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Effect of UV Exposure Time on the Properties of Films Prepared from Biotechnologically Derived Chicken Gelatin

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Abstract: Biomaterials (films, foils, fibers, coatings) based on proteins are becoming increasingly important due to the growing applications for which pork and beef gelatins are used. Alternative types of gelatins (poultry or fish), which have not yet been sufficiently tested, represent a high potential. This study looks at the effect of different UV exposure times on chicken gelatin films with added glycerol. The gelatin was prepared using a unique enzymatic hydrolysis process. The quality of the UV-exposed films was compared with gelatin films not exposed to UV light. Radiation-induced crosslinking improved the mechanical and physical properties of the films. The UV crosslinked films are stabilized at a degree of swelling from 700 to 900%; moreover, they extend their dissolution to more than 7 days while maintaining their original shape. In contrast, non-crosslinked films swell and dissolve in water faster. Further, the effect of UV radiation on the water vapor permeability and color of the films was monitored. Water vapor permeability decreased by 2.5 times with increasing crosslinking time for 30% and 40% glycerol content, and the yellowness of the irradiated samples increased with exposure time in the interval from 24 to 28. Using Fourier transform infrared spectroscopy, the differences in the amount of bonding based on irradiation time were analyzed. As a result of crosslinking, the intensity of existing bonds increased. Thermal properties were verified through differential scanning calorimetry and thermogravimetric analysis. The results proved that chicken gelatin is suitable for preparing films in foods and medicine. Applying UV radiation to crosslink gelatin films is an alternative to traditionally used chemical crosslinkers.

Keywords: chicken gelatin; films; swelling; optical properties; water vapor permeability; UV irradiation; enzymatic processes; gelatin extraction

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1. Introduction

Global consumption of poultry meat is on the rise, with 132.4 million tons consumed in 2021. At the same time, however, the number of by-products not primarily intended for consumption is increasing. These end up in incinerators or, at best, as part of compound feed [1,2]. Poultry by-products contain nutritionally important substances. One of these is collagen, an essential structural protein in all animals. It is contained in the walls of organs and blood vessels, teeth, bones, tendons, and skin and its derivatives (nails, hair,

feathers). In this respect, poultry by-products are of great value because of their high collagen content [3].

Gelatin is a natural polymer that is obtained from collagen through partial hydrolysis. Traditional sources of collagen are of bovine or porcine origin, but lesser-known, unusual sources, such as by-products from the processing of fish or poultry, are increasingly being used. Hydrolysis of collagen has traditionally been carried out via acid or alkaline methods, but these are not gentle on the raw material and the environment due to aggressive chemicals. A unique method of hydrolysis is the involvement of a suitable enzyme [4]. Gelatin has film-forming properties, is non-toxic, and is compatible with other natural substances used to prepare composite materials. Therefore, it is an excellent ingredient for biodegradable films [5,6]. Unfortunately, films prepared from gelatin have poor mechanical and physical properties and are brittle and quickly dissolved. These properties can be significantly improved by crosslinking with chemicals, physical processes, or enzymes [7,8].

Chemically crosslinked gels are created through the radical polymerization of monomers alongside small-molecular-weight crosslinking agents, namely, polymers that dissolve in water and possess a hydroxyl group that can be linked using glutaraldehyde. This linking process occurs solely at elevated temperatures and low pH levels. Glutaraldehyde is also a crosslinking agent for polymers with amine bonds. However, it is a harmful substance that can hinder cell growth, and the possibility of avoiding it is explored [9,10]. EU legislation thus allows a maximum acceptable limit of glutaraldehyde of 2 $mg \cdot kg^{-1}$ of product [11]. US legislation allows the use of glutaraldehyde as a crosslinker for materials coming into contact with food [12]. Non-toxic chemical crosslinking methods include, for example, genipin [13]. Enzymes known as transglutaminases facilitate enzymatic crosslinking, creating a network among free amino groups found in proteins. The connections made by transglutaminases are highly durable against breakdown by proteolytic enzymes. Enzymatic crosslinking presents an ideal food-use option, where the reliance on chemical crosslinking agents, typical in chemical crosslinking, is avoided. The crosslinking process occurs through lysine and glutamine within the collagen structure [14].

Physical crosslinking is based on creating hydrogen or ionic bonds, which can be induced by UV light radiation, plasma, or dehydrothermal methods. While these interchain connections form readily, they do not remain stable because of sol–gel transitions resulting from temperature, pH, or ionic strength variations. For instance, gelatin solutions in water gel are used upon cooling but revert to a sol when the temperature is increased. These types of gels are referred to as reversible. The formation of physical crosslinking through hydrogen bonds is only possible if the carboxyl groups are protonated, making pH a crucial factor. One of the options for crosslinking gelatin chains is based on the use of high-energy light, i.e., UV radiation. This approach uses inter and intramolecular photodimerization techniques to control the crosslinking density [15]. Due to its surface effect, UV radiation can only crosslink thin layers of collagen. The principle is the formation of free radicals in aromatic amino acids (phenylalanine, tyrosine), the content of which is the limiting factor for the UV crosslinking method. More prolonged irradiation forms oxygen radicals from the water molecules that attack the peptide bond. A gradual collapse of the three-dimensional structure occurs, which can be prevented by using antioxidants [16]. Collagen films can also be irradiated with corpuscular particle ionizing β radiation; even in this case, it is not high penetrating radiation, so this method is also unsuitable for crosslinking collagen in more extensive layers [17]. Thermal crosslinking is beneficial due to its economical approach and the absence of the need for extra chemicals or costly machinery. Gelatin that has been thermally crosslinked shows a greater level of crosslinking than plasma methods [18].

To improve the flexibility of films plasticizers are added, which are low-molecular-weight substances. The process is based on limiting the interactions of the polymer chains

by penetrating the plasticizer molecules between the polymer's macromolecular chains, increasing the polymer chains' mobility. The most used are substances with the polyol group, e.g., glycerol, ethylene glycol, propylene glycol, and 1,2,3-propanetriol [19].

Along with crosslinking agents and plasticizers, various other functional materials, such as antioxidants or agents with antimicrobial effects, can be included to enhance specific properties. These include natural oils like tea tree, rosemary, clove, lemon, and oregano, as well as bioactive substances such as bee pollen, ethanol extract from propolis, and dried pomegranate extract [20]. Moreover, phenolic acids such as gallic acid, p-hydroxybenzoic acid, ferulic acid, and flavonoids like catechin, flavone, or quercetin can also be used [21].

Films can be prepared from gelatin alone or with other biopolymers. These may be polysaccharides, lipids, or proteins. Films derived from natural materials are easily degradable. This allows them to be used in food and medical applications [22–24]. Films intended for food applications must meet barrier properties against light or oxygen to extend the shelf life of the wrapped product [25–27]. Films can be prepared using various methods, mainly by casting or extrusion; spreading and layering can also be used. Casting is a cheap, convenient, and standard method used in the food industry to prepare gelatin films. This method involves forming a solution by dissolving gelatin, adding plasticizers, and then crosslinking [28,29].

The author's research team has long been involved in the biotechnological processing of poultry by-products rich in collagen, gelatins, and low-molecular-weight hydrolysates. Applications of chicken collagen hydrolysate in cosmetic matrices [30] and application of chicken gelatins to form coatings on beef sirloin [31] and fibers [32] were verified. The preparation and properties of the chicken gelatin films (foils) are not described. This work first aimed to prepare biodegradable films containing glycerol plasticizer from biotechnologically derived chicken feet gelatins. Secondly, to study the effect of UV exposure at different times on the crosslinking of the films by studying their swelling degree, water vapor permeability, and optical features. Hypothesis: The change in chicken gelatin film properties after UV irradiation would be comparable to the results with samples prepared from commercial gelatin.

2. Materials, Equipment, and Methods

2.1. Raw Materials, Equipment and Chemicals

Materials and equipment include chicken gelatin (197.3 ± 1.6 Bloom) prepared from chicken feet supplied by Raciola Ltd. (Uherský Brod, Czech Republic), ethanol 96 % (Fichema, Brno, Czech Republic), glycerol 99.5% (Fichema, Brno, Czech Republic), petroleum ether p.a. (Sigma-Aldrich, Saint Luis, MA, USA), Xenotest ALPHA+ (Atlas, Mount Prospect, IL, USA), a climate chamber Memmert HPP110 (Memmert, Schwabach, Germany), Scale Kern 440-49 N (Kern & Sohn GmbH, Balingen, Germany), spectrophotometer Ultrascan PRO (HunterLab, Reston, VA, USA), Bruker ALPHA (Bruker, Billerica, MA, USA), Mettler Toledo DSC 1 (Mettler Toledo, Columbus, OH, USA), Netzsch STA449 F1 Jupiter (Netzsch, Selb, Germany), digital caliper Labo Iconic 150 mm (Kinex, Bytca, Slovakia), Protamex®, and a *Bacillus* endopeptidase (Novozymes, Copenhagen, Denmark) to process purified collagen. Activity was 1.5 AU·g^{-1}, with an optimum pH of 5.5–7.5 at 60 °C. The enzyme meets the purity standards set for food-grade enzymes by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC).

2.2. Processing of Chicken Feet into Gelatin

The extraction of soluble proteins (albumins, globulins) from chicken feet was performed according to Du et al. [33]. The tissues were defatted using an equal ratio of

ethanol and petroleum ether. The starting material was agitated with this solution in a 1:6 proportion for three days, with the solvent being replaced every 24 h.

