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The Optimization of Gelatin Extraction from Chicken Feet and the Development of Gelatin Based Active Packaging for the Shelf-Life Extension of Fresh Grapes

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Abstract: Synthetic plastics are causing serious environmental and health problems due to which the concept of developing biodegradable food packaging has gained considerable attention. In this study, extraction of gelatin from chicken feet was optimized followed by characterization of gelatin. Chicken feet gelatin was used to develop biodegradable nanocomposite films by the incorporation of chitosan (CS) and zinc oxide (ZnO) nanoparticles (NPs). Gelatin nanocomposite films were used to increase the shelf-life of fresh grapes by determining the browning index, weight loss, and microbial profile of fresh grapes. A high yield (7.5%) of gelatin and Bloom strength (186 g) were obtained at optimized extraction conditions (pretreatment with 4.2% acetic acid and extraction at 66 °C for 4.2 h). Electrophoretic analysis of gelatin revealed the presence of α (130–140 kDa) and β chains (195–200 kDa), whereas a Fourier transformed infrared (FTIR) spectrometer confirmed the presence of amide A and B and amide I, II, and III. Incorporation of ZnO NPs in a gelatin–CS matrix improved the barrier and the mechanical and the thermal properties of films. Gelatin nanocomposite films with 0.3% ZnO NPs significantly reduced the weight loss (23.88%) and the browning index (53.33%) of grapes in comparison to control treatments. The microbial count in artificially inoculated grapes wrapped in gelatin nanocomposite films remained below 4 log CFU/mL until the fifth storage day in comparison to control treatments. The gelatin from poultry byproducts such as chicken feet can serve as an efficient biopolymer to develop biodegradable food packaging to enhance the shelf-life of perishable food products.

Keywords: gelatin; chicken feet; biodegradable packaging; nanocomposite films; shelf-life

1. Introduction

Gelatin is a natural biopolymer derived from the partial hydrolysis of collagen, which is a major component of skin, bones, and connective tissues [1]. The low melting point and the high gel strength are the key characteristics of gelatin, which make it preferable for food and packaging applications as compared to other plant-based gelling agents [2].

Commonly used gelatins in food exhibit a Bloom value in the range of 125–250 g [3]. Gelatin is recently gaining importance due to its nutritional value and its film forming ability [4]. Poultry processing industries produce a variety of byproducts such as feathers, internal viscera, and chicken feet. Chicken feet are a predominant poultry byproduct, and globally 3.9 million metric tons of chicken feet are produced annually by poultry processing industries [2]. Chicken feet are rich in collagen, which is an excellent source of gelatin, and it is used in food and pharmaceutical products [5]. The use of chicken feet as a raw material for the extraction of gelatin will not only benefit the poultry industry, but it will also lead toward the production of a relatively cheap and widely accepted alternative to mammalian gelatin [6].

Recently, there has been an increasing demand for fresh, safe, and healthy food by consumers. However, most of the fresh food deteriorates during post-harvest handling, packing, and shipping [7]. Synthetic, plastic-based packaging is frequently used to extend the shelf-life of fresh produce. Plastic-based packaging is associated with various environmental and health hazards. Global plastic production reached 380 million metric tons in 2015 and 40% of this plastic production accounted for packaging materials. Approximately 60% of plastic-based packaging material is used for the food sector [8]. Natural polymers, such as gelatin and CS can be used to develop biodegradable packaging to preserve fresh produce [9]. The mechanical and the preservation properties of gelatin based packaging can be improved by the incorporation of other polymers such as CS and metallic NPs [10]. CS is a non-toxic polysaccharide which exhibits good film forming capacity, biodegradability and mechanical and barrier properties [11]. The addition of silver NPs to gelatin composite films has been reported to improve the barrier properties [12]. Incorporation of ZnO NPs improved the barrier properties and the antimicrobial effect of gelatin based food packaging systems; moreover, ZnO NPs were reported to be safe by the Food and Drug Administration (FDA) [1]. The aim of this study was to develop gelatin based active packaging with improved barrier properties. The extraction of gelatin from chicken feet was optimized and chicken feet gelatin composite films reinforced with ZnO NPs were developed to extend the shelf-life of fresh grapes.

2. Materials and Methods

2.1. Materials

Fresh chicken feet were obtained from the local market in the Lahore district of Pakistan and then transported to a laboratory in an ice box. The feet were de-nailed manually, washed with tap water, and stored at −20 °C until further use. Acetic acid and commercial gelatin (bovine source) were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. Extraction of Gelatin from Chicken Feet

The chicken feet were deboned and cut into small pieces (5 cm), followed by grinding by a mincer (Philips, Cucina Series, Shanghai, China). The minced chicken feet were demineralized with 0.5 M sodium hydroxide (1:10, w/v) for 20 h at room temperature, followed by filtration with muslin cloth to remove alkaline material [2]. The residue was washed repeatedly with distilled water until the pH became neutral. The excess water was drained with the help of a muslin cloth. The drained residue was treated with acetic acid (1:1, w/v) for 24 h, separated by filtration, and washed several times with water until a neutral pH. Finally, the residue was soaked in water (1:2, w/v) and extraction was carried out in a water bath (WiseBath, WITEG Labortechnik, Wertheim am Main, Germany) at different temperatures (50, 60, and 70 °C) and different time durations (4, 6, and 8 h), separately [13]. After extraction, the solution was filtered through muslin cloth and Whatman filter paper #4, respectively, followed by freeze drying (Christ Alpha 1-2 LD plus, Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany) to obtain gelatin powder.

