

Article

Development of Biodegradable Alginate-Based Films with Bioactive Properties and Optimal Structural Characteristics with Incorporation of Protein Hydrolysates

Oksana Zinina ¹ , Svetlana Merenkova ^{1,*}  and Damir Galimov ² 

¹ Department of Food and Biotechnology, South Ural State University (National Research University), 76 Lenin Avenue, 454080 Chelyabinsk, Russia; zininaov@susu.ru

² Nanotechnology Research & Education Centre, South Ural State University (National Research University), 76 Lenin Avenue, 16, 454080 Chelyabinsk, Russia; galimovdm@susu.ru

* Correspondence: merenkovasp@susu.ru; Tel.: +7-951-813-70-2

Abstract: Alginate is widely used in the food industry due to its biodegradability, biocompatibility, and non-toxicity. Protein hydrolysates possess properties important for forming the mechanical characteristics, protective, and barrier properties of the films. The aim of the research was to develop biodegradable alginate-based films with bioactive properties and the optimal structural characteristics when protein hydrolysates were incorporated. The microstructure of the cross-sections of films with 0.5 and 1.0% protein hydrolysates was characterized by smoother and homogeneous surfaces, which indicated the compatibility of sodium alginate and protein hydrolysate. The addition of protein hydrolysate significantly increased the thickness of the film by 0.06 mm and reduced the solubility by 49.4% ($p < 0.05$). The results showed the high biodegradability of alginate-based films after 2 weeks of storage. With the introduction of protein hydrolysate, changes occurred in the FTIR patterns due to the interaction between the hydroxyl groups of peptides and the alginate, and, consequently, the thermal stability of the alginate films increased. The alginate films with PH positively affected the storage capacity of sweet cherry berries, both at room temperature and under refrigeration conditions. The alginate-based films with protein hydrolysate have improved properties and can serve as an alternative to polypropylene packaging materials.

Keywords: alginate; film coating; protein hydrolysate; biodegradability; solubility; microstructure; thickness; solubility; chemical relationships; thermal stability; food storage function



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1. Introduction

The problem of polymer package waste disposal has become increasingly urgent due to the harmful impact on surrounding environments. Most traditional package materials do not undergo secondary recycling and are not environmentally friendly. The advanced food packaging sector is in constant development, a considerable number of scientific publications are focused on the implementation of natural and sustainable materials in the production of films and coatings. Biopolymer packaging is an ecological system that prevents the spoilage of food products and improves their quality by protecting against gases and humidity [1,2].

Natural biopolymers are alternative structures, formed when film coatings are produced. Coatings based on biopolymers such as proteins and polysaccharides have good mechanical properties but are permeable to water due to their hydrophilic nature. Composite films and coatings made up of a combination of proteins, polysaccharides and lipids can be categorized as multilayered compositions and conglomerates. Multilayered compositions consist of two or more layers. Structure optimization significantly enhances the final coating's barrier properties; however, its production involves additional technological stages. Conglomerates can be obtained by mixing two or more biopolymers into a single

homogenous layer, allowing it to exhibit all the functional properties of its individual components [3,4].

Alginate, agarose, and carrageenan from marine algae are promising alternatives to traditional plastics used in food packaging [5]. The gel-forming ability, emulsification, and foam formation of various polysaccharides of seaweed are based on their unique structure. These hydrocolloids are added to biocomposites as gelling agents and thickening agents, and they also act as emulsifiers [6]. Alginate has unique colloidal properties thanks to its chemical structure, which enables the creation of transparent and uniform films or coatings.

Alginate-based packaging materials are a sustainable and biodegradable alternative in the food packaging field because they provide quality optimization and waste reduction. However, pure alginate cannot be used to make film materials due to the weak mechanical properties of resulting films. The development of composite films, when alginate is combined with other biopolymers, such as proteins or cellulose, provides an effective solution to the disadvantages of instability to high humidity and mechanical action [7,8]. It was observed that chemically modified film coatings could significantly improve their mechanical properties through crosslinking the polysaccharide with Ca^{2+} and other metal ions [9] since sodium alginate contains a large number of hydroxyl and carboxyl groups, providing excellent adsorption capacity for metal ions. Also, upon interaction with metal ions, non-soluble polymers, impermeable to fats and oils, are formed.

Alginate belongs to the group of substances with proven safety for humans and the environment, so it is a natural choice for packaging materials [10]. The mechanical properties of alginate salts are improved by the addition of plasticizers, the most optimal additive is glycerin because of its excellent compatibility with the polymer matrix.

Recently, researchers have focused on giving packaging materials bioactive properties and improving their barrier, thermal, mechanical, antioxidant, and antimicrobial characteristics, which contribute to prolonging the products' shelf life. The alginate-based packaging materials are promising as they are carriers of active substances (antimicrobial and antioxidant agents) [11,12]. Combinations of plant extracts, metal ions, antioxidants, bacteriocins, propolis, essential oils, and protein hydrolysates are increasingly used as active ingredients in alginate films [13–15]. The antimicrobial activity of alginate films can be enhanced by the incorporation of metal ions or bioactive components [16,17]. Alginate-based edible coatings and films incorporated with the plant extracts have demonstrated pronounced antioxidant properties, as well as significant antifungal and antimicrobial activity [18,19]. These natural antioxidative/antimicrobial compounds are highly valued by consumers because they are perceived to be safer.

Agro-industrial waste is a valuable source of bioactive compounds that can be valorized and used in film and coating production as the bioactive compounds improve the functions properties of the packaging. The incorporation of protein hydrolysates from by-products in edible packaging is a promising strategy to achieve sustainability, circular economy, and to minimize food waste and losses.

Protein hydrolysates are increasingly being used in various industries including the production of bioactive films. Active peptides formed during the process of hydrolysis possess a wide range of properties important for forming the mechanical characteristics of the film and its barrier properties against oxidative and microbiological processes in food products [20–22]. Bioactive peptides and protein hydrolysates have great potential as antioxidant and antimicrobial compounds [23]. Due to their surface active properties, peptides can be located at the interface of the oil–water phases in emulsions, and can create a physical barrier, reducing the contact of lipids with oxidants. Therefore, bioactive films containing these types of peptides may provide additional benefits compared to traditional methods of preservation.

Functional films with added bioactive peptides can be obtained using several methods, as follows: introduction of the peptide in a polymer matrix, peptide coating on a polymer surface, and the immobilization of peptides in polymers. The first method is the most applicable [23].

When mixing components of the biocomposite, antimicrobial peptides should be compatible with used solvents and structural polymers. Biopolymers based on proteins and polysaccharides are a good option because they are water–ethanol soluble, and compatible with bioactive peptides [24]. One advantage of this method is preservation of peptide activity, as no high temperatures are used during film production.

Since microbial contamination mostly occurs on the surfaces of food products, the application of coatings containing antimicrobial peptides may be more effective than directly adding them into the product. Antimicrobial peptides continuously release from the coating onto the surface of the product helping to maintain effective concentrations. Additionally, the use of peptides as active ingredients in thin films requires smaller amounts compared to direct addition into the volume of the product [24].

The development of bioactive films based on protein hydrolysates (bioactive peptides) contributes to sustainable economic development, implementation of resource-saving technologies, and opens new perspectives in obtaining environmentally safe food products without chemicals. Thus, the valorization of agro-industrial waste and byproducts into products with added value used for developing biodegradable packaging material represents a promising practice for sustainability by reducing environmental problems while simultaneously stimulating the circular economy and the rational utilization of secondary resources [25]. An important ecological aspect of this approach is also that some natural polymers are produced by recycling environmentally harmful waste or byproducts of the food industry. The aim of the research was to develop biodegradable alginate-based films with bioactive properties and the optimal structural characteristics when protein hydrolysates were incorporated.

