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Mechanical and Barrier Properties Optimization of Carboxymethyl Chitosan-Gelatin-Based Edible Film Using Response Surface Methodology

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Abstract: Edible coatings have attracted the attention of researchers in recent years due to their degradability, safety, non-toxicity, low cost, good preservation effect, and other advantages. To prepare a new edible film with good mechanical and barrier properties, carboxymethyl chitosan (CMCS) and gelatin (GL) were selected as the film-forming matrix in this experiment, and glycerol, $CaCl_2$, Tween-20, and ascorbic acid (AA) have been added as plasticizers, crosslinking agents, surfactants, and antioxidants. Crosslinking agents and antioxidants first, the film was prepared by the casting method, and single factor tests were used to compare the effects of different CMCS: GL (w:w), glycerol, $CaCl_2$, Tween-20, and AA on mechanical properties (Tensile Strength (TS), Elongation at break (EAB)) and barrier properties (Water Vapor Permeability (WVP), Oxygen Permeability (OP)). Then, the weighting of each performance index is determined by a combination of principal component analysis and the comprehensive membership evaluation method. The formula for calculating the overall rating of edible film performance was determined. Finally, the manufacturing process of edible film with better performance was optimized by a response surface test. The results showed that the influence of each factor on the performance of the edible film was as follows: Glycerol addition > $CaCl_2$ addition > CMCS:GL, Tween-20, and AA had no significant influence on the performance of the edible film. When calculating the overall edible film property score, the weights of TS, EAB, WVP, and OP were 0.251, 0.068, 0.334, and 0.347, respectively. The optimal formulation for an edible film based on CMCS-GL with better properties than pure CMCS and GL film was CMCS:GL = 2:1, with the addition of 1% glycerol, 2% $CaCl_2$, 0.1% Tween-20, and 2% AA. The TS, EAB, OP, and WVP of the film obtained with this formula were: 16.28 MPa, 71.46%, 1.39×10^{-12} g·cm/(cm^2·s·Pa), 5.10×10^{-11} cm^3·cm/(m^2·s·Pa), respectively. This study suggests that CMCS-GL-based edible coatings can be used as a new food packaging material.

Keywords: carboxymethyl chitosan; gelatin; edible film; performance optimization; mechanical and barrier properties; response surface methodology

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1. Introduction

The current widely used food packaging material–plastic–is non-renewable and non-degradable, which not only causes environmental pollution but also poses a risk to human health [1,2]. In order to reduce environmental pollution and improve food safety, biodegradable and food-grade biopolymers (including polysaccharides, proteins, and lipids) are widely used to produce edible coatings [3,4]. In recent years, edible coatings have been widely used for postharvest preservation of fruits and vegetables due to their advantages such as ease of use, safety, nontoxicity, biodegradability, good mechanical properties, and barrier properties. The properties of edible films are closely related to the type of biopolymer chosen [2,5,6].

Among them, chitosan (CS) has the advantages of low price, safety, non-toxicity, biodegradability, good antibacterial activity, film formation, and biocompatibility. A CS-based edible coating has always been the focus of researchers' attention [7] and has been used for the preservation of sweet cherries [8,9]. However, CS can only be dissolved in acidic solutions with a pH below 6.3 and is insoluble in water and general organic solvents, which is not convenient for consumers to clean before consumption. In addition, the bad odor caused by acidic solutions affects the aroma of fruits and vegetables themselves, limiting their use in keeping fruits and vegetables fresh [6].

Carboxymethyl chitosan (CMCS), an amphoteric derivative of CS, is more water-soluble, biocompatible, and biodegradable due to its high content of carboxymethyl groups [10,11]. The film produced with CMCS has some water and oxygen resistance, which can be used for food packaging films. However, the one-component CMCS film is very fragile and hydrophilic and has poor barrier and mechanical properties, which limits its application in the food industry [12]. To improve the physical properties of the CMCS film, it can be mixed with other substances to form membranes. Previous studies have shown that gelatin (GL), as a natural water-soluble protein, can be mixed with CS to improve the properties of edible films [13,14].

In addition, the addition of $CaCl_2$ and/or ascorbic acid (AA) to CS can improve the preservation effect of CS on strawberries [15], pears [16], plums [17], and other fruits and vegetables [18]. $CaCl_2$ can also be used as a crosslinking agent to improve the properties of CS edible films [19,20]. AA as a safe, inexpensive, and efficient antioxidant, $CaCl_2$ can also effectively control the enzymatic browning of fruits and vegetables [17,21]. All these indicate that the addition of $CaCl_2$ and/or AA to edible films not only improves the mechanical properties of the film but also improves the preservation effect on fruits and vegetables.

Thus, the main objectives of this study are to prepare a new edible film with good mechanical and barrier properties using CMCS and GL as film-forming substrates and adding $CaCl_2$ and AA as crosslinking agents and antioxidants, respectively. The film was prepared by casting and evaluated based on its tensile strength (TS), elongation at break (EAB), water vapor permeability (WVP), and oxygen permeability (OP). The effects of the different components on the mechanical properties and barrier properties of the film were compared using a one-factor test. The weights of each performance index were determined by combining principal component analysis and the comprehensive membership evaluation method, and the comprehensive membrane performance evaluation formula was determined. Finally, the film formula with good mechanical and barrier properties was determined by the optimization test of the response surface test to provide a reference for the preparation and application of edible coatings based on CMCS-GL.

Based on the selected edible coating formula in this study, we have published two related papers [22,23]. One paper [22] investigated the impact of different components of CMCS-GL-based edible coating on sweet cherry quality during storage, concluding that AA-$CaCl_2$-CMCS-GL edible coating exhibited superior preservation effects. Subsequently, we examined the preservation effect of the AA-$CaCl_2$-CMCS-GL edible coating on four distinct sweet cherry varieties [23]. The results demonstrated that AA-$CaCl_2$-CMCS-GL coating could be considered a preservation method to enhance postharvest quality and nutritional properties across various sweet cherry cultivars. When utilizing coatings for preserving sweet cherries, it is crucial to consider the specific cultivar and select an appropriate one to achieve optimal preservation outcomes. In our future research, we aim to further investigate the preservation effect and mechanism of the AA-$CaCl_2$-CMCS-GL edible coating on other fruits and vegetables, aiming to facilitate its broader application in postharvest freshness maintenance.

2. Materials and Methods

2.1. Materials

CMCS (white to pale yellow free-flowing powder, deacetylated ≥ 80%, carboxymethylation ≥ 80%), GL (pale yellow solid, ash content < 3%, gel strength ≥ 160 g Bloom), glycerol (purity > 99%), $CaCl_2$ (white powder), and AA (purity > 99%) were from Macklin Biotechnology Co., Ltd. (Shanghai, China). All other chemical reagents were of analytical purity.

