Ranjha et al., 2023). However, the gradient of the pattern observed in the third stage highlighted the favorable impact of the DBD treatment on the thermal stability of the films. Notably, the duration of treatment did not have any influence on the TGA outcomes. Chen et al. (2019) and Zhu et al. (2022) approved the current results. Chen et al. (2019) conducted experiments to improve the thermal stability of chitosan film by cold plasma under three different treatment times (30, 60 and 120s). In a similar method, bio Nano composite films based on cellulose have been treated for 3 and 9min. Although cold plasma declined the attribute of cellulose to the higher surface area, the treatment time did not affect the thermal stability improvements.

Fourier Transform Infrared (FTIR) analysis was performed to assess the impact of cold plasma modification on the molecular structure. The FTIR spectrum of the control films showed the characteristic of saccharide structure peaks at 1037, 1452.6 and 2382 cm^{-1} which shows the C-O stretching, C-N stretching and O-H bending, respectively (Fig. 2.b). The peak around at 2850–3000 cm^{-1} was the O-H stretching and overlaps N-H stretching. In addition, the pick at the 3347 determined the asymmetric C-H vibrations (Chen et al., 2019).

Upon examining the spectrum of the treated films, similar bands to the control sample were observed. However, there was a noticeable difference in the absorption band between the range of 1000–1040 cm^{-1} and 1395–1480 cm^{-1} . Evidently, the cold plasma induced a shift of the carbonyl band towards lower frequencies, suggesting the engagement of this particular group in oxygen-binding interactions with

cellulose groups. Furthermore, the peak intensity of O-containing functional groups was much higher in the spectra of the treated films revealed more oxygen-containing functional groups were created on the surface of nanocomposite film during the treatment. The current results were in agreement with Zhu et al. (2022) and Oberlintner et al. (2022).

Furthermore, X-ray Diffraction (XRD) analysis was carried out to explore the impact of cold plasma on the crystalline structure of cellulose samples (Fig. 2(c)). The broad peak of chitosan at $2\theta = 39.5^\circ$ indicates its semi-formless character (Wildan & Lubis, 2021). The peak has been become more pronounced with increasing nanocomponent at the $2\theta = 48^\circ$. Perhaps transcrystallization affect this enhancement. This phenomenon describes direction of the semicrystalline matrix in the orientation of the nanocomponent (Xu et al., 2021a, b).

The XRD patterns of the treated films were considerably similar to the control film which revealed the cold plasma treatment and its time did not affect the crystallization of the chitosan/cellulose. The statement can be confirmed by the results of Zhu et al. (2022). The crystal structure of the nanocomposite based on the cellulose/chitosan film was evaluated after changing voltage and time treatment of cold plasma. Nonetheless, the crystallization of the control and treated films was similar and they reported the cold plasma did not influence the transfer of more disordered areas of cellulose into aqueous media.

Film Characterization

Figure 3a and b depicted the influence of DBD treatment on the mechanical properties of the film. The 5min treatment could not affect TS and no significant difference was observed with the control film. On the contrary, the TS of treated film for 10 or 15 min, was increased by approximately 70% with respect to the control film. However, there was no significant difference between different treatments on the amount of EAB. Although the DBD treatment (10 and 15min) influenced on the mechanical properties, due to the statistical analysis we cannot claim the DBD treatment significantly affect the tensile strength and elongation at break.

Improvement of mechanical properties of nanocomposite based on chitosan/cellulose films has already been confirmed by Oberlintner et al. (2022). It seems that certain weaker C-N interactions were substituted with potent C-N interactions throughout the treatment procedure, resulting in the reinforcement of the network structure. In addition, cold plasma enhanced inter-fiber hydrogen bonding and could also increase the adsorption of chitosan. Similarly, the cold plasma treatment increased the TS and decreased EAB of different bio nanocomposite films such as zein (Dong et al., 2017), lily polysaccharide (Cui et al., 2022) and casein (Wu et al., 2020) based-films. Furthermore, they recommended

the treatment time influences the cross-linking among their structure bonds and induces them to transformation. They indicated that an extended plasma treatment duration led to a decrease in tensile strength (TS). This could potentially be attributed to a molecular structure fracture phenomenon, which subsequently introduced minor gaps in their network.

Figure 3(c) and (d) illustrates reduction of the water vapor permeability and oxygen transmission rate caused by cold plasma treatment. The DBD-5 min had no significant effect on WVP and OTR parameters compared to the control. In contrast, the treatment of 10 and 15 min had a significant effect ($P < 0.05$) on the WVP. Similarly, a significant difference in the OTR rate was observed in the treatments of 10 and 15 min compared to the control and 5 min.

Since cold plasma affect the formation of hydrogen bonds between chitosan and cellulose hence diminished the hydrophilic properties of the polysaccharide matrix which influence the WVP, efficiently (Chen et al., 2019). Chen et al. (2019) have tried to improve the moisture-resistant function of nanocomposite by cold plasma treatment. The films underwent treatment at various durations, resulting in a varying pattern of water vapor permeability (WVP). Initially, there was a decrease in WVP, indicating potential modification of polysaccharide bonds due to treatment time. Subsequently, WVP increased, which the author attributed to the inherent hydrophilic nature of chitosan. This could imply that chitosan on the surface contributed to the absorption of water vapor.

Also, hydrogen linkage between bacterial cellulose and chitosan probably formed a more arranged structure. This arrangement creates a difficult situation for molecules of gas to diffuse hence the oxygen barrier characteristic was stronger (Ranjha et al., 2023). Also, the plasma treatment affected the higher molecular weights of chitosan which tended to self-accumulate naturally. Thus, the hydrogen bonding was increased and led to decrease oxygen permeability of the films (Bahrami et al., 2022).