Gelatin was produced from chicken feet using a biotechnological method by the authors of patent CZ 307665—Biotechnological production of food gelatin from poultry by-products [4]. Purified collagen was mixed with distilled water in a 1:10 ratio, and the pH was adjusted to 6.5 and 7.0. Afterward, the proteolytic enzyme Protamex® was incorporated at a concentration of 0.4% (based on the dry collagen weight), and the mixture was stirred for 15 h. The altered collagen was then mixed with distilled water in a 1:8 ratio, and gelatin was extracted using hot water at a temperature of 65.0 ± 0.5 °C for 4 h in a batch extractor. Whatman No. 1 filter paper separated the gelatin solution from the undissolved residue. The gelatin solution was poured onto plates covered with non-stick foil in a thin layer (max. 4.0 mm) and dried according to the following procedure: First, the plate with the solution was placed in the refrigerator at 6.0 ± 0.3 °C for 30 min to solidify the liquid film. Then, drying was performed in an air-circulating chamber dryer in two steps: the first for 6 h at 40.0 ± 0.3 °C and the second for 8 h at 65.0 ± 0.3 °C. The prepared gelatin was gradually crushed into particles of 1 mm in diameter. Dry gelatin powder was stored in PE-closed containers in the dark.

2.3. Preparation of Gelatin Films

Gelatin films were prepared by casting 20.0 ± 0.1 g of 20% (*w*/*w*) gelatin solution into a 7.0 × 12.5 cm silicone mold. Two series of films were prepared, differing in the content of the plasticizer (glycerol): 30% or 40% of glycerol, based on gelatin weight. The 20% (*w*/*w*) gelatin solution was prepared using the following procedure: In a 25 mL beaker, 4.0 ± 0.1 g of gelatin and 1.2 g (or 1.6 g) of glycerol were weighed; the glycerol weight corresponds to a 30% or 40% addition of glycerol to the gelatin weight. Then, demineralized water was added to achieve the final weight of 20.0 ± 0.1 g. The mixture was gently stirred in a water bath at 35.0 ± 0.5 °C for 3 min. The gelatin solution was then cast into the silicone mold and dried in the air-circulating oven at 35.0 ± 0.5 °C for 24 h.

2.4. UV Crosslinking

The Xenotest ALPHA+ (Atlas, Mount Prospect, IL, USA) was used for physical crosslinking using UV irradiation. The samples were irradiated with a xenon lamp at 35 ± 0.1 °C and 50% relative humidity. UV exposure in 1 h was 215 kJ·m^{-2}. The films were placed in unique metal holders and exposed to UV light only from one side. Samples were removed at intervals of 0, 1, 2, 4, 8 and 16 h. Each sample was subsequently tested.

2.5. Swelling

First, the samples were dried at 35.0 ± 0.5 °C for 24 h. The dried samples were placed in distilled water at 23.0 ± 0.5 °C and removed after certain intervals (0.5, 1, 2, 4, 10, 24, 36, 48, 96, 168, 216 h). The samples were measured, and the volume was calculated. The degree of swelling (%) was calculated as the increase in the volume of the swollen sample relative to the volume of the original unswollen sample as a percentage using the following equation:

$$\text{Swelling} = \frac{V_t}{V_0} \cdot 100 \quad (1)$$

where V_0 is the initial sample volume [mm^3], and V_t is the sample volume after a specified time [mm^3].

The sample volume was calculated from the sample dimensions, which were determined using a digital caliper. The experiment was performed three times.

2.6. Water Vapor Permeability

Water vapor permeability was determined gravimetrically [34]. Film samples with a diameter of 3.8 ± 0.1 cm were prepared for the water vapor permeability measurements. The samples were not dried in any way; they were only placed in a desiccator (RH 25.0 ± 3.0%) for 1 h after the crosslinking was completed until the test was performed. The cup with a hole was loaded with 25 g of dried silica gel; then, the sample was placed between two rubber seals to cover the entire hole. The cup was sealed with a perforated lid. The silica gel was dried at 150 °C for 20 h. The prepared cups were placed in a climate chamber at 37.0 ± 0.5 °C with a relative humidity of 50.0 ± 1.0%. The samples were removed from the chamber and weighed after 24 h when the weight gain was observed. The experiment was performed three times.

The measured results were plotted on a graph of mass versus time in the climate chamber. The water vapor permeability, q ($g \cdot m^{-2}$), was calculated according to the equation:

$$q = \frac{240 \cdot \Delta m}{S \cdot \Delta t} \quad (2)$$

where Δm is the weight difference over the measurement period [mg], S is the test sample size [cm^2], and Δt is the measurement time [h].

2.7. Optical Features

Using a HunterLab UltraScan Pro D65 spectrophotometer, the color coordinates, yellowness index YI [35], and turbidity [36] were measured for each sample. The color of the samples was expressed in the CIE Lab scale as values L*, a* a b*. Before measurement, the samples were conditioned in an oven at 35 °C for 24 h.

2.8. Vibrational Characterization of Functional Groups

The spectra were detected with a Bruker ALPHA Fourier transform infrared spectroscope using the ATR technique with a platinum crystal for every sample. The samples received infrared illumination within the 400 cm^{-1} to 4000 cm^{-1} wavelength range. During a single measurement, 32 images were captured.

2.9. Differential Scanning Calorimetry

A Mettler Toledo DSC1 differential scanning calorimeter was used for glass transition and melting temperatures. Samples weighing 5 ± 0.1 mg were prepared for testing and placed in aluminum trays. An empty tray was used as a reference. The measurements were conducted in a nitrogen atmosphere with a 20 $mL \cdot s^{-1}$ flow rate. The temperature measurement profile consisted of holding at −25 °C for 5 min and heating from −25 °C to 120 °C at a rate of 10 $°C \cdot min^{-1}$. The experiment was performed three times.

2.10. Thermogravimetric Analysis

Samples weighing 10 ± 0.1 mg were prepared from individual films and subjected to thermogravimetry (TGA), measuring weight loss with temperature. Testing was carried out on a Netzsch STA449 F1 Jupiter apparatus in a simultaneous thermal analyzer with a two-dish system, where the first reference dish is empty and the sample is placed in the second. The measurement was carried out through constant heating at a rate of 10 $°C \cdot min^{-1}$ from 25 to 600 °C in an argon atmosphere. The experiment was performed three times.

2.11. Statistical Analysis

Results were analyzed via two-way ANOVA, with a significance level of $p < 0.05$, using Microsoft Office Excel 2021 (Denver, CO, USA). The tested factors were irradiation time and glycerol content. When there were any significant differences between samples, Tukey's HSD (honestly significant difference) test for paired comparison was performed.

3. Results and Discussion

3.1. Swelling

Table 1 shows the swelling index values for 30 and 40% glycerol samples. Compared to the unirradiated sample, the UV-crosslinked films with 30% glycerol have, with one exception, a similar or lower swelling index. The second set of samples with 40% glycerol had a higher swelling index in all cases. Crosslinking increased the time required for the dissolution of the sample compared to the non-crosslinked sample. Samples with a glycerol content of 40% dissolved in less time than samples with less plasticizer.

Due to the hydrophilic nature of glycerol, films with a higher concentration of glycerol can bind more water into their structure. The decrease in swelling in some cases may be due to the dissolution or dropping of part of the sample.

From the results for the film containing 30% glycerol, it can be observed, except for the sample irradiated for 2 h, that exposure to UV radiation leads to reduced swelling. In all cases of irradiation duration, an equilibrium state of swelling was reached after about 4 days, after which the values did not change much. Regarding the gelatin film containing 40% glycerol, the trend is similar, including the deviating behavior of the sample irradiated for 2 h. The unexposed sample showed the highest swelling after half an hour in the solvent, and after 4 h, the samples were distorted. Even in the case of these films, an equilibrium degree of swelling was reached after about 4 days.

Due to potential applications in food or medicine, resistance to dissolution is an important property. Sample 30/16 (30% glycerol, 16 h exposure interval) could resist water for the longest time of all the samples. This sample lasted 168 h and increased its volume by 787% of its original volume. When the glycerol concentration is increased by 10%, the 3 samples show the longest time to dissolution (96 h): 40/4, 40/8, and 40/16 (40% glycerol, exposure intervals 4, 8, and 16 h). Their swelling rates were 818, 839, and 832%, respectively.

According to the two-way ANOVA, the irradiation time does not have a statistically significant effect on the swelling of the samples; however, the plasticizer content has a statistically significant effect on the swelling.

Ji et al. [37] studied the effect of the gelatin extraction method and gelatin pH during extraction on the swelling of gelatin films. They characterized the swelling as a change in weight. The swelling rate was higher for films of alkaline hydrolyzed gelatin than for enzymatically extracted gelatin. In addition to the different sources of raw material and methods of gelatin extraction, the fact that crosslinking was not performed in this study may have influenced the different results. Gordon et al. [38] investigated the swelling of gelatin prepared from porcine tissues using the fluid dynamic gauging method. The progress of gelatin swelling was monitored at different time intervals, as well as the temperature and pH of the gelatin. Again, no crosslinking was used, and the swelling was higher than in the samples of the presented study. Dang et al. [39] prepared gelatins with varying levels of β-cyclodextrin, acting as a plasticizer and crosslinker. The gelatin was extracted from chromium-tanned skin residues. Among the properties tested was the swelling ability, which was found to be the best in the case of gelatin and β-cyclodextrin content in a ratio of 1:1. In comparable agreement with the studied crosslinked chicken gelatin samples in terms of maximum swelling were samples with a gelatin to β-cyclodextrin ratio of 1:1 and 1:2. However, the swelling process of these samples was faster. Boanini et al. [40] crosslinked pork hides gelatin with alginate dialdehyde. Swelling, in this case, was characterized as the weight gain of the film after immersion in water. In contrast to this research, especially for longer UV exposures, more stable swelling values over more extended observation periods are observed for the prepared samples of crosslinked gelatins, which would correspond to the swelling values containing 1 and 3% alginate dialdehyde from the compared study.

Table 1. Swelling of films expressed as a percentage change in volume.