2. Materials and Methods

2.1. Raw Materials and Ingredients

Sodium alginate (Ingredico LLC, Moscow, Russia), glycerin plasticizer (Iodine Technologies and Marketing LLC, Moscow, Russia) were used as a structure-forming agent. The protein hydrolysate was obtained from poultry byproducts by fermentation, using the previously described technology, and was used as the active component [26].

2.2. Manufacturing of Biodegradable Alginate-Based Films

The objects of the study are samples of films with the addition of different amounts of protein hydrolysate—0.5, 1.0, and 1.5%—and a control sample of the film without the addition of protein hydrolysate. The films were molded by casting according to the instructions of the technology presented in Figure 1.

2.3. Investigation of Thickness, Mechanical Properties, Appearance, and Surface Morphology of Films

Film thickness was measured by a digital micrometer (type KW06-85, Krisbow, Jakarta, Indonesia) at 5 distinct positions with a 0.001 mm accuracy.

Dumbbell-shaped samples were cut from rectangular films using an IDM Instruments C0022 punching press (Pty Ltd.[®], Sydney, NSW, Australia) to determine the mechanical parameters (tensile strength and elongation). The samples had a length of 100 mm, a working part length of 20 mm, and the gripping parts were 15 mm wide. The samples were secured in INSTRON pneumatic grippers (Norwood, MA, USA). The tests were carried out on an INSTRON testing machine with a crosshead speed of 50 mm/min.

Appearance was evaluated visually in daylight. Photos were taken at a magnification of $\times 5$. To study the morphological characteristics, scanning electron microscopy (SEM) was used to visualize the surface topography and cross section of the films. The films were coated with a thin layer of gold in a high-vacuum coating system (MSP-30T, Showa Shinku Devices Inc., Sagamihara, Japan), and were microscoped on a high-resolution transmission electron microscope with scanning transmission electron microscopy (STEM) function (Jeol JEM-2100, Tokyo, Japan) at magnifications of $\times 500$ and $\times 1000$.

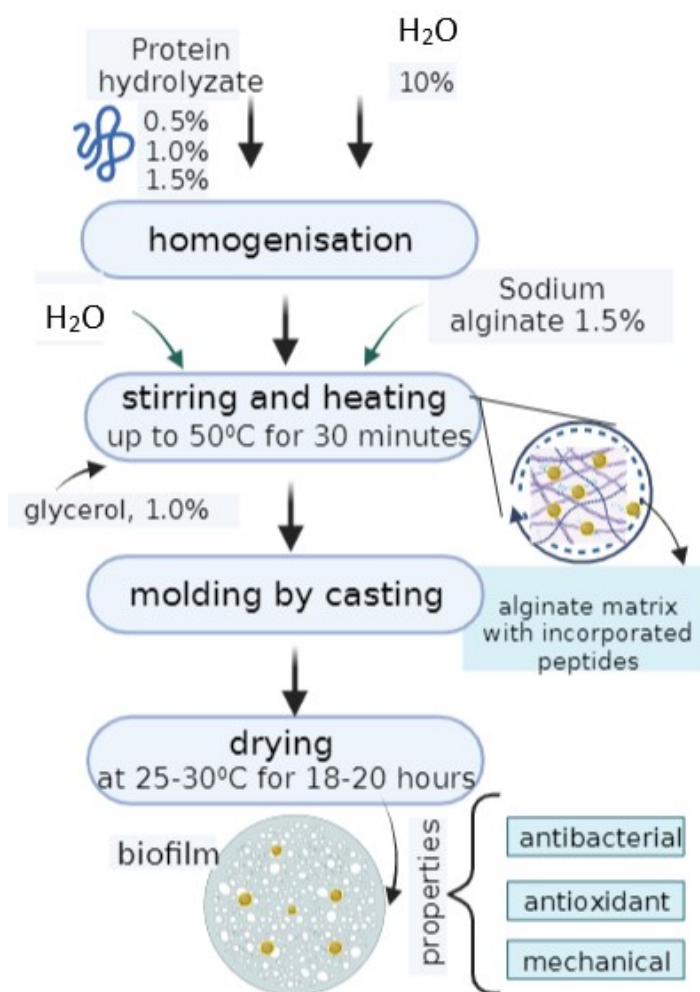


Figure 1. Scheme for obtaining biodegradable film samples based on sodium alginate.

2.4. Analyses of Biodegradability of Films

Biodegradability testing was conducted based on DIN 54900-2 “Testing for complete biological disintegration of polymers in laboratory tests” and DIN 54900-3 “Tests in practical conditions”. Testing was carried out by placing film samples measuring $5 \times 5 \text{ cm}^2$ in bags with biomass compost. Compost samples were analyzed each week, filtering through sieves and checking unbroken components, until the film residues were completely converted into compost. The remaining debris was examined under a microscope.

2.5. Investigation of Water Solubility, Water Vapour, and the Transmission Rate of Films

A modified Farhan-Hani method was used to test the films’ water solubility [27]. Samples were cut into small pieces measuring $2 \times 2 \text{ cm}^2$ and dried at 105°C for 6 h before being weighed (m_1). Each sample was placed in a flask containing 100 cm^3 of distilled water and stirred using a magnetic mixer at 240 rpm. The samples were left in the water bath for 6 h at room temperature (25°C), after which the contents of the flask were filtered and the filter paper with undissolved particles was dried in an oven at 105°C until constant mass. Finally, the weight of the filter paper was measured to determine insoluble dry matter (m_2). Water solubility (%) was calculated according to the following equation:

$$\text{WS \%} = \frac{m_1 - m_2}{m_1} \cdot 100 \quad (1)$$

Water vapor transmission rate (WVTR) was determined gravimetrically using the method described by Dewi et al. [28].

2.6. Investigation of Antioxidant Capacity, Polyphenol Content, and DPPH Antiradical Activity

To evaluate the antioxidant capacity of the films, we performed coulometric titration using an Expert-006 device manufactured by NPK OOO “EcoNix-Expert” (Novosibirsk, Russia). Prior to analysis, researchers prepared a 1% ethanol extract of crushed films and incubated it for 24 h. For testing purposes, aliquots of 1 mL of 0.1% ascorbic acid solution served as reference standards. Antioxidant capacity results were expressed in mg/g polymer.

The antiradical activity was determined by the DPPH method (%). The ethanolic extract of the films was mixed with the DPPH solution (solution of 2,2-diphenyl-1-picrylhydrazyl), incubated for 30 min. The optical density was measured on a spectrophotometer Jenway (6405 UV/Vis, Felstad, UK) at 515 nm [29].

The total phenolic content (TPC) was determined in the film’s ethanol extract using Folin-Chocalteu reagent. The optical density was determined at 700 nm using a Jenway spectrophotometer (6405 UV/Vis, Felstad, UK). The total phenolic content was expressed in gallic acid equivalent mg GAE/g [29].

2.7. FTIR Analysis of Films

Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to determine the chemical structure of the film through wavelengths of 400–4000 cm^{-1} . The study was carried out on a Shimadzu IRAffinity-1S infrared spectrophotometer (Shimadzu, Kyoto, Japan).

2.8. Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis

To determine the thermal effects and mass loss of films during heating differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) were performed using a simultaneous thermal analyzer (TG-DSC) Netzsch STA 449F1 “Jupiter” in corundum crucibles at heating from 30 °C to 500 °C. The heating rate was 10 °C per minute. The measurements were carried out in an argon medium. Using an analog-to-digital converter, curves were obtained in digital form.