Figure 3(e) and (f) showed that plasma treatment had no effect on moisture content and no significant difference was observed between treated and control samples. The moisture content (MC) outcomes were unexpectedly contradictory, as the close proximity of amino groups and hydroxyl groups might have been anticipated to reduce the moisture content of the nanocomposite. The absence of a reduction in water condensation within the film's porous space seems to be primarily attributed to the incorporation of cellulose nanoparticles. These nanoparticles influence the swelling behavior of hydrophilic polymers, which likely played a role in this outcome (Cazón et al., 2020). In addition, cold plasma treatment could decrease the accessibility of hydroxyl components for interchange with water molecules. Thus, the film was more resistant to moisture. The statement can be confirmed by Honarvar et al. (2017) and Oberlintner et al.

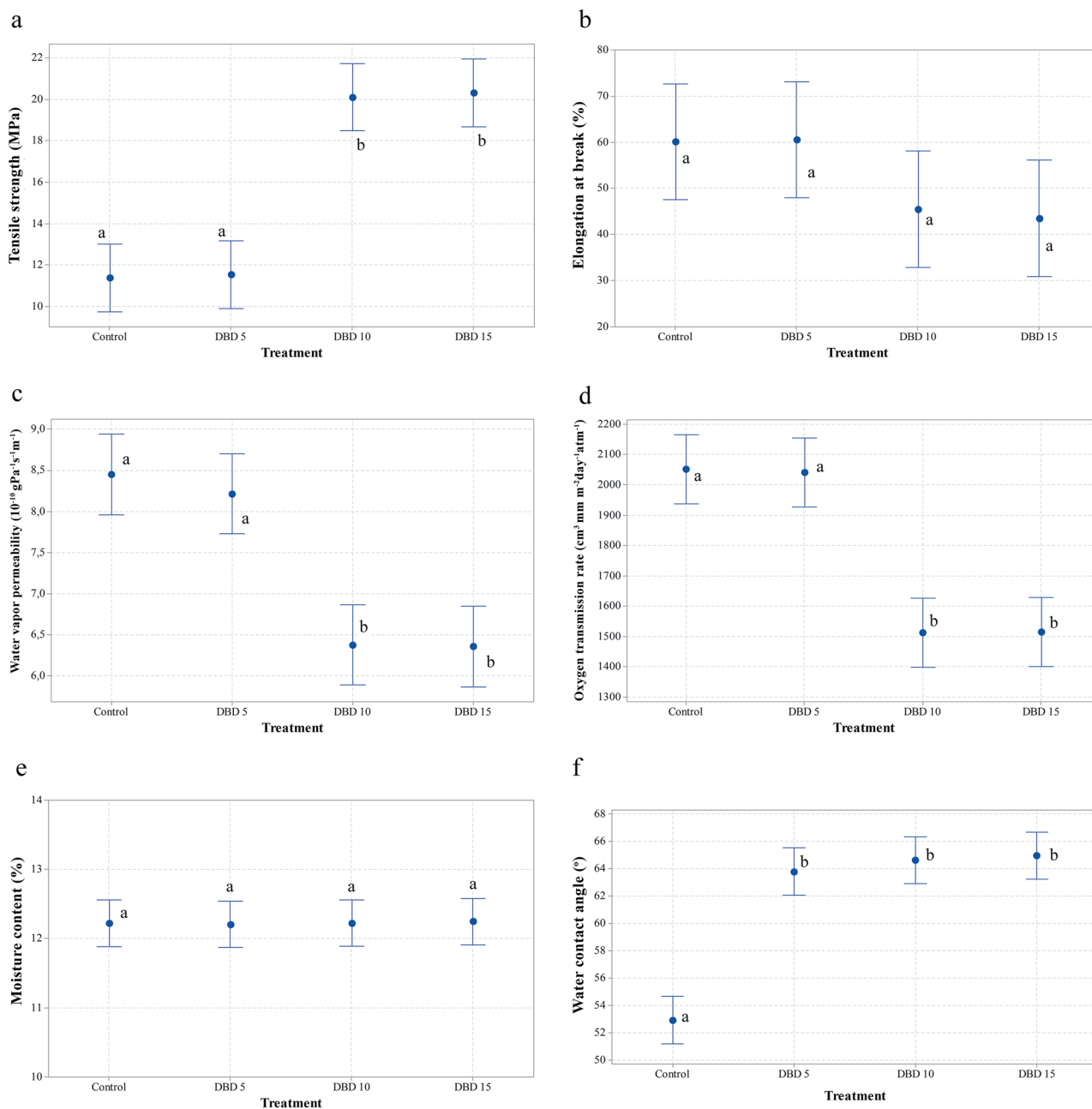


Fig. 3 **a** tensile strength, **b** elongation at break, **c** water vapor permeability, **d** oxygen transmission rate, **e** moisture content and **f** water contact angle parameters of the develop films under different treatment

condition. Different letters indicate significant differences ($p < 0.05$). DBD5, DBD10 and DBD15 was presented the treatment time for 5, 10 and 15 min, respectively

(2022). The reported moisture content of the cold plasma-treated films was lower than the control. The application of cold plasma influenced the establishment of hydrogen bonds between the cellulose and chitosan components, resulting in a modification of the water-resistant film Fig. 4.

By contrast of MC, the amount of water contact angle increased significantly after cold plasma treatment, and compared to the control sample, after 15 min of treatment, the amount of WCA increased from 53° to 65° (Fig. 5(b)).

Utilization of cellulose nano components created an acetate group which led the films into low hydrophilic compared to Xu et al. (2021a, b) results. They recommended the amount of $\text{WCA} < 100^\circ$ to improve the shelf life of the fresh fruits because minimum wettability allows the diffusion of moisture from inside of the package to the environment. The cold plasma treatment improved the wettability of the film by enhancing the oxygen-containing polar groups and modifying hydrophilic characteristics (Honarvar et al., 2017; Oberlintner et al., 2022).