2.2. Experimental Design

2.2.1. Preparation of CMCS-GL-Based Edible Coating Solutions

CMCS-GL-based edible films were prepared according to the method proposed by Martinez Chamacho et al. [24], with some modifications. The schematic representation of the experimental recipe and the chemical interaction between CMCS and GL can be found in Figure 1. Weigh CMCS and GL powders separately and add them to distilled water. Heat them in a constant-temperature water bath of 60 °C, stir continuously until completely dissolved (~30 min), and then cool to 23 ± 1 °C to obtain a final concentration of 2% CMCS and GL solutions. Mix CMCS and GL solutions in a certain ratio, add Tween-20 as a surfactant, AA as an antioxidant, and appropriate amounts of glycerol and $CaCl_2$ powder as plasticizer and crosslinker, respectively. Stir overnight at room temperature, centrifuge at 4000× *g* for 10 min, and recover the supernatant to remove bubbles and insoluble components. Take 20 ± 0.1 g of the supernatant and spread it evenly in a clean and dry organic glass film formed by the tape casting method. Dry it by blowing at 60 °C, cool it, and remove the film. Place it in an incubator with constant temperature and humidity (temperature: 23 ± 1 °C, RH: 50%) for at least 48 h before measuring the various properties of the edible film [14,25].

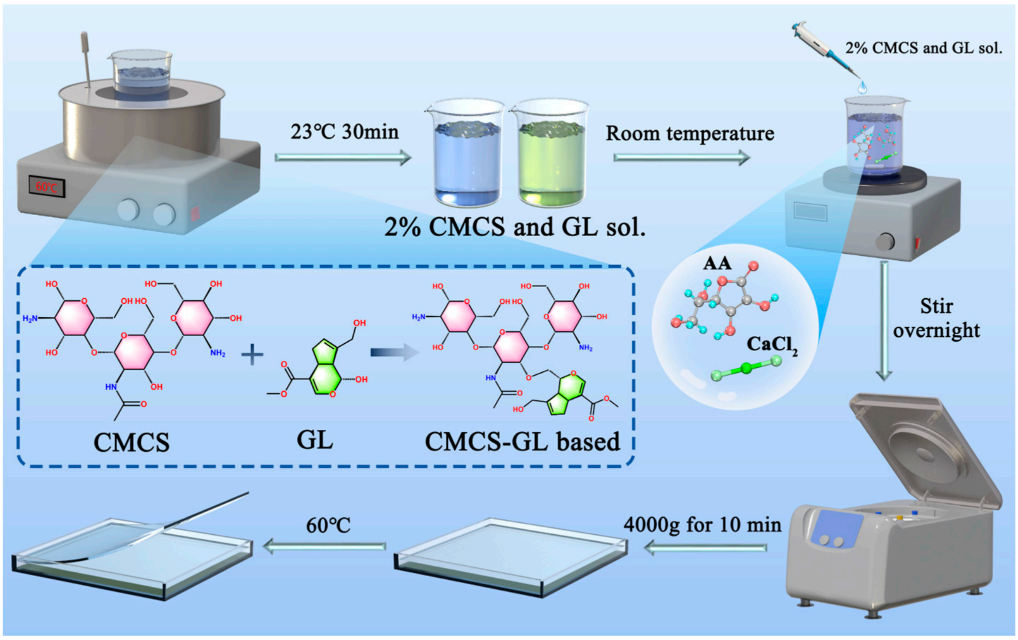

Figure 1. The schematic representation of the experimental recipe and the chemical interaction between CMCS and GL.

2.2.2. Method for Determination of Mechanical Properties and Barrier Properties of CMCS-GL-Based Edible Film

1. Determination of film thickness

Use a handheld digital micrometer to measure the film thickness (accurate to 0.001 mm). Five different points (one in the center and four at the edge) were taken from each membrane and measured. Results were reported as mean (Mean, M) ± standard deviation (SD). The layer thickness was used to calculate the TS, EAB, WVP, and OP of the layer.

2. Determination of TS and EAB

TS and EAB of the film were determined according to the methods of Fan, H.Y. et al. [26] and Yadav, S. et al. [14] with some modifications using an electronic universal testing machine (INSTRON-5544, Instron Corporation, Boston, Massachusetts, USA). The film was cut into a 70 × 10 mm rectangle with an initial standard spacing of 50 mm. The film was stretched to break at a rate of 0.1 mm/min at 23 ± 1 °C and RH 40%–50%. Each sample was measured three times, and the results were averaged.

3. Determination of WVP

The WVP of the film was measured at 23 °C and RH 90% using the water vapor permeability tester. Each membrane sample was tested in triplicate using the weight reduction method, and the results were averaged.

4. Determination of OP

The OP of the film was measured by a gas permeability tester (STG-V1, Guangzhou Xitang Electromechanical Technology Co., Ltd., Guangzhou, China) at 23 °C; the pressure difference between the high- and low-pressure chambers was 50 KPa; and the purity of the oxygen used was 99%. Each membrane was tested in triplicate, and the results were averaged.

5. Determination of comprehensive scores for film mechanical and barrier performance

To obtain a membrane formula with good mechanical and barrier properties, a combination of principal component analysis and a comprehensive membership evaluation method was used in this experiment to determine the comprehensive membrane performance evaluation. Use principal component analysis in SPSS software to determine the weights of each membrane performance index to avoid subjective errors caused by the artificial assignment of weights. Use the comprehensive membership evaluation method to comprehensively evaluate the TS, EAB, WVP, and OP of the slide. In practice, the better the mechanical performance indicators of the film–TS and EAB–the larger they are expected to be. TS and EAB are called positive indicators, and their membership degree is calculated according to Equation (1). However, the smaller the expected barrier performance–WVP and OP–the better. WVP and OP are called negative indicators, and their degree of belonging is calculated according to Equation (2):

$$\text{P} = (A_i - A_{min})/(A_{max} - A_{min}) \tag{1}$$

$$\text{P} = (A_{max} - A_i)/(A_{max} - A_{min}) \tag{2}$$

where P is the degree of membership of a particular indicator; A_i is the indicator value; A_{min} is the minimum value of the same indicator; and A_{max} is the maximum value of the same indicator.

The overall evaluation of the mechanical and barrier properties of the film is calculated using Equation (3):

$$\text{S} = aP_1 + bP_2 + cP_3 + dP_4 \tag{3}$$

where S is the overall rating of the edible film performance; P_1, P_2, P_3, and P_4 are the film grades of TS, EAB, WVP, and OP, respectively; a, b, c, and d are the weights of TS, EAB, WVP, and OP.

2.2.3. Single Factor Test for Performance Optimization of CMCS-GL-Based Edible Film

According to the steps described in Section 2.2.1, the edible film was prepared, and the TS, EAB, WVP, and OP of the film were used as evaluation indices to determine the best values of each factor, and the factors that had less influence on the performance of the film were removed. All experiments were repeated three times, and the results are expressed as M ± SD. The factor levels of each test are shown in Table 1.

Table 1. Factors and levels of a single-factor experiment.

Levels	The Quality Ratio between CMCS and GL	Glycerol Addition Amount/%	$CaCl_2$ Addition Amount/%	Tween-20 Addition Amount/%	AA Addition Amount/%
1	6:0	0	1	0	0
2	4:2	0.5	1.5	0.1	1
3	3:3	1	2	0.2	2
4	2:4	1.5	2.5	0.3	3
5	0:6	2	3	0.4	4

2.2.4. Response Surface Optimization Test of Performance of the CMCS-GL-Based Edible Film

Based on the results of the one-factor experiment, a three-factor, three-level response surface experiment was designed using Design Expert software. CMCS:GL (w:w), glycerol addition, and $CaCl_2$ addition were selected as independent variables, and the comprehensive score of membrane performance Y was used as the response value. The experimental results were fitted into a quadratic regression model, and the optimized membrane formula was finally obtained. The table of response surface analysis factors is shown in Table 2.

