

Article

Gelatine-Based Biopolymer Film Produced from Ozone-Treated Film-Forming Solutions Containing Whey Protein Concentrate: Effects on Physical, Mechanical, and Thermal Characteristics

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Abstract: This study aimed to determine the effects of the ozone treatment of film-forming solutions (FFSs) containing whey protein concentrate (WPC) and gelatine on biopolymer films' physical, mechanical, and thermal properties. Film samples were produced from a FFS that was ozone-treated at 0 (control), 5, 10, and 30 min. Ozone treatment caused an increase in the pH values of the FFS, whereas the zeta potential remained negative. The films became lighter in colour, slightly greenish, and more opaque with longer ozonation times. The control sample showed the highest thermogravimetric weight loss (92.15%). The weight loss of the samples decreased with increasing ozone treatment time. The application of ozone treatment on the FFS enhanced the films' mechanical properties. Increased ozone treatment time improved the film samples' tensile strength, elongation at break, and toughness values. In conclusion, the results of this study demonstrate that the ozone treatment of FFS containing whey protein concentrate and gelatine can significantly enhance the physical, mechanical, and thermal properties of biopolymer films. These results highlight the potential of ozone treatment as a viable method for improving the performance and quality of biopolymer films used in food packaging, offering promising advantages for sustainable and environmentally friendly packaging solutions.



Citation: Mercan, E. Gelatine-Based Biopolymer Film Produced from Ozone-Treated Film-Forming Solutions Containing Whey Protein Concentrate: Effects on Physical, Mechanical, and Thermal Characteristics. *Appl. Sci.* **2024**, *14*, 2176. <https://doi.org/10.3390/app14052176>

Academic Editor: Nikolaos Kopsahelis

Received: 3 February 2024

Revised: 28 February 2024

Accepted: 4 March 2024

Published: 5 March 2024



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Keywords: ozone treatment; whey protein concentrate; edible film; biopolymer film production

1. Introduction

Due to economic constraints, environmental issues and consumer health concerns, biopolymer film is gaining popularity as it has the potential to partially replace traditional synthetic material packaging. Most biopolymer films can protect the packaged product against external influences. Based on their properties, biopolymer films can act as barriers to impede the movement of flavour components, moisture, and gases like oxygen and carbon dioxide.

Renewable components (proteins, polysaccharides, and lipids) can be used to produce biopolymer films. Proteins are frequently utilized in the production of biopolymer films. This is due to their abundance, capacity to form films, and possession of distinctive film properties. Regarding mechanical and barrier characteristics, films made from proteins are often superior to those made from polysaccharides and lipids [1].

Due to their suitable film-forming qualities for producing bio-packaging materials, whey protein isolate (WPI, which contains over 90% protein content) [2], whey protein concentrate (WPC, which contains 35–80% protein content) [3,4], whey powder, ref. [5] and gelatine [6] have been investigated as potential film-forming agents. WPC and WPI have various levels of lipids, carbohydrates (primarily lactose), vitamins, minerals, and different protein concentrations. These differences lead to different molecular structures, which means that films made from WPI or WPC have different properties [7].

Biopolymer films which have low permeability to oxygen have been produced from whey protein (WP), despite their higher permeability to water vapour and poorer ten-

sile strength and elongation at break when compared to plastic films like high-density polyethylene and low-density polyethylene [8].

For use in food packaging, biopolymer films developed through the modification of WP have demonstrated improved physical, optical, morphological, mechanical, and barrier characteristics compared to native WP films. Previous research has indicated the application of some methods that modify whey protein structure to produce modified biopolymer films, such as chemical treatments using carbonyldiimidazole, glutaraldehyde, and formaldehyde [9], the addition of transglutaminase [10], the application of γ -irradiation [11], sonication treatment [12], and UV application [8].

