Influences of four kinds of calcium salts on the functional performances of gelatin composite films

Hong Yu¹, Wenxing Lin¹ and Peng Yu²,a

¹College of Food and Biology Science and Technology, Wuhan Institute of Design and Sciences, Wuhan, Hubei, 430205, People’s Republic of China
²School of Materials Science and Engineering, Xiangtan University, Xiangtan, Hunan, 411105, People’s Republic of China

E-mail: yupeng@xtu.edu.cn

Abstract. Fish gelatin films were prepared with gelatin as the main raw material and four kinds of salts, including CaCl₂, CaSO₄, hydroxyapatite and Ca₃(PO₄)₂, as the auxiliary reagents. The types of the anions and the concentrations of the calcium salts could affect the light transmission, swelling ratio, tensile strength, and elongation at break of gelatin films. The relationship between them was studied in this paper. The experimental data were analyzed and it is noted that the swelling ratio of the gelatin film was improved after adding the calcium salts. The swelling ratio of the hydroxyapatite/gelatin composite film was significantly bigger than that of other composite films. There is no significant effect on the light transmittance of the gelatin film after adding the calcium salts. The improved mechanical performances were found in the composite gelatin films by adding calcium chloride, calcium sulfate, and calcium phosphate. In comparison, calcium phosphate performed the greatest influence on improving the mechanical performances of the composite films.

1. Introduction

Gelatin is a natural protein which can be obtained by the hydrolysis of the collagen in animal skin, bone and other tissues[1]. It is thermo-reversible and can be dissolved in hot water. In addition, some organic solvents including glycerol and acetic acid can dissolve the gelatin while ethanol and ether can not dissolve it. The heated gelatin could form a stable and uniform dispersion with water. If the temperature is lower than the gel point of gelatin, the uniform dispersion is destroyed and a reversible gel is formed[2].

The raw materials used to prepare gelatin could affect the gel points of the resulting gelatin. The range of the gel point could be 30 °C ~ 35 °C for the gelatin prepared from the tissues of warm-blooded animals and 5 °C - 10 °C from the skin of cold-water ocean fish, respectively[3]. Therefore, gelatin is widely used in a lot of fields such as photography, packaging, medicine, and metallurgy due to its excellent performances.

Gelatin is biodegradable and easy to form film, which is expected to replace plastics as a new generation of environmentally friendly packaging[4]. However, some factors including hydrophilicity and poor mechanical performances limit the development of gelatin in food packaging. In order to solve these problems, gelatin is often blended with other polymers or plasticizers, such as chitosan[5], starch[6], carboxymethyl cellulose[7] and pectin[8]. Although these substances can improve some
performances of gelatin film, they still can not meet the needs of food packaging. Hence, there is an urgent need to search the new plasticizer to modify the performances of gelatin films.

Salts are widespread in nature and could be added in gelatin films as plasticizers. The reason is that salts could affect the secondary structure of the gelatin and thus changed the performances of gelatin, such as the melting temperature, gel strength, and storage modulus[9]. A few literature were reported about how the salts influence the performances of gelatin films. Liu group investigated the influence of NaOAc on the functional performances of gelatin films[10]. The performances of the gelatin film including moisture content, swelling ratio, glass transition temperature and mechanical performance were changed after plasticizing with NaOAc. Our group studied the influence of different kinds of chloride salts on the performances of fish gelatin film. In comparison, CaCl₂ exhibited the best compatibility with gelatin and thus improved the mechanical performance (i.e., the elongation at break) of the gelatin composite film[11]. The reason is that CaCl₂ is strongly hydrophilic. Unquestionably, these work has great breakthroughs in understanding the interaction between the inorganic salts and the composite films, but no work has been reported on the influences of different calcium salts on the functional performances of gelatin films.

In this work, four types of calcium salts (calcium chloride, calcium sulfate, hydroxyapatite and calcium phosphate) were used to prepare the composite gelatin films. The interaction between salts and gelatin films were investigated. The instruments such as ultraviolet-visible (UV–vis) spectrophotometer and electric tensile tester were employed to detect the light transmission and mechanical performances of the gelatin composite films. All these results should be of value in further understanding the internal mechanisms of the interaction between the salts and the gelatin molecules.

2. Experimental

2.1. Reagents and equipment
Fish gelatin (FG) powder prepared from grass carp scale (~220 bloom) was purchased from Henan Boyang Biotechnology Co., Ltd (China). The mechanical performances including tensile strength (TS) and elongation at break (EB) were determined by electric tensile tester (ZQ-990, Zhiqu Precision Instrument Co., Ltd, China), which was equipped with tensile load cell of 500 N.