Table 2. Factors and levels of response surface analysis.

The Quality Ratio between CMCS and GL	Glycerol Addition Amount/%	$CaCl_2$ Addition Amount/%
1:1 (1)	0.5	1.5
2:1 (2)	1	2
3:1 (3)	1.5	2.5

2.3. Statistical Analysis

Experimental results were expressed as M ± SD, and software such as Design Expert, SPSS, and Origin were used for experimental design and data processing. Significance analysis of the data were performed using the Duncan New Complex Range method with $p < 0.05$.

3. Results and Discussion

3.1. Univariate Test Results and Analysis

3.1.1. Effect of CMCS:GL (w:w) on Mechanical and Barrier Properties of Edible Film

Table 3 shows the influence of the different factors on the properties of the edible film. Table 3 shows that the TS of the pure CMCS film is significantly higher than that of the pure GL film. With decreasing CMCS:GL (w:w), the TS of the film first increases and then decreases, which might be due to the fact that the addition of GL decreases the crystallization ability of CMCS in the film and makes the composite film softer and more elastic. This is consistent with the result that the addition of GL improves the mechanical properties of CS. However, the EAB of pure GL films was significantly higher than that of pure CMCS films. The EAB first increased and then decreased with decreasing CMCS:GL (w:w), which was attributed to the limited binding sites between CMCS and GL molecules. When the ratio of the two molecules is 4:2, they can be completely bonded together, and the bond between the molecules is the tightest, and the EAB value is the largest. The results showed that the mechanical properties of pure CMCS and GL films were deficient to some extent. The composite membrane prepared by mixing CMCS and GL films in a ratio of 4:2 could overcome the deficiencies of both films and obtain a membrane with better mechanical properties.

Table 3. Effects of different factors on the performance of edible film.

Factors	AA Addition Amount/%	Thickness/mm	TS/MPa	EAB/%	WVP/ 10^{-12} g·cm/ (cm^2·s·Pa)	OP/ 10^{-11} cm^3·cm/ (m^2·s·Pa)
CMCS:GL (w:w)	6:0	0.052 ± 0.006 [a]	17.61 ± 0.57 [a]	41.74 ± 1.77 [a]	1.85 ± 0.12 [a]	6.51 ± 0.19 [a]
	4:2	0.053 ± 0.005 [a]	20.09 ± 0.71 [b]	79.84 ± 2.54 [b]	1.53 ± 0.08 [b]	5.37 ± 0.16 [b]
	3:3	0.053 ± 0.006 [a]	16.03 ± 0.38 [c]	70.95 ± 2.14 [c]	1.38 ± 0.07 [b,c]	5.19 ± 0.15 [b]
	2:4	0.050 ± 0.005 [a]	13.08 ± 0.29 [d]	64.15 ± 2.50 [d]	1.29 ± 0.08 [c]	5.23 ± 0.10 [b]
	0:6	0.053 ± 0.006 [a]	8.40 ± 0.38 [e]	62.15 ± 1.96 [d]	1.23 ± 0.13 [c]	4.52 ± 0.40 [c]
Glycerol addition amount/%	0	0.055 ± 0.004 [a]	20.17 ± 0.63 [a]	45.06 ± 1.99 [a]	1.75 ± 0.09 [a]	6.46 ± 0.09 [a]
	0.5	0.053 ± 0.003 [a]	18.10 ± 0.79 [b]	52.51 ± 1.38 [b]	1.54 ± 0.07 [b]	5.66 ± 0.11 [b]
	1	0.057 ± 0.004 [a]	16.18 ± 0.43 [c]	70.91 ± 1.97 [c]	1.41 ± 0.03 [c]	5.18 ± 0.13 [b]
	1.5	0.055 ± 0.003 [a]	14.36 ± 0.79 [d]	74.64 ± 1.94 [d]	1.58 ± 0.05 [b]	5.84 ± 0.11 [b]
	2	0.054 ± 0.003 [a]	13.06 ± 0.67 [e]	76.20 ± 1.81 [d]	1.81 ± 0.08 [a]	6.38 ± 0.15 [a]
$CaCl_2$ addition amount/%	1	0.055 ± 0.004 [a]	13.37 ± 0.58 [a,b]	78.66 ± 1.93 [a]	1.88 ± 0.07 [a,d]	6.61 ± 0.24 [a]
	1.5	0.052 ± 0.005 [a]	14.84 ± 0.80 [b]	75.68 ± 1.69 [a]	1.61 ± 0.06 [b]	5.66 ± 0.19 [b]
	2	0.055 ± 0.003 [a]	16.22 ± 0.52 [c]	71.92 ± 1.83 [b]	1.45 ± 0.08 [c]	5.20 ± 0.15 [c]
	2.5	0.053 ± 0.004 [a]	14.14 ± 0.73 [b]	57.37 ± 1.46 [c]	1.75 ± 0.08 [a]	5.82 ± 0.25 [b,d]
	3	0.054 ± 0.003 [a]	12.45 ± 0.66 [a]	46.82 ± 1.96 [d]	1.96 ± 0.09 [d]	6.19 ± 0.21 [d]
Tween-20 addition amount/%	0	0.053 ± 0.004 [a]	15.83 ± 0.54 [a]	66.23 ± 1.50 [a]	1.45 ± 0.03 [a]	5.36 ± 0.20 [a]
	0.1	0.054 ± 0.003 [a]	15.75 ± 0.50 [a]	65.61 ± 1.70 [a]	1.47 ± 0.06 [a]	5.25 ± 0.25 [a]
	0.2	0.055 ± 0.003 [a]	15.77 ± 0.93 [a]	66.01 ± 1.61 [a]	1.49 ± 0.08 [a]	5.33 ± 0.20 [a]
	0.3	0.054 ± 0.003 [a]	15.92 ± 0.21 [a]	65.64 ± 1.46 [a]	1.46 ± 0.06 [a]	5.38 ± 0.21 [a]
	0.4	0.055 ± 0.002 [a]	16.06 ± 0.67 [a]	65.20 ± 1.84 [a]	1.42 ± 0.04 [a]	5.35 ± 0.12 [a]
AA addition amount/%	0	0.056 ± 0.004 [a]	15.41 ± 0.31 [a,b]	64.42 ± 1.04 [a]	1.44 ± 0.04 [a]	5.34 ± 0.23 [a]
	1	0.055 ± 0.004 [a]	15.55 ± 0.28 [b]	64.47 ± 0.62 [a]	1.42 ± 0.05 [a]	5.50 ± 0.08 [a]
	2	0.053 ± 0.003 [a]	15.50 ± 0.33 [b]	64.99 ± 1.17 [a]	1.45 ± 0.05 [a]	5.44 ± 0.10 [a]
	3	0.056 ± 0.005 [a]	14.92 ± 0.21 [a,b]	64.30 ± 1.08 [a]	1.47 ± 0.05 [a]	5.46 ± 0.12 [a]
	4	0.057 ± 0.006 [a]	14.73 ± 0.66 [a]	64.38 ± 1.20 [a]	1.49 ± 0.07 [a]	5.47 ± 0.06 [a]

Note: In each single-factor trial, data marked with different letters in the same column indicates a significant difference ($p < 0.05$).