Ozone (O_3) is a form of oxygen that has strong oxidising properties. It naturally decomposes and releases the third atomic oxygen. O_3 is considered safe for use and has been approved by the Food and Drug Administration (FDA) as an antimicrobial ingredient for food processing [13]. Ozone treatment (OT) reduces food production costs without having a hazardous impact on the environment as it prevents hazardous residues from remaining on food or in areas where food is processed. Excess ozone rapidly breaks down into oxygen without causing further pollution [14].

OT provides a broad spectrum of disinfection by increasing the oxidation potential. Bacterial cells, spores, viruses, and fungi can be inactivated and botulinum toxin eliminated during the OT process [15]. In addition to its antimicrobial properties, OT has the potential to alter the structure and functionality of proteins due to its high oxidative potential. Previous studies have focused on the impacts of OT on the chemical, structural, and functional properties of WP [16,17]. The authors specifically reported that WP's structure, solubility, and emulsifying and foaming characteristics were modified by ozone.

The impacts of ozone treatment in film production have been studied in both potato starch [18] and cassava starch [19,20]. In most instances, it was reported that ozone treatment modified the appearance and mechanical characteristics of the films.

Biopolymer films should be suitable to ensure food safety as a food packaging material. Biopolymer films have certain drawbacks compared to synthetic polymers, including less resistance to gas and liquid migration, as well as decreased mechanical strength, particularly in terms of elongation [21]. Biopolymer-based products can be improved with a variety of physical and chemical adjustments (pH changes, heat denaturation, solvent changes, mixing, and cross-linking), as well as new processing methods. Specifically, using advanced methods in the filmmaking process—like ultrasound, ohmic heating, UV light, and ozone treatment—has shown to be an effective alternative for enhancing the properties and composition of biopolymer films and coatings [22].

Gelatine films have been applied to a wide range of food products for shelf-life extension [23,24]. Film production by mixing gelatine and whey protein is an effective strategy to overcome current limitations by improving the functional properties of films [25]. Due to the use of emerging technologies and changes in consumers' preferences for safe food, the application of gelatine-based film as an active and smart biopolymer packaging is proposed [26]. Previous studies researched the effects of ozone treatment on the characteristics of gelatine. These studies reported that ozone treatment did not significantly affect the peptide bonds, protein structure, or thermal characteristics [27–29].

Research on the application of ozone to biopolymer films is scarce. To the best of our knowledge, no studies have investigated the use of OT in film-forming solutions (FFSs) containing WPC for the production of films. Therefore, this research represents the first application of ozonation as an emerging technology to FFSs that contain WPC in biopolymer film production. Ozone treatment has significant potential for the modification of protein structure and the improvement of functional properties of WPC [17]. However, the present study focussed on the effect of WPC, since the effect of ozone on gelatine is negligible, as mentioned in the literature. The aim of this research is to develop and characterise the properties of biopolymer films such as the physico-chemical, thermal, and mechanical produced from ozone-treated FFSs containing blends of WPC, gelatine, and glycerol as plasticiser

2. Materials and Methods

2.1. Materials

WPC with 35% protein was provided from Enka Dairy Company (Konya, Turkey). Bovine gelatine (240 Bloom) was obtained from a local market in Konya, Turkey. The supplier was Tito of Smart Chemical Trading and Consulting Limited Company, Izmir, Turkey. Glycerol (85%, analytical grade) was supplied from Merck Chemicals Co. (Darmstadt, Germany).

2.2. Preparation of Film-Forming Solutions

The FFSs were prepared by dissolving 5% (*w/v*) bovine gelatine powder in distilled water at 60 °C with continuous stirring for 15 min. Then, glycerol as a plasticiser was added into the solutions at a concentration of 10% (*w/v*) and mixed homogeneously for 15 min. WPC was added to the solutions at 5% (*w/v*). For stirring, a magnetic stirrer was used.

2.3. Ozone Treatment of Film-Forming Solutions

After preparing the FFSs, the solutions were exposed to an ozone treatment for 5, 10, and 30 min. In each experiment, 100 mL of FFS were exposed to ozone in a 500 mL Erlenmeyer flask. The ozone treatment was conducted using an ozone generator (Mfresh YL-S3500, M Fresh High-Tech Co., Ltd., Beijing, China), which had a 3.5 g/h ozone capacity. After the FFSs were prepared, the necessary analyses were carried out.