Response surface methodology (RSM) was used to optimize the acid pretreatment and gelatin extraction by using independent variables such as acetic acid concentration (3, 4, and 4.5%), extraction temperature (50, 60, and 70 °C), and extraction time (4, 6, and 8 h), whereas gelatin yield and Bloom strength were used as response variables. The Bloom strength of gelatin was determined by following Sarbon et al. [14]. Briefly, 100 mL of 6.67% (*w*/*v*) gelatin solution was prepared in a Bloom jar and allowed to stand for 3 h, followed by stirring with magnetic stirrer for 20 min at 55 °C. The sample was kept at 25 °C for 15 min and finally placed in refrigerator (5–7 °C) for 18 h to allow the maturation of the gel. The gel was tested on a texture analyzer (IMADA, Toyohashi, Japan) by penetration with a standard cylinder probe to a depth of 4 mm at 1.0 mm/s. The Bloom jar with the gel was placed under the plunger and the maximum resistance to force was recorded as the Bloom strength (g) of the gel.

2.3. Characterization of Gelatin

Proximate analysis of gelatin was carried out for protein content (AOAC 981.10), ash (AOAC 942.05), moisture (AOAC 934.01), and crude fat (AOAC 920.39) by following standard AOAC methods [15]. The emulsifying properties (emulsion stability index and emulsion activity index) and the foaming properties (foam stability and foam capacity) of chicken feet gelatin were evaluated by using a standard method [2,6]. FTIR spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used to determine the chemical finger printing of gelatin. The scans were made in the range of 650–4000 cm^{-1}. SDS-PAGE analysis of gelatin was performed by following the method of Laemmli [16], with slight modifications. The gelatin samples (10–40 µg) were subjected to electrophoresis by using 7.5% (*v*/*v*) running gel and 4% (*v*/*v*) stacking gel at 120 V for 2 h.

2.4. Preparation of Gelatin/Chitosan/ZnO Composite Films

CS, gelatin, and ZnO nanoparticles (NPs) based composite films were developed by the solution casting method, following Kumar et al. [17], with slight modifications. Homogenized aqueous solution of chicken feet gelatin (4%, *w*/*v*) was prepared by continuous stirring, followed by the addition of glycerol (30% based on the gelatin weight). The CS solution (2%, *w*/*v*) was prepared in glacial acetic acid (1%, *v*/*v*) by using a magnetic stirrer at 25 °C for 1 h. Based on the prior optimization process, film forming solution (FFS) was formulated by mixing CS and gelatin solutions in a 1:2 ratio and homogenized by continuous stirring. ZnO NPs were added into the FFS solution at 0% (control), 0.2%, and 0.3%, respectively. FFS was sonicated (Heidolph, Schwabach, Germany) for 10 min to remove air bubbles and casted on polystyrene petri plates followed by drying in the oven (Memmert, ULM 500, Schwabach, Germany) at 35 °C for 48 h. After drying, films were peeled off and stored in zip lock bags at 25 °C and 70% relative humidity until further use.

2.5. Characterization of Chitosan-Gelatin Composite Film

2.5.1. Film Thickness and Total Soluble Matter

A micrometer (model ID-C112PM, Mitutoyo, Japan) was used to measure the thickness of the films at five different points, and average values were reported. The total soluble matter (TSM) of gelatin films was determined by following Nilsuwan et al. [18], with slight modifications. The film samples were dried to initial constant weight in an oven at 105 °C for 24 h and then immersed into 100 mL of water for 24 h in a shaking incubator (150 rpm) at 25 °C. The films samples were removed from the solution by filtration and dried again at 105 °C for 24 h. The final weight of the film was measured and TSM was calculated by using Equation (1).

$$\text{TSM } (\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Intial weight}} \times 100 \quad (1)$$

2.5.2. Mechanical Properties and Water Vapor Permeability (WVP)

Tensile strength (TS) and elongation at break (EAB) of gelatin composite films were determined by following a standard ASTM D882-09 (American Society for Testing and Materials) method by using a universal testing machine (Model 5565, Instron Engineering Corporation, Canton, MA, USA) in tensile mode. Initial grip separation and mechanical cross head speed were set at 40 mm and 5 mm/min, respectively, with a 50 N load cell. Ten WVP of films was determined by Liu et al. [19]. Briefly films of known weight and thickness were fixed onto the openings of glass cells and then placed in desiccators for 7 days at 25 °C. The bottom of the desiccators was filled with water to ensure 100% relative humidity outside the glass cells. The cells were weighed daily and the WVP was calculated by using Equation (2).

$$WVP = Wxt^{-1}\ A^{-1}\Delta P^{-1} \quad (2)$$

where W = weight gain in grams, x = thickness in mm, t = time in hours, A = permeation area in m^2, and ΔP = vapor pressure difference (3.168 kPa at 25 °C).

2.5.3. Biodegradability

Film samples (2 cm × 2 cm) were weighed and buried 2 cm below the soil (bio-compost fertilizer) contained in steel trays. The trays were then incubated in an oven at 25 °C, with 70% relative humidity. The degradation rate was determined in terms of weight loss by removing the film samples from the soil at various intervals (1, 7, 14, and 21 days), dried at 55 °C for 24 h, and the final weight was recorded [4].

2.5.4. FTIR Analysis

Chemical finger printing of gelatin composite films was determined by FTIR (Agilent Technologies, Santa Clara, CA, USA) in the range of 650–4000 cm^{-1}.

2.5.5. Thermogravimetric Analysis (TGA)

The thermal stability of gelatin composite films was determined by thermogravimetric analyzer (SDT Q600, TA Instruments, New Castle, DE, USA). Films (approximately 5 mg) were heated to 30–600 °C at a rate of 20 °C/min and weight loss was recorded as a function of temperature.

