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Production and Testing of Carrageenan-Based Films Enriched with Chinese Hawthorn Extract in Strawberry Packaging

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Abstract: The aim of this study was to develop and characterize carrageenan-based films with the addition of aqueous Chinese hawthorn extract in concentrations of 5%, 10%, and 15%, as well as to examine their application and impact on prolongation of fresh strawberries' shelf life. The films were prepared using the casting method, and their mechanical, physical, structural, chemical, and barrier properties were investigated, along with the antioxidative and antimicrobial properties of the films and the extract. Tests on strawberries included monitoring changes in acidity, visual characteristics, weight loss, ripening, and oxidative status during storage. The results showed that the addition of aqueous hawthorn extract, due to its high total polyphenol content, contributed to the improvement of the films' antioxidant activity but exhibited low antimicrobial potential. Increasing the extract concentration led to higher water content and improved barrier properties at lower concentrations, while excessive concentrations of hawthorn extract (15%) impaired the films' barrier properties. FTIR analysis confirmed the presence of characteristic peaks for the carrageenan spectrum. The carrageenan-based film with the addition of 15% aqueous blackberry extract demonstrated the best efficiency in preserving the post-harvest quality of strawberries. Carrageenan-based films with the addition of aqueous hawthorn extract have significant potential for application in packaging fresh, perishable foods such as strawberries. In addition to providing the necessary protection to the packaged product, they represent a sustainable solution aimed at reducing waste generated by the use of plastic packaging.

Keywords: water vapor permeability; polyphenol content; tensile strength; malondialdehyde reduction; sustainable food packaging

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1. Introduction

Fresh fruits provide a large number of health benefits, as they are a great source of fiber, vitamins, minerals and antioxidants [1]. However, due to their composition and especially high water content, fresh fruits are susceptible to rapid deterioration, which is the main reason for the relatively short shelf life of the fruit. Due to respiration, ethylene production, mechanical damage, the high presence of microorganisms, and many other environmental factors, changes in appearance, texture, taste, aroma, and nutritional value

occur during storage [2]. According to data from the Food and Agriculture Organization (FAO) for 2011 [3], up to 45–50% of fruit "from field to table" ends up as waste. This stems not only from economic problems, but also environmental ones. A large amount of this type of waste leads to unforeseeable consequences for the environment, primarily reflected in the emission of gases that cause the greenhouse effect and the destruction of soil and water resources [4].

Fresh strawberries represent one of the most common fresh fruits in human consumption due to their rich aroma, taste, and nutritional value [5]. However, 20–40% of strawberries end up as waste after harvest [6]. Strawberries are an extremely perishable fruit, and their storage represents a real challenge. The soft and juicy structure of the strawberries allow intense respiration, creating a susceptible environment for microorganism contamination. Mechanical damage that occurs during or after harvest can also shorten the shelf life of strawberries and reduce the quality of the fruit [7,8].

To date, several methods have been applied to extend the shelf life of strawberries. However, some of these methods, such as fungicides, have been proven to negatively impact consumer health [9]. Hence, researchers' attention is focused on discovering new methods, such as the development and application of biodegradable packaging films, for preserving fresh strawberries' nutritional and organoleptic characteristics [7]. Biodegradable films could reduce the respiration rate and microbial growth, and preserve texture and volatile compounds, and thus increase the shelf life of strawberries [5,7].

Carrageenan is one of the polysaccharides with proven potential for food packaging film production. It can be used alone [10,11] or in combination with other biopolymers [10,12,13], with the addition of plasticizers such as glycerol [14], extracts [10], essential oils, [10,15] and another additives [13]. Carrageenan could produce transparent films with good mechanical characteristics [16] with possible application in the packaging of fruits and vegetables [17,18], meat [19], and fish [20], as well as dairy products [21].

Research on the incorporation of plant extracts into biodegradable films has gained much interest since it significantly improves the antioxidant [22,23], antimicrobial [22–24], and mechanical characteristics [25,26] of the films. During the storage period, a continual release of antioxidant compounds from plant extracts ensures the protection of the packaged food from oxidation [27].

Red hawthorn (*Crataegus pinnatifida*) is a species of hawthorn widely distributed in North America, Europe, and Central Asia [28]. It is known for its pharmacological effects and use in medicine and pharmacy [29]. The red hawthorn fruit is extremely rich in flavonoids, organic acids, triterpenoids, lignans, steroids, and nitrogenous compounds [30]. Due to its chemical composition, hawthorn extract was used to produce biodegradable chitosan and gelatin-based films, which resulted in improvement in their mechanical, barrier, and antioxidant properties [27]. In combination with alginate, hawthorn extract improved the mechanical and antioxidant properties of the films and showed good antimicrobial properties [31]. Through the incorporation of hawthorn extract into a composite film based on gelatin, chitosan, and nanocellulose, pH-sensitive packages were developed, enabling the monitoring of shrimp freshness during storage [32]. Addition of hawthorn extract to the 3D-printed corn starch and gelatin films positively affected film tear elasticity and strength, but also showed an effective antimicrobial effect [33]. Although some research has been done with other biopolymers, to the authors' knowledge, there are no data about the application of hawthorn extract in carrageenan film. This study aims to investigate the potential of incorporating hawthorn extract into edible carrageenan films and the possibility of their application in the prolongation of fresh strawberries' shelf life.

2. Materials and Methods

2.1. Red Hawthorn Extract Preparation

Hawthorn (*Crataegus pinnatifida*) extract was obtained according to the method previously described by Norajit et al. (2010) [34], with some modifications. Fresh hawthorn fruits were separated from seeds and stems, and finely chopped. The extraction was carried out with distilled water in a ratio of 1:12 by agitation on a magnetic stirrer (ChemLand, Stargard, Poland) at a temperature of 50 °C for 2 h. The mixture was then filtered through Whatman No. 3 filter paper to remove hawthorn fruit particles, and the obtained extract was stored in a refrigerator at 4 °C.

2.2. Determination of Dry Matter Content

The dry matter content was determined by drying the extract sample at 105 °C until a constant mass was obtained. After drying, the sample was left in a desiccator and weighed on an analytical scale (Joanlab, Ningbo, China), and the content of dry matter was calculated according to the following formula:

$$\% \text{ dry matter} = [(m2 - m0)/(m1 - m0)] \times 100$$

m0—mass of the empty container (g),
m1—mass of container with sample before drying (g),
m2—mass of the container with the sample after drying (g).

2.3. Preparation of the Carragenan-Based Films with Incorporated Hawtorn Extract

The carrageenan-based films were prepared by the casting method according to the modified method of Jancikova and coworkers (2021) [10]. Carrageenan-based films were made by dissolving 0.87 g of carrageenan (Sigma-Aldrich, St. Louis, MO, USA) in 130 mL of distilled water by mixing on a magnetic stirrer at 400 rpm for 10 min at 50 °C. After the addition of 0.45 mL of glycerol, the mixture was stirred for another 10 min. Hawthorn extract was added in three different concentrations: 5, 10, and 15% w/w (gram of dry matter content of extract per gram of dried film) and labelled as k5%CP, k10%CP, and k15%CP, respectively. The concentrations of added extracts were selected according to preliminary studies with respect to the significant increase in total polyphenolic content and antioxidative capacity of the films. After adding the extract, the film solutions were homogenized on an ultrasonic homogenizer (Witeg, Wertheim, Germany) at 13,500 rpm for 3 min and poured into 16 cm diameter Petri plates. The carrageenan-based films were dried for 48 h at room temperature. When the film was completely dry, it was carefully separated from the plates.