2.9. Evaluation of food Storage Function

The effect of films on the storage capacity of food products was determined by assessing the weight loss during storage of sweet cherries at room temperature and in a refrigerator (4 °C) for 9 days. Sweet cherry berries were chosen as a model system convenient for reproducing the results of film studies, however, the established effects can also be observed when packaging other vegetables and fruits with a similar structure. In this study, 10 berries were wrapped in each film. The weight loss during storage was determined as the difference between the weight of sweet cherries without film and the weight of sweet cherries after storage for 3, 6 and 9 days. Also, during storage, the change in the content of vitamin C was evaluated by the spectrophotometric method. To do this, we took a sample of pre-crushed pitted berries weighing 10 g and homogenized them (Stegler S10) in 100 mL of distilled water for 1 min and then filtered and centrifuged. From each supernatant, 2 samples with a volume of 200 μL were taken into centrifuge tubes. One sample was made up to a volume of 5 mL with distilled water. Distilled water, 0.5 mL of 1 N sodium hydroxide solution were added to the second tube to the sample to 1 mL and kept at room temperature for 1 h, then the volume was adjusted to 5 mL. The optical density of these solutions was measured at 265 nm on the Jenway spectrophotometer (6405 UV/Vis, Felstad, UK). The content of vitamin C in the sweet cherry samples was calculated as the difference in absorbance between the test sample and the sample treated with alkali. To construct a calibration curve, the vitamin C was dissolved in distilled water at concentrations from 0 to 60 $\mu\text{g/mL}$ and the optical density of the resulting vitamin C solutions was measured at 265 nm.

2.10. Microbiological Indicators of Sweet Cherry during Storage

The microbiological indicators of the sweet cherries were determined using Petritest™ microbiological rapid tests (NPO Alternativa, Moscow, Russia). To prepare the initial dilutions, 10 g of the crushed pulp of the cherry berries was taken and 90 mL of sterile saline was added. A series of tenfold dilutions was prepared from the initial dilution of the pulp.

Petritest™ containing an indicator for staining enterobacteria colonies in red was used, to determine the content of the total amount of coliforms (TCC) in 1 g. After adding 0.2 cm³ of the dilution to the surface of the substrate, the Petritests were placed in a thermostat and incubated at a temperature of $(36 \pm 1)^\circ\text{C}$ for (12–24) h. Petritests were selected, on which from 15 to 300 colonies were grown and counted. The result was multiplied by the value of the appropriate dilution to give the total number of viable bacteria, or the total number of coliforms, in 0.2 cm³ of the sample. According to the manufacturer's recommendations, the counting results were multiplied by 5 to obtain a result of 1 cm³.

The samples were incubated at a temperature of $(24 \pm 1)^\circ\text{C}$ for 24 h (for preliminary accounting) and 120 h (for final accounting) when determining yeast and mold fungi. Petritests were selected, on which from 15 to 150 yeast colonies and from 5 to 50 mold colonies were grown and counted. The result was multiplied by the value of the appropriate dilution and the amount of yeast or mold in 0.2 cm³ of the sample, and then multiplied by 5 in order to obtain the final result.

2.11. Statistical Analyses

All investigations were performed in three replications. The three repetitions were made to measure each indicator. The results were expressed as an average value $\pm$ standard deviation. The probability values $p \leq 0.05$ were taken to indicate statistical significance. The data were analyzed using one-way ANOVA analysis using free web-based software offered by Assaad et al. [30].

3. Results and Discussion

3.1. Physical Properties and Surface Morphology of Films

The visual appearance and microstructure of alginate films with varying levels of protein hydrolysate are presented in Figure 2. Films containing protein hydrolysate were visually transparent and not sticky, whereas the control film without the added hydrolysate was less transparent and more adhesive. The observed changes were caused by the effective incorporation of peptides and amino acids into the alginate matrix, which is confirmed by the results of the microstructure study. To study the internal structure of the films with the addition of protein hydrolysates, SEM (scanning electron microscopy) was applied. As shown in Figure 2, the cross sections of films with different concentrations of hydrolysates had smoother and more uniform surfaces compared to the control sample, which demonstrated excellent compatibility between alginate sodium and protein hydrolysate. Granulated particles (agglomerates) ranging from 1 to 10 μm observed in the cross-sections of the control films, which led to an uneven structure, reducing the mechanical properties and increasing the solubility and brittleness. During the visualization of the microstructure, it was determined that films with a 1.0% addition of protein hydrolysate were more homogeneous, with hydrolysate particles being evenly distributed within the alginate matrix.

Riahi et al. noted a more homogeneous structure of sodium alginate-based films with the inclusion of the hydrophilic molecules of sulfur quantum dots, which is explained by the effective uniform dispersion of molecules in the alginate matrix [31]. Zhang et al. found that, at high concentration of hydrophobic nanocrystalline, cellulose in the composition of alginate–carrageenan films causes the agglomeration of cellulose particles, leading to multiple small cracks and holes that negatively impact the mechanical properties of composite films [9]. Bishnoi et al., when investigating the microstructure of alginate-

based films, observed a more compacted cross-section and homogeneous surface with an increasing concentration of wheat proteins in the film's composition [7].

In this research, we also investigated the effect of different concentrations of protein hydrolysate on film physical properties such as thickness, solubility, water vapor transmission rate, tensile strength, and elongation. The results, as shown in Table 1, indicated that the addition of 1.5% protein hydrolysate significantly increased the film thickness by 0.06 mm compared to the control group ($p < 0.05$), this indicated an increase in the amount of dissolved solid material presented in the matrix. This finding could be explained by the dispersion and swelling of the hydrophilic molecules, as suggested by Farhan and Hani [27]. These hydrophilic molecules may have interacted with the alginate matrix, causing an expansion of the distance between polymer chains, resulting in increased film thickness. Protein hydrolysates are amino acid-peptide combinations that consist of oligopeptides and free amino acids. It was found that the functional groups of polar amino acids and amino acid residues in the composition of peptides were hydrophilic and capable of forming hydrogen bonds with water molecules [26].