2.5.6. Antimicrobial Activity

The antibacterial activity of the gelatin composite films was evaluated against *Escherichia coli* (ATCC# 8739), *Staphylococcus aureus* (ATCC # 25923), and *Salmonella typhimurium* (ATCC# 14028) by disc diffusion assay. Briefly the bacterial culture (10^6 CFU/mL) was spread on the surface of Muller–Hinton agar (Sigma-Aldrich, St. Louis, MO, USA) plates and gelatin composite film (20 mm discs) were placed on the surface of plates. The plates were then incubated at 37 °C for 24 h and, after incubation, results were recorded as diameter of inhibition zone.

2.6. Preservation Studies

Fresh grapes of approximately the same size, color, and shape were selected for preservation studies. The grapes were washed with sterile water and after drying packed in gelatin–CS composite films containing different concentrations (0.2% and 0.3%) of ZnO NPs. Gelatin, CS, and polythene films were used as controls. The packed grapes were stored at 20 °C and evaluated for weight loss and browning index [10,20]. The weight loss of the grapes was determined after 14 days of storage by using Equation (3).

$$\text{Weight loss } (\%) = \frac{\text{Initial weight} - \text{Final Weight}}{\text{Initial weight}} \times 100 \quad (3)$$

The browning index was determined visually according to the scale, 1 = no browning, 2 = less than 25% browning, 3 = more than 25% browning, 4 = less than 50%, and 5 = more than 50% browning, respectively. The browning index was calculated by using Equation (4).

$$\text{Browning index } (\%) = \frac{1 \times N1 + 2 \times N2 + 3 \times N3 + 4 \times N4 + 5 \times N5}{5 \times N} \times 100 \quad (4)$$

where N = total number of fruits measured and N1, N2, N3, N4, and N5 were the number of fruits showing the different degrees of browning.

Microbiological Analysis

The microbial analysis of grapes was performed by following Mehmood et al. [10], with slight modifications. The grapes were washed twice with sterilized water and dried at room temperature. The grapes were disinfected by UV light for 15 min and dipped in a microbial suspension of *S. aureus* (10^4 CFU/mL) for 2 min. After draining, the fruit samples were kept at 25 °C for 1 h to facilitate drying and bacterial adhesion. The inoculated grapes were wrapped in gelatin composite films and stored at 20 °C ± 2 for 5 days. Neat gelatin, CS and polythene films were used as controls. During storage, everyday grapes from each treatment were homogenized with peptone saline (0.1%, *w*/*v*), subjected to plate count, and results were reported as CFU/g of grapes.

2.7. Statistical Analysis

All experiments were carried out in triplicates, and results are expressed as mean values with standard deviation (±SD) of three replicates. One-way analysis of variance (ANOVA) with Tukey's test was used for characterization of gelatin and packaging films, whereas two-way ANOVA with Tukey's test was used for antimicrobial and preservation assays to determine significant differences ($p < 0.05$) among treatments by using the SPSS statistical software package (SPSS, version 22.0, IBM, Chicago, IL, USA).

3. Results

3.1. Optimized Extraction of Gelatin

By using RSM, gelatin yield and Bloom strength were obtained in the range of 3.5–7.65% and 101.3–186 g, respectively (Table 1). The quadratic model was used for the optimization of gelatin extraction from chicken feet and R^2 values (coefficient of determination) for gelatin yield and gel strength were 0.928 and 0.870, respectively. The acetic acid concentration and extraction time did not show a significant ($p > 0.05$) effect on gelatin yield, whereas the extraction temperature significantly ($p < 0.05$) influenced the yield (Supplementary Materials, Table S1). The extraction time and temperature had a significant effect ($p < 0.05$) on the gel strength of chicken feet gelatin. The optimized extraction conditions were obtained by using the desirability function of RSM as pretreatment with 4.2% acetic acid and extraction at 66 °C for 4.2 h. The optimized extraction resulted in 7.5% gelatin yield and 186 g Bloom strength, which were similar to predicted values of gelatin yield (7.67%) and Bloom strength (187.3 g).

Table 1. Effect of extraction parameters on gelatin yield and gel strength.

No	Acetic Acid (%)	Temperature (°C)	Time (h)	Yield (%)	Gel Strength (g)
1	4.0	50	4	3.50	101.3
2	4.5	70	6	6.34	183.0
3	4.0	60	6	7.65	178.5
4	4.0	70	4	6.77	181.0
5	3.5	50	6	4.0	172.5
6	4.5	60	4	7.35	186.0
7	4.0	60	6	7.54	179.3
8	4.5	60	8	7.40	179.8
9	4.0	60	6	7.50	182.0
10	4.5	50	6	3.53	122.3
11	3.5	70	6	6.45	182.5
12	4.0	50	8	6.75	167.8
13	3.5	60	8	7.30	183.0
14	3.5	60	4	7.55	132.5
15	4.0	60	6	7.6	177.4
16	4.0	60	6	7.65	184.5
17	4.0	70	8	7.52	163.1

3.2. Characterization of Gelatin

3.2.1. Proximate Analysis, Emulsifying and Foaming Properties

In chicken feet gelatin, protein content was 85.62 ± 2.34%, whereas the moisture, ash, and fat contents were 8.93 ± 1.13%, 2.01 ± 0.74%, and 2.99 ± 0.40%, respectively. The chicken feet gelatin had an emulsion activity index and emulsion stability index values of 16.04 ± 1.32 m^2 g^{-1} and 26.36 ± 2.33 min, respectively, whereas the values for foam capacity and foam stability were 197.3 ± 4.73% and 87.8 ± 1.30%.