2.2. Fabrication of gelatin composite films plasticized with different calcium salts
At first, the solution for film-forming (FFS) was prepared by dissolving gelatin powder in ultrapure water. The concentration of gelatin in FFS was 4% (w/v). Different amounts of salts were then added into the FFS with a volume of 50 mL. The final concentrations of salts in FFS were 0.2, 0.4, 0.6, 0.8, 1 mM, respectively. After that, 0.5 mL of HCl solution (1 M) was slowly dropped into the FFS for improving the dissolution of the salts under the continuous agitation at 50 °C for 10 min. 4.5 mL of the resulting FFS was evenly cast on a 30 mm × 100 mm polypropylene plate and allowed to dry in an oven at 35 °C for 15 h[12]. The peeled films were placed in a dry dish containing discolored silica gel for 48 h before testing. The control film without the incorporation of calcium salt was prepared and labeled as FG. FG films plasticized with calcium chloride, calcium sulfate, hydroxyapatite and calcium phosphate were marked as CC/FG, CS/FG, HP/FG, CP/FG, respectively.

2.3. The test of the light barrier performances
The light barrier performances of the composite films were determined at the wavelength range of 280 ~ 600 nm. The films were cut into rectangular pieces (8 × 100 mm) and placed in a UV–vis spectrophotometer test cell, using a 754PC UV-Vis spectrophotometer (Spectrum, Shanghai, China).

2.4. The test of TS and EB
The electric tensile tester was applied to test the mechanical performances such as TS and EB. The composite films were cut into rectangles with the size of 8 mm × 100 mm by a scissors and then fixed
on the equipment. The initial gauge and the stretching speed were 35 mm and 50 mm/min, respectively. The maximum load and spacing were recorded during the stretching.

2.5. The test of swelling ratio

The prepared film was cut into small strips with a width of 2 cm and a length of 3 cm. The weight of film strip was measured and recorded as \( m_1 \). After that, the film strip was immersed in the ultrapure water at 10 °C for 24 h. Then, the film strip was blotted with filter paper to remove excessive water. The weight of the film strip was measured again and recorded as \( m_2 \). The swelling ratio was calculated using the equation listed below:

\[
\text{Swelling} = \frac{m_2 - m_1}{m_1}
\]

2.6. Statistic analysis

Statistical analysis was performed using a SPSS computer program (SPSS 13.0, SPSS Inc., Chicago, IL, USA). Duncan’s multiple range tests were used to compare the difference among the mean values at the level of 0.05. The data was expressed as mean ± SD (standard deviation). In the tables shown in this paper, within each row, means with different lowercase letters are significantly different (p < 0.05) among different groups. Within each column, means with different capital letters are significantly different among different concentration points (p < 0.05).

3. Results and discussion

3.1. Swelling ratio of the composite films

| Film type | 0 mM | 0.2 mM | 0.4 mM | 0.6 mM | 0.8 mM | 1 mM |
|-----------|------|--------|--------|--------|--------|------|
| CC/FG     | 3.51±0.08a | 4.43±0.17ac | 4.73±0.41ab | 4.71±0.75ab | 4.75±0.31ab | 4.85±0.12ab |
| HP/FG     | 3.51±0.08c | 7.18±0.25AA | 6.52±0.47ab | 5.87±0.06cA | 5.94±0.27Aa | 5.39±0.26ab |
| CS/FG     | 3.51±0.08c | 4.19±0.09bc | 4.36±0.14ab | 4.66±0.10ab | 5.12±0.11ab | 5.04±0.18AB |
| CP/FG     | 3.51±0.08b | 4.93±0.15ab | 5.03±0.31ab | 5.04±0.20ab | 5.02±0.18ab | 5.10±0.24ab |

The swelling ratio of gelatin films incorporated with various levels of calcium salts is presented in Table 1. With a contrast to the control film, the swelling values of films with salt were significantly bigger than that of the control film due to the interactions between salt and gelatin in the films. Since the salts interspersed in the network of gelatin, the spacing of the gelatin chains became bigger and the interactions between gelatin molecules became weaker. This point was also validated by the work done by Liu group, who studied the interaction between NaOAC and gelatin [10]. The higher capacity to absorb water was found in NaOAC/gelatin composite films compared to the control film.

The swelling values of HP/FG at all levels were significantly bigger than that of other salts. These results indicated that the incorporation of hydroxyapatite reduced the intermolecular interactions of gelatin seriously. No significant difference was found among swelling values of CC/FG, CS/FG and PC/FG when the level of salt was 0.4 mM or higher (p ≥ 0.05).