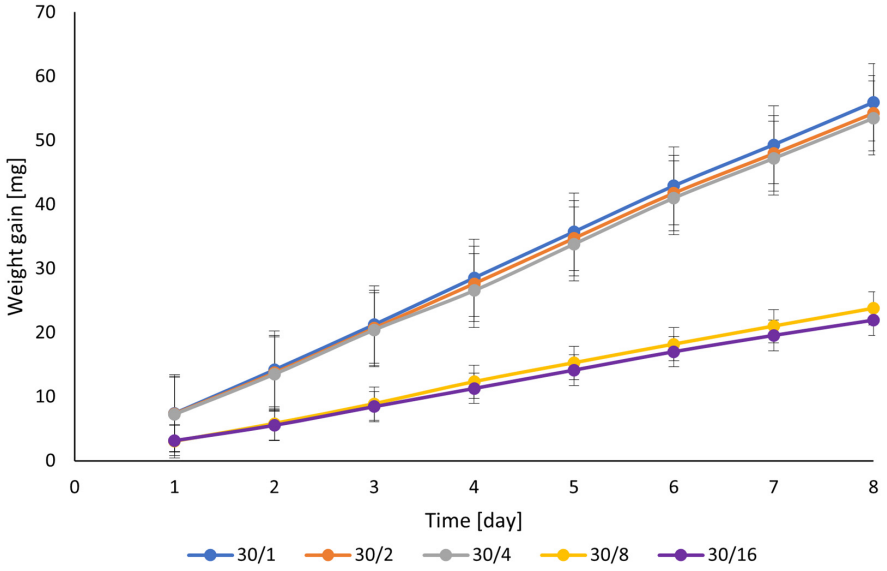
Time	Glycerol Addition [wt%]/UV Irradiation Time [h]											
	30/0	**30/1**	**30/2**	**30/4**	**30/8**	**30/16**	**40/0**	**40/1**	**40/2**	**40/4**	**40/8**	**40/16**
0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
0.5	552 ± 14 ^{a}	460 ± 11 ^{b}	586 ± 24 ^{a}	382 ± 11 ^{c}	318 ± 15 ^{c}	333 ± 19 ^{c}	549 ± 25 ^{a}	440 ± 25 ^{b}	452 ± 32 ^{b}	400 ± 7 ^{b}	449 ± 26 ^{b}	439 ± 19 ^{b}
1	615 ± 31 ^{a}	546 ± 37 ^{b}	733 ± 29 ^{c}	532 ± 25 ^{b}	477 ± 26 ^{b}	482 ± 28 ^{b}	551 ± 28 ^{b}	554 ± 29 ^{b}	673 ± 10 ^{a}	507 ± 41 ^{b}	581 ± 34 ^{a}	627 ± 6 ^{a}
2	651 ± 47 ^{a}	616 ± 24 ^{b}	928 ± 15 ^{c}	611 ± 37 ^{b}	485 ± 32 ^{e}	559 ± 37 ^{d}	618 ± 37 ^{b}	573 ± 43 ^{d}	774 ± 29	595 ± 27 ^{b}	669 ± 28 ^{a}	739 ± 42 ^{f}
4	822 ± 25 ^{a}	621 ± 13 ^{b}	979 ± 9 ^{c}	695 ± 27 ^{d}	592 ± 34 ^{b}	578 ± 24 ^{b}	0 ^{e}	590 ± 57 ^{b}	863 ± 36 ^{a}	591 ± 23 ^{b}	756 ± 31 ^{f}	756 ± 30 ^{f}
10	850 ± 19 ^{a}	641 ± 18 ^{b}	962 ± 32 ^{c}	813 ± 41 ^{a}	575 ± 38 ^{d}	660 ± 29 ^{b}	0 ^{e}	678 ± 46 ^{b}	912 ± 42 ^{c}	604 ± 42 ^{b}	783 ± 46 ^{f}	762 ± 39 ^{f}
24	0 ^{a}	689 ± 52 ^{b}	1071 ± 63 ^{c}	824 ± 35 ^{d}	615 ± 52 ^{b}	679 ± 37 ^{b}	0 ^{a}	686 ± 41 ^{b}	943 ± 65 ^{e}	683 ± 27 ^{b}	729 ± 29 ^{f}	720 ± 28 ^{f}
36	0 ^{a}	686 ± 29 ^{b}	947 ± 12 ^{c}	830 ± 42 ^{d}	625 ± 48 ^{b}	657 ± 42 ^{b}	0 ^{a}	0 ^{a}	0 ^{a}	707 ± 51 ^{e}	736 ± 40 ^{e}	719 ± 51 ^{e}
48	0 ^{a}	0 ^{a}	991 ± 43 ^{b}	804 ± 12 ^{c}	655 ± 29 ^{d}	661 ± 45 ^{d}	0 ^{a}	0 ^{a}	0 ^{a}	746 ± 26 ^{e}	762 ± 37 ^{e}	737 ± 31 ^{e}
72	0 ^{a}	0 ^{a}	1163 ± 87 ^{b}	846 ± 26 ^{c}	624 ± 25 ^{d}	687 ± 36 ^{d}	0 ^{a}	0 ^{a}	0 ^{a}	759 ± 39 ^{e}	767 ± 24 ^{e}	740 ± 54 ^{e}
96	0 ^{a}	0 ^{a}	0 ^{a}	881 ± 47 ^{b}	690 ± 42 ^{c}	628 ± 62 ^{c}	0 ^{a}	0 ^{a}	0 ^{a}	819 ± 25 ^{d}	839 ± 54 ^{d}	832 ± 49 ^{d}
168	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	787 ± 23 ^{b}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}	0 ^{a}
216	0	0	0	0	0	0	0	0	0	0	0	0

Different letters in the same row indicate significant differences ($p < 0.05$) between samples based on glycerol content via Tukey's test.

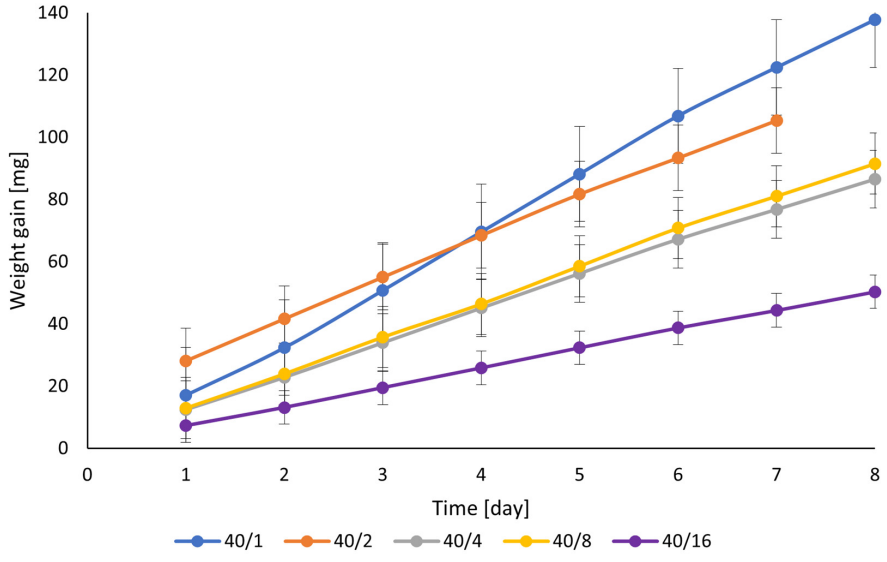
3.2. Water Vapor Permeability

The permeability to water vapor depends mainly on the structure of the polymers and the thickness of the film. Both gelatin and glycerol are hydrophilic substances. When exposed to UV light, the free volume decreases, and bonds are formed between the individual gelatin chains. The structure of the gelatin film is strengthened, and its brittleness and water vapor permeability are reduced due to the reduced mobility of the gelatin chains.

Given the high initial brittleness and uneven surface of the gelatin film, placing the undegraded film into the cup for measurement was impossible. Therefore, the effect of different glycerol concentrations and UV exposure time is compared. From Figure 1a,b, a constant increase in weight can be observed for all samples throughout the measurement time.



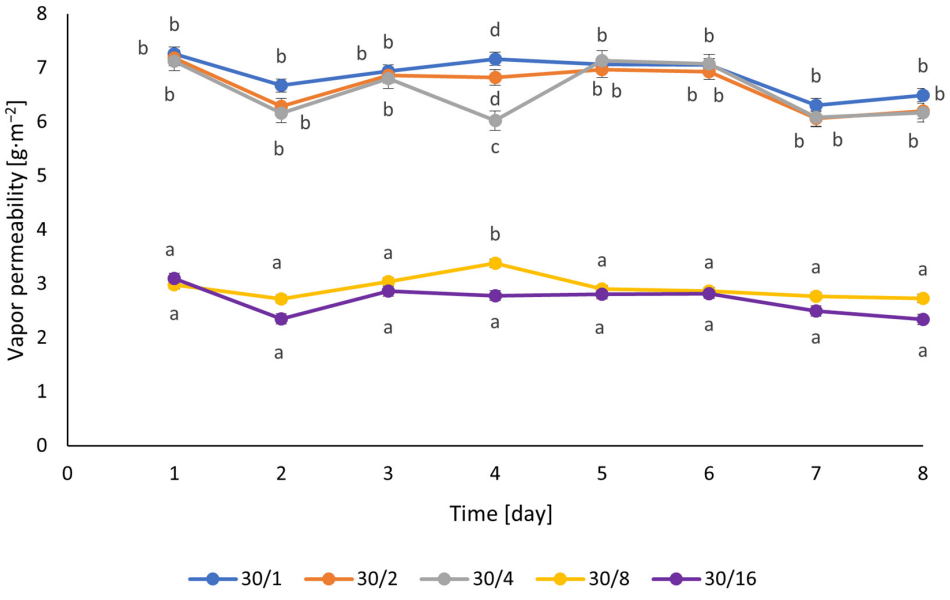
(**a**)