3.2.2. FTIR and SDS-PAGE Analysis

FTIR spectra of chicken feet gelatin and bovine gelatin are shown in Figure 1a. The secondary structure and functional groups present in chicken feet gelatin were similar to commercial bovine gelatin. Electrophoretic analysis of chicken feet gelatin revealed the presence of α (130–140 kDa) and β chains (195–200 kDa) (Figure 1b).

3.3. Characterization of Gelatin Composite Films

3.3.1. Film Thickness, Biodegradability and Total Soluble Matter

The thickness of the gelatin films increased with an increase in the ZnO NPs concentration (Table 2). The thickness of film with 0.3% ZnO NPs was more (0.143 ± 0.01mm) than the control (without NPs) gelatin and CS films (0.087 ± 0.01 and 0.113 ± 0.03mm, respectively). The thickness of the gelatin nanocomposites films increased due to an increase in the viscosity and the solid content of the FFS. Control gelatin films were completely dissolved in water (100% TSM), whereas TSM was 28.22 ± 2.28% in the control CS films. TSM was significantly decreased when gelatin was blended with CS and NPs (Table 2).

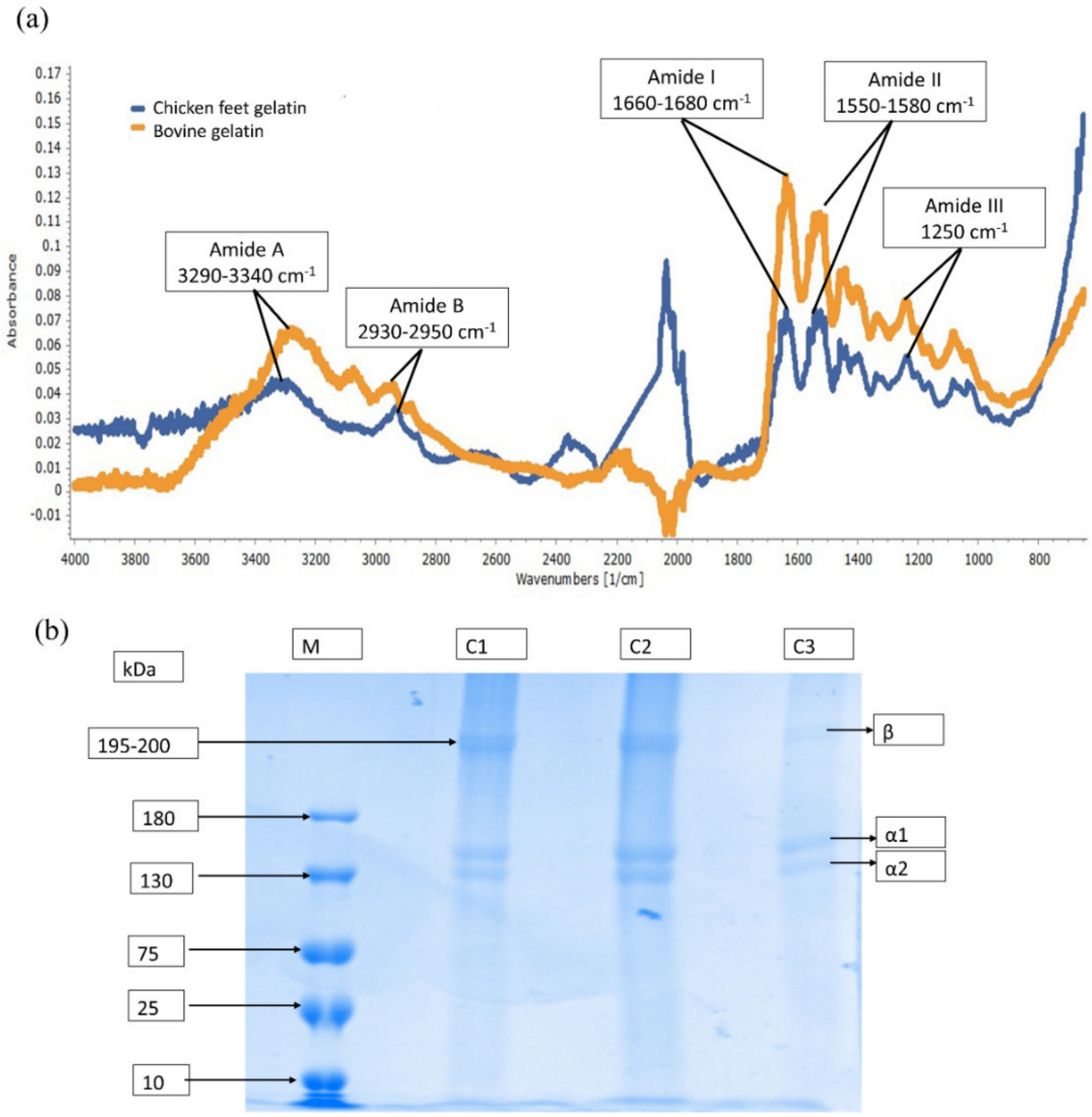

Figure 1. (**a**) FTIR spectra of chicken feet and bovine gelatins; (**b**) SDS-PAGE profile of chicken feet gelatin.

Table 2. Thickness, total soluble matter, water vapor permeability, biodegradability, and mechanical properties of gelatin nanocomposite films.