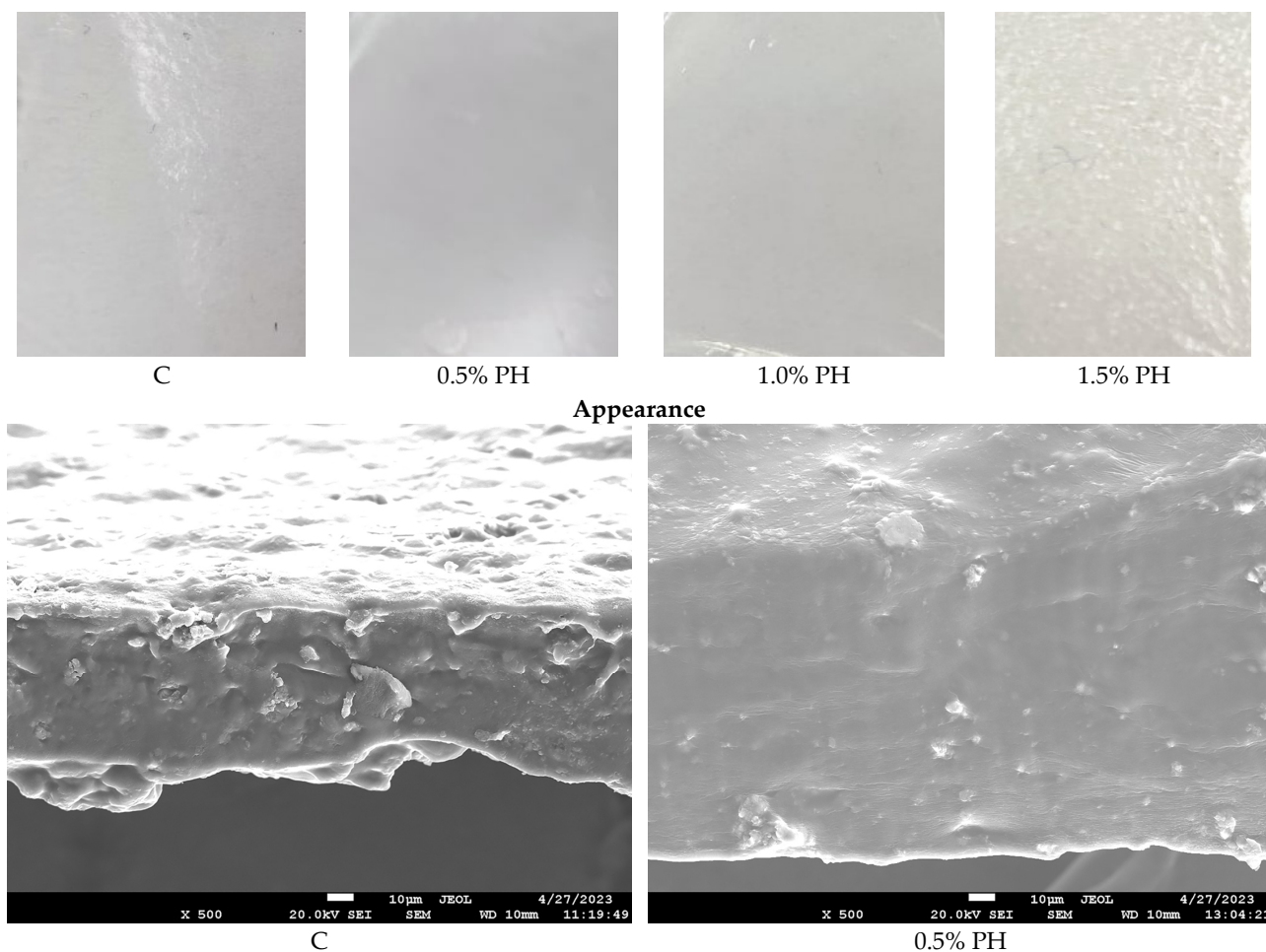


Figure 2. Cont.

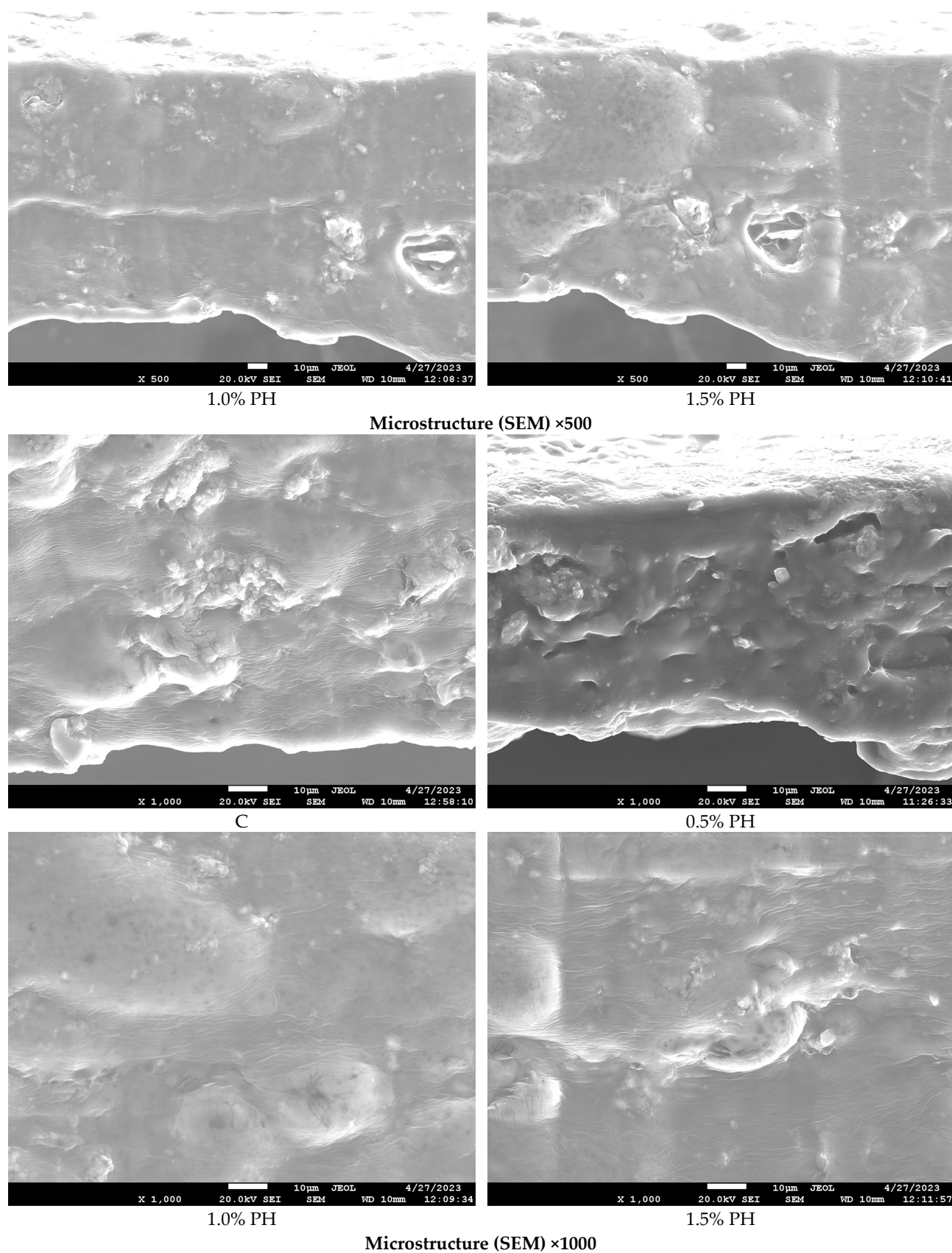


Figure 2. Structure of alginate films. Designation of samples: C—control alginate film; 0.5% PH—alginate films with 0.5% protein hydrolysate; 1.0% PH—alginate films with 1.0% protein hydrolysate; 1.5% PH—alginate films with 1.5% protein hydrolysate.

Table 1. Physical properties of films.

Indicator	Sample of the Film			
	Control	0.5% PH	1% PH	1.5% PH
Thickness, mm	0.23 ± 0.02 ^a	0.24 ± 0.01 ^a	0.26 ± 0.01 ^{ab}	0.29 ± 0.03 ^b
Solubility, %	68.42 ± 0.23 ^b	61.11 ± 0.10 ^b	41.70 ± 0.14 ^a	34.63 ± 0.18 ^a
WVTR, g/mm ² s	212.08 ± 2.25 ^b	210.26 ± 2.05 ^b	175.31 ± 1.55 ^a	173.19 ± 1.25 ^a
Tensile strength, MPa	7.53 ± 0.20 ^a	10.56 ± 0.35 ^b	11.80 ± 0.30 ^b	8.18 ± 0.28 ^a
Elongation, %	45.60 ± 0.55 ^{ab}	48.50 ± 0.64 ^b	53.65 ± 0.48 ^b	37.3 ± 0.33 ^a

The values are means (M) ± standard deviation of three replicates (s). Different letters in the same column (M^{a, b}) refer to a significant difference at ($p \leq 0.05$).

Solubility is also an important indicator for films, and depending on their purpose, preference is given to composite materials with the lowest or highest solubility. For example, edible food films require high solubility, while packaging materials used for products with high moisture content should not possess excessive solubility or hygroscopicity [32].

Highly soluble films are not suitable for use as primary packaging of food products with high water activity. However, they can be used in the form of soluble sachets for the preparation of individual portions of food and as active packaging for the release of antioxidant and antimicrobial compounds.