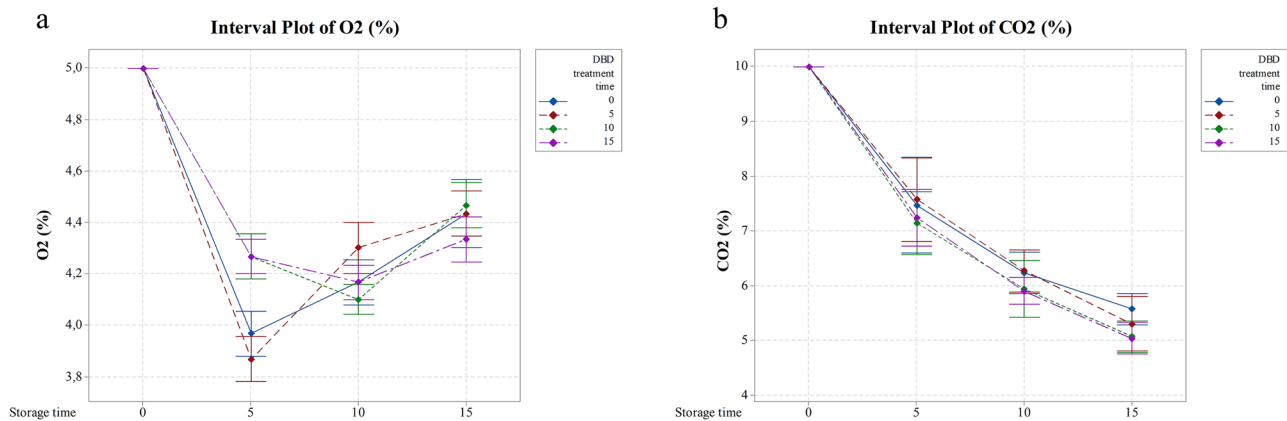


Fig. 4 **a** Evolution of O₂% and **b** CO₂ during the storage. Error bars represent the standard error of the mean

Gas Concentration

The O₂% and CO₂% content in the sealed package over the storage were depicted in Fig. 6. The levels of both CO₂ and O₂ declined during storage, although they never reached an anoxic state. It's widely recognized that in the respiration process crucial for the preservation of fruits and vegetables, oxygen is utilized and carbon dioxide is generated. These characteristics are contingent on various intrinsic and external factors. The chosen packaging strategies can set gas levels suitable for preservation, a pivotal aspect for maintaining product quality. Two-way ANOVA, carried out within the MAP DBD-treated and untreated products to determine the effect of the treatments and storage time within the MAP, suggested as the DBD treatment time affect only the CO₂ content ($P=0.000$), whilst the O₂ was affected by the storage time ($P=0.000$) and its interaction with the duration of DBD treatment ($P=0.017$).

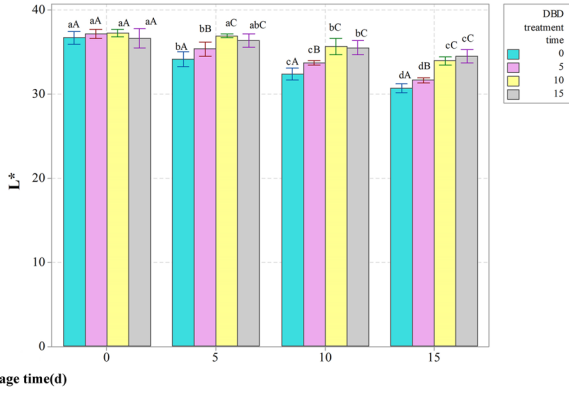
The positive effect of developed nanocomposite film based on chitosan/cellulose on CO₂ production and O₂ consumption can be found by de Oliveira Filho et al. (2022) and Lee et al. (2022) reports. They report using cellulose nanocomponents in the film, excessive gas transportation was barricade and respiration rate was decreased. In addition, the polysaccharides bonds improved gas diffusion which led to decline CO₂ production. Also, due to the effect of cold plasma treatment on the barrier properties of the film, the dehydration process was restrained and CO₂ production was delayed during storage time (Misra et al., 2014). In addition, the influence of cold plasma treatment on the O₂ consumption and/or CO₂ production for different fresh fruits during storage time such as mango (Yi et al., 2022), blueberry and apple (Zhang et al., 2023) were confirmed.

Properties of the Samples

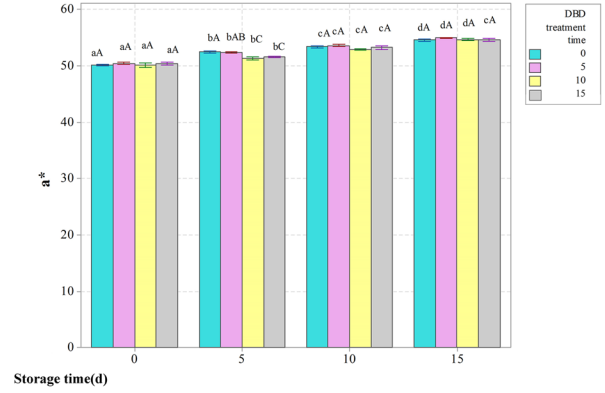
The major changes detected in strawberry fruits' colour concern the intensity of red tone, along with lightness drop. ANOVA results indicates that a* and L* parameters were affected by treatment time and storage time ($P=0.000$), but their interaction did not affect a* (Fig. 5(a), (b)). L* and a* vary significantly during storage but with characteristics depending on the treatments. In general, the control batch exhibited the most pronounced rate of variation, manifesting an increase in a* and a decrease in L*. However, the application of DBD treatment for a minimum of 10 min proved effective in diminishing the measured color parameters. On the initial day, immediately after the treatments, there were no notable distinctions in values between the treated and untreated products. This observation aligns with the findings of Giannoglou et al. (2021), who employed surface Dielectric Barrier Discharge (SDBD) plasma on unpackaged strawberries. They utilized sinusoidal high voltage signals of 6 kVpp at a frequency of 42 kHz for 10 min, and the color preservation was upheld post-treatment. However, their study did not identify any changes in the a* parameter over a 14-day storage period.