Films	Thickness (mm)	TSM (%)	TS (MPa)	EAB (%)	WVP (g mm m^{-2} h^{-1} kPa^{-1})	Biodegradability (%)
Gel	0.087 ± 0.01 ^{b}	100% ± 0.0 ^{a}	17.4 ± 0.78 ^{e}	33.97 ± 0.92 ^{a}	0.42 ± 0.01 ^{a}	70.02 ± 2.51 ^{a}
Chi	0.113 ± 0.03 ^{ab}	28.22 ± 2.28 ^{b}	34.26 ± 0.66 ^{a}	20.86 ± 1.06 ^{c}	0.33 ± 0.03 ^{b}	39.66 ± 2.69 ^{b}
Gel + Chi	0.100 ± 0.01 ^{b}	27.72 ± 2.63 ^{b}	27.33 ± 0.80 ^{b}	23.73 ± 0.75 ^{b}	0.30 ± 0.02 ^{bc}	31.97 ± 1.83 ^{c}
Gel + Chi 0.2% ZnO NPs	0.110 ± 0.01 ^{ab}	21.19 ± 2.41 ^{c}	23.76 ± 0.45 ^{c}	18.1 ± 0.40 ^{d}	0.28 ± 0.02 ^{c}	30.63 ± 0.86 ^{c}
Gel + Chi 0.3% ZnO NPs	0.143 ± 0.01 ^{a}	20.86 ± 2.10 ^{c}	21.21 ± 0.48 ^{d}	15.16 ± 0.50 ^{e}	0.20 ± 0.02 ^{d}	19.72 ± 1.42 ^{d}

Different superscript letters ($^{a–e}$) indicate significant ($p < 0.05$) differences among mean observations of three samples. TSM = total soluble matter, TS = tensile strength, EAB = elongation at break, WVP = water vapor permeability.

Significantly high ($p < 0.05$) biodegradability was observed in neat gelatin film (70.02 ± 2.51%) compared to the films containing 0.2% (30.63 ± 0.86%) and 0.3% (19.72 ± 1.42%) ZnO NPs. The degradation rate was high in neat gelatin film due to the hydrophilic nature of the gelatin which resulted in the increased moisture content and elevated the rate of microorganism attack on the film surface during the burial in soil. The inclusion of NPs in gelatin films decreased the moisture content and the microbial activity, which accounted for low biodegradability.

3.3.2. Mechanical Properties and WVP

The neat gelatin film exhibited significantly low TS (17.4 MPa) compared to other composite films. The blending of gelatin with CS significantly increased the TS of the gelatin composite film (27.33 MPa), however incorporation of ZnO NPs into gelatin-CS composite films gradually decreased the TS (27.33 to 21.21 MPa with 0.3% NPs) (Table 2). Neat gelatin film was found with significantly high EAB (33.97%) and WVP (0.42 g mm m^{-2} h^{-1} kPa^{-1}) compared to other composite films.

3.3.3. FTIR and TGA Analysis of Films

The FTIR spectra of the gelatin films are shown in Figure 2a. The gelatin film showed absorption peaks at 3283.1 cm^{-1}, 2938.5 cm^{-1}, 1632.2 cm^{-1}, 1547.6 cm^{-1}, and 1035.1 cm^{-1}. The FTIR spectra of gelatin/chitosan hybrid sample film showed strong absorption at 1552.8 cm^{-1} and 1407.6 cm^{-1}.

TGA thermograms of gelatin composite films are shown in Figure 2b. The thermal degradation of films was categorized into three stages. Initial weight loss of films (30–110 °C) was due to moisture evaporation, followed by degradation in the range of 110–240 °C and 240–400 °C, respectively. The wight loss of neat gelatin film was more than the composite films, which might be due to high moisture content in gelatin films.

3.3.4. Antibacterial Activity

The control gelatin and CS films did not show antibacterial activity against test bacteria. The gelatin nanocomposite films containing 0.3% ZnO NPs showed antibacterial activity against *Salmonella* (33 ± 1.32 mm), *E. coli* (35 ± 1.53 mm), and *S. aureus* (34 ± 2.52 mm). Overall, nanocomposite films with 0.3% ZnO NPs showed high antibacterial activity compared to films with 0.2% NPs (Table S2). However, in this study there was no significant difference between inhibition zones of nanocomposite films against Gram +ve and Gram −ve bacteria. Two-way ANOVA revealed that the association between antibacterial activity and different packaging films was significant; however, there was no significant difference in the inhibition of different bacteria, when subjected to particular antimicrobial film (Table S3).

3.4. Preservation Studies

3.4.1. Weight Loss and Browning Index of Grapes

After 14 days of storage, the weight loss was significantly high in the fruit samples that were unwrapped (70.41%), packed in neat gelatin film (57.41%), CS film (53.97%), and gelatin–CS composite film (50.25%) in comparison to the fruits packed in nanocomposite films containing ZnO NPs (Figure 3a). The grapes packed in gelatin nanocomposite films with 0.3% NPs showed significantly less weight loss (23.88%) after 14 days of storage.