Compared to CMCS-only films, GL-only films exhibited lower WVP and OP, which was consistent with the results of Pereda et al. [27]. Moreover, the WVP and OP-values of edible films showed a downward trend with the decrease of CMCS:GL (w:w), which could be due to GL molecules entering the film cavity and the hydrogen and covalent interactions between the CMCS and the GL network reducing the availability of hydrophilic groups, making the film more compact, decreasing the transfer rate of H_2O and O_2 in the film, and decreasing the WVP and OP values [14,25]. When the CMCS:GL ratio was 4:2 and 3:3, there was no significant difference in the WVP of the films. There was no significant difference between CMCS:GL 3:3, 2:4, and pure GL films. At CMCS:GL ratios of 4:2, 3:3, and 2:4, there was no significant difference in the OP values of the prepared edible films; however, they were all lower than those of the pure CMCS films.

In summary, CMCS:GL = 4:2 (2:1) was chosen as the central level for the response surface test.

3.1.2. The Effect of Glycerol Addition on the Mechanical and Barrier Properties of Edible Films

From Table 3, it can be seen that the TS of the film decreases with increasing amounts of added glycerol. This is because glycerol is a small molecule that can easily be inserted into the chains of CMCS and GL molecules, leading to the destruction of the dense structure of the film. This effect weakens the interaction between or within the molecules of CMCS and GL, resulting in a decrease in TS [27]. However, the EAB of the membrane increased with the increase in glycerol supply because glycerol softened the rigid structure of the CMCS-GL film and increased the fluidity in the chain. The structure of the film was loosened to some extent, which improved the flexibility of the membrane and increased the EAB.

The WVP and OP of the film first decreased and then increased with the increase in glycerol addition. At 1% glycerol addition, the WVP and OP reached the minimum values ((1.41 ± 0.03) × 10^{-12} g·cm/(cm·s·Pa), (5.18 ± 0.13) × 10^{-11} cm^3·cm/(m·s·Pa)). This is due to the formation of a large number of hydrogen bonds in the molecular structure of the film with appropriate glycerol addition, and the bond between CMCS and GL molecules is stronger, which hinders the penetration of H_2O and O_2. However, when the addition of glycerol further increases, the WVP of the film shows an increasing trend, which is due to the change in hydrogen bonds between and within the molecules of CMCS and GL caused by too much glycerol. This leads to more voids in the film structure, a loosened film structure, and an increased WVP and OP of the film [14,28,29].

In summary, 1% glycerol was selected as the central value for the response surface experiment.

3.1.3. Effect of $CaCl_2$ Addition on the Mechanical and Barrier Properties of Edible Films

Table 3 shows that the TS of the film first increases and then decreases with increasing $CaCl_2$ addition. When $CaCl_2$ addition increases to 2%, TS reaches its maximum value (16.22 ± 0.49 MPa). However, when the $CaCl_2$ addition continues to increase, the TS of the film decreases. This is because $CaCl_2$ as a crosslinking agent at a suitable concentration can make the connection between CMCS and GL molecules tighter, increase the crosslinking density between the molecular chains, and increase in TS of the film. However, if the added amount of $CaCl_2$ is too high, the film becomes brittle and hard, and TS decreases [30]. The EAB of the film decreases with increasing $CaCl_2$ addition, which is due to the fact that the ductility of the membrane decreases with increasing $CaCl_2$ addition, leading to a decrease in membrane flexibility and EAB. At 1%–2% addition, the EAB value of the film decreased slightly (from 78.66% ± 1.83% to 71.92% ± 1.74%), but with increasing addition, the EAB value of the film decreased rapidly (from 71.92% ± 1.74% to 46.82% ± 1.86%). In addition, studies have shown that the floating powder phenomenon occurs when the added amount of $CaCl_2$ is too large, which affects the appearance of the film [15,20]. Therefore, the appropriate amount of added $CaCl_2$ should be selected by combining various factors.

The WVP and OP of the film showed a trend that first decreased and then increased with increasing $CaCl_2$ addition. When the $CaCl_2$ addition reached 2%, the WVP and OP values both reached the minimum value of $(1.45 \pm 0.07) \times 10^{-12}$ g·cm/(cm·s·Pa), $(5.20 \pm 0.12) \times 10^{-11}$ cm^3·cm/(m·s·Pa)). This is because when an appropriate amount of $CaCl_2$ is added as a crosslinking agent, a dense network structure is formed between CMCS and GL molecules, which reduces the diffusion rate of H_2O and O_2 and reduces the WVP and OP values of the film. Excess $CaCl_2$ also damages the dense structure of the film, again increasing the WVP and OP of the film [15,20]. In summary, adding an appropriate amount of $CaCl_2$ as a crosslinking agent can improve the mechanical and barrier properties of the film. However, if too much $CaCl_2$ is added, it will have negative effects on the properties of the film.

Therefore, after extensive consideration, 2% $CaCl_2$ was selected as the central value for the response surface experiment.

3.1.4. Effect of Tween-20 Addition on the Mechanical and Barrier Properties of Edible Films

Table 3 shows that there is no significant difference in the thickness, TS, EAB, WVP, and OP of the edible membrane films with different Tween-20 addition amounts, i.e., the addition amount of Tween-20 does not have a great influence on the mechanical and barrier properties of the film, which are determined by its own properties. As a surfactant, Tween-20 can be added to the edible coating solution to reduce the surface tension of the coating solution so that the coating solution can be evenly applied to the surface of fruits and vegetables with low surface tension [27]. The results of the contact angle test of different coating solutions on the surface of sweet cherries show that the contact angle of the coating solution on the surface of sweet cherries can be reduced from 86.7° to 63.6° when 0.1% Tween-20 is added to the coating solution so that the coating solution can spread better on the epidermis of sweet cherries with strong hydrophobicity. In addition, Tween-20 has some inherent odor, so under the premise of reducing the surface tension of the film coating solution, the less Tween-20 is added, the less the effect on the sensory properties of the film. Therefore, in conjunction with the conclusion of this test, the amount of Tween-20 added in this test was set at 0.1%. In addition, the amount of added Tween-20 was no longer considered a response surface factor for further analysis.

3.1.5. Effect of AA Addition on the Mechanical and Barrier Properties of Edible Films

Table 3 shows that there is no significant difference in the thickness, EAB, WVP, and OP of films prepared with different AA addition amounts. When the additional amount of AA was 4%, the TS of the film was significantly lower than that of the film with an additional amount of AA of 1 and 2%, indicating that increasing the amount of AA to 4% would decrease the TS of the film. There was no significant difference in the TS of the films prepared with 0, 1%, 2%, and 3% AA addition, and there was no significant difference between the films prepared with 0, 3%, and 4% AA addition, indicating that the amount of AA addition had little effect on the properties of the edible membrane. This is due to the fact that AA was added to the edible membrane as an antioxidant and did not have much effect on the mechanical and barrier properties of the membrane. Therefore, after reviewing the relevant literature [17,21,31] and combining the results of this one-factor test, the added amount of AA was fixed at 2%, and the added amount of AA was no longer used as a factor in the response surface test for further analysis.