2.4. Preparation of Biopolymer Films

After ozone treatment, the FFSs were poured into polythene dishes (d: 11.8 cm) at a density of around 0.38 g/cm² on a dry basis and allowed to dry for 24 h at 24 °C at 50 ± 5% RH to prepare the films. After the drying process was completed, the obtained films were manually detached and employed for subsequent analyses. Films from FFSs that were not ozone-treated were used as a control sample. The films from FFSs exposed to ozone treatment durations of 5, 10, and 30 min were designated as O-5, O-10, and O-30, respectively. Triplicate production were carried out for each film sample.

2.5. Characterisation of Film-Forming Solutions

A pH metre (315i/SET, WTW GmbH, Weilheim, Germany) was used to determine the film-forming solutions' pH. The measurement of pH was performed at room temperature. A dynamic light scattering instrument (Micromeritics NanoPlus-3, Norcross, GA, USA) was used to measure the zeta potential and average particle diameter of FFSs. Particle size and interfacial charge characteristics were measured at a temperature of 40 °C [5]. Triplicate measurements were conducted for these analyses.

2.6. Characterisation of Biopolymer Films

2.6.1. Thickness and Density

The thickness of the films was obtained from measurement with a micrometre (Mitutoyo Corporation, SC-6'', Tokyo, Japan). The overall thickness of each film sample was calculated by averaging the measurements from five randomly selected locations [19]. The density of the film was determined by dividing its weight by its volume. The weight was calculated by multiplying the area of the film by its thickness. The results were grams per cubic centimetre (g/cm³).

$$\rho = \frac{m}{A \times \delta}$$

where A is the film area (cm²), δ the film thickness (cm), m the film weight (g), and ρ the density of the film (g/cm³).

2.6.2. Biopolymer Film Colour

The colour parameters of the films were measured with a colourimeter (Konica Minolta, CR-400, Osaka, Japan). The instrument was calibrated before the measurements, using a

white reference tile with $L^* = 97.10$, $a^* = 4.88$, and $b^* = 7.04$. The colour parameters were expressed on the CIELab scale as L^* , a^* , and b^* , representing lightness (ranging from 0 for black to 100 for white), green to red, and blue to yellow, respectively. The CIELab scale is a standardised method for colour measurement. Each sample was subjected to five measurements. The following equation was used to figure out the total colour change (ΔE):

$$\Delta L^* = L^* - L_0^*$$

$$\Delta a^* = a^* - a_0^*$$

$$\Delta b^* = b^* - b_0^*$$

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

where L_0^* , a_0^* , and b_0^* were the colour values of the control film produced from non-ozonated FFSs [30].

2.6.3. Opacity

To evaluate biopolymer film opacity, a UV–visible spectrophotometer (Biochrom Libra S22, Cambridge, UK) was used. The film was cut into rectangular strips before placement in the test cell of a spectrophotometer. An empty test cell was used to provide a reference for the measurements. In this analysis, triplicate measurements were conducted. The following equation was used to calculate film opacity [31]:

$$O = \frac{Abs_{600}}{x}$$

where Abs_{600} stands for the absorbance at 600 nm, while x represents the thickness of the film in millimetres. A higher O value indicates a less transparent and more opaque object.

2.6.4. Contact Angle

The surface hydrophilicity of the biopolymer films was evaluated through contact angle analyses, performed at room temperature (24 ± 2 °C), using the sessile drop technique (Attension-Theta Lite, Biolin Scientific, Espoo, Finland), as described in a previously published study [5]. Three measurements were applied for contact angle analysis.

2.6.5. Thermo-Gravimetric Analysis (TGA)

TGA was carried out using a TGA instrument (Setaram LabSys Evo, Caluire, France). Thermal characteristics were evaluated by scanning films from 25 to 900 °C at a rate of 10 °C/min under a nitrogen flow of 20 mL/min [5]. Duplicate measurements were carried out.