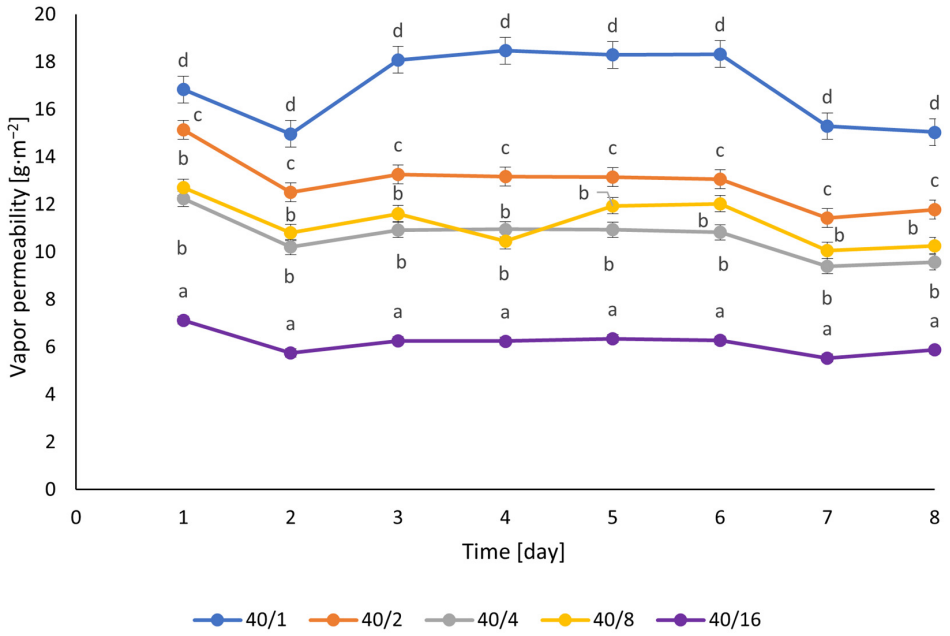
(**b**)

Figure 1. (**a**) Weight gain of films with 30% glycerol with 1–16 h UV irradiation intervals for vapor permeability measurements. (**b**) Weight gain of films with 40% glycerol with 1–16 h UV irradiation intervals for vapor permeability measurements.

As can be seen from the graphical representation (Figure 2a,b) for gelatin films with 30% glycerol and 40% glycerol, increasing the UV exposure time decreased the water vapor permeability due to the crosslinking of the structure. Water vapor permeability was higher for samples with higher glycerol concentrations. This was true at all irradiation intervals, probably due to the increased mobility of the gelatin chains and more significant gaps between the polymer chains after adding more plasticizer. According to two-way ANOVA, a statistically significant effect of irradiation time and plasticizer content was found in the case of water vapor permeability.



(**a**)



(**b**)

Figure 2. (**a**) Water vapor permeability of films containing 30% glycerol after 1–16 h of UV irradiation. According to Tukey's test, different letters indicate significant differences ($p < 0.05$) between samples. (**b**) Water vapor permeability of films containing 40% glycerol after 1–16 h of UV irradiation. According to Tukey's test, different letters indicate significant differences ($p < 0.05$) between samples.

Avena-Bustillos et al. [41] evaluated the water vapor permeability of gelatin films of cold- and warm-water fish skin. They compared it with films prepared from different types of mammalian gelatins. The water vapor permeability of gelatin films from cold-water fish was significantly lower than that from warm-water fish and mammals. Our study's results were different, probably due to the different gelatin sources, the addition of plasticizer, and crosslinking. Tyuftin et al. [42] tested the vapor permeability using the ASTM F1249 method. The films were prepared from bovine gelatin. This method showed that the water vapor permeability increased with increasing thickness of the gelatin film, similar to the samples prepared in this study. Better results were obtained for irradiated films containing 30% glycerol for 8 and 16 h. Suhaima et al. [43] compared the properties of films prepared from fish gelatin and mammalian tissue gelatin; it was shown that fish gelatin films had significantly lower water vapor permeability compared to mammalian gelatin films, with which the prepared chicken gelatin films were in agreement. Loo et al. [23] used a mixture of chicken gelatin and tapioca starch to prepare the films. The films were prepared with different starch concentrations (0–25%) by casting. The physical and mechanical properties of each film were evaluated. The addition of tapioca starch increased the thickness and improved the water resistance of the films. Adding tapioca starch in quantities greater than 5% did not improve the water barrier properties but increased the water vapor permeability. The values of these samples were comparable to the results of the presented experiment.

3.3. Optical Features

The measured L*, a* b* coordinates for samples with 30% glycerol differed minimally. In the case of brightness, the values ranged from 88.50 to 90.00, and the brightness values decreased with increasing UV exposure time. The a* value remained similar in the interval from −0.85 to −0.80, with no apparent effect of UV exposure time. The b* value remained from 12.00 to 15.00, increasing with higher irradiation time. The yellowness index also increased with the crosslinking time, correlating with the b* value shift to higher values. As well as crosslinking, electromagnetic radiation causes a gradual decomposition of the native gelatin structure, increasing the yellowness index [44]. The turbidity of the films increases slightly after an hour of irradiation but does not change after that, as seen in Table 2.

Table 2. The L*, a*, and b* coordinates, yellowness, and turbidity of films with 30 and 40% glycerol depending on the UV exposure time.

Parameter	Glycerol Addition [wt%]/UV Irradiation Time [h]					
	30/0	**30/1**	**30/2**	**30/4**	**30/8**	**30/16**
L*	89.62 ± 0.31	89.39 ± 0.64	89.05 ± 0.82	88.73 ± 0.25	87.94 ± 0.63	87.66 ± 0.74
a*	−0.85 ± 0.02	−0.83 ± 0.05	−0.81 ± 0.03	−0.82 ± 0.02	−0.80 ± 0.01	−0.82 ± 0.04
b*	12.17 ± 0.11	13.12 ± 0.09	13.69 ± 0.65	14.30 ± 0.49	14.37 ± 0.27	15.00 ± 0.36
Yellowness	22.41 ± 0.13 [a]	24.15 ± 0.75 [b]	25.16 ± 0.64 [c]	26.36 ± 0.36 [c]	26.64 ± 0.44 [c]	27.92 ± 0.96 [d]
Turbidity	91.20 ± 0.41	94.00 ± 0.09	93.90 ± 0.27	93.60 ± 0.63	94.10 ± 0.51	94.30 ± 0.86
	40/0	**40/1**	**40/2**	**40/4**	**40/8**	**40/16**
L*	88.91 ± 0.37	88.69 ± 0.86	89.53 ± 0.77	88.27 ± 0.61	88.01 ± 0.78	87.92 ± 0.39
a*	−0.33 ± 0.06	−0.30 ± 0.09	−0.42 ± 0.05	−0.26 ± 0.07	−0.28 ± 0.02	−0.28 ± 0.05
b*	12.26 ± 0.12	13.13 ± 0.19	13.27 ± 0.25	13.98 ± 0.31	14.30 ± 0.54	14.95 ± 0.67
Yellowness	23.13 ± 0.28 [a]	24.91 ± 0.17 [b]	25.11 ± 0.90 [b]	25.63 ± 0.87 [b]	26.36 ± 0.61 [c]	28.11 ± 0.07 [d]
Turbidity	93.60 ± 0.81	93.90 ± 0.56	94.10 ± 0.23	93.60 ± 0.76	94.50 ± 0.53	93.70 ± 0.89

Different letters in the same row indicate significant differences ($p < 0.05$) between samples based on glycerol content according to Tukey's test.

The obtained of L*, a* b* coordinates met our expectations as the measures for the samples with the addition of 40% (wt) glycerol also varied only slightly depending on the irradiation time. The brightness values ranged from 88.50 to 90.00, decreasing with

increasing UV exposure time. The a* value remained similar from −0.85 to −0.25, with no apparent effect of UV exposure time. The b* value remained from 12.00 to 15.00, increasing with increasing UV exposure time. For none of these parameters was there a statistically significant influence of both UV exposure time and the amount of glycerol found.

For other applications, such as packaging materials or medicine, changes in the intensity of turbidity and yellowness are of the greatest importance. A significant increase can, e.g., indicate material degradation due to excessive UV radiation. The yellowness index also increases with crosslinking time, correlating with a shift in the b* value to higher values. The glycerol content is statistically significant for the yellowness of the samples, but the UV exposure time is not.

The turbidity of films with 30% plasticizer before and after crosslinking increased. No such difference was observed for films with a higher glycerol content (see values in Table 2). The measured turbidity values do not differ significantly from the results of gelatins prepared by our team [4]. According to the two-way ANOVA, neither the irradiation time nor the plasticizer content have a statistically significant effect on the turbidity of the samples.

Stevenson et al. [45] crosslinked fish-derived gelatin using ribose. The Maillard reaction produced compounds with color pigments. The degree of crosslinking was monitored according to the intensity of the color change. The pigments formed due to the Maillard reaction resulted in more pronounced color changes than in the case of our sample measurements. Kim et al. [46] also worked with fish gelatin from trout skins. The brightness and a* parameter values were consistent with the published values. Still, the b* parameter results were lower than the values in Table 2, which may be due to a different source and processing method. The gelatin was not crosslinked; glycerol was added as a plasticizer. Yap et al. [47] investigated the effect of *Garcinia atroviridis* plant extract on the properties of gelatin films prepared by 3D printing. The brightness was around 30, a* was even slightly negative, and b* was close to zero, in contrast to the results obtained on crosslinked films of chicken gelatin. Rawdkuen et al. [48] compared the properties of bovine and fish gelatin. The color was also one of the monitored parameters. The brightness values were slightly higher than our samples, i.e., around 90. Also, the a* values were very similar in the negative spectrum interval from −1.10 to −1.30. The values of the b* coordinate differed the most, which was approximately 10 lower than in our samples. Similar values were also achieved by samples from Pranoto et al. [49], who compared the properties of fish gelatin films with the addition of gellan and κ-carrageenan. Ekielski et al. [50] studied the effect of adding digestate sludge on the properties of thermoplastic starch coatings plasticized with glycerol. The color was also one of the monitored parameters. The sample without the addition of digestate had low turbidity (lightness value of 77) compared to the samples in the present study. The samples with the addition of digestate had a much lower brightness in the interval from 25 to 48. The a* parameter also changed with the addition of digestate. Adding digestate shifted the values from the green spectrum to the red. The value of the b* parameter increased with two exceptions, but in all cases, they remained in the yellow spectrum. The differences between the referenced study and tested chicken gelatin films can be attributed to different biopolymer and the processing method. Modification by UV radiation did not have such an effect on changes in color coordinates as the addition of digestate to a thermoplastic starch coating.