In summary, CMCS:GL (*w*/*w*) and the addition of glycerol and $CaCl_2$ have a great influence on the mechanical properties and barrier properties of the edible membrane. Therefore, these three factors were selected as objects for the analysis of the response surface test.

3.2. Determination of the Comprehensive Scores of the Mechanical and Barrier Properties of the Edible Film

3.2.1. Results of Principal Component Analysis

Using the four membrane performance indicators (TS, EAB, WVP, and OP) as objects of analysis, two data sets were randomly selected from three single-factor test results (CMCS:GL (w:w), the amount of added glycerol, and the amount of added $CaCl_2$. The SPSS software was used to perform principal component analysis for the six selected data sets (see Table 4). Due to the different dimensions of the four indicators and the inclusion of two positive indicators (TS, EAB) and two negative indicators (WVP, OP), the four indicators were standardized according to the Formulas (1) and (2) before analysis. The standardized data are shown in Table 4, and the eigenvalues and contribution rates of the relevant components are shown in Table 5.

Table 4. Raw and standardized test data of contribution rate by principal component analysis.

Types	Number	TS/MPa	EAB/%	WVP/ 10^{-12} g·cm/(cm^2·s·Pa)	OP 10^{-11} cm^3·cm/(m^2·s·Pa)
Raw test data	1	17.61 ± 0.57	41.74 ± 1.77	1.85 ± 0.12	6.51 ± 0.19
	2	20.09 ± 0.71	79.84 ± 2.54	1.53 ± 0.08	5.37 ± 0.16
	3	18.10 ± 0.79	52.51 ± 1.38	1.54 ± 0.07	5.66 ± 0.11
	4	16.18 ± 0.43	70.91 ± 1.97	1.41 ± 0.03	5.18 ± 0.13
	5	16.22 ± 0.52	71.92 ± 1.83	1.45 ± 0.08	5.20 ± 0.15
	6	14.14 ± 0.73	57.37 ± 1.46	1.75 ± 0.08	5.82 ± 0.25
Standardized Test data	1	0.55 ± 0.08	0.04 ± 0.04	0.20 ± 0.19	0.11 ± 0.11
	2	0.90 ± 0.24	0.93 ± 0.49	0.73 ± 0.19	0.79 ± 0.45
	3	0.82 ± 0.24	0.63 ± 0.47	0.51 ± 0.29	0.64 ± 0.36
	4	0.65 ± 0.10	0.51 ± 0.06	0.80 ± 0.14	0.67 ± 0. 09
	5	0.73 ± 0.21	0.72 ± 0.40	0.78 ± 0. 06	0.71 ± 0.09
	6	0.59 ± 0.15	0.45 ± 0.42	0.77 ± 0.03	0.69 ± 0.03

Table 5. Eigenvalues and contribution rates of related components.

Component	Eigenvalues	Variance Contribution Rate/%	Cumulative Variance Contribution Rate/%
Z_1	2.619	65.475	65.475
Z_2	1.008	25.188	90.663
Z_3	0.280	6.993	97.656
Z_4	0.094	2.344	100.000

Table 5 shows that the eigenvalues (2.619, 1.008) of the first two principal components are greater than one, the variance contribution of the first principal component (Z_1) is 65.475%, the variance contribution of the second principal component (Z_2) is 25.188%, and the cumulative variance contribution of the two principal components is 90.663%, exceeding 85%. Therefore, the first two principal components can essentially reflect the overall information of the film performance index and replace the original four indicators.

The factor loading matrix of the two principal components is shown in Table 6. The magnitude of the factor loading may reflect the contribution of each index to the principal components. The magnitude of Z_1 is mainly determined by TS and OP, with OP having the largest loading on Z_1. The magnitude of Z_2 is mainly determined by TS and EAB, with EAB having the largest loading on Z_2.

Table 6. Factor loading matrix of two principal components.

Component	TS (X_1)	EAB (X_2)	WVP (X_3)	OP (X_4)
Z_1	0.922	0.186	0.914	0.948
Z_2	0.044	0.981	−0.030	−0.206

According to the factor loading matrix of principal components, the linear relationship between Z_1, $Z_{2.}$ and the performance indices of CMCS-GL film can be constructed as follows: Equations (4) and (5)

$$Z_1 = -0.922\ X_1 + 0.186\ X_2 + 0.914\ X_3 + 0.948\ X_4 \tag{4}$$

$$Z_2 = 0.044\ X_1 + 0.981\ X_2 - 0.030\ X_4 - 0.206\ X_4 \tag{5}$$

3.2.2. Determination of Comprehensive Scores of Mechanical and Barrier Properties of Edible Membranes

Using the factor loading matrix of the two principal components (Table 6) and the eigenvalues of the two principal components (Table 5), the coefficient Y was calculated in the linear combination (= the number of loadings of the index/square root of the corresponding eigenvalues of the principal components). Then, the coefficient H in the comprehensive score model was calculated from the variance contribution fraction of Y and the principal components: (Z_1 variance contribution fraction × Y_1 + Z_2 variance contribution fraction × Y_2)/(Z_1 variance contribution fraction + Z_2 variance contribution fraction)). After normalizing H for each index, the weight W for each index is obtained; see Table 7. The weights of TS, EAB, WVP, and OP were 0.251, 0.068, 0.334, and 0.347, respectively.

Table 7. Coefficients Y, H, W in the process of calculating the comprehensive score of edible film.

Component	TS	EAB	WVP	OP
Y_1	0.570	0.115	0.565	0.948
Y_2	0.044	0.997	−0.030	−0.205
H	0.423	0.115	0.565	0.586
W	0.251	0.068	0.334	0.347

According to Formula (3), the calculation formula for the overall grade of the film's performance is as follows:

$$S = 0.251P_1 + 0.068P_2 + 0.334P_3 + 0.347P_4 \tag{6}$$

3.3. Response Surface Optimization Test Results and Analysis

Based on the results of the one-factor experiment, CMCS:GL (w:w, X_1), glycerol additive (X_2), and $CaCl_2$ additive (X_3) were selected as independent variables, and the comprehensive values of the mechanical properties and barrier of the film were used as response values (Y). A three-factorial and three-stage response surface experiment was conducted. The experimental design and results are shown in Table 8.

Table 8. Design and result of the response surface methodology experiment.