3.2. Light barrier performance of the composite films

| Film type | 0 mM | 0.2 mM | 0.4 mM | 0.6 mM | 0.8 mM | 1 mM |
|-----------|------|--------|--------|--------|--------|------|
| CC/FG     | 25.83±1.38a | 22.89±1.17aA | 24.12±1.41aA | 21.91±1.75aA | 25.94±2.31aA | 25.53±1.12Aa |
| HP/FG     | 25.83±1.38a | 24.53±2.25aA | 25.53±1.47aA | 20.89±3.06aA | 21.36±2.27aA | 20.97±3.46aA |
| CS/FG     | 25.83±1.38a | 21.83±2.09aA | 23.09±2.18aA | 22.95±1.19aA | 22.42±2.53aA | 23.42±1.68aA |
| CP/FG     | 25.83±1.38a | 24.30±2.39aA | 22.36±3.11aA | 20.54±2.25aA | 22.95±2.18aA | 24.12±1.59aA |
The similar conclusion was also obtained in his work, which is consistent with our results[14]. FG film was transparent and had a high transmittance at 600 nm. The incorporation of four different kinds of salts had no significant influence on the transmittance of FG films under UV and visible light (p ≥ 0.05).

### 3.3. TS and EB values of the composite films

The influence of salts on the mechanical performances of the FG films were also investigated. The results were shown in Table 4 and Table 5. The TS and EB values of HP/FG were significantly lower than those of other films. The similar conclusion was also obtained in Table 1. The reason is the presence of hydroxyapatite destroys the force between gelatin molecules. The TS and EB of CP/FG at 0.8 mM level were significantly bigger than those of other films.

### Table 3. Light barrier performances of FG plasticized with calcium salts at 600 nm.

| Film type | Light transmittance (%) |
|-----------|-------------------------|
|           | 0 mM | 0.2 mM | 0.4 mM | 0.6 mM | 0.8 mM | 1 mM   |
| CC/FG     | 86.21±1.38<sup>a</sup> | 89.10±1.31<sup>AA</sup> | 88.59±0.61<sup>aA</sup> | 89.65±0.75<sup>aA</sup> | 90.17±0.31<sup>aA</sup> | 89.65±0.12<sup>aA</sup> |
| HP/FG     | 86.21±1.38<sup>a</sup> | 88.52±0.75<sup>aA</sup> | 89.40±1.17<sup>aA</sup> | 89.54±1.21<sup>aA</sup> | 90.47±0.67<sup>aA</sup> | 90.39±1.15<sup>aA</sup> |
| CS/FG     | 86.21±1.38<sup>a</sup> | 88.90±1.09<sup>AA</sup> | 86.82±1.14<sup>aA</sup> | 89.57±1.35<sup>aA</sup> | 88.77±2.11<sup>aA</sup> | 89.50±0.88<sup>aA</sup> |
| CP/FG     | 86.21±1.38<sup>a</sup> | 87.65±1.06<sup>aA</sup> | 89.53±1.26<sup>aA</sup> | 89.39±1.21<sup>aA</sup> | 89.64±1.15<sup>aA</sup> | 86.68±0.84<sup>aA</sup> |

The effect of different kinds of calcium salts on light transmission of FG composite films was investigated by UV–vis spectrometry analysis. The results of the light transmittance of FG films plasticized with calcium salts are presented in Table 2 and Table 3. It is noted that all the FG composite films had the strong absorption at 280 nm. The reason is that there is a lot of the aromatic amino acid residues in gelatin, which could effectively prevent the UV light transmission[13]. Shankar group obtained the similar result in his work, which is consistent with our results[14]. FG film was transparent and had a high transmittance at 600 nm. The incorporation of different kinds of salts had no significant influence on the transmittance of FG films under UV and visible light (p ≥ 0.05).

### Table 4. TS of FG plasticized with calcium salts.

| Film type | TS (MPa) |
|-----------|----------|
|           | 0 mM | 0.2 mM | 0.4 mM | 0.6 mM | 0.8 mM | 1 mM   |
| CC/FG     | 46.67±5.16<sup>b</sup> | 45.32±6.89<sup>BB</sup> | 73.21±7.6<sup>bb</sup> | 75.52±13.7<sup>aa</sup> | 49.16±6.36<sup>BC</sup> | 58.92±5.64<sup>BA</sup> |
| HP/FG     | 46.67±5.16<sup>a</sup> | 45.03±5.58<sup>AB</sup> | 54.98±6.62<sup>AC</sup> | 56.18±6.27<sup>aa</sup> | 58.21±6.02<sup>bA</sup> | 46.72±8.59<sup>AB</sup> |
| CS/FG     | 46.67±5.16<sup>b</sup> | 52.75±4.54<sup>bA</sup> | 27.92±2.08<sup>bA</sup> | 54.35±8.11<sup>bA</sup> | 67.87±6.92<sup>abA</sup> | 55.67±4.91<sup>abA</sup> |
| CP/FG     | 46.67±5.16<sup>b</sup> | 62.90±5.39<sup>AB</sup> | 86.12±8.19<sup>bB</sup> | 55.72±4.12<sup>abA</sup> | 93.86±5.26<sup>baA</sup> | 56.47±2.58<sup>bbA</sup> |