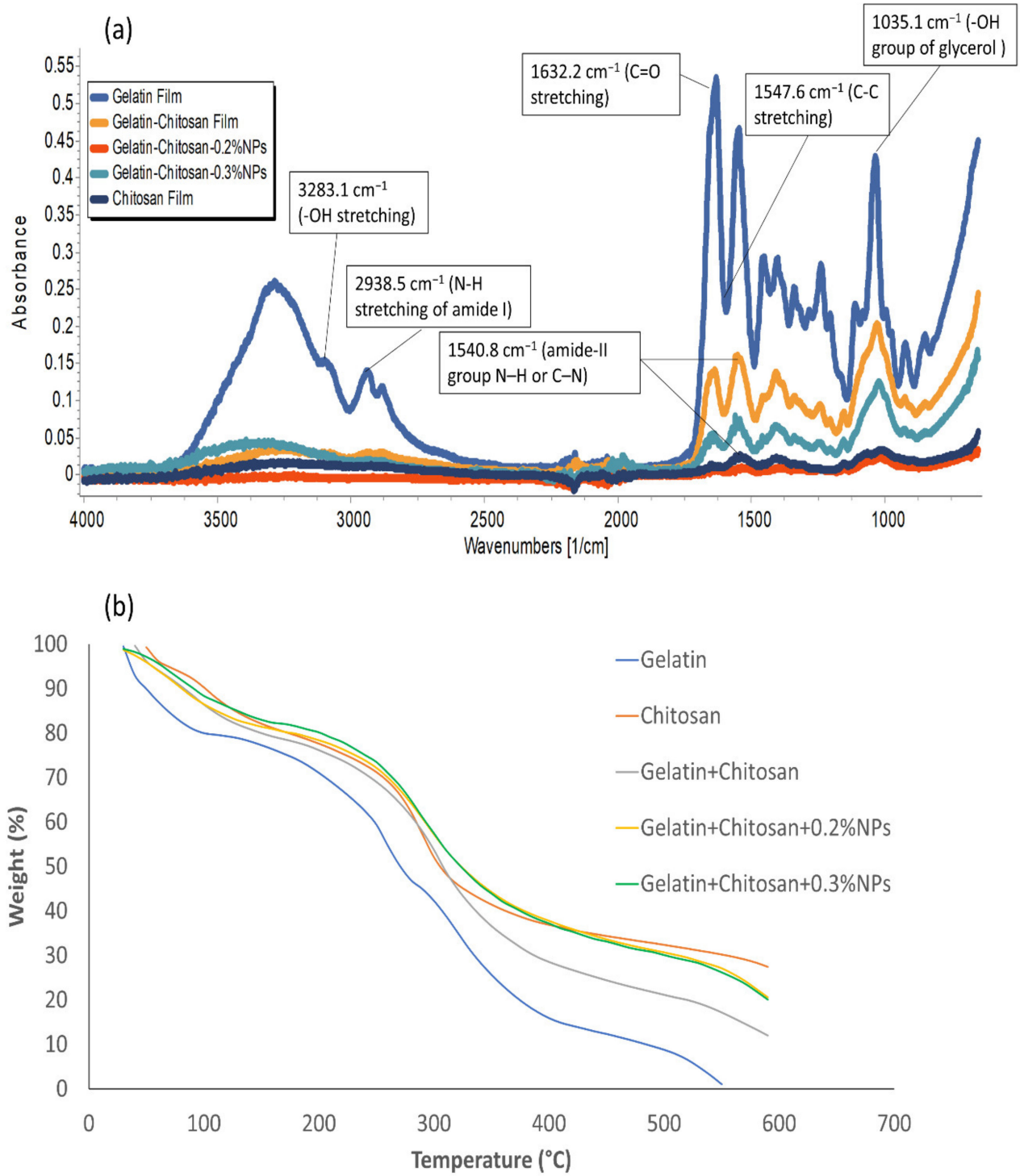

Figure 2. (**a**) FTIR spectra; (**b**) thermogravimetric curves of chicken feet gelatin–chitosan based nanocomposite films.

After 14 days of storage, the highest browning index was observed in the control treatment (unwrapped grapes, 100%), followed by grapes wrapped in plastic and gelatin (97.78%). The lowest browning index was observed in grapes wrapped in nanocomposite film containing 0.3% ZnO NPs (53.33%) after 14 days of storage (Figure 3b).