Number	CMCS:GL (w:w, X_1)	Glycerol Addition Amount/% (X_2)	$CaCl_2$ Addition Amount/% (X_3)	Comprehensive Score Y
1	0	0	0	0.76
2	−1	0	0	0.62
3	0	1	−1	0.45
4	0	−1	−1	0.40
5	0	−1	1	0.49
6	0	0	0	0.75
7	1	−1	0	0.38
8	0	0	0	0.74
9	1	1	0	0.33
10	−1	−1	0	0.42
11	0	0	0	0.71
12	−1	0	−1	0.41
13	−1	1	0	0.36
14	0	0	0	0.73
15	1	0	−1	0.56
16	1	0	−1	0.37
17	0	1	0	0.31

Applying a Design Expert to perform a multiple regression fit on the experimental data in Table 8 yielded the following regression model:

$$Y = 0.74 - 0.018X_1 - 0.03X_2 - 8.663 \times 10^{-3}X_3 + 2.5 \times 10^{-3}X_1X_2 - 0.09X_1X_3 - 0.058X_2X_3 - 0.22X_2^2 - 0.11X_3^2 \tag{7}$$

Perform an analysis of variance and significance test for the above regression model, and the results are shown in Table 9. From $p < 0.0001$, it can be seen that the regression of this model is highly significant. From the p-value of the misfit term = 0.8240 (>0.05), it can be seen that the misfit of the model is not significant, which indicates that other factors have less influence on the model. The experimental results are in good agreement with the regression model [26]. From $R_2 = 0.9959$, $R^2_{Adj} = 0.9906$, and the coefficient of variation CV = 3.11%, it can be seen that the predicted value of this experiment has a high correlation with the experimental value and the error is small. Therefore, this model can be used to analyze and predict the comprehensive result Y of edible film performance based on CMCS-GL. According to the F value of each factor ($X_2 > X_1 > X_3$), the factors affecting the overall evaluation of the mechanical and barrier performance of the membrane are ranked as follows: X_2 (addition of glycerol) > X_1 (addition of $CaCl_2$) > X_3 (CMCS:GL). From the significance results of each element of the regression model, the primary term X_1 ($p = 0.0248 < 0.05$) has a significant effect on membrane performance, X_2 ($p = 0.0012 < 0.01$) has a very significant effect, and X_3 ($p = 0.2427 > 0.05$) has no significant effect. The interaction between X_1X_2 ($p = 0.7652 > 0.05$) was not significant, while the interaction between X_1X_3 ($p < 0.0001$) and X_2X_3 ($p = 0.0002$) was significant.

Table 9. Analysis of variance for the fitted regression model.

Source of Variance	Sum of Squares	DF	Mean Square	F Value	p Value	Significance
Model	0.44	9	0.049	188.15	<0.0001	**
X_1	2.1×10^{-3}	1	2.1×10^{-3}	8.10	0.0248	*
X_2	7.2×10^{-3}	1	7.2×10^{-3}	27.78	0.0012	**
X_3	4.22×10^{-3}	1	4.22×10^{-3}	1.63	0.2427	
X_1X_2	2.5×10^{-3}	1	2.5×10^{-3}	0.096	0.7652	
X_1X_3	0.018	1	0.018	68.01	<0.0001	**
X_2X_3	0.013	1	0.013	51.02	0.0002	**
X_1^2	0.085	1	0.085	325.98	<0.0001	**
X_2^2	0.18	1	0.18	690.45	<0.0001	**
X_3^2	0.040	1	0.040	155.97	<0.0001	**
Residual	1.815×10^{-3}	7	2.592×10^{-3}			
Lack of fit	3.345×10^{-3}	3	1.115×10^{-3}	0.30	0.8240	no
Pure Error	1.48×10^{-3}	4	3.7×10^{-3}			
Cor total	0.44	16				
R^2	0.9959					
R^2_{Adj}	0.9906					
CV/%	3.11					

Note: ** represent an extremely significant effect at $p < 0.01$; * represents a significant effect at $p < 0.05$.

3.4. Response Surface Optimization Test Graph Analysis

The three-dimensional surface and contour plots of the reaction surface for the interaction of the various factors are shown in Figure 2. From Figure 2a,c,e, the interaction of the three experimental factors can be intuitively seen. In the three-dimensional surface plot, the change in color from blue to red indicates a change in response value from small to large. The faster the change, the steeper the slope of the reaction surface and the flatter the reaction surface. This indicates that this factor has a smaller effect on the overall evaluation of the mechanical and barrier performance of the membrane. Conversely, the steeper the reaction surface, the greater the impact this factor has on the overall evaluation of membrane performance. In the contour plots, the center of the smallest circle is the maximum value of the response value, while the circle represents a weak interaction between factors and the ellipse represents a strong interaction between factors [32]. The contour lines in Figure 2b are nearly circular, indicating a weak interaction between CMCS:GL and the amount of glycerol addition, while the contour plots in Figure 2d,e are elliptical, indicating a strong interaction between CMCS:GL and $CaCl_2$ addition, glycerol addition, and $CaCl_2$ addition.

3.5. Determination and Verification of the Optimal Formulation of CMCS-GL-Based Edible Film

The regression model was analyzed using response surface analysis. When the mechanical and barrier properties of the edible membrane based on CMCS-GL were better, the formula was CMCS:GL = 2:1, the addition of glycerol was 1%, the addition of $CaCl_2$ was 2.002%, and the predictive value of the comprehensive score of membrane performance was 0.741. Under these conditions, the film was prepared, and three parallel tests were performed. The TS, EAB, WVP, and OP were measured as follows: an amount of 16.28 MPa, 71.46%, 1.39×10^{-12} g·cm/(cm^2·s·Pa), 5.10×10^{-11} cm^3·cm/(m^2·s·Pa). After standardization according to Formula (6), the comprehensive evaluation of membrane performance was 0.73, which shows that the verification test results were close to the predicted value of the model. It can be seen that the model can well simulate and predict the comprehensive evaluation of the edible membrane based on CMCS-GL. The formulation of the edible film obtained by response surface optimization has some practical significance when its mechanical and barrier properties are good.