2.4. Mechanical Properties of Carragenan-Based Films

The thickness of the carrageenan-based films was measured using a thickness gauge INSIZE 2364-10 (Precision measurement, Suzhou, China), according to the ISO 4593:1993 standard [35], at five different places as already described [2].

Determination of tensile strength (Mpa) and elasticity (%) was performed according to the already described procedure [13]. Measurements were made using a Texture Analyzer Texture Exponent 32 (Stable Micro Systems, Goldalming, UK). Five 15 × 1 cm strips were cut from each carrageenan-based film, and measurements were made according to the international test method ASTM D882-02 [36].

2.5. Determination of Frictional Properties of Carrageenan-Based Films

To determine the frictional properties of biodegradable films, the Texture Analyzer Texture Exponent 32 (Stable Micro System, Godalming, UK), equipped with a coefficient

of sliding friction attachment (A/FR), was used. This method complies with ASTM D 1894-14 (Standard Test Method for Static and Kinetic Coefficients of Friction of Plastic Film and Sheeting) [37]. The biodegradable films were securely attached to the aluminum base of the texture analyzer, while the reference material was mounted on a so-called sled (63.5 mm × 63.5 mm, weighing 200 ± 5 g). The sled was pulled over the biodegradable films at a constant speed, and the force was measured.

2.6. Determination of Water Content, Degree of Swelling, and Solubility of Carrageenan-Based Films

Water content, degree of swelling, and solubility were determined according to a modified method published by Souza et al. (2017) [38]. Each carrageenan-based film sample was cut into 2 cm × 2 cm squares and weighted on an analytical scale (Joanlab, Ningbo, China) (W1). Carrageenan-based film samples were dried for 2 h at 105 °C in a laboratory dryer (Sutjeska, Belgrade, Serbia) and then measured again (W2). Then, 25 mL of distilled water was poured over the samples, and they were left for 24 h at room temperature. After 24 h, carrageenan-based film samples were dried on filter paper and measured again (W3), then transferred to a laboratory dryer (Sutjeska, Belgrade, Serbia) at 105 °C. Carrageenan-based film samples were measured again after 24 h (W4). All analyses were performed in triplicate. The water content, solubility, and degree of swelling of the carrageenan-based films were calculated according to the following formulas:

$$\text{Water content (\%)} = [(W1 - W2)/W1] \times 100$$

$$\text{Solubility (\%)} = [(W2 - W3)/W2] \times 100$$

$$\text{Degree of swelling (\%)} = [(W3 - W2)/W2] \times 100$$

2.7. Water Vapor Permeability of Carrageenan-Based Films

Water vapor permeability was determined using a modified gravimetrical method [39]. A carrageenan-based film sample was placed on the opening of the vial filled with silica gel and secured with parafilm. The vials were weighed on an analytical scale and left in a desiccator filled with distilled water at room temperature. The change in the weight of the vials was monitored for three consecutive days, and the water vapor permeability was determined by the following formula:

$$WVP = (W \times x)/(A \times t \times \Delta P)$$

W—sample weight increase (g),
x—film thickness (m),
t—time (s),
A—leakage area (m^2),
ΔP—the difference in partial pressure of pure water vapor and dry atmosphere (2339 Pa at 20 °C).

The results are expressed in $g \times m^{-1} \times s^{-1} \times Pa^{-1}$.

2.8. Antioxidant Activity of Carrageenan-Based Films

Antioxidant activity was determined by the DPPH method [24]. The samples of carrageenan-based films were ground. The ground samples 0.1 g of carrageenan-based films and 0.1 mL of extract were immersed in 20 mL ethanol. The samples were sonicated 30 min, and the solution was filtrated. A quantity of 3 mL of each sample was mixed with 1 mL of 0.1 mM DPPH radical solution (Sigma Aldrich, St. Louis, MO, USA) in ethanol and left to incubate in the dark for 30 min. The absorbance was measured at 517 nm using

a UV-VIS spectrophotometer UV-M51 (Bel Engineering, Monza, Italy), and pure ethanol was used as a blank. The DPPH radical neutralization capacity is determined according to the formula

$$\text{DPPHscavenging activity [\%]} = [(\text{AbsDPPH} - \text{Abssample})/\text{AbsDPPH}] \times 100$$

AbsDPPH—absorbance of DPPH solution,
Abs sample—absorbance of sample.

2.9. Determination of the Migration of Bioactive Components from Carrageenan-Based Films

The migration of bioactive components was determined according to the method of Dordevic et al. (2021) [26]. The carrageenan-based films were cut into 1 cm × 1 cm squares and placed in a pre-labelled test tube with 2.5 mL of 10% ethanol. The samples were stored at 30 °C, and sampling for determination of the total content of polyphenol was carried out over ten days.

2.10. Determination of Total Polyphenol Content in Carrageenan-Based Films

Determination of total polyphenols was performed according to the modified method described by Matshediso et al. (2015) [40]. The ground samples (0.1 g of carrageenan-based films or 0.1 mL of extract) were submerged in distilled water at a ratio of 1:40. After 10 min, 1 mL of each sample was mixed with 5 mL of Folin–Cicalteau reagent dissolved in distilled water at a ratio of 1:10 and 4 mL of 7.5% Na_2CO_3. The mixture was incubated in the dark for 30 min, and then the absorbance was measured at 765 nm. A solution of distilled water, Folin–Cicolteau (1:10) and 7.5% Na_2CO_3 was used as a blank. The total polyphenol content was expressed as mg of gallic acid per g of the sample.

2.11. Determination of Antimicrobial Activity of Carrageenan-Based Films

Antimicrobial activity was performed according to the modified disk diffusion method recommended by the European Committee for Antimicrobial Susceptibility Testing (EUCAST). Discs with a diameter of 5 mm were cut from the carrageenan-based films and exposed to UV light (260 nm) for 60 s for disinfection. Antimicrobial activity against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATC 27853, *Proteus vulgaris* ATCC 8427, Staphylococcus aureus ATCC 25923, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* ATCC 700603, *Candida albicans* ATCC 2091, and *Listeria monocytogenes* ATCC 15313 was determined. The inoculum concentration was approximately 1–2 × 10^8 CFU/mL, according to the McFarland 0.5 standard. The inoculum was swabbed by sterile swabs on the surface of the agar plates with nutrient agar (Torlak, Belgrade, Serbia) for bacteria, and Sabouraud maltose agar (Torlak, Belgrade, Serbia) for yeast. Discs were placed on the surface of the plates and incubated for 24 h at 37 °C for bacteria and 72 h at 25 °C for yeast. The antimicrobial activity was evaluated by the presence of the zones of inhibition and expressed in mm.