The effect of cold plasma treatment on the color features of this study was in agreement with Misra et al. (2014) and Ziuzina et al. (2020). The color changes under different MAP condition and fixed treatment time was evaluated by Misra et al. (2014). The lightness parameter of treated and untreated samples was found to be significantly different ($p \leq 0.05$). In contrast, no significant difference in a* was observed. This can be described considering the pink and dark red colors can have similar values for chroma. Similarly, Ziuzina et al. (2020) reported although the value of color features showed a fluctuating trend, the value of L, a, and b have declined during the storage time. The reduction

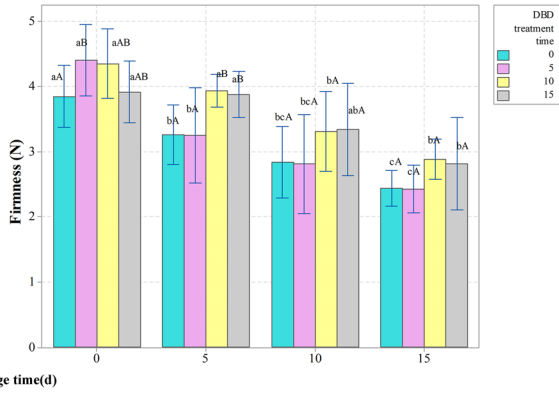
a



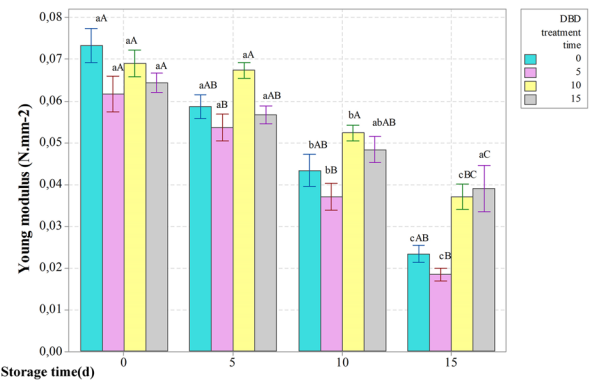
b



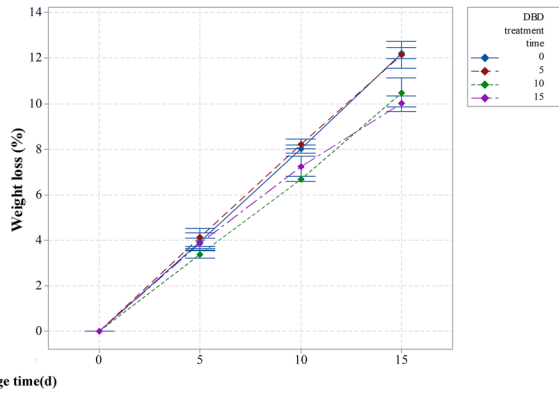
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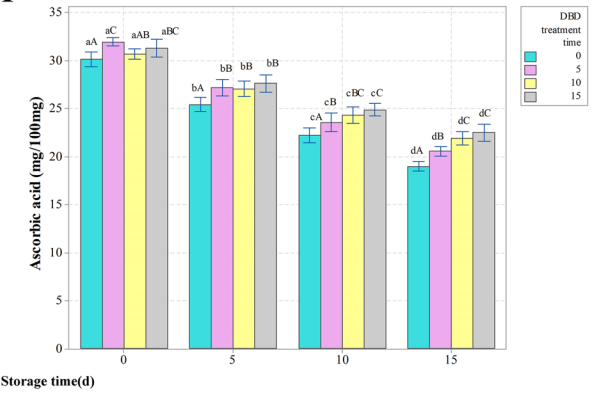
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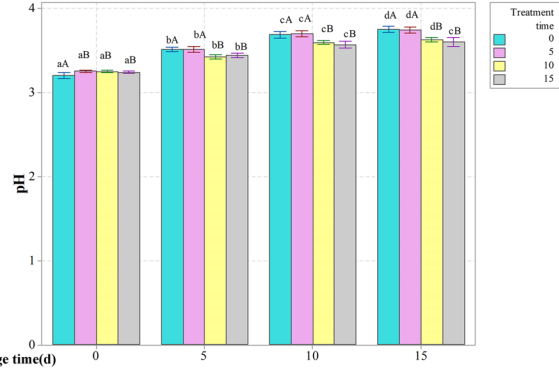
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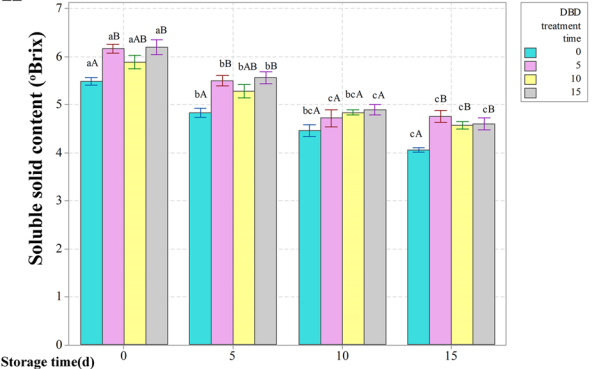


Fig. 5 Evolution of CIELAB coordinates **a** L*, **b** a* detected, Evolution of **c** Firmness, **d** Young modulus, **e** weight loss, **f** ascorbic acid content, **g** pH and **h** SSC for the strawberries. Different uppercase letters indicate a significant difference between DBD treatments at that storage time. Different lowercase letters indicate a significant difference between storage days at that DBD treatment. ($p \leq 0.05$). Error bars represent the standard error of the mean

of lightness parameters for control samples was more apparent rather than the treated samples due to the fungal growth.