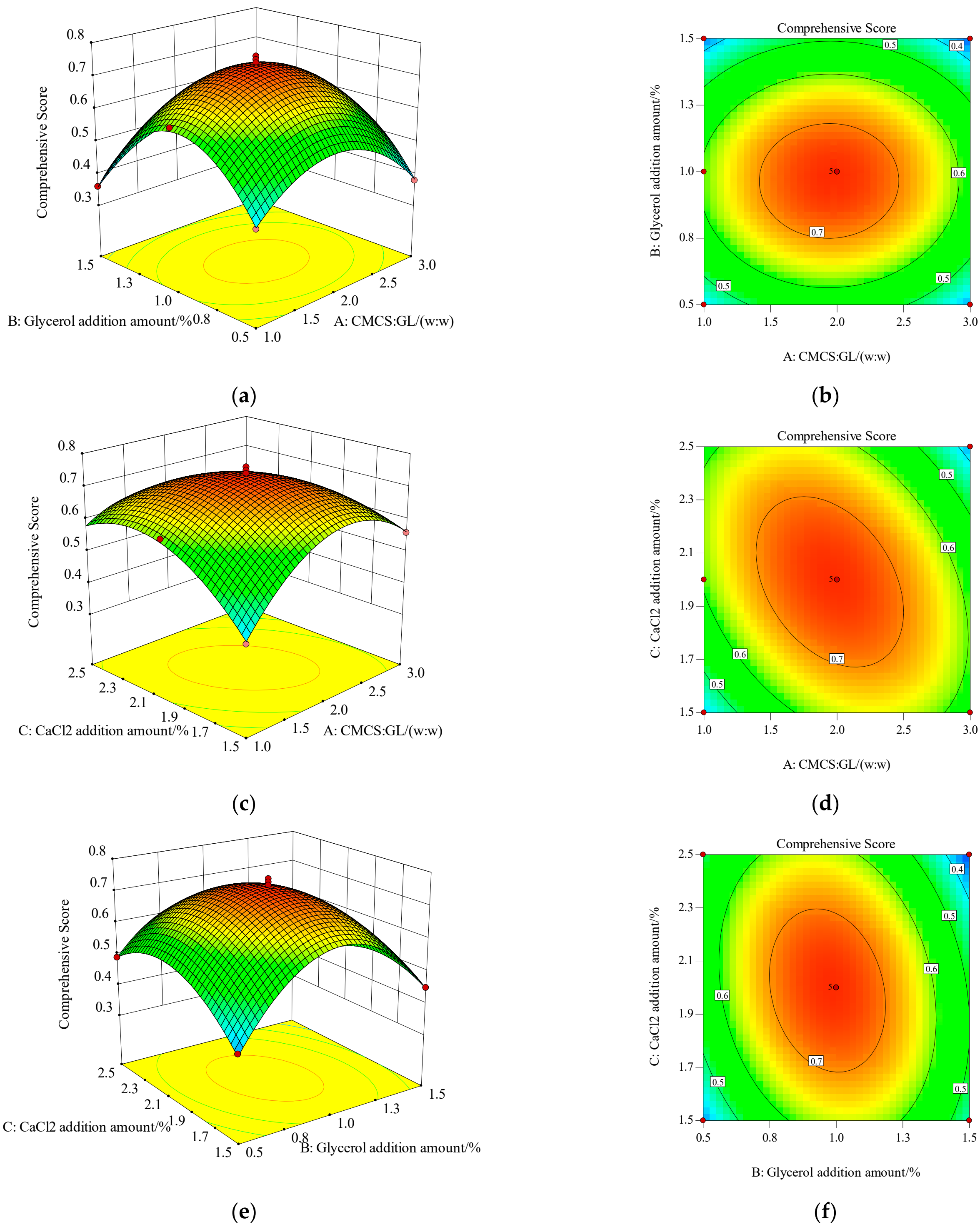

Figure 2. Response surface and contour plot of the interaction between three factors. (**a**) Response surface plot between CMCS:GL and glycerol addition, (**b**) Contour plot between CMCS:GL and glycerol addition, (**c**) Response surface plot between CMCS:GL and $CaCl_2$ addition, (**d**) Contour plot between CMCS:GL and $CaCl_2$ addition, (**e**) Response surface plot between $CaCl_2$ addition and glycerol addition, (**f**) Contour plot between $CaCl_2$ addition and glycerol addition.

4. Conclusions

Based on the single-factorial test, this study developed a three-factorial test at three levels using the response surface method. Considering the overall evaluation of the mechanical and barrier properties of the edible film as the response value, the order of the factors affecting the overall evaluation of the edible membrane (glycerol addition > $CaCl_2$ addition > CMCS:GL) and the formula with the best performance of the edible membrane were determined as follows: CMCS:GL (w:w) = 2:1, and the addition amounts of glycerol, $CaCl_2$, Tween-20, and AA were 1%, 2%, 0.1%, and 2%, respectively. Under these conditions, the average values for TS, EAB, WVP, and OP of the edible film were as follows: 16.28 MPa, 71.46%, 1.39×10^{-12} g·cm/(cm^2·s·Pa), 5.10×10^{-11} cm^3·cm/(m^2·s·Pa), respectively. The comprehensive score was 0.73, which was close to the predicted value of 0.74. This suggests that the formulation of an edible film based on CMCS-GL with good mechanical and barrier properties obtained by the response surface method has some practical significance. This study suggests that the edible coating based on CMCS-GL can be used as a new food packaging material. Further properties of this edible coating and its application to fruits and vegetables need to be further investigated.

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References

1. Mkandawire, M.; Aryee, A.N.A. Resurfacing and modernization of edible packaging material technology. *Curr. Opin. Food Sci.* **2018**, *19*, 104–112. [CrossRef]
2. Nešić, A.; Cabrera-Barjas, G.; Dimitrijević-Branković, S.; Davidović, S.; Radovanović, N.; Delattre, C. Prospect of Polysaccharide-Based Materials as Advanced Food Packaging. *Molecules* **2020**, *25*, 135. [CrossRef] [PubMed]
3. Petkoska, A.T.; Daniloski, D.; D'Cunha, N.M.; Naumovski, N.; Broach, A.T. Edible packaging: Sustainable solutions and novel trends in food packaging. *Food Res. Int.* **2021**, *140*, 109981. [CrossRef]
4. Amin, U.; Khan, M.U.; Majeed, Y.; Rebezov, M.; Khayrullin, M.; Bobkova, E.; Shariati, M.A.; Chung, I.M.; Thiruvengadam, M. Potentials of polysaccharides, lipids and proteins in biodegradable food packaging applications. *Int. J. Biol. Macromol.* **2021**, *183*, 2184–2198. [CrossRef] [PubMed]
5. Yousuf, B.; Qadri, O.S.; Srivastava, A.K. Recent developments in shelf-life extension of fresh-cut fruits and vegetables by application of different edible coatings: A review. *LWT Food Sci. Technol.* **2018**, *89*, 198–209. [CrossRef]
6. Cazón, P.; Velazquez, G.; Ramírez, J.A.; Vázquez, M. Polysaccharide-based films and coatings for food packaging: A review. *Food Hydrocoll.* **2017**, *68*, 136–148. [CrossRef]
7. Mujtaba, M.; Morsi, R.E.; Kerch, G.; Elsabee, M.Z.; Kaya, M.; Labidi, J.; Khawar, K.M. Current advancements in chitosan-based film production for food technology; A review. *Int. J. Biol. Macromol.* **2019**, *121*, 889–904. [CrossRef]
8. Zhang, C.; Gong, H.; Liu, Y. Effects of postharvest coating using chitosan combined with natamycin on physicochemical and microbial properties of sweet cherry during cold storage. *Int. J. Biol. Macromol.* **2022**, *214*, 1–9. [CrossRef]
9. Tokatlı, K.; Demirdöven, A. Effects of chitosan edible film coatings on the physicochemical and microbiological qualities of sweet cherry (*Prunus avium* L.). *Sci. Hortic.* **2020**, *259*, 108656. [CrossRef]
10. Zhao, J.; Jiang, H.; Huang, Q.; Xu, J.; Duan, M.; Yu, S.; Zhi, Z.; Pang, J.; Wu, C. Carboxymethyl chitosan incorporated with gliadin/phlorotannin nanoparticles enables the formation of new active packaging films. *Int. J. Biol. Macromol.* **2022**, *203*, 40–48. [CrossRef]