The results presented in Table 1 showed that the incorporation of protein hydrolysate had a significant impact on film solubility. As the protein hydrolysate concentration increased, the solubility of the film decreased, despite the high solubility of the hydrolysate itself (more than 90%). Alginate formed linear polysaccharides with moderate branching, which contributed to the formation of strong films upon reaction with the polyvalent metal cations (such as calcium, magnesium, manganese, iron, etc.) that were present in the protein hydrolysate. The resulting conglomerates enhanced the film resistance to water [33]. Bishnoi et al. demonstrated that the addition of protein to alginate improves film stability in water; alginate–protein films become partially soluble at a wheat protein concentration of 4–6%; and, at a protein content of 8%, the films are insoluble [7]. Presepiando et al. confirmed an increase in solubility with the introduction of high concentrations of honey into the biofilm in their studies, which is probably due to the plasticizing properties of this component [13].

The results of determining the mechanical characteristics of the films showed that the experimental samples of films with protein hydrolysate have exhibited greater strength compared to the controls. The relative elongation of the sample with 1.5% hydrolysate turned out to be lower than that of the control sample and the two other experimental film samples.

The detected decrease in water vapor transmission rate in the experimental samples with the addition of protein hydrolysate was consistent with the results obtained by Dewi et al. and Wulandari et al., who also noted that the WVTR was affected by the ratio between hydrophobic and hydrophilic components, as well as the thickness of the film, on which the diffusion time of water vapor depends [28,34]. The test sample with the greatest introduction of protein hydrolysate (1.5%) had the greatest thickness (0.29 mm) and the lowest WVTR value (173.19 g/mm²s).

The combination of biopolymers allowed us to overcome the disadvantages of single-component films and achieved the required functional and mechanical characteristics. The material properties of biodegradable films could be modified based on the hydrophobic and hydrophilic properties of the applied biopolymers. The hydrophobic molecules positively affected the water vapor permeability, while the hydrophilic molecules increased material strength and reduced oxygen permeability [33].

Alginate-based films were distinguished by their high resistance to fat and low permeability to oxygen. In a number of studies, alginates were used as the main component of films. For example, films were obtained from sodium alginate with a glycerin cross-linked

with calcium chloride and citric acid; from sodium alginate cross-linked with calcium chloride; from sodium alginate plasticized with glycerin [33,35].

3.2. Antioxidant Activity of Alginate Films

The results of determining the antioxidant properties of alginate films are given in Table 2.

Table 2. Antioxidant activity of alginate films.

Indicator	Sample of the Film			
	Control	0.5% PH	1% PH	1.5% PH
Antioxidant capacity, mg-eq. ascorbic acid/g	2.82 ± 0.12 ^a	3.41 ± 0.12 ^{ab}	3.62 ± 0.15 ^b	3.90 ± 0.16 ^b
DPPH activity, %	52.53 ± 2.50 ^a	66.76 ± 2.60 ^{ab}	68.43 ± 3.10 ^{bc}	74.74 ± 3.70 ^c
TPC, mg GAE/g	2.55 ± 0.09 ^a	2.87 ± 0.15 ^a	3.05 ± 0.12 ^{ab}	3.34 ± 0.22 ^b

The values are means (M) ± standard deviation of three replicates (s). Different letters in the same column (M^{a, b, c}) refer to a significant difference at ($p \leq 0.05$).

Antioxidant capacity and DPPH antiradical activity was high across all three film samples with hydrolysate. When we increased the protein hydrolysate concentration, the antioxidant capacity of the film significantly improved ($p \leq 0.05$). The highest DPPH activity (74.74%) and polyphenol content (3.34 mg GAE/g) were established for samples with 1.5% protein hydrolysate. The protein fermentation by microorganisms led to the accumulation of several compounds, including exopolysaccharides, polyphenols, and bioactive peptides. These molecules played an important role in conferring antioxidant properties to the generated protein hydrolysates [36], and the ability to form film matrices. The literature has shown that marine polysaccharides, particularly sodium alginate, also possess inherent antioxidative properties through natural polyphenol content [5]. Due to the excellent compatibility of sodium alginate and protein hydrolysate, the bioactive components embedded in the polysaccharide matrix were progressively released and exhibited antiradical properties. The DPPH activity, reducing power, and TBARS activity of alginate films and coatings can be enhanced by the incorporation of tannic acid or plant extracts as bioactive components [17–19].

3.3. Biodegradability of Films

When assessing the degradability of polymeric materials derived from natural polymers, it was important to determine the time required for material degradation under exposure to the microorganisms and enzymes present in the environment. A film was considered biodegradable when 90% of its material has decomposed due to biological action within a period of up to six months [37]. The experiment showed that all samples of the films had a good degree of biodegradation, but strong connections between alginate particles and protein hydrolysate fragments prolonged the degradation time of film samples. After one week all film samples were shriveled and partially covered with mold. After 2 weeks, the control sample and the sample with 0.5% protein hydrolysate were completely decomposed into humus. Samples with the addition of 1.0 and 1.5% hydrolysate were completely decomposed in humus after three weeks. The natural polymers, which served as nutrient sources for soil microorganisms, degraded under the influence of a combination of factors such as moisture, oxygen deficiency, and temperature (Figure 3).