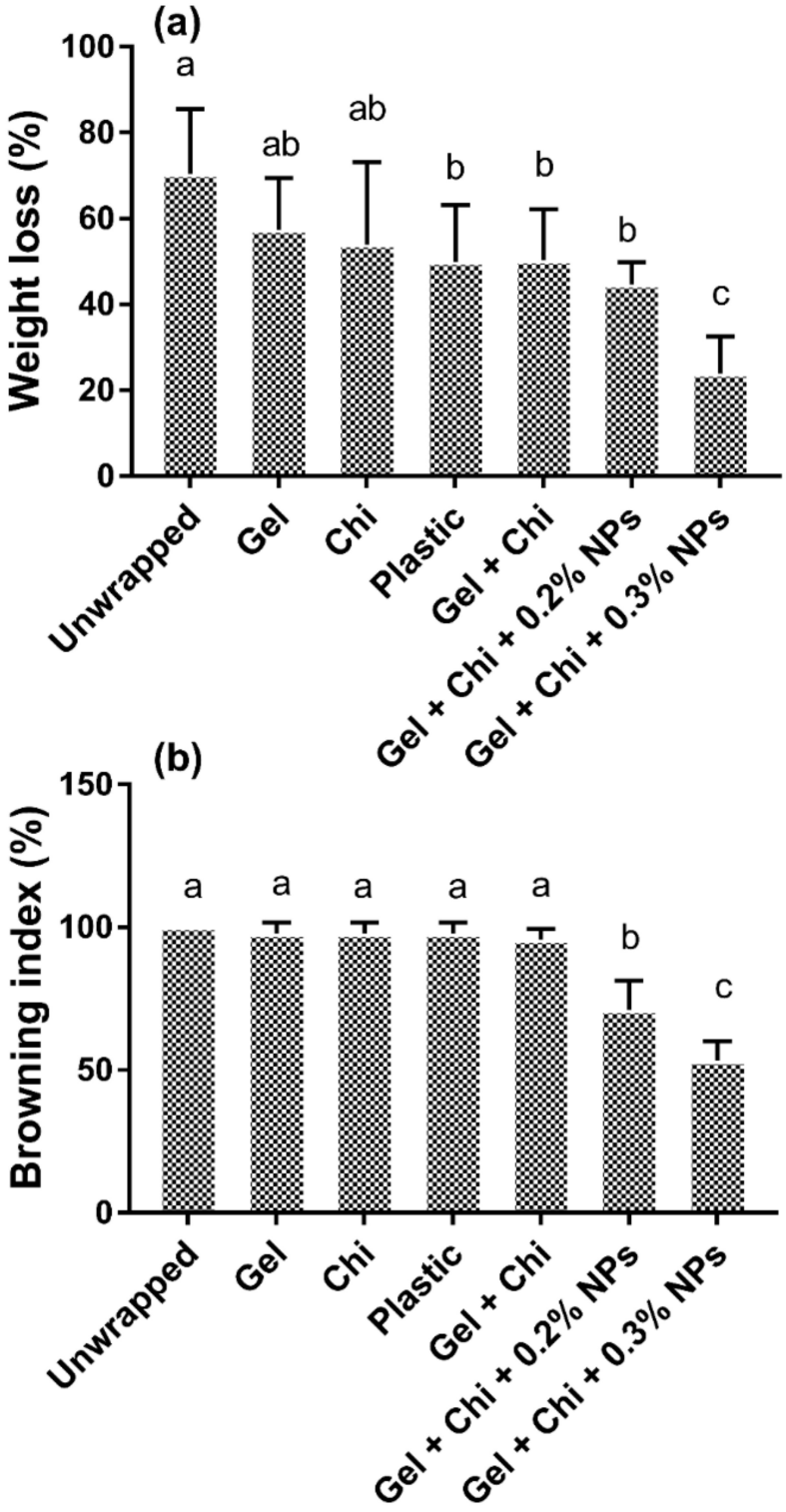

Figure 3. (**a**) Weight loss (%); (**b**) browning index of grapes packed in gelatin–chitosan nanocomposite films. Different superscript letters (a–c) above the bars indicate significant ($p < 0.05$) differences among mean observations.

3.4.2. Microbial Analysis of Grapes

The gelatin nanocomposite films restricted the growth of *S. aureus* in grapes until the third day of storage in comparison to control treatments (unwrapped and plastic wrapped grapes inoculated with *S. aureus*). Two-way ANOVA revealed that there was significant difference ($p < 0.05$) in bacterial count when grapes were subjected to different packaging

treatments, similarly the bacterial count was significantly different at different storage days (Table S4). After the fourth day of storage, grapes packed in nanocomposite films with 0.3% NPs presented significantly low bacterial count (2.39 log CFU/g) in comparison to control treatments (Table 3). After the fifth day of storage, the bacterial count in unwrapped and plastic wrapped grapes was 6.97 and 6.07 log CFU/g, respectively. However, the microbial count in grapes wrapped in the nanocomposite films containing 0.2% and 0.3% ZnO NPs remained below 4 log CFU/mL until day 5, which is the maximum acceptable microbial limit for fruits suggested by the FDA.

Table 3. Microbial analysis of *Staphylococcus aureus* inoculated grapes (log CFU/g) packed in gelatin composite films.

Days	Uninoculated Unwrapped Grapes ^{C}	Inoculated Unwrapped Grapes ^{D}	Inoculated Grapes Wrapped in Plastic ^{C}	Inoculated Grapes Wrapped in Gelatin + Chitosan Film ^{B}	Inoculated Grapes Wrapped in Gelatin + Chitosan +0.2% NPs Film ^{A}	Inoculated Grapes Wrapped in Gelatin + Chitosan +0.3% NPs Film ^{A}
1 ^{a}	2.30 ± 0.30	3.87 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2 ^{b}	3.08 ± 0.14	4.02 ± 0.03	3.95 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3 ^{c}	3.76 ± 0.06	4.26 ± 0.05	4.07 ± 0.04	3.21 ± 0.10	0.00 ± 0.00	0.00 ± 0.00
4 ^{d}	5.04 ± 0.03	6.03 ± 0.05	4.16 ± 0.03	3.91 ± 0.04	3.05 ± 0.10	2.39 ± 0.36
5 ^{e}	6.15 ± 0.06	6.97 ± 0.06	6.07 ± 0.04	3.97 ± 0.04	3.90 ± 0.11	3.81 ± 0.05

Different capital superscript letters ($^{A–D}$) indicate packaging treatments in which bacterial count was significantly different ($p < 0.05$), whereas packaging treatments with similar superscript letters indicate that the bacterial count was not significantly different within the treatments. Small superscript letters ($^{a–e}$) indicate different days at which bacterial count was significantly different ($p < 0.05$). NPs = nanoparticles.

4. Discussion

The optimization of gelatin extraction revealed that gelatin yield from chicken feet was significantly influenced by the extraction temperature; however, the gel strength of gelatin was influenced by both extraction time and temperature. Proximate analysis of chicken feet gelatin was in accordance with the previous report of da Almeida et al. [21], who reported protein (84.96%), ash (1.91%), moisture (10.39%), and fat content (2.71%) of gelatin extracted from chicken feet skin and tendon. Gelatin with a low moisture content (6–8%) is hydroscopic and gelatin with a high moisture (>15%) content is not characterized as edible [22]. In this study, the moisture content of gelatin was within the edible range. FTIR analysis of gelatin presented characteristic peaks corresponding to amide A, amide B, amide I, amide II, and amide III in the ranges of 3290–3340 cm^{-1}, 2930–2950 cm^{-1}, 1660–1680 cm^{-1}, 1550–1580 cm^{-1}, and 1250 cm^{-1}, respectively [2,21]. The molecular distribution of chicken feet gelatin was similar to previous reports on gelatin from chicken feet and skin [6,23]. The molecular weight of the gelatin is directly influenced by the hydrolysis process which breaks the peptide chains [14]. The good emulsifying properties and the foaming stability of chicken feet gelatin revealed that it can be used in the development of products with desired physicochemical characteristics, such as stable emulsions [2].