2.12. Packing of the Strawberries

Fresh strawberries were obtained from a local producer one day after harvest, washed with a 1% sodium hypochlorite solution, and then air-dried in sterile conditions. Packaging was performed according to the modified method of Dong et al. (2020) [41]. The washed and dried strawberries were wrapped in carragenan-based films and stored at 4 °C for 10 days. Sampling was performed after each two days.

2.13. Determination of Total Soluble Particles and pH Value of Packaged Strawberries

The content of total soluble particles and the pH value were analyzed according to a modified previously described method of Dong et al. (2020) [41]. To determine the content of total soluble particles, the juice of 5 strawberries was extracted with a hand mixer and filtered. The analysis was carried out with a refractometer. The results were indicated by Brix and three measurements were made for each sample. To measure the pH value, 5 g of strawberries were dissolved in 50 mL of water and stirred for 15 min on a magnetic stirrer. Masuring of pH value was performed by a pH meter (HANNA HI 9318, Leighton Buzzard, UK).

2.14. Titrable Acidity of Packaged Strawberries

Titrable acidity was determined according to the method of Dong et al. (2020) [41] by titrating 10 mL of prepared and filtered strawberry juice with 0.1 N NaOH. Phenolphthalein was used as an indicator, and the results were expressed as a percentage of citric acid:

$$TA = V1 \times N/V2 \times K \times 100 \times f$$

V1—volume of NaOH consumed (mL),
V2—sample volume (mL),
K—equivalent-weight of citric acid (0.064 g/milligram equivalent),
N—standard concentration of NaOH (0.1-milligram equivalent/mL),
F—dilution factor.

2.15. Maturity Index of Packaged Strawberries

The maturity index was determined according to the method of Dong et al. (2020) [41], calculated as the ratio of total soluble solids to titratable acidity.

2.16. Weight Loss of Packaged Strawberries and Spoilage Rate

Weight loss of strawberries was determined according to the modified method of Dong et al. (2020) [41]. The percentage of weight loss was calculated for each sample during storage, and the result was expressed according to the following formula:

$$\text{Weight loss rate (\%)} = (W0 - Wt)/W0 \times 100\%$$

W0—weight of fresh strawberries,
Wt—weight of stored strawberries.

The rate of decay was accessed for six strawberries. Fruits which showed visual decay were removed and counted. The rate of decay was calculated by dividing the number of decayed fruits and initial number of fruits.

2.17. Determination of Malondialdehyde Content in Packaged Strawberries

The determination of malondialdehyde was performed according to the modified method previously described by Dordevic et al. (2024) [42]. A sample of 3 g of strawberry was weighed, homogenized by mixing with 15 mL of 10% trichloroacetic acid, and centrifuged at 7000 rpm for 20 min. A volume of 1 mL of the supernatant was mixed with 3 mL of 0.5% 2-thiobarbituric acid and heated for 10 min at 95 °C. The samples were cooled with water, and the absorbance was measured at 532 nm. A mixture of 1 mL of 10% trichloroacetic acid and 3 mL of 0.5% 2-thiobarbituric acid are used as a blank.

2.18. FTIR Analysis of Carrageenan-Based Films

The FTIR spectra were measured using a BOMEM MB-100 (Hartmann & Braun, Brampton, ON, Canada) spectrometer coupled with a KBr detector, in the range of 400 to 4000 cm^{-1}. It was used with 32 scans per spectrum with a resolution of 4 cm^{-1}.

2.19. Statistical Analysis

All analyses were performed in triplicate, and the results were expressed as mean value with standard deviation. Statistically significant differences among the results were determined by one-way ANOVA followed by the Tukey test with the use of the software SPSS 21.0 (IBM, New York, NY, USA, SAD). The results were labeled as statistically different when the p value was lower than 0.5.

3. Results

3.1. Extract Characterisation

Characterization of hawthorn fruit extract included the determination of dry matter content, antioxidant activity, and total polyphenol content, as presented in Table 1.

Table 1. Characteristics of the hawthorn water extract.

Dry matter content (%)	1.15 ± 0.29
Total polyphenol content (mg GAE/g)	2.99 ± 0.01
Capacity of DPPH radical neutralization (%)	60.02 ± 0.002

The total polyphenol content in the analyzed extract was 2.99 mg GAE/g, which is higher than the results of 1.86 mg GAE/g reported by Miao et al. (2016) [43] for water extract of hawthorn fruits. Such high differences in the characteristics of the hawthorn extracts may occur due to the extraction conditions or the characteristics of the raw material [24].

3.2. Development of the Carrageenan-Based Films with Incorporated Hawthorn Extract

Four different types of carrageenan-based films were prepared including the control without the addition of hawthorn extract, and three carrageenan-based films with 5%, 10%, and 15% of hawthorn extract. The resulting carrageenan-based films were transparent, and the visual appearance of the films with the addition of hawthorn extract indicated the increase in yellow color with the increase in hawthorn extract concentration.

3.3. Mechanical Properties of Carrageenan-Based Films

The analysis of the mechanical properties of carrageenan-based films (Table 2) prepared with different concentrations of hawthorn extract indicated that film thickness was in the range of 0.06–0.09 mm. The addition of hawthorn extract in the concentrations of 5% and 10% did not significantly affect the thickness of the carrageenan-based film, while the thickness of the carrageenan-based film with the addition of hawthorn extract in a concentration of 15% was statistically significantly different ($p < 0.05$) from the other samples.

The weak impact of lower concentrations of the extract on the film thickness and significant difference with higher concentrations were also reported in carrageenan film with the addition of Beluga black *Lentil leachate* [44] and *Zataria multiflora* extract [43]. The differences in the thickness of the samples are probably caused by the formation of additional bonds in the matrix of the film due to the presence of the extract [45]. Also, some studies showed that the thickness of the film may depend on the amount of added plant extract [10,46,47]. Yan et al. (2021) [32] reported that the thickness of films based on chitosan, gelatin, and cellulose nanoparticles with incorporated 10, 15, and 20% hawthorn

extract was significantly different from the control film made without added extract. The obtained results are in accordance with the results of Kan et al. (2019) [28], who reported that the addition of hawthorn extract significantly increased the thickness of the chitosan–gelatine films. In a study by Lim et al. (2021) [31], it was observed that the incorporation of hawthorn extract into alginate-based films resulted in significant differences in thickness compared to carrageenan-based films supplemented with the same extract. The difference in the biopolymer matrix results in variations in the thickness of films containing the same extract [48].

Table 2. Mechanical characteristics of carrageenan-based films with the addition of hawthorn extract.

	kC	k5%CP	k10%CP	k15%CP
Film thickness (mm)	0.06 ± 0.06 [a]	0.06 ± 0.005 [a]	0.07 ± 0.001 [a]	0.09 ± 0.002 [b]
Tensile strength (MPa)	0.136 ± 0.019 [a]	0.109 ± 0.01 [a]	0.126 ± 0.004 [a]	0.115 ± 0.012 [a]
Elongation to break (%)	262.93 ± 1.12 [a]	264.41 ± 1.26 [a]	263.55 ± 0.86 [a]	263.64 ± 0.85 [a]
Toughness (MJ/m^3)	0.001 ± 0.0005 [a]	0.001 ± 0.0 [a]	0.002 ± 0.0 [a]	0.003 ± 0.001 [a]

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10 and 15% of hawthorn extract, respectively; a,b—different letters indicate statistically significant differences among the values in the same row ($p < 0.05$).