Figure 5(c) and (d) shows the evolution of Firmness and Young's modulus during storage. The observed pattern indicates an inevitable reduction in these mechanical parameters for the product. Nonetheless, several experimental conditions contributed to decelerating the progression of these phenomena. Statistically, both storage time and the duration of DBD treatment exerted an impact on firmness and Young's modulus. Nonetheless, only products treated for 10 or 15 min exhibited superior attributes. This difference was especially evident for firmness within the first 5 days, where the 10 and 15 min DBD treatments resulted in enhanced outcomes compared to the control and the product treated for 5 min. The young modulus of the samples was between 0.06 and 0.07 N mm² in the 0 d, and after the 5, 10 and 15 treatments DBD did not undergo significant changes. The DBD effect was more evident during the cold storage at 10 °C, since after 5 days in the product treated with DBD for 10 and 15 min, the Young's modulus was not significantly different from day 0, while the products untreated or treated for 5 min had a similar trend and suffered a more significant reduction.

The acceptable effect of the developed nanocomposite on the mechanical properties of strawberries during storage time can be confirmed by comparing it to Kang et al. (2021) and Pasha et al. (2023) reports. They developed nanocomposite films for strawberry preservation and increasing shelf life. To prevent the decline in firmness over storage duration, they suggested that the packaging film design should consider factors such as moisture retention and the presence of extracellular pectolytic enzymes. Based on the mentioned statement, the developed film of this research affected the softening phenomenon by cell wall component degradation. The cold plasma treatment caused barricade water vapor and oxygen from the porous space of the film hence the respiration and other metabolic reactions of the strawberry were decreased. As a result, the reduction of the treated samples' firmness was lower than the untreated samples during the storage time (Li et al., 2019; Ziuzina et al., 2020).

Weight loss serves as an indicator of water loss during product storage and is notably influenced by both packaging systems and environmental storage conditions. The nano-material-based film that was developed exhibited the ability to readily release CO₂ and uptake O₂. Following a 5-day storage period, all products experienced an approximate 4%

reduction in weight. Subsequently, this weight loss escalated to a range between 10 and 12% after 15 days of storage (Fig. 5(e)). At this stage, the impact of DBD time treatment became apparent, as the untreated and DBD-5min treated products experienced more significant water loss compared to DBD-10 and 15 min treated products. The current results compared to the Robles-Flores et al. (2018) and Pasha et al. (2023) showed that the developed film improved strawberry water evaporation which is the main attribute for weight loss. Given that strawberries are prone to dehydration owing to the rheological properties of their skin, the barrier attributes of the film indirectly influence weight loss by affecting the respiration and transpiration rates of the strawberries. The result of cold plasma treatment revealed the treatment time affected the porous spaces of the film hence the barrier's ability to delay the water interchange was shifted (Zhang et al., 2023).

Ascorbic acid (Vitamin C) is a crucial nutritional component that plays a role in human metabolism and can be beneficial in certain disease conditions. The content in untreated product was close to 30 mg/100 g, and it undergone to slight decreasing during the storage in all conditions, but in particular in untreated product (Fig. 5(f)). As for the other measured parameters, the interaction of DBD-time treatment and storage duration was particularly evident, and after 10 and 15 days storage the ascorbic acid content of DBD-5 min products was lower than DBD-10 and 15 min.

It seems the amount of O₂ inside the package affects the reduction of AA. As mentioned previously (3.3. Gas concentration), the developed bio-nano composite film had potential to control the permeability of O₂ and CO₂ which led to decreasing the autoxidation of ascorbic acid. Sogvar et al. (2016) highlighted that a film exhibiting low O₂ permeability led to a slowdown in the oxidative deterioration reaction, thereby affecting the ascorbic acid (AA) content of strawberries. Due to the effect of the cold plasma treatment on the O₂ permeability of the film, the reduction trend of AA was affected. The observation was in agreement with Misra et al. (2015). The AA of in-packaged strawberries which were treated by a dielectric barrier discharge system was analyzed. The voltage and treatment time had a significant effect on the AA. The AA of treated samples was more than the control due to the reaction of singlet oxygen and excited molecular oxygen.

The initial average pH of untreated strawberry was 3.21, and after the DBD treatments, slightly but significantly was risen (Fig. 5(g)). Cold storage had an additional impact on the pH increase, particularly noticeable in the untreated and DBD-5 min treated products. Following a 15-day storage period, the pH of samples treated for 10 and 15 min ranged from 3.5 to 3.6, whereas the pH of the untreated or DBD-5 min treated products ranged from 3.7 to 3.8. The pH of strawberries is influenced by factors such as maturity

level, microbial contamination, and storage conditions. The respiration rate of strawberries within the packaging generally leads to changes in pH, TA, and TSS during the storage period. The results revealed that the pH of the samples during the storage time was 3–4 which presented the nanocomposite film could maintain the pH of the strawberry in an acceptable range (Pasha et al., 2023). However, similar to Rana et al. (2020) results no significant effect of cold plasma treatment on the pH value was shown.

The SSC of untreated product was close to 5.5°Brix, and it worth to note, that it rose after the DBD treatment. There was no evidence of the effect of DBD time treatment on the SSC increasing rate, as its content was similar in all the treatment at time 0 and the treated samples was not significantly different at every sampling. However, the SSC exhibited a decrease throughout the course of the experiment across all samples, with a more pronounced decline observed in the untreated product. This decrease could be attributed to elevated microbial metabolism taking place in the control samples. SSC can be described by the hydrolysis of sugars to keep respiration during the WL process of strawberries (Robles-Flores et al., 2018). The utilized film was successful to reduce the respiration rate of the strawberry hence the gas concentration could preserve the SSC content of the samples. The results are consistent with Kang et al. (2021) and Pasha et al. (2023). However, the increasing and decreasing trends of SSC were different from the current study. They reported during the initial days the SSC was enhanced and then sharply fell down. While the SSC decreased slightly during the storage time for both of treated and untreated samples, similar to Li et al. (2019) and Rana et al. (2020) results.