2.6.6. Mechanical Characteristics

The mechanical characteristics of the biopolymer films were determined using a Texture Analyser TAXT-plus (Stable Micro Systems, Surrey, UK) equipped with tensile grips (A/MTG, Stable Micro Systems). As specified by ASTM D882-02, the films were slit into strips 10 mm wide and 80 mm long [32]. During analysis, the crosshead speed and the initial grip separation were set to 60 mm/min and 50 mm, respectively. Six samples of each film type were examined for the strength at break (tensile strength, TS), elongation at break (percentage of elongation, E%), and toughness.

2.7. Statistical Analysis

For this study's data analysis, a one-way ANOVA was performed. In addition, the post hoc Tukey's test was used to examine statistically significant differences between groups' means. Statistical analyses were performed at a 95% level of confidence. All statistical analyses were conducted in Minitab 18 (Minitab LLC, State College, PA, USA).

3. Results and Discussion

3.1. pH, Zeta Potential, and Average Particle Diameter of Film-Forming Solutions

The pH, zeta potential and average particle diameter of the ozone-treated film-forming solutions (FFSs) are given in Table 1. The pH values of the FFSs ranged from 5.71 to 5.96. Ozone treatment increased the FFSs' pH values ($p < 0.05$). No significant differences were found among samples with O-10 and O-30 ($p \geq 0.05$). Previous research revealed that the pH of FFSs influences films' tensile strength, colour, transparency, and water vapour permeability [33,34]. The stability of films can be affected by the pH of solutions of whey protein films plasticised with glycerol. When pH falls below the isoelectric point, film formation deteriorates since the SH group's reactivity decreases significantly [35]. Previous research reported that films at low pH and high pH are characterised by more brittle and rubber-like film formation, respectively [36,37].

Table 1. pH, zeta potential, and average particle diameter parameters of the ozone-treated film-forming solutions (FFSs).

Sample	pH	ζ -Potential (mV)	Average Diameter (nm)
Control	5.71 $\pm$ 0.01 c	−0.92 $\pm$ 0.03 a	684 $\pm$ 19.8 ab
O-5	5.85 $\pm$ 0.01 b	−1.24 $\pm$ 0.04 b	727.5 $\pm$ 27.6 a
O-10	5.95 $\pm$ 0.02 a	−0.99 $\pm$ 0.01 a	593.0 $\pm$ 9.9 c
O-30	5.96 $\pm$ 0.03 a	−0.88 $\pm$ 0.03 a	638.5 $\pm$ 16.3 bc

Data are the mean $\pm$ standard deviation ($n = 3$). Different letters within the same column show the significant differences between the samples ($p < 0.05$). Control: non-ozonated FFS; O-5: ozone-treated FFS for 5 min; O-10: ozone-treated FFS for 10 min; O-30: ozone-treated FFS for 30 min.

The zeta potential is calculated by measuring the response of charged particles to an electric field. When subjected to a continuous electric field, particles move at a constant velocity. Using this velocity, the charge and the zeta potential can be calculated [38]. While some samples showed statistically significant differences in the determined zeta potential values ($p < 0.05$), it is essential to note that the results were numerically very similar. The results indicate that the zeta potential values for all the samples were negative, suggesting that the particles' surfaces carried a negative charge. Jiang et al. [39] reported negative zeta potentials of WPC samples and suggested that the protein composition and applied treatments may influence these potentials.

The average particle diameters of the FFSs varied from 593.0 to 727.5 nm, where ozone treatment considerably affected them ($p < 0.05$). The average particle diameters of the O-10 and O-30 samples were lower than the control, whereas those of O-5 were higher than the control. The larger particle diameter of O-5 might be due to the lower zeta potential. Sert et al. [5] reported that the average particle diameters of FFSs from whey powders at various demineralisation ratios ranged from 1569.95 to 2989.25 nm. The smaller average particle size could indicate the increased molecular interactions [40], and therefore increased film strength, which is in agreement with the results found in this work.

