The Influence of Cysteine on the Performances of Gelatin Film

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Abstract. In order to explore the cysteine as a potential plasticizer for gelatin films, the influences of the amount of cysteine, pH and reaction temperature on the functional performances including water absorption, mechanical performances, and chromaticity of gelatin films have been investigated. The results indicated that the amount of cysteine, pH and reaction temperature did not affect the water absorption and chromaticity of the gelatin film. However, the mechanical performances of the gelatin film were influenced greatly by the amount of cysteine and water bath temperature. With the concentration of cysteine ranging from 0 to 0.02%, the tensile strength (TS) of the gelatin film had no significant change but the elongation at break (EB) increased gradually. When the concentration of cysteine ranged from 0.01% to 0.02%, the largest value of the EB of gelatin film was obtained. When the concentration of cysteine was greater than or equal to 0.04%, the TS and EB of the gelatin film decreased significantly. When the reaction temperature was 60 ~ 90°C, the largest values of the EB and TS of the gelatin film were obtained. The addition of cysteine as a plasticizer can improve the EB of gelatin film, but has no influence on other performances. Therefore, there is a promising application value for cysteine in food preservation packaging film, due to its advantages of biodegradability and wide source.

Keywords: Gelatin film, cysteine, performance, chromaticity, mechanical performances, water absorption.

1. Introduction
The raw material of the traditional plastic packaging is from oil, which is non-renewable and non-degradable. The plastic packaging has caused the serious white pollution to the environment. Therefore, it is urgent to develop the non-toxic, biodegradable and renewable packaging materials to replace the traditional plastic packaging materials. In recent years, the preparation of the edible films from natural polymers has become a hot topic all over the world. According to the substrates used, the edible membranes can be divided into polysaccharide films, protein films, lipid films and composite films. Protein films exhibit certain mechanical performances and good resistance, which can prevent fat oxidation and the volatilization of the volatile components [1]. Thus, protein is considered the most promising material of the edible film.

Proteins are heteropolymers with multiple functional groups. The performances of the materials made by protein can be adjusted by enzyme and functional modifications in order to get the required performances and meet the specific needs. Accordingly, protein as a matrix can provide the valuable performances for food packaging [2]. Under the interactions of bonds and groups, the protein forms a three-dimensional network structure, and then forms a film with certain mechanical strength and barrier...
performances. Collagen is a protein whose partial hydrolysis results in the production of gelatin [3]. The formation of gelatin will destroy the original three-dimensional spiral structure of collagen. As a result, gelatin performs completely different performances from collagen, including good adhesion, surface activity, sol-gel reversible exchangeability, and film formation ability [4]. Besides, gelatin is a good dispersant and film-forming agent. During the process of film formation, the gelatin will reproduce the three-dimensional spiral structure of collagen [5, 6]. In recent years, the gelatin based films have been studied widely because of its low cost, non-toxicity, good film-forming performance and biocompatibility.

Amino acids are the basic components of protein but whether the amino acid affects the performances of the gelatin film has not been studied. Therefore, it is important to study the influence of cysteine on the performances of gelatin films. In this work, we used gelatin as the raw material and cysteine as the additive to study the influence of amino acids on the performances of the gelatin film to find the new plasticizer.

2. Experimental

2.1. Chemicals and Instruments

Gelatin is of food grade and was bought from Henan Boyang Biotechnology Co., Ltd. (China). Hydrochloric acid was provided by Xinyang Chemical Reagent Co., Ltd. (China). L- Cysteine (biological grade) and sodium hydroxide were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The commercially available polyethylene preservation bag was purchased from Kaishun Commodity Co., Ltd. (Zhongshan, China).

Analytical Balance (FA 1004) was provided by Shanghai Yueping Scientific Instrument Co., Ltd. (China). Electrothermal blast dryer (DHG-9240A) was bought from Shanghai Jinghong Experimental Equipment Co., Ltd. (China). UV-Vis spectrophotometer (754PC) was purchased from Shanghai Spectrometer Co., Ltd. (China). Electronic tensile machine (ZQ-990) was provided by Dongguan Zhidai Precision Instrument Co., Ltd. (China). Intelligent biochemical incubator (SPX) was bought from Ningbo Jiangnan Instrument Factory. Color difference meter (WSF) was bought from Shanghai Instrument Electrophysical Optical Instrument Co., Ltd. (China).

2.2. Preparation of the Gelatin Films with Different Concentrations of Cysteine

Gelatin (2 g) was dissolved in the deionized water with a volume of 50 mL. At pH 7, different concentrations of cysteine were added in the gelatin solution including 0.005%, 0.01%, 0.02%, 0.04%, 0.08%, 0.16%, 0.25% and 0.50% (according to the percentage of gelatin mass). Then the solution was heated in water-bath at 50 ℃ and stirred until the gelatin was completely dissolved. 4.5 mL of the solution was pipetted to the polypropylene plate (3 cm × 10 cm). After that, the polypropylene plate was dried at 30 ℃ for 12 h. At last, the gelatin film was removed from the polypropylene plate and preserved in a self-sealing bag before testing [7].