3.4. Vibrational Characterization of Functional Groups

Using an FTIR spectrometer, the effect of UV irradiation on functional groups in gelatin films with the addition of glycerol as a plasticizer was studied. The measured spectra are shown in Figure 3. The graph shows that no new peaks were formed. All prepared films

show peaks in the same wavelength regions regardless of the plasticizer concentration and the length of UV exposure, differing only in intensity.

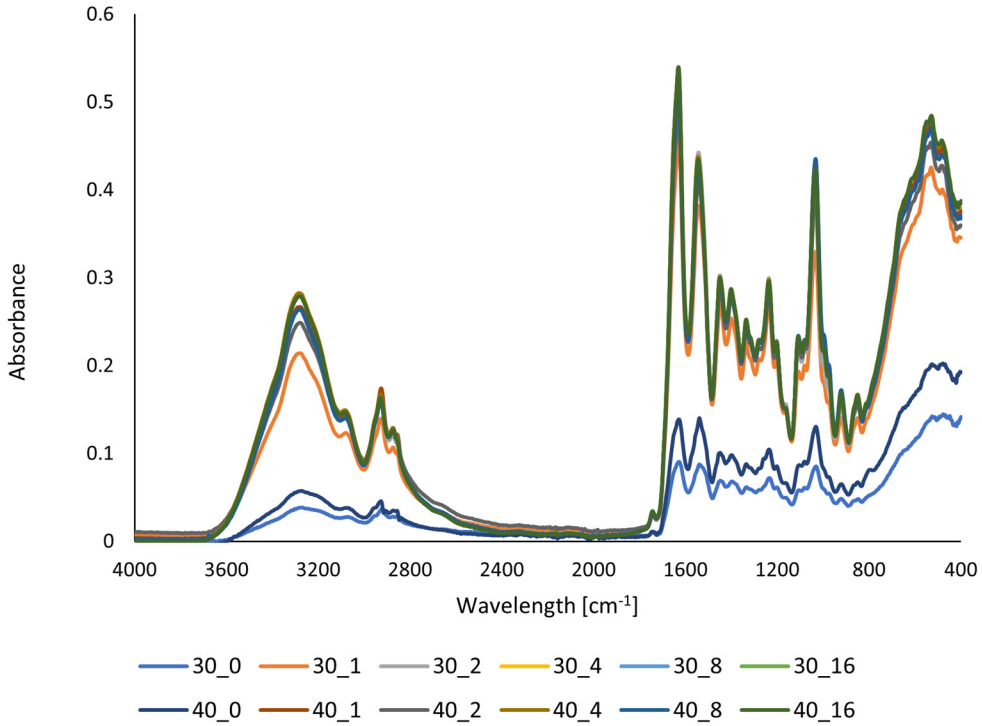


Figure 3. Vibrational characterization of functional groups of gelatin films containing 30% and 40% glycerol after UV irradiation at 0–16 h intervals.

Table 3 shows the values of the areas under the important peaks from the measured spectra.

Table 3. Amide A, I, II, and III peak areas of samples before and after UV irradiation.

Band	Glycerol Addition [wt%]/UV Irradiation Time [h]											
	30/0	**30/1**	**30/2**	**30/4**	**30/8**	**30/16**	**40/0**	**40/1**	**40/2**	**40/4**	**40/8**	**40/16**
Amid A	4.725	30.605	39.379	43.027	39.056	39.999	8.455	35.246	39.778	42.994	39.138	42.717
Amid I	3.244	17.726	21.150	20.067	19.978	19.425	5.132	18.808	20.605	21.341	20.590	21.326
Amid II	1.944	10.580	12.510	11.756	11.746	11.608	3.417	11.111	12.055	12.208	11.807	12.121
Amid III	0.334	1.734	2.026	1.890	1.898	1.861	0.627	1.759	1.944	1.978	1.864	1.940

The peaks in the spectrum are located in bands around 3288, 1631, 1544 a 1243 cm^{-1} corresponding to amide A (stretching and oscillation of N-H), amide I (oscillation and stretching of C=O and C-N bonds), amide II (bending of N-H bonds) and amide III (bending of N-H bonds). If glycerol is added to the film, another characteristic peak is formed around 1045 cm^{-1}, and its intensity increases with higher amounts of glycerol [51,52].

The peak in the amide A region should also be more intense and broader and grow sharper with higher glycerol content, according to the study by Nor et al. This fact should be related to the -OH group, introduced into the film by adding a plasticizer [52].

The increase in the amide I peak intensity with the addition of glycerol is due to the ease of formation of intermolecular hydrogen bridges between the C=O and N-H groups with O-H from glycerol. Amide I is the most effective band for examining protein structures through infrared spectroscopy. The precise location of the amide I peak is influenced by the hydrogen bonds and the protein's shape. Typically, many proteins exhibit various forms of

secondary structures (α-helix, β-sheet, or random structure) simultaneously, which is why the peak in the amide I region frequently displays several branches [51].

The range and intensity of the amide II peak are generally much more sensitive to hydration than to changes in secondary structure. Although the films were dried for 24 h at 35 °C, the differences could be due to different water contents. The peak around 1045 cm^{-1} probably relates to the interaction between glycerol and the film structure [52].

FTIR spectra of gelatin films with 30% glycerol and 40% glycerol (Table 3) after UV irradiation at different time intervals show an increase in the intensity of all peaks. The increase in intensity indicates the formation and presence of multiple bonds characterized by specific wavelengths. This fact may indicate the samples' crosslinking after exposure to UV irradiation. A dramatic increase in intensity occurred after one hour of UV exposure, and no further significant change in intensity was observed as the UV exposure was prolonged.

All prepared films show peaks in the same wavelength regions regardless of the plasticizer concentration and the length of UV exposure, differing only in intensity.

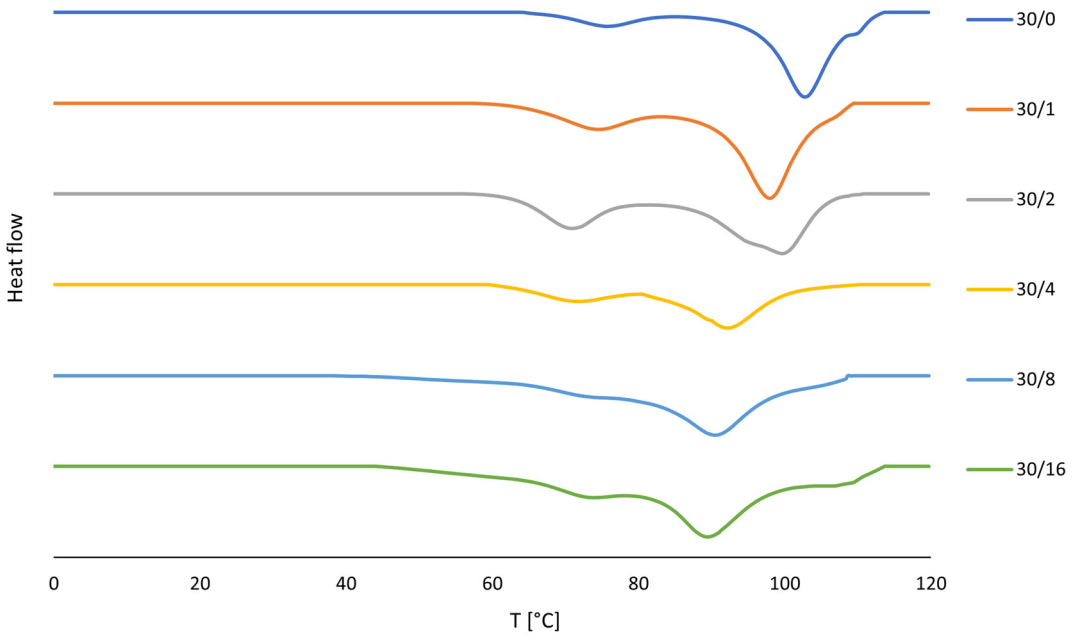
3.5. Thermal Analysis

The heating of samples with 30% and 40% glycerol is shown in Figure 4a,b. Two critical transitions can be seen here: the glass transition temperature, characterized by a change in baseline position (change in heat capacity); and in this case, endothermic recovery (glass transition and relaxation). T_g is followed by an endothermic peak corresponding to the spiral structure's melting temperature.

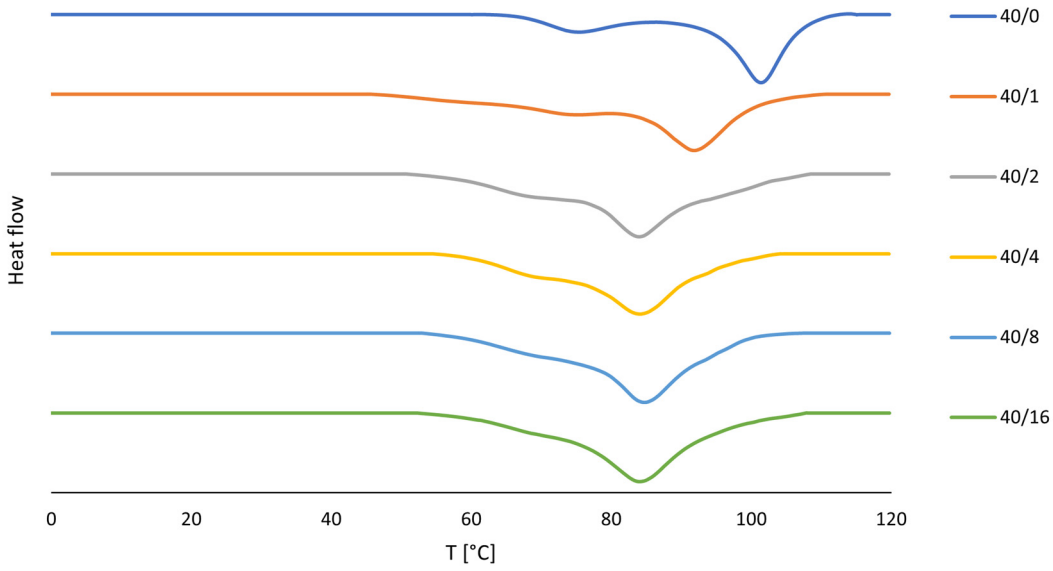
With heating, the film between T_g and T_m becomes elastic and contains a semi-crystalline structure formed by the presence of triple helices. Above T_m, all helices are melted, and the film transitions to a viscous liquid state with minimal solvent. When the sample is cooled at 10 °C/min, the helices cannot form the crystalline phase again, so the gelatin is only purely amorphous during the second heating. The cause of the T_g differences and shifts may be water immobilized between the sample peptide chains forming the triple helix during the first heating.