3.2. Thickness, Density, and Contact Angle Parameters of Films

Table 2 shows the thickness, density, and contact angle parameters of the films produced from ozone-treated FFSs. The ozone treatment did not significantly affect the thickness ($p \geq 0.05$). The film samples had a thickness ranging from 0.31 to 0.38 mm. Due to the insignificant differences in the thickness of the films, the mechanical and colour properties could be determined without the effect of thickness [19].

The density of the film samples was between 1.00 and 1.13 g/cm³. The density of films from ozone-treated FFSs was lower than the control sample ($p < 0.05$). However, similar density values were observed between the O-10 and O-30 samples ($p \geq 0.05$).

Table 2. Thickness, density, and contact angle parameters of biopolymer films produced from the ozone-treated film-forming solutions (FFSs).

Sample	Thickness (mm)	Density (g/cm ³)	θ_{60s} (°)
Control	0.31 ± 0.01 ns	1.13 ± 0.04 a	104.30 ± 0.99 ns
O-5	0.30 ± 0.02	1.00 ± 0.02 b	101.54 ± 2.06
O-10	0.34 ± 0.01	1.04 ± 0.02 ab	101.10 ± 1.56
O-30	0.38 ± 0.03	1.07 ± 0.03 ab	96.92 ± 2.72

Data are the mean ± standard deviation ($n = 3$). Different letters within the same column show the significant differences between the samples ($p < 0.05$). Control: biopolymer film from non-ozonated FFS; O-5: biopolymer film from ozone-treated FFS for 5 min; O-10: biopolymer film from ozone-treated FFS for 10 min; O-30: biopolymer film from ozone-treated FFS for 30 min.

The water resistance of biopolymer films is crucial for preserving food when coated items come into contact with water [41]. The contact angle (CA) is the angle between the tangent line touching the water drop at the point of contact and the baseline of the film surface (Ojagh et al. [42]; Khazaei et al. [43]). The contact angle (CA) parameter indicates a surface's hydrophobicity level. A high CA indicates surface hydrophobicity, while a low CA indicates surface hydrophilicity (Karbowski et al. [44]). According to Ma et al. [45], a material is called “hydrophilic” if the CA is smaller than 90° and “hydrophobic” if the CA is greater than 90°. The CA values of the samples varied from 96.92 to 104.30°; the increased ozonation time decreased this value. The CA values obtained were similar to those of gelatine-based edible films [46,47]. According to the results of the CA analysis, all samples showed hydrophobic characteristics. Film affinity with water may or may not be desirable. Potential applications may require water insolubility to improve product integrity and water resistance [22].

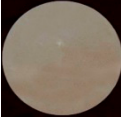
All film samples produced in this study were hydrophobic because the CA values of films were higher than 90°. Although there was a significant difference between samples, the results revealed that the hydrophobicity of samples was decreased depending on the ozonation time. Similar to this study, a previous study reported that cassava starch ozonation reduced the biopolymer films' CA values [20].

3.3. Colour Characteristics and Opacity Parameter of Biopolymer Films

Table 3 presents the colour characteristics and opacity of biopolymer films produced from ozone-treated FFSs. Ozone treatment significantly affected all colour parameters and the opacity of films ($p < 0.05$). One of the most important factors in edible films developed for food products is colour [48].

The L^* values of the films ranged from 80.10 to 89.77; ozone treatment increased this value depending on the treatment time. The highest a^* value was determined in the control film, and ozone treatment significantly decreased the a^* value. However, similar a^* values were obtained in the films from ozone-treated WPC ($p \geq 0.05$). On the contrary, ozone treatment increased the b^* value of the films depending on the increasing treatment time. Hence, the films became slightly greenish ($-a^*$), yellowish ($+b^*$), and lighter ($+L^*$) with the increased ozone treatment of the WPC. Similar to the present study, Sert and Mercan [49] concluded that ozone treatment increased the lightness, yellowness, and greenness of the whey powder samples. This situation might be due to the carotenoid degradation in WPC depending on ozone treatment, which could cause a higher L^* value and lower a^* value in the samples [50]. Moreover, when milk-based products are utilised, the colour values are frequently impacted in this manner. A previous study reported that the colour differences in gelatine films with kefir addition were similar to the present study [51]. The colour difference (ΔE^*) indicates the degree of the total colour difference from the control sample. The ozone treatments of the FFSs increased the ΔE^* value compared to the control. The highest ΔE^* was determined in the O-30.