2.3. Preparation of the Gelatin Films under Different pH

The pH of the distilled water was adjusted to a certain value by HCl (0.01 M) or NaOH (0.01 M). Then gelatin (2 g) was added into the distilled water (50 mL) with a certain pH and heated in a constant temperature water bath at 50 ℃. The solution was stirred until the gelatin was completely dissolved. After that, cysteine (0.02%) was added in the solution and stirred until the protein was completely dissolved. 4.5 mL of the resulting solution was pipetted to a polypropylene plate (3 cm × 10 cm) and put into an oven at 30 ℃. After 12 h, the polypropylene plate was took out. The gelatin film was uncovered and stored into a self-sealing bag before testing.

2.4. Preparation of the Gelatin Films under Different Water Bath Temperature

The experimental steps were almost the same as the previous section. After adding cysteine (0.02%) in the solution, different temperatures including were 50 ℃, 60 ℃, 70 ℃, 80 ℃, 90 ℃ and 100 ℃ were
applied for the water bath. The influence of the reaction temperature on the performances of the gelatin film was investigated.

2.5. *The Chromaticity Analysis of the Gelatin Films*

The color of the film (100 mm×30 mm) was measured by a color meter. The colorimeter could record the values of the three parameters (i.e., L (black-white), a (green-red), and b (blue-yellow)). At first, the background whiteboard was used to calibrate the colorimeter. Then each film was spread on the whiteboard. Three random points on the film were chosen to be measured and the average value was calculated.

2.6. *The Assay of the Mechanical Performance of the Gelatin Films*

The film was cut into the rectangular strip (100 mm ×9 mm). The tensile strength (TS) and elongation at break (EB) of the gelatin film were measured by a tensile force machine. The parameters were set as follows: the standard distance of the film 35 mm, the tensile force 100 N and the tensile speed 50 mm/min [8].

2.7. *Determination of Water Absorption of the Gelatin Film*

The gelatin film was cut into a long strip (30 mm ×25 mm). The weight of the strip was measured accurately with an analytical balance and recorded as \( m_0 \). Then the strip was placed in distilled water at 5 ℃ for 24 h for complete water absorption. The surface moisture of the gelatin film was absorbed with the filter paper. Finally, the strip was weighed again. The weight was recorded as \( m_1 \) when the constant weight was reached [9]. The water absorption \( Q \) was calculated using equation (1).

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Q = \frac{(m_1 - m_0)}{m_0} \times 100\%.
\]

\[ (1) \]

2.8. *Determination of the Thickness of the Gelatin Film*

The thickness of the gelatin film was measured by a digital micrometer at the four corners and the center of the square film, and then the average value was taken as the thickness of the film. The minimum precision of the micrometer was 0.001 mm.

2.9. *The Analysis of the Data*

The IBM SPSS Statistics 23 software was applied to carry out the statistical analysis of the data. The significant difference between the data were analyzed by the Duncan multiple comparison. The data were represented by mean ± standard deviation.

3. *Results and Discussion*

3.1. *The Influence of Cysteine Concentration on the Performances of Gelatin Film*

Chromaticity is recognized as an important index of consumer satisfaction with food packaging. It is noted from figure 1 that the cysteine addition had no significant influence on the chromaticity of gelatin film. With the increase of cysteine concentration \( (C_{\text{cysteine}}) \), no significant change was found in the chromaticity parameters of the gelatin film.
The packaging films are usually required to perform high mechanical performances to bear the pressure during the transportation, usage, and food treatment. The films with poor mechanical performances can produce cracks or holes, thus reducing the barrier performances. It is noted from figure 2 that the TS and EB of the control group were 61.36 MPa and 4.05%, respectively. With the increase of cysteine concentration, the EB of gelatin film first increased and then decreased. When the cysteine concentrations were 0.01% and 0.02%, the values of the EB were largest (9.04% and 11.29%, respectively) and there was no significant difference. When the concentration of cysteine was less than or equal to 0.02%, no significant difference was found in the TS of the gelatin film with a comparison to the control film. However, when the concentration of cysteine was 0.04% ~ 0.5%, the TS of gelatin film decreased significantly. The reason was that the interaction force between cysteine and gelatin molecule broke the original interaction force between gelatin molecules and reduced the van der Waals force [10]. No significant change was found in the water absorption and thickness of gelatin film at various concentrations of cysteine. All these results demonstrated that the mechanical strength of gelatin film was best when the concentration of cysteine was 0.01~0.02%.

Figure 1. The chromaticity of the gelatin films with the addition of different concentrations of cysteine.

Figure 2. The mechanical performances and water absorption of the gelatin films prepared with different concentrations of cysteine.

3.2. The Influence of pH on the Performances of Gelatin Film
The influence of pH on the chromaticity of the gelatin film was shown in figure 3. The results indicated that pH had no significant influence on the chromaticity parameters of the gelatin film. Furthermore, the influences of pH on the TS and EB of gelatin film were studied as well and the results were presented in figure 4. As the pH increased, the EB, TS and water absorption of gelatin film did not change.
significantly, indicating that pH had no significant influence on the mechanical performances and water absorption of the gelatin film.