In the case of films with 30% glycerol, the glass transition temperature increased with increasing UV exposure time. The opposite trend was observed for samples with 40% glycerol content, where the glass transition temperature decreased. In the study by Coppola et al. [53], it was found that samples with higher plasticizer (glycerol) content showed lower glass transition temperatures. The melting temperature also decreased again with the addition of glycerol.

For most of the samples studied, it can be observed that the higher the glycerol content, the lower the melting point and, generally, the lower the glass transition temperature. The different values could be due to the homogeneity of the samples. A different film sample was used for each UV-irradiation time, which may have caused some anomalies in the characterization. Although the samples for the measurements were taken from a single film, its structure may not have been uniform. The film portion of 5 mg, which was cut for the measurement, could have contained tiny microscopic air capsules or cracks distorting the measurement result. For example, sample 40_2 also showed a deviating behavior in the FTIR analysis and swelling test. Statistical analysis using a two-way ANOVA did not reveal a statistically significant effect of UV exposure time or glycerol content on the glass transition or melting temperature.



(**a**)



(**b**)

Figure 4. (**a**) DSC heating curves of gelatin films with 30% glycerol after irradiation at 0–16 h intervals. (**b**) DSC heating curves of gelatin films with 40% glycerol after irradiation at 0–16 h intervals.

Ekielski et al. [50] observed a melting temperature of 150 °C for a thermoplastic starch coating without the addition of digestate, and this temperature shifted to 180 °C for samples with digestate. In all cases, the melting temperature is higher than that of our samples.

Thermogravimetric analysis (TGA) gives information on the thermal stability of samples by using the relationship between mass loss and temperature.

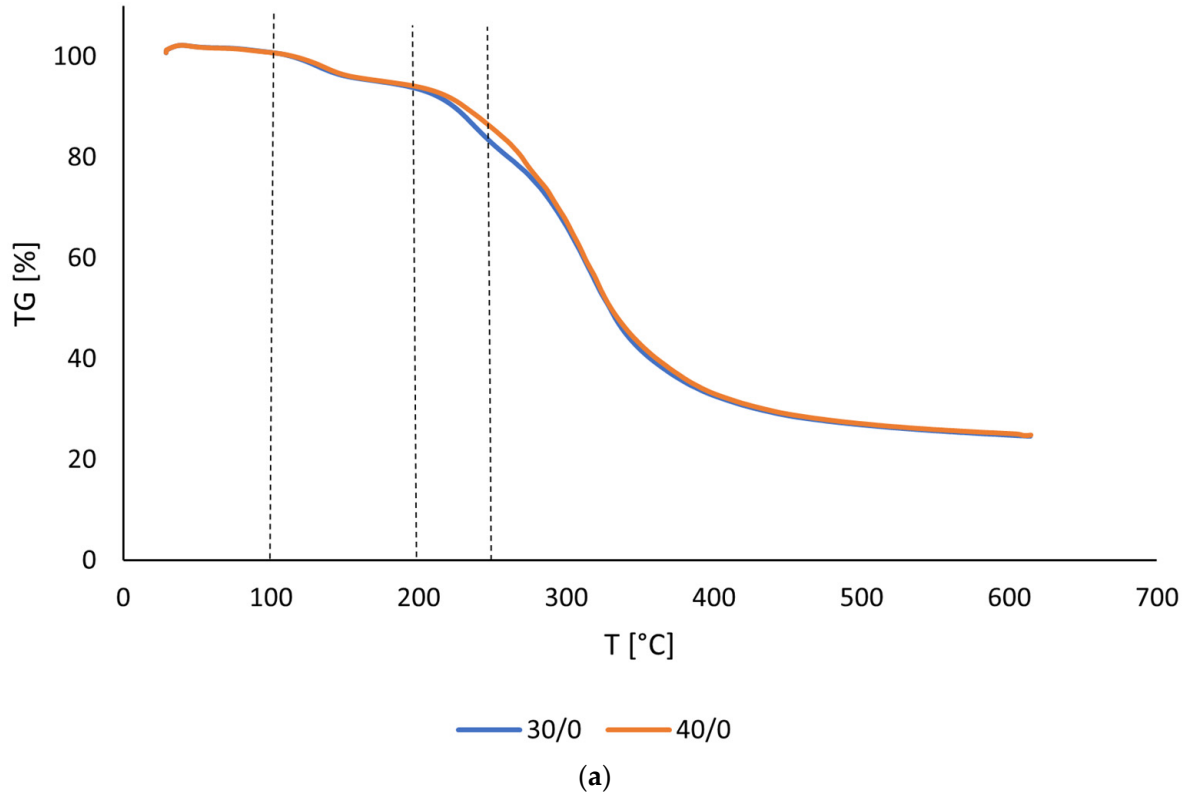
The TGA curve shows three regions of decomposition. The first stage is associated with the disruption of hydrogen bridges and the loss of surface and internally bound water. It can be observed around 100 °C. The second phase of decomposition started around 200 °C; at this point, degradation and molecular modification of the components in the

gelatin film—glycerol and less-stable shorter gelatin chains—occurs. The last phase occurs around 250 °C, marking the depolymerization of the longer gelatin macromolecules [54].

The measured results of the uncrosslinked samples with 30 and 40% glycerol (Figure 5a) show the same trend as mentioned above. The first stage, up to about 180 °C, is associated with removing residual water, which was 6.27% for the sample with 30% glycerol and 5.21% for the sample with 40% glycerol. In the region of the onset of degradation of glycerol and shorter gelatin chains around 200 °C, it can be seen that the sample with the higher glycerol content shows a 3% higher residual mass. The third stage of degradation of the long gelatin chains lasted until a temperature of about 500 °C. When the temperature reached 600 °C, the measurements came to an end. The residual amount was about 24% for both samples. This amount contained carbon residues that could not decompose at this temperature in argon. If the sample had been exposed to oxygen, these carbon residues would have been able to burn off, and only the ash content would have remained.

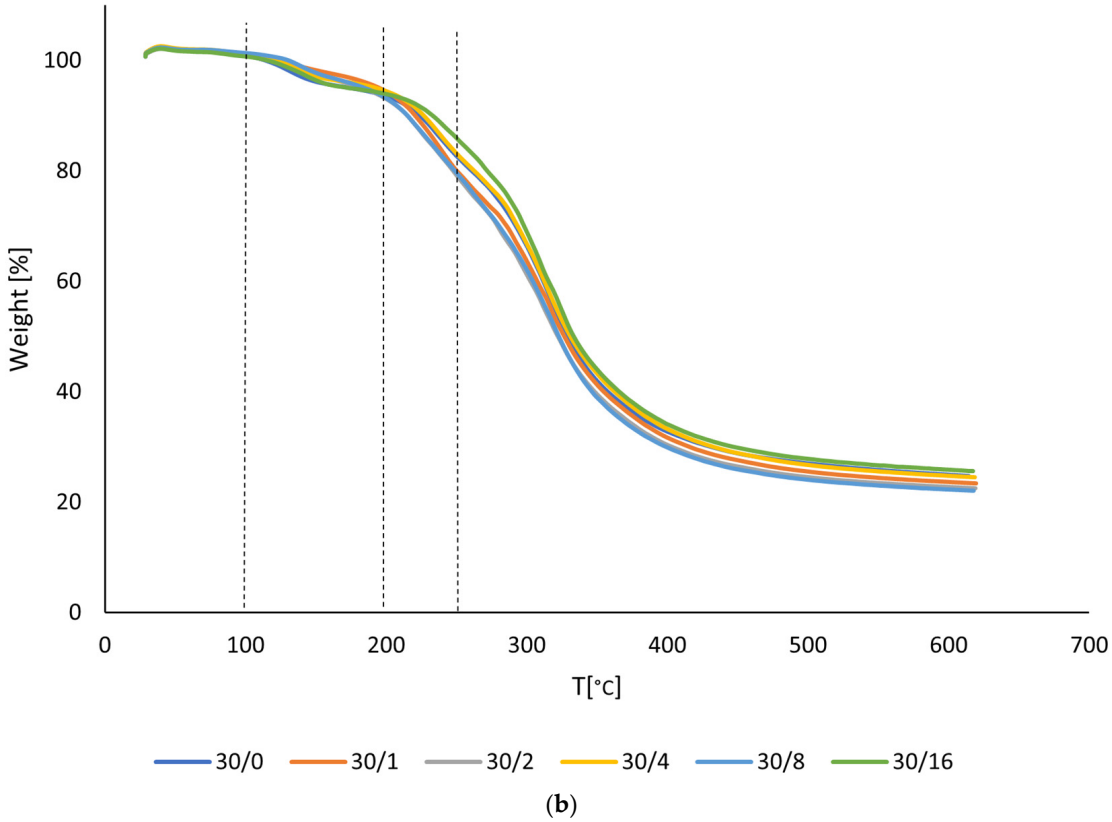
When comparing the samples before and after irradiation, the curves of the 30% and 40% samples (Figure 5b,c) after irradiation show a very similar pattern to the non-irradiated ones. Again, three stages of decline can be observed around the same temperatures, with a comparable course for all tested samples.