### Table 5. EB of FG plasticized with calcium salts.

| Film type | EB (%) |
|-----------|--------|
|           | 0 mM | 0.2 mM | 0.4 mM | 0.6 mM | 0.8 mM | 1 mM   |
| CC/FG     | 4.56±0.62<sup>b</sup> | 2.77±0.13<sup>BB</sup> | 3.81±0.41<sup>BB</sup> | 6.26±0.93<sup>AA</sup> | 2.62±0.09<sup>BC</sup> | 4.69±0.26<sup>BA</sup> |
| HP/FG     | 4.56±0.62<sup>b</sup> | 1.92±0.24<sup>BC</sup> | 2.36±0.35<sup>BC</sup> | 3.38±0.56<sup>AB</sup> | 5.70±0.42<sup>BB</sup> | 3.38±0.31<sup>AB</sup> |
| CS/FG     | 4.56±0.62<sup>a</sup> | 4.32±0.54<sup>AB</sup> | 1.92±0.19<sup>AA</sup> | 3.62±0.39<sup>BB</sup> | 3.85±0.34<sup>AB</sup> | 3.33±0.71<sup>bB</sup> |
| CP/FG     | 4.56±0.62<sup>b</sup> | 4.59±0.21<sup>bA</sup> | 4.84±0.42<sup>bA</sup> | 4.11±0.24<sup>bB</sup> | 6.61±0.19<sup>BA</sup> | 3.11±0.47<sup>bB</sup> |

### 4. Conclusion

In conclusion, the effects of four different kinds of calcium salts on the light transmission, swelling and mechanical performances of gelatin films were studied. The addition of calcium salts had no influence on the light transmission of gelatin films. The improved swelling of FG films was obtained by adding calcium salts. The CP/FG film had lower swelling and higher TS and EB than FG films incorporated with other salts. This work is a rare example of studying calcium salt-gelatin composite film. More importantly, this study provides ideas for finding the new plasticizers to realize the early application of the gelatin in food packaging.

### Acknowledgments
The authors are sincerely grateful to the anonymous referees. The valuable comments from referees improved the quality of the paper remarkably.

References
[1] Ottani, V., Raspani, M., Ruggeri, A. (2001) Collagen structure and functional implications. Micron, 32(3): 251-260.
[2] Nijenhuis, K. (2007) On the nature of crosslinks in thermoreversible gels. Polym. Bull., 58: 27-42.
[3] Suderman, N., Isa, M.I.N., Sarbon, N.M. (2018) The effect of plasticizers on the functional properties of biodegradable gelatin-based film: A review. Food Biosci., 24: 111-119.
[4] 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(4), 1753-1762.
[5] Abruzzo, A., Bigucci, F., Cerchiara, T., Cruciani, F., Vitali, B., Luppi, B. (2012) Mucoadhesive chitosan/gelatin films for buccal delivery of propranolol hydrochloride. Carbohydr. Polym., 87(1), 581-588.
[6] Soo, P.Y., Sarbon, N.M. (2018) Preparation and characterization of edible chicken skin gelatin film incorporated with rice flour. Food Packaging Shelf, 15, 1-8.
[7] Nazmi, N.N., Isa, M.I.N., Sarbon, N.M. (2017) Preparation and characterization of chicken skin gelatin/CMC composite film as compared to bovine gelatin film. Food Biosci., 19, 149-155.
[8] Farris, S., Schaich, K.M., Liu, L., Cooke, P.H., Piergiovanni, L., Yam, K.L. (2011) Gelatin–pectin composite films from polyion-complex hydrogels. Food Hydrocolloid., 25(1), 61-70.
[9] Sow, L.C., Yang, H. (2015) Effects of salt and sugar addition on the physicochemical properties and nanostructure of fish gelatin. Food Hydrocolloid., 45, 72-82.
[10] Liu, F., Antoniou, J., Li, Y., Ma, J., Zhong, F. (2015) Effect of sodium acetate and drying temperature on physicochemical and thermomechanical properties of gelatin films. Food Hydrocolloid., 45, 140-149.
[11] 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.
[12] 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(6): 1447-1455.
[13] Ma, L., Yang, H., Ma, M., Zhang, X., Zhang, Y. (2018) Mechanical and structural properties of rabbit skin gelatin films. Int. J. Food Prop., 21(1): 1203-1218.
[14] Shankar, S., Teng, X., Li, G., Rhim, J. (2015) Preparation, characterization, and antimicrobial activity of gelatin/ZnO nanocomposite films. Food Hydrocolloid., 45: 264-271.