The mechanical properties tested were tensile strength (MPa), elongation to break (%), and toughness (MJ/m^3). According to the obtained results, carrageenan-based film samples with different concentrations of hawthorn extract did not show a significant difference. The values for tensile strength ranged from 0.098 to 0.136 MPa. The values for elongation to break were similar ($p > 0.05$) for all carrageenan-based film samples with different extract concentrations, ranging from 262.93% to 264.41%. Similar values for all carrageenan-based films were also obtained for toughness, with values ranging from 0.001 to 0.003 MJ/m^3. The results of this study show that the mechanical properties of carrageenan-based films with different concentrations of hawthorn extract are significantly higher compared to literature data. In the study by Jancikova et al. (2020) [49], tensile strength values ranged from 0.14 to 0.42 MPa, while elongation to break ranged from 72.0% to 113.5%. Similar results were reported by Dordevic et al. (2023) [13], in which the tensile strength values ranged from 0.25 to 0.53 MPa, while elongation to break was between 74.47% and 85.83% for carrageenan-based films with added coffee grounds.

3.4. Frictional Properties of Carrageenan-Based Films

The stiction values of carrageenan-based films with the addition of hawthorn extract did not significantly differ ($p > 0.05$) for the carrageenan-based film samples (Table 3). The value for the control film without hawthorn extract was 477.05 N, while the samples with the addition of hawthorn extract had values ranging from 458.94 to 494.16 N. The results suggest a potential interaction between carrageenan and compounds from the hawthorn extract at higher concentrations. The results for friction showed a difference for carrageenan-based films with the addition of different concentrations of hawthorn extract. The value for the film without hawthorn extract was 474.68 N, which was significantly higher than the values for the other samples. The values for the films with the addition of 5%, 10%, and 15% extract ranged from 464.47 to 475.68 N. The film with the addition of 10% hawthorn extract had a higher friction value compared to the other samples, indicating that this is the optimal concentration of hawthorn extract for preserving frictional properties.

Table 3. Frictional properties of carrageenan-based films with the addition of hawthorn extract.

	Stiction (N *)	Friction (N)
kC	477.05 ± 32.41 [a]	474.68 ± 1.5 [b]
k5%CP	458.94 ± 25.57 [a]	465.32 ± 3.83 [a]
k10%CP	484.14 ± 4.87 [a]	475.68 ± 7.43 [b]
k15%CP	494.16 ± 7.62 [a]	464.47 ± 6.64 [a]

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10, and 15% of hawthorn extract, respectively; a,b—different letters indicate statistically significant differences among the values in the same row ($p < 0.05$); * Newton.

3.5. Determination of Water Content, Degree of Swelling, and Solubility of Films

The results of the determination of water content, solubility, and degree of swelling of the carrageenan-based films with different concentrations of hawthorn extract are shown in Table 3.

The value of water content ranged from 17.3 for the control carrageenan-based film to 25.8 for the film with the addition of 5% of hawthorn extract. The addition of hawthorn extract in the concentrations of 5 and 10% did not have a statistically significant ($p < 0.05$) effect on the water content of the carrageenan-based film. However, the carrageenan-based film with 15% extract had a statistically lower water content than the other samples with added extract, but a higher content than the control carrageenan-based film. Based on the results, it can be concluded that the addition of water extract of hawthorn leads to an increase in water content, but that influence decreases with an increase in the concentration of added extract. The results are consistent with the results of Lim et al. (2021) [31], who reported that incorporating hawthorn extract in concentrations of 1–3% into alginate-based films did not show significant differences in water content. A study by Yong et al. (2019) [50] showed that chitosan-based films had higher moisture content with increasing amounts of added blue and black eggplant extracts. The hydrophilic nature of the anthocyanins from the added plant extracts probably affects the increase in the moisture content of the films [50]. On the other hand, some data indicated the reduction of the water content with the addition of hawthorn extract to gelatin- and chitosan-based films [27] and films based on gelatin, chitosan, and nanocellulose.

The results for the water solubility of carrageenan-based films were 100%, regardless of the addition of hawthorn extract, which is probably a consequence of the highly hydrophilic character of carrageenan [51]. All carrageenan-based films dissolved in distilled water within a few hours. According to that, it was not possible to determine the degree of swelling for the analyzed samples. The obtained results are in accordance with the results of Jancikova et al. (2020) [49].

Water vapor permeability (WVP) is a key parameter in the development of biodegradable films. Control of the gas permeability is essential to prevent harmful consequences such as more intensive respiration, fungal spoilage, and impairment of nutritional and organoleptic characteristics [52]. Table 3 shows the water vapor permeability of carrageenan-based films with different concentrations of hawthorn extract. Based on the results, the film based on carrageenan with the addition of hawthorn extract in a concentration of 15% has the highest water vapor permeability. The addition of hawthorn extract significantly affected the water vapor permeability of the carrageenan-based films. The carrageenan-based film with 5% and 10% extract had a lower WVP compared to the carrageenan-based film without extract, probably due to well dispersion of the extract in the matrix of the film, and thus reduced water vapor transport [27]. However, the carrageenan-based film with 15% extract was significantly different from the others ($p < 0.05$), with the increase in WVPa values. This can be explained by the possible aggregation of the extract which can

occur with the increase in its concentration and result in increased permeability to water vapor [27]. Based on the results, it can be concluded that the addition of 10% of hawthorn extract to the carrageenan-based films was the best in the prevention of the transport of water vapor. These results are in agreement with the research of Khan et al. (2019) [27]. Their results showed that the addition of hawthorn extract in the amount of 4% w/w to chitosan–gelatin-blend film resulted in better barrier performance of film than the addition of 2 or 6% of extract. As Martiny et al. (2020) [53] reported, the addition of olive leaf extract to carrageenan-based films led to the construction of a compact structure, which affected the transport of moisture through the polymer matrix and improved the barrier properties of the films. On the opposite, Yan et al. (2021) [32] reported that there was no significant difference in terms of WVP of the films based on grafted cellulose nanocrystals and hawthorn anthocyanin extract in the concentration range of 0–15%.

3.6. Antioxidant Activity and Total Polyphenol Content of Carrageenan-Based Films

The antioxidant activity of plant extracts is based on the presence of phenols and phenolic acids [24]. Table 4 summarizes the values of total polyphenol content and degree of free radical neutralization of carrageenan-based films with different concentrations of hawthorn extract.

Table 4. Water content, solubility, and degree of swelling of carrageenan-based films with the addition of hawthorn extract.