The thermoplastic starch coating [50] showed a similar decomposition process to that of our samples. Baggio et al. [55] studied the effect of the amount of transglutaminase on the properties of gelatin films. In addition to mechanical properties, they verified the extent of film degradation in soil by thermogravimetry. As the amount of transglutaminase increased, there was greater crosslinking and thus more excellent resistance to biodegradation. Chiono et al. [56] tested films based on bovine gelatin crosslinked with genipin for medical applications. One of the tests used was thermogravimetric analysis, which showed pyrolysis of the samples at temperatures similar to our measurements.

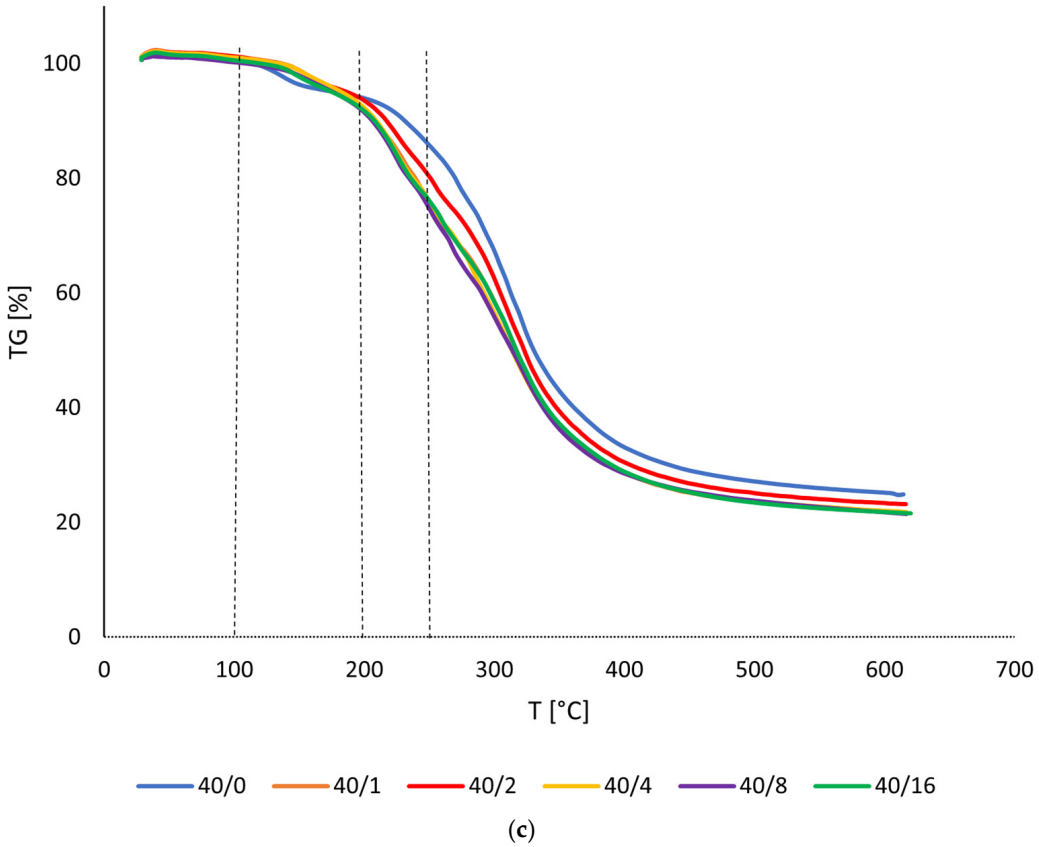


(**a**)

Figure 5. *Cont.*



(**b**)



(**c**)

Figure 5. (**a**) Thermal analysis curve for uncrosslinked samples with 30% and 40% glycerol. (**b**) Thermal analysis curve for samples with 30% glycerol with 0–16 h UV irradiation intervals. (**c**) Thermal analysis curve for samples with 40% glycerol with 0–16 h UV irradiation intervals. Dashed lines at 100 °C indicate evaporation of free water from samples, at 200 °C degradation of short gelatin chains and glycerol, and at 250 °C degradation of long gelatin chains.

4. Conclusions

The work contributes to expanding the potential applications of gelatins prepared from the unused raw material source of collagen. Physical crosslinking of the films with UV irradiation positively reduced the swelling of the samples and increased the bond intensity compared to the uncrosslinked samples. The film structure was strengthened by UV crosslinking, and with increasing time, the maximum swelling degree of the film was achieved. The IR spectra did not differ between the tested samples by forming new bonds, but in all cases, the crosslinked samples showed an increase in the intensity of the original bonds. Irradiation of the sample after the shortest time increased the intensity of existing bonds; with prolonged irradiation, the intensities increased slightly. UV radiation positively affected the structural cohesion of the gelatin films; even after 16 h of exposure, no degradation was observed. Yellowness and turbidity were unaffected by UV exposure. In addition to irradiation, the plasticizer content lowers the glass transition and melting temperatures. Chicken by-products such as feet represent an unconventional and still little-used source for preparing gelatin that has yet to be studied on a large scale. The results showed that UV-crosslinked chicken gelatin biodegradable films could be a good alternative to gelatin from traditional sources in producing food packaging materials (e.g., for fresh fruit and vegetables, meat, or baking products) or for medical applications (tissue engineering, drug delivery, etc.).

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References

1. Zhang, Y.Z.; Venugopal, J.; Huang, Z.-M.; Lim, C.T.; Ramakrishna, S. Crosslinking of the electrospun gelatin nanofibers. *Polymer* **2006**, *47*, 2911–2917. [CrossRef]
2. Gómez-Guillén, M.C.; Giménez, B.; López-Caballero, M.E.; Montero, M.P. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocoll.* **2011**, *25*, 1813–1827. [CrossRef]
3. Rigueto, C.V.T.; Rosseto, M.; Krein, D.D.C.; Ostwald, B.E.P.; Massuda, L.A. Alternative uses for tannery wastes: A review of environmental, sustainability, and science. *J. Leather Sci. Eng.* **2020**, *2*, 21. [CrossRef]
4. Mokrejš, P.; Gál, R.; Mrázek, P. Biotechnology-Based Production of Food Gelatine from Poultry By-Products. WO2019091502, 16 May 2019.
5. Vandervoort, J.; Ludwig, A. Preparation and evaluation of drug-loaded gelatin nanoparticles for topical ophthalmic use. *Eur. J. Pharm. Biopharm.* **2004**, *57*, 251–261. [CrossRef]
6. Rigueto, C.V.T.; Rosseto, M.; Alessandretti, I.; De Oliveira, R.; Wohlmuth, D.A.R.; Zanella, B.B.; Dettmer, A. Gelatin films from wastes: A review of production, characterization, and application trends in food preservation and agriculture. *Int. Food Res.* **2022**, *162*, 112114. [CrossRef]
7. Jafari, M.; Afkhami, R.; Sedaghat, N. Preparation and characterization of active Cirish fructans–fish gelatin film: Physicochemical, antioxidant, and antimicrobial properties. *Food Sci. Nutr.* **2023**, *11*, 709–721. [CrossRef]