Table 3. Colour characteristics and opacity parameter of biopolymer films produced from the ozone-treated film-forming solutions (FFSs).

Sample	Colour				Opacity	Photograph
	L^*	a^*	b^*	ΔE^*		
Control	80.10 $\pm$ 0.14 c	2.01 $\pm$ 0.02 a	7.58 $\pm$ 0.11 b	0	0.990 $\pm$ 0.014 c	
O-5	81.28 $\pm$ 0.40 c	1.88 $\pm$ 0.03 b	7.96 $\pm$ 0.09 b	1.25 $\pm$ 0.24 c	0.107 $\pm$ 0.005 d	
O-10	83.90 $\pm$ 0.42 b	1.87 $\pm$ 0.05 b	8.46 $\pm$ 0.01 a	3.91 $\pm$ 0.57 b	1.120 $\pm$ 0.007 a	
O-30	89.77 $\pm$ 0.45 a	1.77 $\pm$ 0.02 b	8.70 $\pm$ 0.13 a	9.74 $\pm$ 0.59 a	1.027 $\pm$ 0.005 b	

Data are the mean $\pm$ standard deviation ($n = 3$). Different letters within the same column show the significant differences between the samples ($p < 0.05$). Control: biopolymer film from non-ozonated FFS; O-5: biopolymer film from ozone-treated FFS for 5 min; O-10: biopolymer film from ozone-treated FFS for 10 min; O-30: biopolymer film from ozone-treated FFS for 30 min.

The opacity of biopolymer films used in food packaging is crucial; films with higher opacity can restrict light transmission [52]. The opacity of film samples ranged from 0.107 to 1.120. The ozone treatment of the FFSs increased the opacity of the samples depending on the increasing treatment time. This could be attributed to the protein development in WPC by ozone treatment, which formed a denser network structure. Hence, the denser the cross-linking of disulphide bonds, the stronger the ability of the films to act as a barrier to UV light [53]. This was coherent with the higher opacity of gelatine film produced from ozone-treated FFSs containing WPC. A higher opacity of films depending on the ozone treatment could protect packaged foods from light. The exposure of foods to light can alter their sensory and nutritional properties due to photocatalytic reactions that produce activated free radicals [54]. Therefore, this situation was advantageous for food packaging.

3.4. Thermal Characteristics of Films

A thermogravimetric analysis was carried out to characterise the structural differences and thermal stability of films produced from ozone-treated FFSs containing WPC. The thermal degradation temperatures and weight loss (%) of gelatine-based biopolymer films from ozone-treated FFSs are shown in Table 4. The onset degradation temperature of samples ranged from 152.05 to 158.12 °C. The control sample had the lowest onset temperature, whereas O-30 had the highest one. Ozone treatment increased the degradation starting temperature depending on the increasing ozonation time. The degradation of the ozone-treated samples at higher temperatures was lower as compared to the control. The highest maximum degradation temperature (238.50 °C) was observed in the control. The maximum degradation temperature of the ozone-treated samples was lower than the control. However, ozone treatment increased the maximum degradation temperature among the ozone-treated samples. According to the results, a slightly higher temperature was necessary to decompose the control sample. The formation of covalent cross-links and hydrophobic interactions between different protein components induced by ozone treatment may be the main contributor to this increase in thermal stability [55]. The highest weight loss (93.56%) was determined in the control film. The weight loss of films from the

ozone-treated FFSs was between 89.78 and 92.15%. The ozone treatment of the FFSs decreased the weight loss of the film samples. This is thought to be beneficial for biopolymer film technologies. Previous studies have reported similar values for weight loss [5,51].