The incorporation of NPs in chicken feet gelatin–CS FFS increased the viscosity of films and decreased the TSM. Mehmood et al. [10] also reported an increase in thickness of gelatin nanocomposites after incorporation of 20% magnetic iron oxide NPs. Kumar et al. [17] also described that by the integration of ZnO NPs the viscosity of FFS was increased. Nilsuwan et al. [18] reported a 99.55% TSM of fish gelatin film which decreased significantly after the incorporation of phenolic compounds. Ahmadi et al. [24] described that pure gelatin exhibits hydrophilic properties due to which electrostatic interactions and hydrogen bonding occur between the amine group of gelatins and the OH group of water. Mehmood et al. [10] explained that the inclusion of NPs into the gelatin matrix resulted in a network rearrangement, and new hydrogen bonds were formed between NPs and

the gelatin matrix, thus decreasing TSM. ZnO NPs decrease the biodegradability of the gelatin–CS films by decreasing the moisture and restricting microbial growth. Ediyilyam et al. [25] reported a decrease in the biodegradability of gelatin–CS composite films after the incorporation of silver NPs, which was attributed to the antimicrobial effect of silver NPs. Similar results were reported by Kumar et al. [4], who found a decrease in biodegradability of gelatin films with an increase in the concentration of citric acid. Incorporation of NPs in gelatin–CS nanocomposite films significantly decreased EAB and WVP. The inclusion of ZnO NPs in a gelatin–CS matrix weakened the hydrogen bonds between the gelatin and the CS due to the formation of bonds between NPs and gelatin, which accounted for the decrease in the TS and the EAB of gelatin nanocomposite films [26]. The gelatin exhibits hydrophilic properties due to which electrostatic interactions and hydrogen bonding occur between the amine group of gelatins and the OH group of water, which contribute to an increase in the WVP of neat gelatin films [24,27]. The addition of NPs creates an impermeable medium by the entrapment of NPs within the gelatin matrix to decrease the WVP of nanocomposite films [28].

FTIR analysis of gelatin composite films revealed that infra-red absorption signal intensities were changed upon incorporation of ZnO NPs in films. The characteristic bands shifted to lower wave numbers. Moreover, the peaks in the fingerprint region from 1558–653.86 cm^{-1} became complex, indicating there was strong interaction between gelatin/CS and ZnO NPs [26]. TGA thermograms of gelatin composite films revealed that the initial weight loss of films (30–110 °C) was due to moisture evaporation. The degradation of films at 110–240 °C was associated with the removal of the structural water and the decomposition of the plasticizer [29]. The thermal degradation of films at 240–400 °C was linked to the degradation of CS and of gelatin chains. The high thermal stability of gelatin nanocomposite films was attributed to the presence of NPs in a gelatin–CS matrix, which increased the interaction among polymer chains and hindered the thermal degradation [17]. The antibacterial activity of nanocomposite films was attributed to the presence of ZnO NPs. Ahmadi et al. [24] and Kumar et al. [17], highlighted that the bactericidal effect was increased with an increase in the concentration of ZnO NPs into FFS. A fruit preservation study demonstrated that the weight loss and the browning index of grapes were reduced by gelatin nanocomposite films due to the presence of NPs, which improved the barrier properties and the antioxidant and antimicrobial potential of films [10]. Fakhouri et al. [27] and Mehmood et al. [10] reported a high browning index in unwrapped fruits due to the oxidative reaction of the polyphenol oxidase (PPO) enzyme, which is involved in the browning reaction. In case of nanocomposite films, ZnO NPs act as an active component and retard the activity of PPO, and they increased the withholding capacity of phenolic compounds. NPs also maintain the activity of naturally present anti-oxidative enzymes and agents in order to prevent browning [20]. Liu et al. [26] reported that the bactericidal effect of ZnO NPs is associated with the release of Zn^{+2} ions, which bind with the negatively charged bacterial cell wall and compromise microbial membrane integrity. Moreover, the generation of reactive oxygen species and interaction with the bacterial cell wall, cell membrane, enzymes, and other essential biomolecules result in bacterial cell death [10,17,26].

5. Conclusions

By optimizing pretreatment and extraction conditions, gelatin with a high yield and Bloom strength was obtained from chicken feet. Chicken feet gelatin–CS and ZnO NPs based active packaging reduced the browning index and weight reduction of fresh grapes during the storage period in comparison to control treatments. Incorporation of ZnO NPs improved the barrier properties and the antimicrobial effect of gelatin composite films. Gelatin based nanocomposite films restrict the microbial growth <4 log CFU/g in artificially inoculated fresh grapes stored for 5 days. Chicken feet and other poultry byproducts can be used as a sustainable and a cheap source for the optimized extraction of gelatin and its application in developing low-cost biodegradable food packaging.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14137881/s1, Table S1: Analysis of variance (ANOVA) results for the effect of extraction conditions on gelatin yield and gel strength; Table S2: Antibacterial activity (diameter of inhibition zone) of gelatin composite films. Table S3: Two-way ANOVA results for antibacterial activities of gelatin composite films. Table S4: Two-way ANOVA results for microbial analysis of Staphylococcus aureus inoculated grapes (log CFU/g).

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