Microbial Analysis

The findings of the microbiological analysis, which included monitoring the levels of yeasts, moulds, and total mesophilic bacterial counts, are shown in Table 1. The average bacterial and fungi count in the fresh-untreated product were 2.69 and 2.18 log cfu/g, respectively. After the DBD-treatment, at every duration, the contamination was significantly not different from the untreated product. The microbial counts increased during storage with features that varied depending on the treatment, indicating that the interaction between DBD-time and storage length has a significant impact on contamination ($P=0.001$). The growth rate of TBC was higher in the untreated and 5 min DBD- treatment products, rose up to 4 log cfu/g after 10 days' storage, whereas those treated at least up to 10 min where lower than 4 log cfu/g throughout the experiment duration. Moulds, in particular *Botrytis cynerea*, represent the main spoilage agent in strawberry storage. As observed

for TBC, the yeast and mould cells were not damaged by DBD duration treatment, being similar the counts of untreated and treated products. Yeast and mould growth rate was lower rather TBC, but more affected by storage duration rather than DBD-time treatment. Moreover, over the five days of storage, no signs of decay were detected in either the control group or the treated samples. Initial indications of decay was emerged after the 10th day of storage, and subsequently, the occurrence of decay increased as the storage duration extended. However, the decay ratio of the treated samples under 10 and 15min presented less decay ratio compare to the control.

Similar to Robledo et al. (2018) and Liu et al. (2021) reports, the application of developed bio nanocomposite film to diminish the microbial decay indexes was well-proven. One of the main reasons for increasing aerobic bacteria, yeast and mould is water activities inside the package. Considering to improved WCA and MC parameters of the film, oxidative stress was decreased which led to degrade the microbial activities.

The utility of the cold plasma treatment on the bacteria, yeast and mould activities of the strawberry can be confirmed by Rana et al. (2020) and Belay et al. (2022). The Polar groups of cellulose compounds interact with hydrogen bond caused reduction of bacteria activities. Rana et al. (2020) reported that cold plasma significantly affected the yeast and mould during the storage time (9d). In contrast the treatment times (10, 15 and 30min) did not influence on the reduction of yeast and mould. In addition, they recommended the temperature condition should be considered and as much as possible, avoid the laboratory at a temperature above 25°C. Therefore, further investigation into the effect of different temperatures on the performance of improved film under cold plasma treatment is required.

PCA Analysis

Multivariate analysis was performed using principal component analysis. Figure 6 shows the biplot graph (score plot + loading plot) of multivariate statistical processing obtained from the relationship matrix with the measured parameters and the product at every sampling. The model succeeded in explaining about 94% of the variance and separated the samples mainly according to the storage time rather than the DBD time treatment.

The variables that correlate the most with first component (PC1, 91.3%) were microbiological counts and weight loss (0.32), a^* and pH (0.31), whilst the variables that correlate the most with second component (PC2, 3.7%) were Young modulus (0.50), L^* (0.44) and a^* (0.20). Furthermore, the coordinate values of the samples on the PCA graphs were employed to compute the Pythagorean distance between the samples on day 0 and during storage for each tested DBD treatment. This approach was utilized to assess the overall