Table 4. Thermal characteristics of biopolymer films produced from the ozone-treated film-forming solutions (FFSs).

Sample	Onset Temp. T_0 (°C)	Degradation Temp. T_{max} (°C)	Weight Loss (%)
Control	152.05	238.50	93.56
O-5	154.62	209.20	92.15
O-10	156.78	214.50	90.35
O-30	158.12	227.65	89.78

Data are the mean ($n = 3$). Control: biopolymer film from non-ozonated FFS; O-5: biopolymer film from ozone-treated FFS for 5 min; O-10: biopolymer film from ozone-treated FFS for 10 min; O-30: biopolymer film from ozone-treated FFS for 30 min.

3.5. Mechanical Characteristics of Biopolymer Films

The mechanical properties of biopolymer films must meet a specific strength requirement for their use as packaging. Table 5 presents the mechanical characteristics of biopolymer films produced from the ozone-treated film-forming solutions (FFSs). The ozone treatment of the FFSs significantly affected the tensile strength, toughness, and breaking strain of the films ($p < 0.05$). The highest tensile stress that a film can withstand during testing without rupturing is referred to as its tensile strength. This characteristic affects whether edible coatings are suitable for use as packaging material [51]. Tensile strength is of great interest because maintaining the film's integrity is crucial to food quality and safety [56]. The tensile strength of films from ozone-treated FFSs ranged from 0.986 to 1.130 MPa, where ozone treatment increased this value. The tensile strength of the control sample was the lowest at 0.986 MPa, whereas the O-30 sample exhibited the highest tensile strength at 1.130 MPa. The observed values may be attributed to the highest stability and integrity of the films. The trend indicates that the use of ozone treatment has a considerable effect on improving the tensile strength of biopolymer films. One of the potential causes behind the improved tensile strength is the formation of cross-links between the polymer chains in the FFS. Ozone treatment can introduce oxygen-containing functional groups into the polymer matrix, facilitating cross-link formation. These cross-links are physical bridges between polymer chains, making the film more cohesive and structurally robust. Izzi et al. [57] reported that the increase of tensile strength related to the formation of intermolecular hydrogen bonds. Deng et al. [53] revealed that the disulphide bond cross-linking of gelatine can significantly improve the mechanical strength of films.

Table 5. Mechanical characteristics of biopolymer films produced from the ozone-treated film-forming solutions.

Sample	Tensile Strength (MPa)	Elongation at Break "Breaking Strain" (%)	Toughness (MJ/m ³)
Control	0.986 ± 0.005 c	139.72 ± 1.80 b	0.480 ± 0.006 c
O-5	1.093 ± 0.009 b	143.16 ± 1.19 b	0.566 ± 0.008 b
O-10	1.110 ± 0.007 ab	156.62 ± 2.29 a	0.588 ± 0.003 ab
O-30	1.130 ± 0.008 a	162.21 ± 2.53 a	0.611 ± 0.008 a

Data are the mean ± standard deviation ($n = 3$). Different letters within the same column show the significant differences between the samples ($p < 0.05$). Control: biopolymer film from non-ozonated FFS; O-5: biopolymer film from ozone-treated FFS for 5 min; O-10: biopolymer film from ozone-treated FFS for 10 min; O-30: biopolymer film from ozone-treated FFS for 30 min.

Elongation at break measures the ability of biopolymer films to stretch prior to breaking. A higher elongation at break value indicates that the film is more flexible and stretchable. The high elasticity of the films is advantageous as it facilitates their use as coatings.