11. Bai, R.; Zhang, X.; Yong, H.; Wang, X.; Liu, Y.; Liu, J. Development and characterization of antioxidant active packaging and intelligent Al^{3+}-sensing films based on carboxymethyl chitosan and quercetin. *Int. J. Biol. Macromol.* **2019**, *126*, 1074–1084. [CrossRef] [PubMed]
12. Dayarian, S.; Zamani, A.; Moheb, A.; Masoomi, M. Physico-Mechanical Properties of Films of Chitosan, Carboxymethyl Chitosan, and Their Blends. *J. Polym. Environ.* **2014**, *22*, 409–416. [CrossRef]
13. Fan, S.; Wang, D.; Wen, X.; Li, X.; Fang, F.; Richel, A.; Xiao, N.; Fauconnier, M.-L.; Hou, C.; Zhang, D. Incorporation of cinnamon essential oil-loaded Pickering emulsion for improving antimicrobial properties and control release of chitosan/gelatin films. *Food Hydrocoll.* **2023**, *138*, 108438. [CrossRef]
14. Yadav, S.; Mehrotra, G.; Bhartiya, P.; Singh, A.; Dutta, P. Preparation, physicochemical and biological evaluation of quercetin based chitosan-gelatin film for food packaging. *Carbohydr. Polym.* **2020**, *227*, 115348. [CrossRef] [PubMed]
15. Nguyen, V.T.; Nguyen, D.H.; Nguyen, H.V. Combination effects of calcium chloride and nano-chitosan on the postharvest quality of strawberry (Fragaria x ananassa Duch.). *Postharvest Biol. Technol.* **2020**, *162*, 111103. [CrossRef]
16. Kou, X.-H.; Guo, W.-L.; Guo, R.-Z.; Li, X.-Y.; Xue, Z.-H. Effects of Chitosan, Calcium Chloride, and Pullulan Coating Treatments on Antioxidant Activity in Pear cv. "Huang guan" During Storage. *Food Bioprocess Technol.* **2014**, *7*, 671–681. [CrossRef]
17. Liu, K.; Yuan, C.; Chen, Y.; Li, H.; Liu, J. Combined effects of ascorbic acid and chitosan on the quality maintenance and shelf life of plums. *Sci. Hortic.* **2014**, *176*, 45–53. [CrossRef]
18. Aleryani-Raqeeb, A.; Mahmud, T.M.; Omar, S.S.; Za, A.M. Effects of Calcium Infiltration and Chitosan Coating on Storage Life and Quality Characteristics During Storage of Papaya (*Carica papaya* L.). *Int. J. Agric. Res.* **2008**, *3*, 296–306. [CrossRef]
19. Tian, Z.; Guo, Y.; Yang, X.; Guo, K.; Ji, J.; Hao, S. Nano Calcium-Deficient Hydroxyapatite/O-carboxymethyl Chitosan-$CaCl_2$ Microspheres Loaded with Rhein for Bone Defect Repair. *J. Bionic Eng.* **2022**, *19*, 1087–1099. [CrossRef]
20. Mohamood, N.F.A.-Z.T.; Halim, A.H.A.; Zainuddin, N. Carboxymethyl Cellulose Hydrogel from Biomass Waste of Oil Palm Empty Fruit Bunch Using Calcium Chloride as Crosslinking Agent. *Polymers* **2021**, *13*, 4056. [CrossRef]
21. Özdemir, K.S.; Gökmen, V. Effect of Chitosan-Ascorbic Acid Coatings on the Refrigerated Storage Stability of Fresh-Cut Apples. *Coatings* **2019**, *9*, 503. [CrossRef]
22. Zhang, Y.-L.; Cui, Q.-L.; Wang, Y.; Shi, F.; Liu, Y.-P.; Liu, J.-L.; Nie, G.-W. Effect of carboxymethyl chitosan-gelatin-based edible coatings on the quality and antioxidant properties of sweet cherry during postharvest storage. *Sci. Hortic.* **2021**, *289*, 110462. [CrossRef]
23. Zhang, Y.-L.; Cui, Q.-L.; Wang, Y.; Shi, F.; Fan, H.; Zhang, Y.-Q.; Lai, S.-T.; Li, Z.-H.; Li, L.; Sun, Y.-K. Effect of Edible Carboxymethyl Chitosan-Gelatin Based Coating on the Quality and Nutritional Properties of Different Sweet Cherry Cultivars during Postharvest Storage. *Coatings* **2021**, *11*, 396. [CrossRef]
24. Martínez-Camacho, A.; Cortez-Rocha, M.; Ezquerra-Brauer, J.; Graciano-Verdugo, A.; Rodriguez-Félix, F.; Castillo-Ortega, M.; Yépiz-Gómez, M.; Plascencia-Jatomea, M. Chitosan composite films: Thermal, structural, mechanical and antifungal properties. *Carbohydr. Polym.* **2010**, *82*, 305–315. [CrossRef]
25. Haghighi, H.; De Leo, R.; Bedin, E.; Pfeifer, F.; Siesler, H.W.; Pulvirenti, A. Comparative analysis of blend and bilayer films based on chitosan and gelatin enriched with LAE (lauroyl arginate ethyl) with antimicrobial activity for food packaging applications. *Food Packag. Shelf Life* **2019**, *19*, 31–39. [CrossRef]
26. Fan, H.Y.; Duquette, D.; Dumont, M.-J.; Simpson, B.K. Salmon skin gelatin-corn zein composite films produced via crosslinking with glutaraldehyde: Optimization using response surface methodology and characterization. *Int. J. Biol. Macromol.* **2018**, *120*, 263–273. [CrossRef]
27. Ziani, K.; Oses, J.; Coma, V.; Maté, J.I. Effect of the presence of glycerol and Tween 20 on the chemical and physical properties of films based on chitosan with different degree of deacetylation. *LWT Food Sci. Technol.* **2008**, *41*, 2159–2165. [CrossRef]
28. Rocha, G.O.; Farias, M.G.; de Carvalho, C.W.P.; Ascheri, J.L.R.; Galdeano, M.C. Filmes compostos biodegradáveis a base de amido de mandioca e proteína de soja. *Polim. Cienc. Tecnol.* **2014**, *24*, 587–595. [CrossRef]
29. Xu, J.; Wei, R.; Jia, Z.; Song, R. Characteristics and bioactive functions of chitosan/gelatin-based film incorporated with ε-polylysine and astaxanthin extracts derived from by-products of shrimp (*Litopenaeus vannamei*). *Food Hydrocoll.* **2020**, *100*, 105436. [CrossRef]
30. Zugravu, M.V.; Smith, R.A.; Reves, B.T.; Jennings, J.A.; Cooper, J.O.; Haggard, W.O.; Bumgardner, J.D. Physical properties and in vitro evaluation of collagen–chitosan–calcium phosphate microparticle-based scaffolds for bone tissue regeneration. *J. Biomater. Appl.* **2012**, *28*, 566–579. [CrossRef]
31. Maringgal, B.; Hashim, N.; Tawakkal, I.S.M.A.; Mohamed, M.T.M. Recent advance in edible coating and its effect on fresh/fresh-cut fruits quality. *Trends Food Sci. Technol.* **2020**, *96*, 253–267. [CrossRef]
32. Davidović, S.; Miljković, M.; Tomić, M.; Gordić, M.; Nešić, A.; Dimitrijević, S. Response surface methodology for optimisation of edible coatings based on dextran from Leuconostoc mesenteroides T3. *Carbohydr. Polym.* **2018**, *184*, 207–213. [CrossRef] [PubMed]