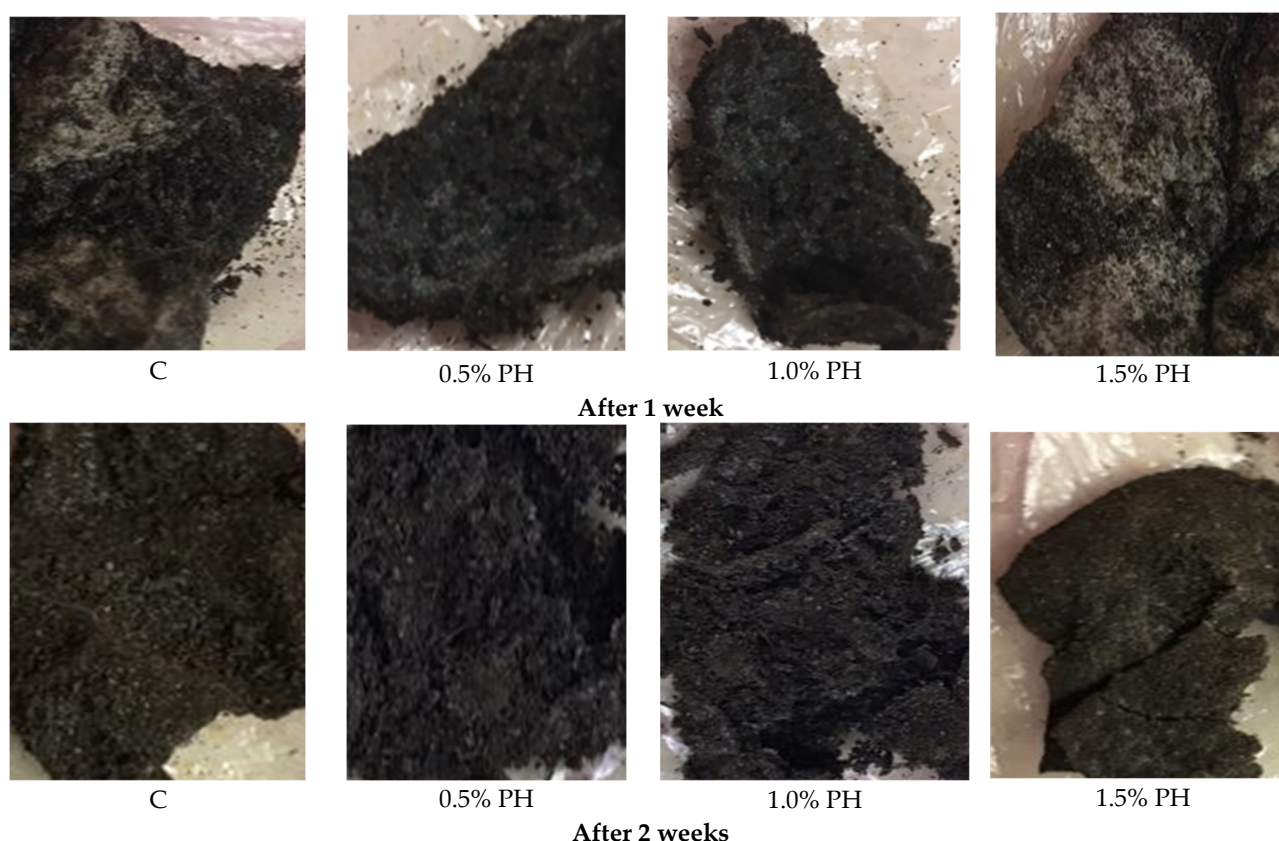


Figure 3. Biodegradability of alginate biofilms. Designation of samples: C—control alginate film; 0.5% PH—alginate films with 0.5% protein hydrolysate; 1.0% PH—alginate films with 1.0% protein hydrolysate; 1.5% PH—alginate films with 1.5% protein hydrolysate.

The results of the experiment showed that alginate-based film materials were biodegradable. After 2 weeks, biohumus was formed from more homogeneous and porous film samples, since under these conditions the decomposition reaction was faster. The mycelium mold, which formed on the samples, used thin cracks and pores for its growth, with the proliferation of mycelium, mechanical destruction of the material occurred. In addition, mold in the process of metabolism formed organic acids, which were aggressive agents for films. The data obtained were consistent with the results obtained by Santos et al., who also established the decomposition period of alginate films in the soil and sand about 15 days [38].

The results obtained were consistent with the higher stability of films with the addition of protein hydrolysate. The high crosslinking ability of alginate films with metal cations contained in hydrolysates contributed to greater resistance to biodegradation factors presented in the soil. In any case, with the slightly higher degree of biodegradability of films with protein hydrolysate, all samples were characterized by relatively rapid biodegradability and can be considered as an alternative to synthetic polymer materials.

3.4. FTIR Spectroscopy and Thermal Stability of Films

The effects induced by the protein hydrolysate addition to the alginate matrix were investigated by FTIR spectroscopy. The peaks corresponding to the main absorption capacity of the biofilms are presented in Figure 4. The protein-containing biofilms showed all the characteristic FTIR absorption bands reported for alginate [31]. The broad area from $3273\text{--}3420\text{ cm}^{-1}$ attributed to the stretching vibrations of the hydroxyl group in the alginate polymer. The absorption band at $2931\text{--}2941\text{ cm}^{-1}$ corresponded to the $\text{Csp}_3\text{-H}$ symmetric vibrations of the alginate. The intense $1618\text{--}1629\text{ cm}^{-1}$ peaks are attributed to the asymmetric and symmetric vibrations of the C-O in the carboxylic group. The

1028–1112 cm^{-1} peaks are correlated to the glycoside bond in the sodium alginate. The observed oscillations may reflect the interaction between the alginate negative charged -COO- groups and functional groups of peptides [16]. However, with the incorporation of the peptides, significant changes occurred in the FTIR patterns of the alginate films. For film samples, containing protein hydrolysate, the less pronounced peaks at 3331 cm^{-1} and between 1154 and 975 cm^{-1} indicated the presence of hydrophilic compounds (e.g., polyphenols) [37]. For the biofilm with protein hydrolysate, broadening of the absorption band was observed at 3550–3100 cm^{-1} due to the interaction between hydroxyl groups of peptides and the alginate structure. The characteristic absorption zones corresponding to the formation of S-O bonds and S-S bonds was observed in the low-frequency region, between 582 cm^{-1} and 553 cm^{-1} (Figure 4) [39].

A typical alginate film degradation curve was obtained with two thermal events [31,40] starting at a maximum temperature of 190 °C for the 1.5% hydrolysate film and 184.2 °C for the control film. At the first stage of thermal decomposition, weight loss was maximum 11.93% for the sample with 1.5% PH and minimum 10.51% for the control sample, during this period free and weakly bound moisture evaporated. The next thermal effect occurred when reaching 270.5 °C for the control sample and 306.3 °C for the sample with 1.5% PH. At the same time, the maximum weight loss was 82.25% in the control sample. It was noted that during degradation at temperatures of about 200 °C, the evaporation of glycerol and the base polymer occurs [41]. The addition of a protein hydrolysate in the composition of the films contributed to an increase in the thermal stability of the films; a tendency to increase with an increase in the content of protein hydrolysate in the films was noted. The results obtained confirmed the establishment of strong bonds between the alginate and the peptides of the protein hydrolysate (Figure 4).

3.5. Food Storage Function of films

3.5.1. Sweet Cherry Storage Capacity

The skin on the sweet cherries is a natural barrier to moisture loss during storage. As shown by the results in Figure 5, weight loss of the sweet cherries without a film turned out to be higher both when stored in room conditions and in a refrigerator. In general, the weight loss of the berries was higher when stored in the room condition, which could be due to higher shrinkage. In appearance, the sweet cherry clearly showed a difference when stored in a packaged form, in contrast to berries without films. By the 5th day of storage in the room condition, the berries without a film began to dry out and deteriorate significantly, in contrast to the experimental samples of berries in films with PH, which remained visually fresh. Similar results were obtained by Zhang et al. (2022) when studying changes in the mass of cherry tomatoes packed in films with different structure formers [9]. The results of determining the vitamin C in the cherries stored in the refrigerator (Figure 6) showed that its losses differ slightly between test samples and do not depend on the amounts of protein hydrolysate introduced into the film, in contrast to the control sample. The loss of vitamin C during storage was due to its ability to quickly oxidize [42], so the amount of loss would depend on the gas permeability of film coatings and possibly the manifestation of the antioxidant effect by protein hydrolysate particles.