8. Rosseto, M.; Rigueto, C.V.T.; Krein, D.D.C.; Massuda, L.A.; Balbé, N.P.; Colla, L.M.; Dettmer, A. Combined effect of transglutaminase and phenolic extract of *Spirulina platensis* in films based on starch and gelatin recovered from chrome III tanned leather waste. *Biofuels Bioprod. Biorefin.* **2021**, *15*, 1406–1420. [CrossRef]
9. Sun, H.W.; Feigal, R.J.; Messer, H.H. Cytotoxicity of glutaraldehyde and formaldehyde in relation to time of exposure and concentration. *Pediatr. Dent.* **1990**, *12*, 303–307. [PubMed]
10. Gough, J.E.; Scotchford, C.A.; Downes, S. Cytotoxicity of glutaraldehyde crosslinked collagen/poly(vinyl alcohol) films is by the mechanism of apoptosis. *J. Biomed. Mater. Res.* **2002**, *61*, 121–130. [CrossRef] [PubMed]
11. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a Request from the Commission Related to Flavouring Group Evaluation 10: Aliphatic Primary and Secondary Saturated and Unsaturated Alcohols, Aldehydes, Acetals, Carboxylic Acids and Esters Containing an Additional Oxygenated Functional Group and Lactones from Chemical Groups 9, 13 and 30 (Commission Regulation (EC) No. 1565/2000 of 18 July 2000). Available online: https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2005.246 (accessed on 13 March 2022).
12. Code of Federal Regulations. Available online: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-172#p-172.230(a)(2) (accessed on 13 March 2022).
13. Bigi, A.; Cojazzi, G.; Panzavolta, S.; Roveri, N.; Rubini, K. Stabilization of gelatin films by crosslinking with genipin. *Biomaterials* **2002**, *23*, 4827–4832. [CrossRef]
14. Babczyk, P.; Conzendorf, C.; Klose, J.; Schulze, M.; Harre, K.; Tobiasch, E. Stem cells on biomaterials for synthetic grafts to promote vascular healing. *J. Clin. Med.* **2014**, *3*, 39–87. [CrossRef] [PubMed]
15. Ko, J.H.; Yin, H.Y.; An, J.H.; Chung, D.J. Characterization of crosslinked gelatin nanofibers through electrospinning. *Macromol. Res.* **2010**, *18*, 137–143. [CrossRef]
16. Xuejing, Z.; Ying, P.; Jie, L.; Kun, W.; Keyong, T. Influence of UV irradiation on the properties of goatskin collagen matrices. *J. Soc. Leather Technol. Chem.* **2017**, *101*, 66–71.
17. Jiang, B.; Wu, Z.; Zhao, H.; Tang, F.; Lu, J.; Wei, Q.; Zhang, X. Electron beam irradiation modification of collagen membrane. *Biomaterials* **2006**, *27*, 385–408. [CrossRef] [PubMed]
18. Gungor, M.; Sagirli, M.N.; Calisir, M.D.; Sule, S.; Kilic, A. Developing centrifugal spun thermally crosslinked gelatin based fibrous biomats for antibacterial wound dressing applications. *Polym. Sci. Eng.* **2021**, *61*, 2311–2322. [CrossRef]
19. Vieira, M.G.A.; Da Silva, M.A.; Dos Santos, L.O.; Beppu, M.M. Natural-based plasticizers and biopolymer films: A review. *Eur. Polym. J.* **2011**, *47*, 254–263. [CrossRef]
20. Suput, D.; Lazic, V.; Popovic, S.; Hromis, N. Edible films and coatings: Sources, properties and application. *Food Feed. Res.* **2015**, *42*, 11–22. [CrossRef]
21. Alvarez, M.V.; Ponce, A.G.; Moreira, M.D.R. Antimicrobial efficiency of chitosan coating enriched with bioactive compounds to improve the safety of fresh cut broccoli. *LWT-Food Sci. Technol.* **2013**, *50*, 78–87. [CrossRef]
22. Arcan, I.; Yemenicioğlu, A. Incorporating phenolic compounds opens a new perspective to use zein films as flexible bioactive packaging materials. *Int. Food Res.* **2011**, *44*, 550–556. [CrossRef]
23. Loo, C.P.Y.; Sarbon, N.M. Chicken skin gelatin films with tapioca starch. *Food Biosci.* **2020**, *35*, 100589. [CrossRef]
24. Qiao, C.; Xianguang, M.A.; Zhang, J.; Yao, J. Molecular interactions in gelatin/chitosan composite films. *Food Chem.* **2017**, *235*, 45–50. [CrossRef] [PubMed]
25. Zheng, H.; Zhao, M.; Dong, Q.; Fan, M.; Wang, L.; Li, L. Extruded transglutaminase-modified gelatin–beeswax composite packaging film. *Food Hydrocoll.* **2022**, *132*, 107849. [CrossRef]
26. Thomazine, M.; Carvalho, R.A.; Sobral, P.J. Physical properties of gelatin films plasticized by blends of glycerol and sorbitol. *J. Food Sci.* **2005**, *70*, E172–E176. [CrossRef]
27. Suderman, N.; Sarbon, N.M. Optimization of chicken skin gelatin film production with different glycerol concentrations by response surface methodology (RSM) approach. *J. Food Sci. Technol.* **2020**, *57*, 463–472. [CrossRef] [PubMed]
28. Sancakli, A.; Başaran, B.; Polat, O. Effects of bovine gelatin viscosity on gelatin-based edible film mechanical, physical and morphological properties. *Discov. Appl. Sci.* **2021**, *3*, 8. [CrossRef]
29. Lv, L.C.; Huang, Q.Y.; Ding, W.; Xiao, X.H.; Zhang, H.Y.; Xiong, L.X. Fish gelatin: The novel potential applications. *J. Funct. Foods* **2019**, *63*, 103581. [CrossRef]
30. Prokopová, A.; Pavlačková, J.; Mokrejš, P.; Gál, R. Collagen hydrolysate prepared from chicken by-product as a functional polymer in cosmetic formulation. *Molecules* **2021**, *26*, 2021. [CrossRef] [PubMed]
31. Martinek, J.; Gál, R.; Mokrejs, P.; Sucháčková, K.; Pavlačkova, J.; Kalendová, A. The effect of application of chicken gelatin on reducing the weight loss of beef sirloin after thawing. *Polymers* **2022**, *14*, 3094. [CrossRef]
32. Martinek, J.; Mokrejš, P.; Pavlačková, J.; Hřibová, M.; Pokorný, P.; Janáčová, D.; Gál, R. Characterization of fibers prepared by centrifugal spinning from biotechnologically derived chicken gelatin. *Foods* **2024**, *13*, 2630. [CrossRef]
33. Du, L.; Khiari, Z.; Pietrasik, Z.; Betti, M. Physicochemical and functional properties of gelatins extracted from turkey and chicken heads. *Poult. Sci.* **2013**, *92*, 2463–2474. [CrossRef]

34. *ASTM D 1653-03*; Standard Test Methods for Water Vapor Transmission of Organic Coating Films. ASTM International: West Conshohocken, PA, USA, 2010.
35. *ASTM E313-20*; Standard Practice for Calculating Yellowness and Whiteness Indices from Instrumentally Measured Color Coordinates. ASTM International: West Conshohocken, PA, USA, 2020.
36. *ASTM D1003-21*; Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics. ASTM International: West Conshohocken, PA, USA, 2021.
37. Ji, F.; Zhou, W.; Zhang, Z.; Zhang, B. Effects of relative molecular weight distribution and isoelectric point on the swelling behavior of gelatin films. *Front. Chem.* **2022**, *10*, 857976. [CrossRef] [PubMed]
38. Gordon, P.W.; Brooker, A.D.M.; Chew, Y.M.J.; Wilson, D.I.; York, D.W. Studies into the swelling of gelatine films using a scanning fluid dynamic gauge. *Food Bioprod. Process.* **2010**, *88*, 357–364. [CrossRef]
39. Dang, X.; Shan, Z.; Chen, H. Biodegradable films based on gelatin extracted from chrome leather scrap. *Int. J. Biol. Macromol.* **2018**, *107*, 1023–1029. [CrossRef] [PubMed]
40. Boanini, E.; Rubini, K.; Panzavolta, S.; Bigi, A. Chemico-physical characterization of gelatin films modified with oxidized alginate. *Acta Biomater.* **2010**, *6*, 383–388. [CrossRef] [PubMed]
41. Avena-Bustillos, R.J.; Olsen, C.W.; Olson, D.A.; Chiou, B.; Yee, E.; Bechtel, P.J.; McHugh, T.H. Water vapor permeability of mammalian and fish gelatin films. *J. Food Sci.* **2006**, *71*, E202–E207. [CrossRef]
42. Tyuftin, A.A.; Pecorini, F.; Zanardi, E.; Kerry, J.P. Parameters affecting the water vapour permeability of gelatin films as evaluated by the infrared detecting method ASTM F1249. *Sustainability* **2022**, *14*, 9018. [CrossRef]
43. Suhaima, N.R.; Suyatma, N.E.; Hunaefi, D.; Jayanegara, A. Comparison of fish and mammalian gelatin film properties: A meta-analysis. *AIMS Agric. Food* **2022**, *7*, 461–480. [CrossRef]
44. Perkasa, D.P.; Erizal, E.; Abbas, B. Polymeric biomaterials film based on poly(vinyl alcohol) and fish scale collagen by repetitive freeze-thaw cycles followed by gamma irradiation. *Indones. J. Chem.* **2013**, *13*, 221–228. [CrossRef]
45. Stevenson, M.; Long, J.; Seyfoddin, A.; Guerrero, P.; Caba, d.l.K.; Etxabideet, A. Characterization of ribose-induced crosslinking extension in gelatin films. *Food Hydrocoll.* **2020**, *99*, 105324. [CrossRef]
46. Kim, D.; Min, S.C. Trout skin gelatin-based edible film development. *J. Food Sci.* **2012**, *77*, E240–E246. [CrossRef] [PubMed]
47. Yap, K.L.; Kong, I.; Abdul Kalam Saleena, L.; Pui, L.P. 3D Printed gelatin film with Garcinia atroviridis extract. *J. Food Sci. Technol.* **2022**, *59*, 4341–4351. [CrossRef] [PubMed]
48. Rawdkuen, S.; Sai-Ut, S.; Benjakul, S. Properties of gelatin films from giant catfish skin and bovine bone: A comparative study. *Eur. Food Res. Technol.* **2010**, *231*, 907–916. [CrossRef]
49. Pranoto, Y.; Lee, C.M.; Park, H.J. Characterizations of fish gelatin films added with gellan and κ-carrageenan. *LWT-Food Sci. Technol.* **2007**, *40*, 766–774. [CrossRef]
50. Ekielski, A.; Żelaziński, T.; Mishra, P.K.; Skudlarski, J. Properties of biocomposites produced with thermoplastic starch and digestate: Physicochemical and mechanical characteristics. *Materials* **2021**, *14*, 6092. [CrossRef]
51. Peña-Rodriguez, C.; Martucci, J.F.; Neira, L.M.; Arbelaiz, A.; Eceiza, A.; Ruseckaite, R.A. Functional properties and in vitro antioxidant and antibacterial effectiveness of pigskin gelatin films incorporated with hydrolysable chestnut tannin. *Food Sci. Technol. Int.* **2015**, *21*, 221–231. [CrossRef]
52. Nor, M.H.M.; Nazmi, N.N.M.; Sarbon, N.M. Effects of plasticizer concentrations on functional properties of chicken skin gelatin films. *Int. Food Res. J.* **2017**, *24*, 1910–1918.
53. Coppola, M.; Djabourov, M.; Ferrand, M. Phase diagram of gelatin plasticized by water and glycerol. *Macromol. Symp.* **2008**, *273*, 56–65. [CrossRef]
54. Rosseto, M.; Rigueto, C.V.T.; Krein, D.D.C.; Massuda, L.A.; Ostwald, B.E.P.; Colla, L.M.; Dettmer, A. Accelerated aging of starch-gelatin films with enzymatic treatment. *J. Polym. Environ.* **2021**, *29*, 1063–1075. [CrossRef]
55. Baggio, E.; Scopel, B.S.; Rosseto, M.; Rigueto, C.V.T.; Dettmer, A.; Baldasso, C. Transglutaminase effect on the gelatin-films properties. *Polym. Bull.* **2022**, *79*, 7347–7361. [CrossRef]
56. Chiono, V.; Pulieri, E.; Vozzi, G.; Ciardelli, G.; Ahluwalia, A.; Giusti, P. Genipin-crosslinked chitosan/gelatin blends for biomedical applications. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 889–898. [CrossRef]