	Water Content %	**Water Solubility %**	**Water Vapor Permeability $\times$ 10^{-10}, g/(m $\times$ s $\times$ Pa)**
kC	17.30 ± 0.39 ^{a}	100 ± 0	7.0 ± 0.1 ^{bc}
k5%CP	25.83 ± 0.94 ^{c}	100 ± 0	4.8 ± 0.3 ^{a}
k10%CP	24.47 ± 0.35 ^{c}	100 ± 0	5.8 ± 0.4 ^{ab}
k15%CP	21.56 ± 1.20 ^{b}	100 ± 0	7.7 ± 0.1 ^{c}

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10, and 15% of hawthorn extract, respectively; a–c—different letters indicate statistically significant differences among the values in the same row ($p < 0.05$).

The analysis showed that all the carrageenan-based films differ significantly ($p < 0.05$) in the total polyphenolic content and degree of neutralization of DPPH radicals. According to the high antioxidative activity of the extract, which is presented in Table 1, both parameters related to the antioxidative activity of the films increase with the increase in the extract content. Literature data showed that the chemical composition of hawthorn extract is very rich, with over 150 compounds with a great range of biological effects [30]. Most of them belong to the group of procyanidins (procyanidin B2, B5, and C1), flavonoids (epicatechin, hyperoside, quercetin, rutin, and isoquercitrin), and triterpenoid acid (ursolic acid, corosolic acid, oleanolic acid, and maslinic acid). According to the literature, major compounds of hawthorn fruit extract are epicatechin, procyanidin B2, hyperoside, isoquercitrin, and chlorogenic acid [54,55], but the composition may vary depending on the characteristics of hawthorn and extraction procedures. Compounds which can have great antioxidative potential are epicatechin, isoquercetin, hyperoside, quercetin, rutin, protocatechuic acid, and chlorogenic acid [55,56].

As expected, carrageenan-based film k15%CP had the highest content of total polyphenols, and therefore the highest antioxidative activity. The antioxidant potential decreased proportionally as the concentration of hawthorn extract decreased, so film with the addition of only 5% hawthorn extract showed the lowest degree of free radical neutralization of 50%. The control film also showed a certain content of polyphenols, as well as antioxidant activity, which can be explained by the presence of sulfate groups in carrageenan [57].

It is interesting that hawthorn extract showed a lower content of total polyphenols, as well as antioxidant activity, compared to film with 15% of extract. According to Khan et al. (2019) [27], this could be the consequence of the interaction of the plant extract and the polysaccharide matrices and the possibility that carrageenan induced a controlled release of antioxidant components and increased their bioavailability. The obtained results are in agreement with the results of Khan et al. (2019) [27]. Addition of hawthorn extract to chitosan- and gelatin-based films significantly improved the antioxidant properties of the films. The values of the degree of neutralization of free radicals were 33.42–84.40% at a concentration of film of 5 mg/mL. The reason for such differences in antioxidative activity is probably due to the use of different extraction conditions, the solvent, or the characteristics of the raw material itself (Lapornik et al., 2005) [58]. The results are consistent with the results of Jancikova et al. (2020) [49]. As they reported, the carrageenan-based control film also showed weak antioxidative properties, which increased with the increase in concentration of lapacho tea extract.

The migration of bioactive components from packaging, such as polyphenols, can contribute to improving the characteristics of packaged products [59]. As can be seen from the results (Table 5), the carrageenan-based control film with 0% extract showed the lowest amount of migrated polyphenols. The highest amount of bioactive components migrated from the film k15%CP, with the highest concentration of hawthorn extract.

Table 5. Total polyphenol content, degree of neutralization of free radicals, and migration of bioactive components of carrageenan-based films with the addition of hawthorn extract.

	Total Polyphenol Content (mg GAE/g)	Degree of Neutralization of DPPH Radicals (%)	Migration of Bioactive Components (mg GAE/g)
kC	1.12 ± 0.03 ^{a}	48.50 ± 0.3 ^{a}	1.26 ± 0.05 ^{a}
k5%CP	1.30 ± 0.01 ^{b}	50.50 ± 0.4 ^{b}	2.48 ± 0.04 ^{b}
k10%CP	1.39 ± 0.01 ^{c}	52.27 ± 0.1 ^{c}	3.10 ± 0.01 ^{c}
k15%CP	3.97 ± 0.02 ^{d}	63.69 ± 0.2 ^{d}	8.64 ± 0.03 ^{d}

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10, and 15% of hawthorn extract, respectively; a–d—different letters indicate statistically significant differences among the values in the same row ($p < 0.05$).

3.7. Antimicrobial Activity of Carrageenan-Based Films

The shelf life of food largely depends on its microbiological integrity. Very often, when making biodegradable packages, plant extracts are incorporated with the aim to evince their antimicrobial effects [60]. The antimicrobial potential of carrageenan-based films with the addition of hawthorn extract is shown in Table 6.

Determination of antimicrobial activity indicated low activity of all analyzed samples against treated bacterial and yeast strains. None of the inhibition zones exceeded a diameter of 6 mm, while the samples showed no activity against Gram-positive bacteria *S. aureus*, as well as yeasts *C. albicans*. Additionally, there was no statistically significant difference among different analyzed samples. The obtained results are not consistent with the results of Leaw et al. (2021) [33], who reported that the incorporation of aqueous hawthorn extract into starch and gelatin-based films improves the antimicrobial effect, and the zones of inhibition are in the range 9–13 mm. On the other hand, research of Tadić et al. (2008) [61] showed very low antimicrobial activity of hawthorn extract against *C. albicans*, *E.coli*, *S.aureus*, *and P. aeruginosa*. Differences in results can be explained by characteristics in the concentration of the extract. A lower concentration of the extract affects the lower antimicrobial activity of the carrageenan-based films. Also, the differences may be a consequence of the different preparation of the extract.

Table 6. Antimicrobial activity of carrageenan-based films with different concentrations of hawthorn extract.

	kCP	k5%CP	k10%CP	k15%CP
Escherichia coli ATCC 25922	5.08 ± 0.2 [a]	5.08 ± 0.2 [a]	5.08 ± 0.2 [a]	5.41 ± 0.4 [a]
Pseudomonas aeruginosa ATCC 27853	5.16 ± 0.4 [a]	5.16 ± 0.2 [a]	5.25 ± 0.4 [a]	5.33 ± 0.4 [a]
Proteus vulgaris ATCC 8427	5.00 ± 0.0 [a]	5.08 ± 0.2 [a]	5.41 ± 0.4 [a]	5.41 ± 0.3 [a]
Staphylococcus aureus ATCC 25923	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]
Bacillus subtilis ATCC 6633	5.1 ± 0.2 [a]	5.08 ± 0.2 [a]	5.16 ± 0.4 [a]	5.25 ± 0.2 [a]
Klebsiella pneumoniae ATCC 700603	5.08 ± 0.2 [a]	5.16 ± 0.3 [a]	5.00 ± 0.0 [a]	5.33 ± 0.5 [a]
Candida albicans ATCC 2091	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]	0.0 ± 0.0 [a]
Listeria monocytogenes ATCC 15313	5.00 ± 0.0 [a]	5.00 ± 0.0 [a]	5.08 ± 0.2 [a]	5.3 ± 0.5 [a]

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10, and 15% of hawthorn extract, respectively; a—different letters indicate statistically significant differences among the values in the same row ($p < 0.05$).