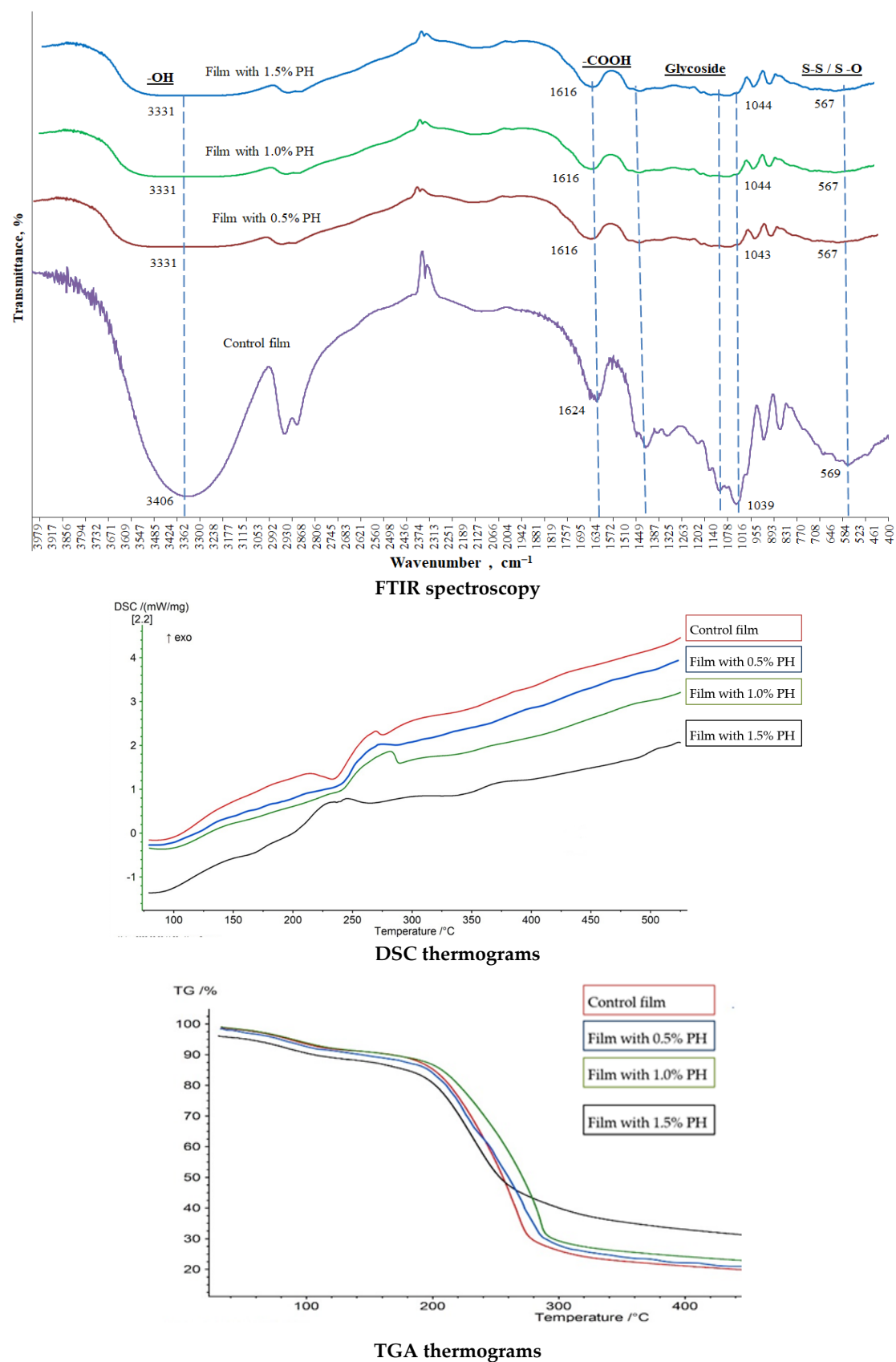


Figure 4. Results of FTIR spectroscopy and thermal stability of films. Designation of samples: C—control alginate film; 0.5% PH—alginate films with 0.5% protein hydrolysate; 1.0% PH—alginate films with 1.0% protein hydrolysate; 1.5% PH—alginate films with 1.5% protein hydrolysate.

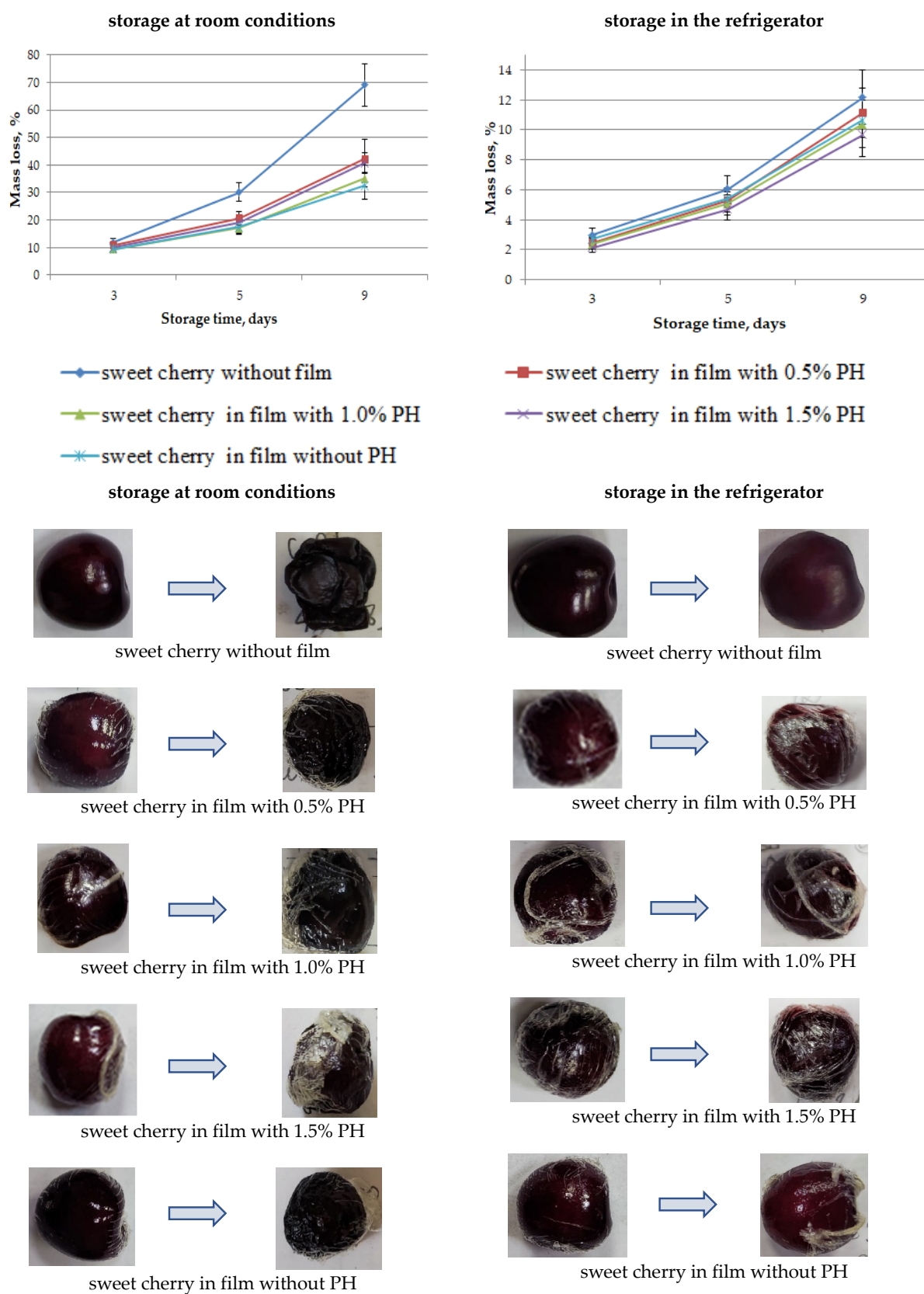


Figure 5. Dynamics of changes in the appearance of sweet cherry berries during the storage period. Designation of samples: C—control alginate film; 0.5% PH—alginate films with 0.5% protein hydrolysate; 1.0% PH—alginate films with 1.0% protein hydrolysate; 1.5% PH—alginate films with 1.5% protein hydrolysate.