**Figure 3.** The chromaticity analysis of the gelatin films prepared under different pH.

**Figure 4.** The mechanical performances and water absorption of the gelatin films prepared under different pH.

### 3.3. The Influence of Reaction Temperature on the Performances of Gelatin Film

The influence of the reaction temperature on the performances of the gelatin film was studied. It is noted from figure 5 that the temperature of the water bath had no significant influence on the chromaticity of the gelatin film. With the increase of the reaction temperature, there was no significant change in the chromaticity of the gelatin film. As shown in figure 6, with the increase of reaction temperature, no significant change was found in the EB of the gelatin film, while the TS of the gelatin film first increased and then decreased. The TS of gelatin film was lowest at 50 ℃. When the temperature ranged from 60 ℃ to 90 ℃, the higher values of the TS of gelatin film were obtained. No significant change was found in the water absorption of the gelatin film at different reaction temperatures.

**Figure 5.** The chromaticity of the gelatin films prepared under different reaction temperature.

**Figure 6.** The mechanical performances and water absorption of the gelatin films prepared under different reaction temperature.

### 3.4. The Comparison with Plastic Packaging

The thickness of the cysteine/gelatin film was revealed to be about 0.06 mm. Such thickness of the film (0.06 mm) was chosen because it was suitable to food packaging [11]. The parameters of the commercially available plastic bag were also determined and were compared with the properties of the cysteine/gelatin film. It is noted from table 1 that there was no difference in color between the plastic bag and the cysteine/gelatin film. The EB of the plastic bag was higher than cysteine/gelatin film, while the TS of the plastic bag was lower than the cysteine/gelatin film. The water absorption of the plastic bag was lower than cysteine/gelatin film, suggesting that gelatin film was hydrophilic while the plastic bag was hydrophobic.
4. Conclusion

The influence of the plasticizers on the gelatin film depends on their physical and chemical performances (such as size, chemical composition, molecular weight, shape and functional groups) and their compatibility with the polymer (such as solubility, hydrogen bonding and polarity). Chromaticity and mechanical performances of the gelatin film are the two important factors of the food packaging evaluation for consumers. The cysteine concentration, pH and reaction temperature had no significant influence on the chromaticity and water absorption of the gelatin film. However, the mechanical performances of the gelatin film were influenced by the concentration of cysteine and water bath temperature. The optimized conditions were the concentration of cysteine (0.01% ~ 0.02%) and the reaction temperature (60 ~ 90°C), under which the best mechanical performances of the gelatin film were obtained. In conclusion, cysteine can be used as a natural plasticizer for gelatin film and has the potential application value in food preservation packaging film. This study provided a new perspective for the application of plasticizer in the gelatin film.

References

[1] Khiair Z, Rico D, Martin-Diana A B, Barry-Ryan C 2013 Comparison between gelatins extracted from mackerel and blue whiting bones after different pretreatments Food Chem. 139 347-354.
[2] Etxabide A, Uranga J, Guerrero P, De la Caba K 2017 Development of active gelatin films by means of valorisation of food processing waste: A review Food Hydrocolloid 68 192-198.
[3] Ottani V, Raspanti M, Ruggeri A 2001 Collagen structure and functional implications Micron 32 251-260
[4] Nijenhuis K On the nature of crosslinks in thermo reversible gels, Polymer Bull. 58 (2007) 27-42
[5] Ahmad M, Hani N M, Nirmal N P, Fazial F F, Mohtar N F, Romli S R 2015 Optical and thermo-mechanical properties of composite films based on fish gelatin/rice flour fabricated by casting technique Prog. Org. Coat. 84 115-127
[6] Bakry N F, Isa M I N, Sarbon N M 2017 Effect of sorbitol at different concentrations on the functional properties of gelatin/carboxymethyl cellulose (CMC)/ chitosan composite films Int. Food Res. J. 24 1753-1762
[7] Xiao J, Zhang M, Wang W, Teng A, Liu A, Ye R, Liu Y, Wang K, Ding J, Wu X 2019 An attempt of using β-sitosterol-corn oil oleogels to improve water barrier properties of gelatin film J. Food Sci. 84 1447-1455.
[8] Zhang X, Ma L, Yu Y, Zhou H, Guo T, Dai H, Zhang Y 2019 Physico-mechanical and antioxidant properties of gelatin film from rabbit skin incorporated with rosemary acid Food Packaging Shelf 19 121-130
[9] Yu H, Peng C, Li F C, Yu P 2019 Effect of chloride salt type on the physicochemical, mechanical and morphological properties of fish gelatin film Mater. Res. Express 6 126414.
[10] Vieira M G A, da Silva M A, dos Santos L O, Beppu M M 2011 Natural-based plasticizers and biopolymer films: A review Eur. Polym. J. 47 254-263.
[11] Xiao J, Zhang M, Wang W, Teng A, Liu A, Ye R, Liu Y, Wang K, Ding J, Wu X 2019 An attempt of using β-sitosterol-corn oil oleogels to improve water barrier properties of gelatin film J. Food Sci. 84 1447-1455.