3.8. FTIR Spectrums of Carrageenan-Based Films

During the FTIR analysis of the films (Figure 1), characteristic absorption bands for carrageenan were observed, indicating the presence of specific chemical groups in the carrageenan-based films. In the range of 3300–3400 cm^{-1}, a broad, strong absorption band attributed to O–H vibrations was noted. Hydroxyl groups are found in all carrageenan-based film components, although this band may also be associated with residual moisture within the film. In the range between 2800 cm^{-1} and 3000 cm^{-1}, two bands were recorded, corresponding to the asymmetric and symmetric stretching vibrations of C–H bonds in the methylene groups of carrageenan. The medium-intensity absorption band observed at 1640 cm^{-1} corresponds to water vibrations which allow distinguishing of OH groups and H_2O within a material, thus indicating the presence of free water molecules in the film, likely as a result of interactions between the film components. Deformation vibrations of C–H bonds were observed in the range of 1400–1500 cm^{-1}. Additionally, absorption bands in the range of 1000–1100 cm^{-1} were assigned to the stretching vibrations of C–O bonds, characteristic of polysaccharides. The presence of bands specific to $–SO_3$ group vibrations in carrageenan molecules was noted in the range of 800–900 cm^{-1}. It is important to note that peaks specific to the hawthorn extract were not particularly visible in the spectrum, likely due to overlap with carrageenan peaks, further confirming the dominance of characteristic vibrations in the carrageenan molecule. Similar results were reported by Jancikova et al. (2021) [10] for carrageenan-based films, although in films containing essential oils and emulsifiers (Tween 20 and Tween 80), these specific peaks were very pronounced.

3.9. Application Carrageenan-Based Films on Strawberries

Strawberries are a very sensitive fruit subject to spoilage in the post-harvest period [7]. In this study, the influence of biodegradable films based on carrageenan with the addition of hawthorn extract on the extension of the shelf life of fresh strawberries was analyzed. Over 11 days, the amount of total soluble particles, titration acidity, pH value, decay rate, weight loss, and malondialdehyde content were monitored (Table 7).

A gradual increase in the percentage of total soluble solids can be seen (Table 7). The highest increase of 10.5% was observed in unwrapped strawberries (control). In the case of unpackaged strawberries, the metabolic processes took place much faster, so the loss of moisture was greater, which results in an increase in the content of total soluble solids [62]. The percentage of total soluble solids differed significantly ($p < 0.05$) between the samples wrapped in carrageenan-based films and the control sample of strawberries without film for

10 days. As the percentage of total soluble solids decreased with increasing concentration of incorporated hawthorn extract, the obtained results suggest that carrageenan-based films with the addition of hawthorn extract prevent moisture loss and slow down the respiration of packaged strawberries.

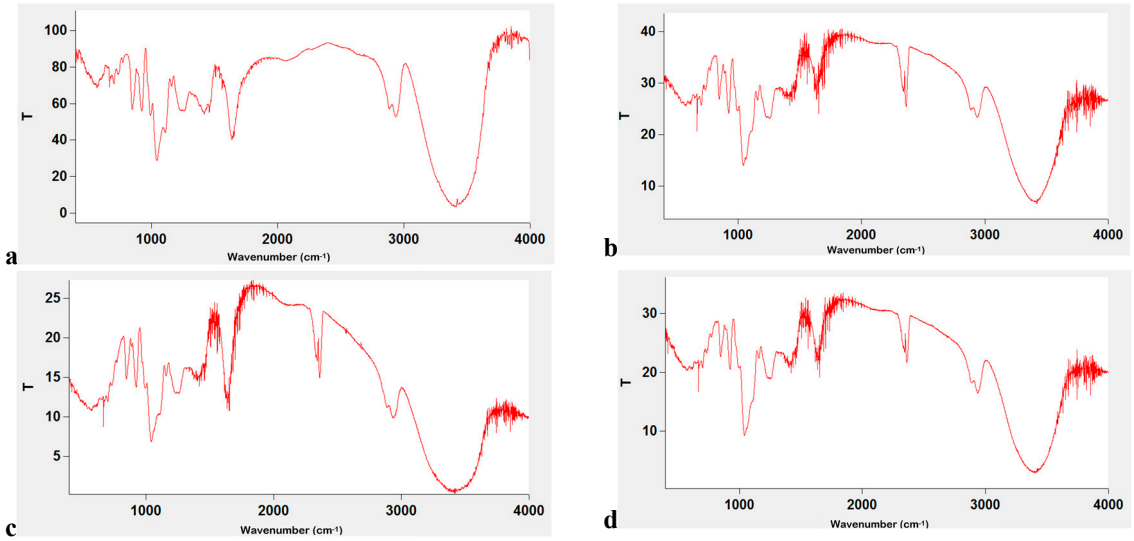

Figure 1. FTIR spectrums of carrageenan-based films with the addition of (**a**) 0%, (**b**) 5%, (**c**) 10%, (**d**) 15% hawthorn extract.

Table 7. Content of total soluble solids in fresh strawberries packed in carrageenan-based films with different concentrations of hawthorn extract.

Total Soluble Solids (°Brix)	Control	kC	k5%CP	k10%CP	k15%CP
0	7.2 ± 0.05^{bA}	7.2 ± 0.05^{dA}	7.2 ± 0.05^{cA}	7.2 ± 0.05^{aA}	7.2 ± 0.05^{cA}
2	7.9 ± 0.06^{cB}	7.8 ± 0.06^{cB}	7.4 ± 0.06^{bB}	7.2 ± 0.06^{aB}	7.5 ± 0.04^{bB}
4	8.5 ± 0.05^{cC}	8.6 ± 0.06^{cC}	8.2 ± 0.06^{bC}	7.4 ± 0.06^{aC}	8.1 ± 0.04^{bC}
6	9.6 ± 0.05^{cD}	9.0 ± 0.05^{bD}	8.5 ± 0.05^{aD}	8.5 ± 0.05^{aD}	8.5 ± 0.05^{aD}
8	9.8 ± 0.1^{dE}	9.5 ± 0.06^{cE}	8.7 ± 0.04^{aE}	8.9 ± 0.05^{bE}	9.0 ± 0.05^{bE}
10	10.5 ± 0.05^{bF}	10.0 ± 0.06^{aF}	9.8 ± 0.05^{aF}	9.8 ± 0.05^{aF}	9.6 ± 0.05^{aF}

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10, and 15% of hawthorn extract, respectively; a–d—different letters in the same row indicate statistically significant difference differences among the values in the same row ($p < 0.05$); A–F—different uppercase letters indicate statistically significant differences among the values in the same column ($p < 0.05$).