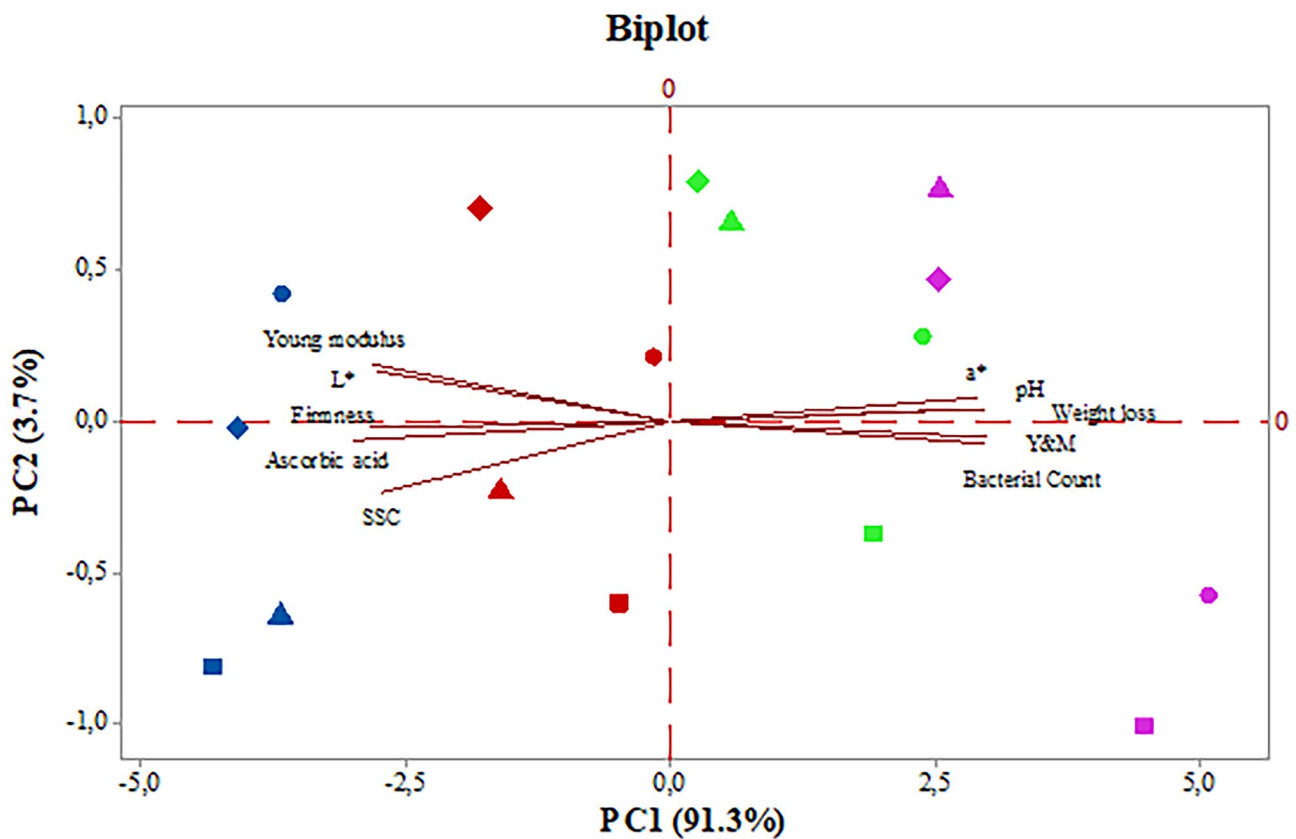


Fig. 6 Biplot graph (score plot+loading plot) of multivariate statistical processing obtained from the relationship matrix with the Young modulus, weight loss, ascorbic acid, solid content soluble (SSC),

colour indexes (L* and a*), pH, on sampled day 0 (blue), 5 (red), 10 (green), 15 (magenta)

efficacy of each treatment in relation to the parameters utilized in the correlation matrix. The lower the distance the lower are the changes detected respect to the product

as soon as the treatment at day 0. Therefore, through this approach, is noticeable as the DBD treatment for 15 min the most contribute to the quality maintenance of the product.

Table 1 Results of microbiological analysis respect to total bacterial count (TBC) and Mould and Yeast. Data are expressed as mean ± standard deviation. Mean that no shear the same letters are significantly different. Different uppercase letters indicate a significant difference between DBD treatments at that storage time. Different lowercase letters indicate a significant difference between storage days in every DBD treatment

DBD treatment time (min)	Storage time (days)	TBC (Log10 CFU g-1)	Mould and yeast (Log10 CFU g-1)	Decay rate (%)
0	0	2.69 ± 0.07 ^{aA}	2.18 ± 0.03 ^{aA}	-
	5	3.61 ± 0.05 ^{bcA}	2.63 ± 0.04 ^{bcA}	-
	10	4.05 ± 0.07 ^{bA}	2.9 ± 0.08 ^{bA}	1.65 ± 0.12 ^{bA}
	15	4.65 ± 0.06 ^{cA}	3.48 ± 0.05 ^{cA}	5.20 ± 0.15 ^{cA}
5	0	2.62 ± 0.07 ^{aA}	2.20 ± 0.02 ^{aA}	-
	5	3.48 ± 0.06 ^{aB}	2.59 ± 0.07 ^{aA}	-
	10	4.07 ± 0.13 ^{aA}	2.99 ± 0.09 ^{aA}	1.66 ± 0.14 ^{aA}
	15	4.67 ± 0.13 ^{bA}	3.49 ± 0.09 ^{bA}	5.25 ± 0.13 ^{bA}
10	0	2.66 ± 0.06 ^{aA}	2.16 ± 0.01 ^{aA}	-
	5	3.14 ± 0.04 ^{aC}	2.33 ± 0.02 ^{aB}	-
	10	3.50 ± 0.07 ^{aB}	2.67 ± 0.04 ^{bB}	1.02 ± 0.03 ^{bB}
	15	3.99 ± 0.10 ^{bB}	3.05 ± 0.06 ^{cB}	3.95 ± 0.08 ^{cB}
15	0	2.67 ± 0.04 ^{aA}	2.19 ± 0.01 ^{aA}	-
	5	3.14 ± 0.06 ^{abC}	2.34 ± 0.03 ^{aB}	-
	10	3.49 ± 0.12 ^{bB}	2.66 ± 0.08 ^{bB}	1.02 ± 0.04 ^{bB}
	15	3.97 ± 0.06 ^{cB}	3.04 ± 0.05 ^{cB}	3.90 ± 0.09 ^{cB}

Morphological Analysis of the Film and Strawberry

The surface morphology of the control and treated films were shown in Fig. 7. The control film represented a smooth surface with minimum porous spaces. This smooth structure was appropriate for postponing the water and gaseous interchange and decreasing the respiration of fruits and vegetables (Liu et al., 2021). In addition, using nano cellulose in the film caused effective interactions between hydroxyl groups of protein isolate and chitosan (Li et al., 2023a, b). It seems the cold plasma treatment improved the compatibility of the chitosan and nano cellulose surface as well as the roughness of the film which led to enhancing the surface hydrophobicity. This high compatibility can be described by appropriate hydrogen bond interaction between the film components which was useful for loading functional molecules (Hosseini et al., 2022; Oberlintner et al., 2022). Hosseini et al. (2022) investigated the effect of DBD cold plasma on the nanocomposite film based on cellulose. They reported that increasing the treatment time to 10min caused the homogeneous distribution of cellulose particles on the surface that affected the water vapor and oxygen permeability. While there was no microstructural difference observed under 15min treatment.

The morphological deformation in the cells and disruption of the surface microorganisms of the untreated/treated samples which were caused by bacteria and yeast activities at

the end of the storage time were shown. Increasing the bacteria count leads to colonization and also altered the structure of the pathogen. Although some researchers reported the bacterial area was able to generate some kind of bioactive compounds, this research could not claim due to the requirement of chromatography analysis combined with mass spectrometry (de Andrade et al., 2019; de Moura et al., 2021). Also, the microstructure of the control samples showed the cells lost their well-defined boundary membrane.