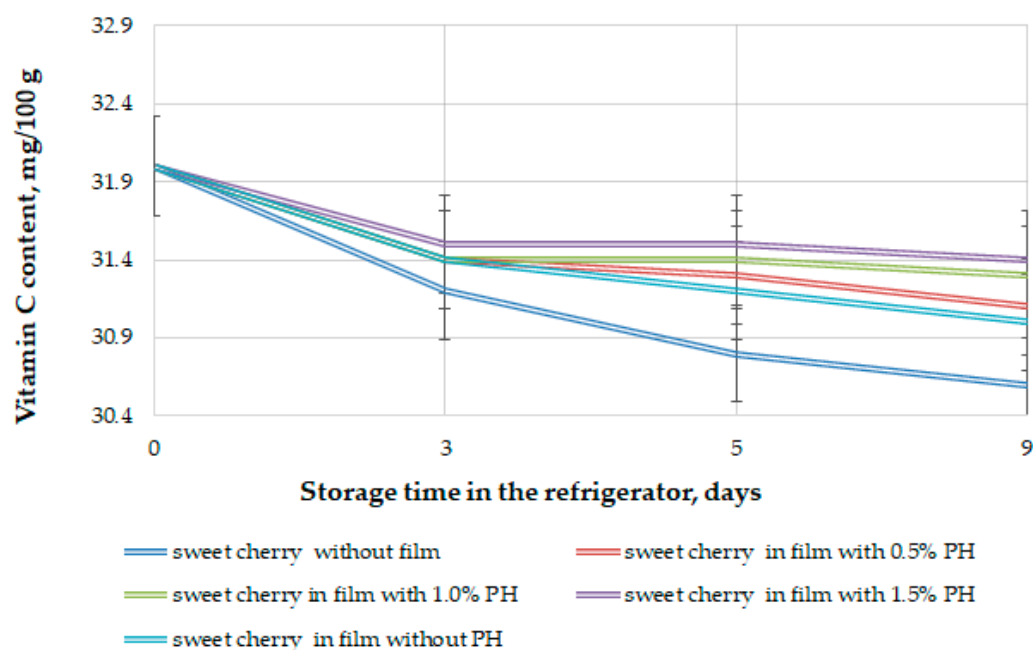


Figure 6. Changes in vitamin C content in cherries during refrigerated storage. Designation of samples: C—control alginate film; 0.5% PH—alginate films with 0.5% protein hydrolysate; 1.0% PH—alginate films with 1.0% protein hydrolysate; 1.5% PH—alginate films with 1.5% protein hydrolysate.

3.5.2. Microbiological Indicators of Sweet Cherry during Storage

The results of studies of microbiological indicators showed that films with the addition of protein hydrolysate, either when stored in a refrigerator or when stored at room temperature, had an inhibitory effect on the growth of microorganisms and molds. More active growth of mold on the sweet cherries was observed when stored in the refrigerator (Table 3). The antimicrobial activity of PH is due to electrostatic interaction between bioactive peptides and cell membrane of microorganisms causing its disruption [23]. Similar results were obtained in microbiological studies of cherries coated with natamycin-chitosan. The authors found that when storing cherries in the coating, the number of aerobic mesophilic bacteria, yeasts, and molds, including pathogenic fungi that cause berry rotting, decreased [43].

In this study, we proved the potential of biodegradable films based on sodium alginate with the inclusion of protein hydrolysate as a functional and bioactive component. The selected PH concentrations promoted the formation of homogeneous uniform film structure and improved its structural and mechanical characteristics, antioxidant and antimicrobial properties.

However, further research is needed to optimize the production process and improve the properties of the resulting material. In addition, further investigations are essential to establish the functionality of films in various conditions and applications.

The following areas of future research are prospective: inclusion of a combination of protein hydrolysate with essential oils or metal cations in the composition of alginate matrix, and complex analysis of structural and bioactive properties of composite materials [16,33]. Another promising area of research is the study of the potential of biodegradable films with the inclusion of protein hydrolysates as sources of bioactive peptides, as bioactive coatings for the storage of perishable raw materials, raw meat, or fish [11,28].

Table 3. Microbiological indicators of sweet cherry during storage.

Microbiological Indicator during Storage	Sample of the Film			
	Control	0.5% PH	1% PH	1.5% PH
at room conditions				
AMB (log CFU/g)				
0 d	1.94 ± 0.08 ^a	1.92 ± 0.10 ^a	1.92 ± 0.10 ^a	1.93 ± 0.09 ^a
3 d	2.74 ± 0.15 ^a	2.41 ± 0.12 ^a	2.40 ± 0.12 ^a	2.38 ± 0.10 ^a
5 d	3.88 ± 0.22 ^b	3.56 ± 0.23 ^{ab}	3.42 ± 0.20 ^{ab}	3.16 ± 0.21 ^a
9 d	5.19 ± 0.31 ^b	4.81 ± 0.28 ^{ab}	4.60 ± 0.25 ^a	4.41 ± 0.24 ^a
Yeast and mold (log CFU/g)				
0 d	2.10 ± 0.11 ^a	2.08 ± 0.09 ^a	2.09 ± 0.09 ^a	2.09 ± 0.10 ^a
3 d	3.19 ± 0.13 ^a	2.84 ± 0.12 ^a	2.79 ± 0.12 ^a	2.78 ± 0.10 ^a
5 d	3.60 ± 0.14 ^b	2.88 ± 0.13 ^a	2.91 ± 0.13 ^a	2.90 ± 0.11 ^a
9 d	3.98 ± 0.16 ^b	2.90 ± 0.12 ^a	3.04 ± 0.13 ^a	2.98 ± 0.10 ^a
in the refrigerator				
AMB (log CFU/g)				
0 d	1.96 ± 0.09 ^a	1.96 ± 0.11 ^a	1.94 ± 0.08 ^a	1.93 ± 0.10 ^a
3 d	2.15 ± 0.14 ^a	2.10 ± 0.08 ^a	2.08 ± 0.12 ^a	2.08 ± 0.12 ^a
5 d	3.42 ± 0.18 ^b	3.02 ± 0.16 ^{ab}	2.96 ± 0.14 ^a	2.81 ± 0.14 ^a
9 d	4.91 ± 0.28 ^b	4.63 ± 0.21 ^b	4.18 ± 0.18 ^a	4.02 ± 0.15 ^a
Yeast and mold (log CFU/g)				
0 d	2.11 ± 0.10 ^a	2.10 ± 0.09 ^a	2.10 ± 0.08 ^a	2.09 ± 0.08 ^a
3 d	3.28 ± 0.21 ^a	2.96 ± 0.18 ^a	2.82 ± 0.18 ^a	2.80 ± 0.16 ^a
5 d	3.80 ± 0.24 ^b	3.12 ± 0.20 ^a	3.10 ± 0.18 ^a	2.98 ± 0.18 ^a
9 d	4.24 ± 0.26 ^b	3.84 ± 0.23 ^{ab}	3.61 ± 0.21 ^a	3.42 ± 0.20 ^a

Designation of samples: C—control alginate film; 0.5% PH—alginate films with 0.5% protein hydrolysate; 1.0% PH—alginate films with 1.0% protein hydrolysate; 1.5% PH—alginate films with 1.5% protein hydrolysate. The values are means (M) ± standard deviation of three replicates (s). Different letters in the same column (M^{a, b} refer to a significant difference at ($p \leq 0.05$)).

4. Conclusions

In conclusion, experimental samples of alginate films with the inclusion of protein hydrolysate were obtained in this study. The experiment demonstrated that 0.5% and 1.0% concentrations of protein hydrolysate could improve the appearance and transparency of the film and allowed to obtain its more homogeneous microstructure. The addition of protein hydrolysate to the composition of alginate films positively affected the water solubility, biodegradability, and antioxidant properties of the obtained films. According to the investigated parameters, the optimal properties were exhibited by the sample with 1.0% protein hydrolysate. The sample with a protein hydrolysate content of 1.5% had the greatest thickness and the lowest water vapor transmission rate value. FTIR spectroscopy and thermograms reflected the interaction between the alginate and peptide functional groups, as well as increasing in the thermal stability of the films upon addition of protein hydrolysate. Alginate films containing protein hydrolysate prevented weight loss and inhibited microbiological spoilage of cherry berries during storage period of 9 days.

The research results demonstrated that biocomposites based on sodium alginate with the inclusion of protein hydrolysate distinguished by improved characteristics compared to mono-component films and could serve as an alternative to synthetic packaging materials, which meets the principles of sustainability for the agro-industrial complex.

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