This is in relation with previously presented results in this paper (Table 3) that the addition of hawthorn extract to carrageenan-based film decreases the water vapor permeability of the film. The results are consistent with the results of Wani et al. (2021) [63], who indicated that the values of total soluble solids in carrageenan-coated strawberries were significantly lower during storage compared to uncoated strawberries, as well as with the results of Mali and Grossman (2003) [64], which showed that the use of starch-based films delays the increase in the percentage of total soluble solids of fresh strawberries. Robles-Flores et al. (2019) [65] also reported that the use of films and coatings derived from *Cajanus cajan* seeds slows the ripening and respiration of packaged strawberries.

Changes in pH value and titrable acidity, determined with phenolphthalein, of fresh strawberries packed in carrageenan-based films with the addition of hawthorn extract were monitored during 10 days of storage (Table 8).

Table 8. pH value and titrable acidity (phenolphthalein) in fresh strawberries packed in carrageenan-based films with different concentrations of hawthorn extract.

pH	Control	kC	k5%CP	k10%CP	k15%CP
0	2.60 ± 0.01^{A}	2.60 ± 0.01^{A}	2.60 ± 0.01^{A}	2.60 ± 0.01^{A}	2.60 ± 0.01^{A}
2	3.21 ± 0.01^{dB}	3.17 ± 0.02^{dB}	3.09 ± 0.02^{cB}	2.71 ± 0.01^{aB}	2.77 ± 0.01^{bB}
4	3.46 ± 0.01^{dC}	3.32 ± 0.03^{cC}	3.22 ± 0.01^{bC}	2.93 ± 0.01^{aC}	2.93 ± 0.01^{aC}
6	3.86 ± 0.02^{dD}	3.72 ± 0.02^{cD}	3.61 ± 0.02^{bD}	3.17 ± 0.03^{aD}	3.21 ± 0.01^{aD}
8	4.61 ± 0.03^{dE}	4.12 ± 0.01^{cE}	4.09 ± 0.01^{cE}	3.35 ± 0.03^{bE}	3.32 ± 0.01^{aE}
10	5.37 ± 0.02^{eF}	4.61 ± 0.01^{dF}	4.21 ± 0.01^{cF}	3.86 ± 0.01^{bF}	3.77 ± 0.04^{aF}
Titrable acidity with phenolphthalein (%)					
0	1.44 ± 0.02^{F}	1.44 ± 0.02^{F}	1.44 ± 0.02^{F}	1.44 ± 0.02^{F}	1.44 ± 0.02^{dE}
2	1.31 ± 0.02^{aE}	1.32 ± 0.02^{aE}	1.3 ± 0.01^{aE}	1.33 ± 0.02^{aE}	1.46 ± 0.02^{bE}
4	1.22 ± 0.03^{aD}	1.25 ± 0.01^{abD}	1.27 ± 0.02^{bD}	1.32 ± 0.03^{bD}	1.39 ± 0.03^{cD}
6	1.15 ± 0.01^{aC}	1.18 ± 0.01^{aC}	1.22 ± 0.02^{bC}	1.25 ± 0.02^{bcC}	1.26 ± 0.03^{cC}
8	0.92 ± 0.04^{aB}	0.99 ± 0.01^{aB}	1.15 ± 0.01^{bB}	1.2 ± 0.02^{cB}	1.21 ± 0.03^{cB}
10	0.84 ± 0.01^{aA}	0.91 ± 0.01^{bA}	1.01 ± 0.01^{cA}	1.05 ± 0.01^{dA}	1.12 ± 0.02^{eA}
Maturity index					
0	5.00 ± 0.03^{A}	5.00 ± 0.03^{A}	5.00 ± 0.03^{A}	5.00 ± 0.03^{A}	5.00 ± 0.03^{A}
2	5.99 ± 0.03^{cB}	6.01 ± 0.1^{cB}	5.70 ± 0.05^{bB}	5.51 ± 0.09^{bB}	5.21 ± 0.07^{aB}
4	6.88 ± 0.08^{dC}	6.94 ± 0.06^{dC}	6.41 ± 0.04^{cC}	5.55 ± 0.05^{aB}	5.76 ± 0.06^{bC}
6	8.31 ± 0.04^{dD}	7.69 ± 0.06^{cD}	6.91 ± 0.06^{bD}	6.75 ± 0.05^{aC}	6.67 ± 0.08^{aD}
8	10.25 ± 0.1^{cE}	9.59 ± 0.01^{bE}	7.62 ± 0.06^{aE}	7.35 ± 0.06^{aD}	7.32 ± 0.1^{aE}
10	12.40 ± 0.1^{eF}	11.16 ± 0.2^{dF}	9.64 ± 0.06^{cF}	9.32 ± 0.01^{bE}	8.45 ± 0.1^{aF}

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10, and 15% of hawthorn extract, respectively; a–e—different letters in the same row indicate statistically significant difference differences among the values in the same row ($p < 0.05$); A–F—different uppercase letters indicate statistically significant differences among the values in the same column ($p < 0.05$).

At the very beginning of storage, the pH value was 2.49–2.71 for all analyzed samples. However, on the tenth day of storage, the highest increase in pH value, as much as 5.37, was recorded in the control sample, while in the packaged strawberries, pH ranged from 3.77 to 4.61. A statistically significant difference ($p < 0.05$) was observed between the samples regardless the amount of added hawthorn extract. During the storage, the incorporation of hawthorn extract had a significant effect on the changes in the pH value in all packed samples. The increase in pH value caused a decrease in titratable acidity, which was expressed as a percentage of citric acid. The initial values of titratable acidity in all the samples ranged from 1.39 to 1.49%. The largest decrease was observed in unwrapped strawberries, reaching the value of 0.84%, while the film with the addition of 15% hawthorn extract showed the smallest decrease: to 1.12%. At the end of the storage period, the titration acidity of carrageenan-based films with different concentrations of hawthorn extract was in the range of 1.01–1.12%, while the film without added extract was slightly lower: 0.91%. Such results suggest that carrageenan-based films have the ability to slow down the metabolism of organic acids in strawberries, and the incorporation of hawthorn extract into the films significantly contributes to this [66]. The results are consistent with the results of Mali and Grossman (2003) [64], who reported that starch-based films slow

down the metabolism of organic acids in fresh strawberries. According to the results of Wani et al. (2021) [63], carrageenan-based films significantly prevented the decrease in the percentage of titratable acidity of strawberries compared to films based on gum arabic and xanthan, as well as compared to the unpacked sample. A study conducted by Dong et al. (2020) [41] also observed that the use of chitosan-based coatings significantly affected the stability of titration acidity in fresh strawberries during storage.

Due to the lack of protection from respiration processes and enzymes that lead to the softening of the strawberry fruit, the maturity index in unpacked samples increased from 4.79 to 12.50 during storage. A similar result was observed in samples packed in carrageenan-based films without added extract, where the maturity index increased from 5.25 to 10.99. On the other hand, the application of carrageenan-based films with different concentrations of added hawthorn extract had a positive effect on slowing down the respiration process of strawberries. The maturity index of the packed strawberries ranged from 8.57 to 9.70 on the tenth day of storage. The lowest maturity index was recorded for the carrageenan-based film with 15% added hawthorn extract. Similar results were reported by Dong et al. (2020) [41], where an edible chitosan-based coating was applied for strawberry packaging.