The elongation at break of the samples was between 139.72 and 162.21%. The data show that the ozone treatment of the FFSs for 10 and 30 min produced biopolymer films with higher elongation at break values compared to the control and O-5. The extended ozone treatment time significantly improved the flexibility of the biopolymer films. The more prolonged ozone treatment likely promoted the formation of additional intermolecular interactions and cross-linking within the film matrix. These interactions can enhance the film's structural integrity and flexibility, allowing it to stretch further before breaking. The longer treatment time may have also led to some chain scission or rearrangement, resulting in a more elastic film. The obtained values for elongation at break in this study were significantly higher than those in films from WPC observed by Guimarães et al. [58,59] (around 50 and 43%, respectively). In addition to the ozone treatment's effect on elongation at break, when gelatine is used with glycerol in composite film formulations, it may serve as a "co-plasticizer", increasing the flexibility and reducing the fragility of the films [57].

The toughness of a material is a mechanical property that describes its ability to absorb energy without fracturing. It is a measure of the material's resistance to crack propagation and is often used to characterise materials that undergo significant deformation before failure [60]. The toughness of the biopolymer films increased with increasing ozone treatment time. The control sample had the lowest toughness value (0.480 MJ/m^3), while the O-30 sample (ozonated for 30 min) had the highest toughness value (0.611 MJ/m^3). The formation of cross-links between polymer chains can enhance the resulting film's mechanical strength, rigidity, and toughness. Therefore, the observed increase in toughness with longer ozone treatment durations aligns with increased cross-linking expectations. A higher value of toughness indicates a film that can absorb more energy before it breaks, providing better protection for the food product. Proteins were found to have an important role in the strength and flexibility of films because they engage with surrounding molecules via various types of linkages, generating films with strength, cohesion, and viscoelasticity [61].

Overall, the results indicate that treating film-forming solutions with ozone positively impacts the mechanical properties of biopolymer films. The films showed higher tensile strength, improved flexibility (higher elongation at break), and increased toughness as the ozone exposure time increased. The improved mechanical properties have the potential to create more durable and flexible biopolymer films that can be used in a variety of food and packaging industries. The data show that using ozone to treat film-forming solutions could be a potential approach to improve the mechanical properties of biopolymer films, especially their tensile strength. In conclusion, the fabricated biopolymer films produced by the ozone-treated FFSs containing WPC provide good mechanical properties to cope with external forces and can meet the practical application requirements for food packaging films.

4. Conclusions

This study aimed to investigate the impact of the ozone treatment of film-forming solutions (FFSs) on the quality characteristics of biopolymer films containing whey protein concentrate (WPC). The study's findings demonstrated the significant impact of ozone treatment on various features of the biopolymer films. The treatment of FFSs with ozone resulted in an increase in pH values and average particle diameter. In contrast, the zeta potential remained negative. The proposed modifications may affect film formation and stability, resulting in improved film characteristics. Changes in the colour characteristics and opacity were observed in the ozone-treated films. The films became lighter in colour, slightly greenish, and more opaque as the ozonation time increased. These changes may affect the UV protection and visual appearance of food packaging. The application of ozone treatment resulted in improvements to the thermal stability and mechanical properties of the films. Increased ozone treatment time improved the films' tensile strength, elongation at break, and toughness. This suggested that the films demonstrated enhanced strength, flexibility, and energy absorption capacity prior to fracturing. The potential modification of WPC and gelatine was significant in the improvement of the films.

To conclude, the findings indicate that the ozone treatment of FFSs which contain WPC can potentially improve the quality characteristics of biopolymer films utilised in food packaging. These films might enhance mechanical performance and thermal stability, and provide UV protection, making them appropriate for various food packaging applications.

One of the limitations of this study is that it focused only on the effect of ozone treatment on film properties without considering its effects on food preservation or shelf life. Future research could investigate the combined effects of ozone treatment and food packaging on food quality and safety. Furthermore, investigating these ozone-treated biopolymer films' long-term stability and environmental impacts would provide valuable information on their feasibility and sustainability as packaging materials. Furthermore, optimizing ozone treatment process parameters such as concentration and duration could further improve film properties and potential cost savings in production. Therefore, future research efforts should address these limitations and optimize the ozone treatment process to develop more sustainable and environmentally friendly packaging solutions.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data are available in the article.

Conflicts of Interest: The author declares no conflicts of interest.

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