In contrast, the samples that were placed in the treated films show the bacterial cells have been decreased and were surrounded by cellular debris. This disturbance could be attributed to the decay of the organic material and the effect of reactive oxygen species (ROS) on the cell wall of the microorganisms (Ahmadnia et al., 2021). Furthermore, by increasing the treatment time of the films, the amount of bacteria accumulated on the strawberry surface was lower. In the literature, the morphological properties of the different nanocomposite films were discussed rather than the morphological characteristics of the packed fruit hence comparison of current results with other research was not possible. However, some researchers investigated the direct treatment of cold plasma with various products such as strawberries (Ahmadnia et al., 2021), apricot (Hua et al., 2022) and mulberry fruit (Yinxin et al., 2022), and the application of cold plasma to reduce the bacteria, yeast and mould activities was approved.

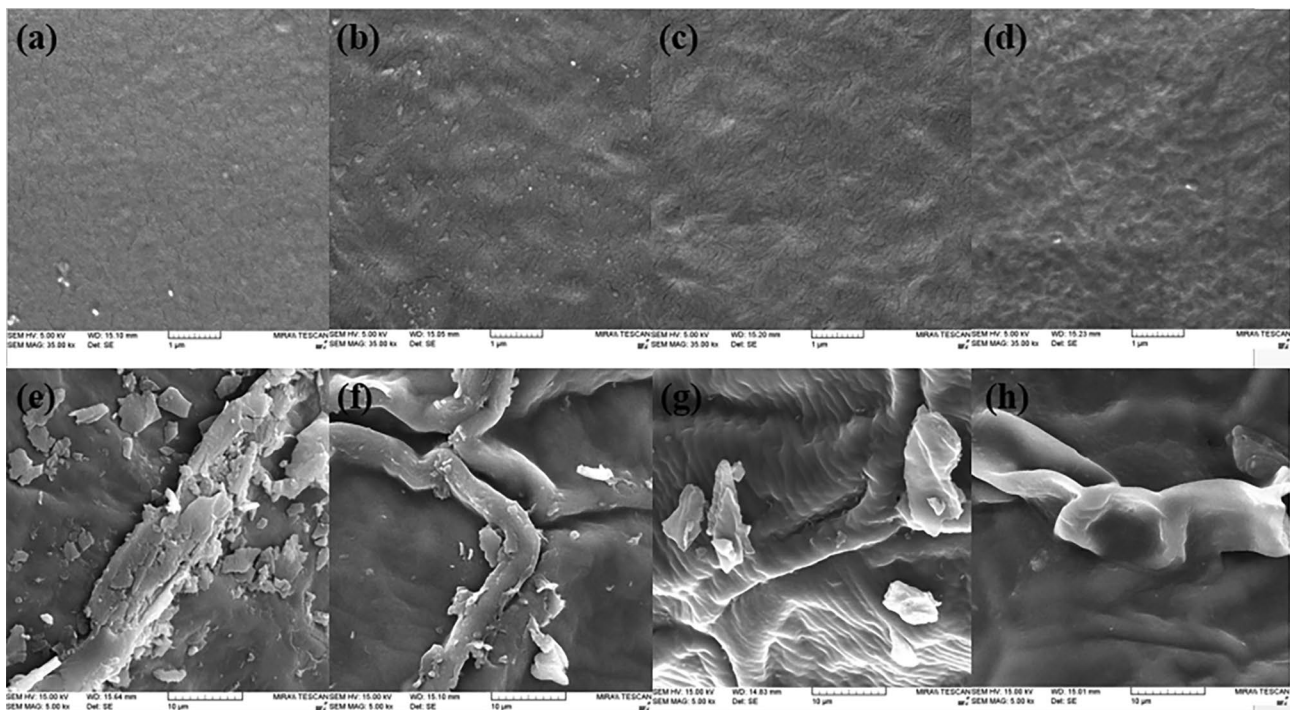


Fig. 7 Surface morphology of the film **a** control **b** DBD-5min **c** DBD-10 min **d** DBD-15min and the surface morphology of the strawberry which the samples packed by the **e** control **f** DBD-5min **g** DBD-10 min **h** DBD-15min treated films

Conclusion

In current study, bio nanocomposite film was improved using the selection of proper ratio nanocomponent by casting method. Dielectric barrier discharge cold plasma was utilized to modify the surface of the film. The slope of the trend in different stages from TGA analysis and evaluation of semicrystalline matrix direction in the orientation of the nanocomponent from XRD analysis revealed the cold plasma treatment affects the thermal stability and crystallized structure of the film. In addition, the plasma treatment increased the water contact angle of the film due to the formation of polar groups, which was in agreement with FTIR analysis. The moisture content, water vapour and oxygen permeability parameters that influence the respiration rate and enzyme activities of the fresh products were decreased. Although the films treated for 15min had better results on the chemical attributes of the packed strawberries, the energy consumption for industrial scale should be considered. Moreover, the efficiency of the cold plasma and modified atmosphere systems are sensitive to environmental conditions. For further study, it is suggested the effect of low and high temperatures on the performance of the developed film, dielectric barrier discharge cold plasma and modified atmosphere packaging be investigated.

Acknowledgements The authors would like to thank Dr. Rouzbeh Abbaszadeh from Iranian research organization for science and technology institute (IROST) for his guidance in methodology.

Authors' Contribution **Mahdi Rashvand:** Writing- original draft, Methodology, Software. **Giuseppe Altieri:** Investigation, Validation. **Mehrad Nikzadfar:** Investigation, Writing- review & editing. **Attilio Matera:** Visualization, Writing- review & editing. **Francesco Genovese:** Visualization, Writing- review & editing. **Aberham Hailu Feyissa:** Investigation, Writing- review & editing. **Giovanni Carlo Di Renzo:** Project administration, Supervision.

Funding Open access funding provided by Università degli Studi della Basilicata within the CRUI-CARE Agreement. This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Data Availability Statement The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

Conflict of Interest The authors have declared no conflicts of interest in this article.

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