When it comes to the degree of deterioration (Table 9), the control showed the highest percentage of fruit decay of as much as 83.3%. Based on the results, it can be concluded that the use of carrageenan-based films significantly ($p < 0.05$) reduced the degree of deterioration of packed strawberries. The film k15%CP showed the best properties in terms of extending the shelf life of fresh strawberries, because even on the eleventh day of sampling, the percentage of strawberries decaying was only 16.7%. The results are consistent with those of Wani et al. (2021) [63], where carrageenan-based films showed the lowest deterioration rate of 14.29%. Also, Feng et al. (2024) [67] reported that the decay rate of fresh strawberries was significantly reduced by using different chitosan-based coatings with the addition of hawthorn leaf extract.

Table 9. Degree of deterioration of fresh strawberries packed in carrageenan-based films with different concentrations of hawthorn extract.

Degree of Deterioration (%)	Control	kC	k5%CP	k10%CP	k15%CP
0	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}
2	50.0 ± 0.1^{cB}	16.7 ± 0.0^{bB}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}
4	50 ± 0.2^{cB}	33.3 ± 0.1^{bC}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}	0.0 ± 0.0^{aA}
6	66.7 ± 0.3^{cC}	33.3 ± 0.2^{bC}	16.7 ± 0.1^{aB}	16.7 ± 0.1^{aB}	16.7 ± 0.1^{aB}
8	83.3 ± 0.4^{dD}	66.7 ± 0.1^{cD}	66.7 ± 0.2^{cC}	50 ± 0.1^{bC}	16.7 ± 0.2^{aB}
10	83.3 ± 0.3^{cD}	83.3 ± 0.2^{cE}	83.3 ± 0.3^{cD}	66.7 ± 0.4^{bD}	16.7 ± 0.1^{aB}

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10, and 15% of hawthorn extract, respectively; a–d—different letters in the same row indicate statistically significant difference differences among the values in the same row ($p < 0.05$); A–E—different uppercase letters indicate statistically significant differences among the values in the same column ($p < 0.05$).

3.10. Malondialdehyde Content in Packaged Strawberries

Oxidative stress, which occurs after harvest, can lead to membrane damage in fruit. One of the indicators of damage to membrane integrity is malondialdehyde [61]. Table 10 shows the content of malondialdehyde in packaged strawberries, as well as in the control sample.

Table 10. Malondialdehyde content in fresh strawberries packed in carrageenan-based films with different concentrations of hawthorn extract.

Malondialdehyde Content (mg/kg)	Control	kC	k5%CP	k10%CP	k15%CP
0	0.064 ± 0.0003^{A}	0.064 ± 0.0003^{A}	0.064 ± 0.0003^{A}	0.064 ± 0.0003^{A}	0.064 ± 0.0003^{A}
2	0.123 ± 0.0001^{dB}	0.113 ± 0.0003^{cB}	0.099 ± 0.0004^{bB}	0.076 ± 0.0001^{aB}	0.076 ± 0.0003^{aB}
4	0.132 ± 0.0001^{eC}	0.124 ± 0.0004^{cC}	0.131 ± 0.0004^{dC}	0.101 ± 0.0001^{bC}	0.088 ± 0.0004^{aC}
6	0.313 ± 0.0003^{eD}	0.305 ± 0.0001^{dD}	0.199 ± 0.0004^{cD}	0.193 ± 0.0003^{bD}	0.174 ± 0.0005^{aD}
8	0.526 ± 0.0001^{eE}	0.513 ± 0.0003^{dE}	0.223 ± 0.0001^{cE}	0.197 ± 0.0003^{bE}	0.177 ± 0.0004^{aE}
10	0.547 ± 0.0001^{eF}	0.515 ± 0.0005^{dF}	0.239 ± 0.0001^{cF}	0.233 ± 0.0005^{bF}	0.197 ± 0.0001^{aF}

kC, k5%CP, k10%CP, k15%CP—carrageenan-based film with the addition of 0, 5, 10, and 15% of hawthorn extract, respectively; a–e—different letters indicate statistically significant differences among the values in the same row ($p < 0.05$); A–F—different uppercase letters indicate statistically significant differences among the values in the same column ($p < 0.05$).

The initial value of malondialdehyde in all the samples was 0.063–0.065 mg/kg. After 3 days of storage, the content of malondialdehyde was the highest in the unwrapped strawberries compared to the samples wrapped in carrageenan-based films. Malondialdehyde accumulated less in strawberries packed in carrageenan-based films. On the eleventh day, the samples differed significantly ($p < 0.05$) in terms of malondialdehyde content. At the end of the storage period, the lowest content was recorded in samples packed in carrageenan-based films with the addition of 15% hawthorn extract, namely 0.197 mg/g. Accumulation of malondialdehyde in samples is a consequence of deoxygenation of polyunsaturated fatty acids, resulting in the formation of toxic hydroxyl fatty acids and membrane damage [68]. Therefore, the highest antioxidative activity which was observed in film k15%CP (Table 7) resulted in the best reduction of the malondialdehyde content in wrapped strawberries. The results are in agreement with the results of Saleem et al. (2021) [69], who reported that the highest malondialdehyde accumulation in strawberries was observed in the control sample, while chitosan-coated samples showed significantly lower malondialdehyde content during storage. Also, the results are similar to the results obtained by Li et al. (2023) [70] during their research. Feng et al. (2024) [60] determined that the application of chitosan in combination with hawthorn leaf extract for packaging significantly reduced the content of malondialdehyde in fresh strawberries.

4. Conclusions

In this study, biodegradable films based on carrageenan with the addition of hawthorn extract were investigated for their physical, mechanical, antioxidant, and antimicrobial properties. The results showed that the addition of hawthorn extract influenced the thickness, moisture content, and water vapor permeability of the films, with films containing 10% hawthorn extract exhibiting the best properties in terms of preventing water vapor transmission. The mechanical properties of the films did not vary significantly with increasing concentrations of hawthorn extract, while films with higher concentrations showed improved antioxidant activity and higher polyphenol content.

Although the film with 15% hawthorn extract exhibited the best antioxidant properties in terms of free radical reduction, the antimicrobial activity results did not show a significant effect against the tested bacterial and yeast strains. FTIR analysis confirmed the presence of characteristic absorption peaks for carrageenan, while specific peaks for the hawthorn

extract were not clearly visible, indicating the dominance of carrageenan vibrations in the film matrix.

The results of the study show that carrageenan-based films with the addition of hawthorn extract significantly extend the shelf life of fresh strawberries. During 10 days of storage, the application of these films slowed metabolic processes and reduced moisture loss in strawberries, which led to a decrease in total soluble solids and malondialdehyde content, an indicator of oxidative stress. The film with 15% hawthorn extract showed the best efficiency in preserving the quality of strawberries, as it exhibited the smallest increase in pH value and titratable acidity, as well as the lowest degree of deterioration (only 16.7% on day 10).

This study confirms the potential of carrageenan-based films with hawthorn extract as eco-friendly and functional packaging materials for fresh fruit. Further research could focus on optimizing the concentration of the added extract and its application to other seasonal fruits to extend the freshness of such products.

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