Effect of Storage Conditions on the Physicochemical Characteristics of Bilayer Edible Films Based on Iron Yam–Pea Starch Blend and Corn Zein

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Abstract: Edible iron yam–pea starch-based bilayer films with a water and oil proof layer of corn zein were prepared by casting method and stored under normal temperature (25 °C; relative humidity (RH): 43%, 54%, 65%), refrigeration (4 °C) and freezing (−17 °C) for 150 days. The mechanical properties, scanning electron microscope (SEM) micrographs, oxygen and water vapor permeability, color, transmittance, haze, water content were systematically evaluated after 0, 30, 60, 90, 120 and 150 days. The transmittance, haze, and water content of bilayer films changed greatly within 150 days, which indirectly indicated the changes of the internal microstructure of the film matrix. The results were further verified by SEM analysis. Water and oxygen resistance gradually become worse. At 25 °C and 54% RH, the barrier performances were relatively strong. Films had relatively good tensile strength at normal temperature and high humidity, and relatively good elongation at break at low temperature and high humidity. SEM observation showed that there was no interlayer separation during storage. The internal network structure disappeared and reappeared again. The changes of internal microstructure also verified the changes of barrier and mechanical properties of bilayer films.

Keywords: bilayer edible film; iron yam–pea starch; corn zein; physicochemical characterization; storage

1. Introduction

With the increasing demand of consumers for green and healthy food, the research on degradable and edible packaging materials has become a hot spot [1–7]. Biodegradable polymers have been widely used in film production [8–13]. The enhancement of people’s environmental protection concept has led to changes in the manufacturing methods of food packaging materials [14–17]. However, both physical and chemical properties of edible films based on biological macromolecules, are easily affected by the changes of the external environmental conditions. These changes may affect its practical application and consumer acceptability [18,19]. Therefore, for a long storage time, the high stability of film performance is usually required. Studies on the behavior of edible films during storage have been reported in the last years. Such as caseinate-based films [20], whey protein isolate-based films [21], cassava starch films [22], jumbo squid myofibrillar protein-based films [23], gellan gum edible films [24], prebiotic edible films [25], fish gelatin edible films [26], triticale flour films [27], glycercol plasticized–soybean protein films [28], whey protein edible films [18,29–31], chitosan films [32], squid mantle muscle films [19], Soybean protein films [28], glutenin films [33].

To the best of our knowledge, however, there is no work related to the storage-induced changes of bilayer edible films based on iron yam–pea starch blend and corn zein. Among various natural polymeric materials, starch is considered as a good edible film material because of its wide source, low price and biodegradability [34]. However, the starch based biodegradable film has the disadvantages of brittleness and low water resistance [14,35,36]. Corn zein films can compensate for these shortcomings of starch films through hydrophobic amino acids [37,38], excellent film forming ability [39], low oxygen
permeability [40]. However, poor mechanical properties and high cost of zein limit its practical application [41]. In short, the inherent properties of starch and protein films restrict the use of starch and protein in pure form for intended purpose [42,43]. The preliminary work of our research team shows that the preparation of starch and protein bilayer film, making full use of the advantages of the two film-forming substrates, can be used as one of the choices of food inner packaging materials (such as instant food seasoning bags) [44]. The iron yam (*Dioscorea opposita Thunb*) is the only Chinese yam variety protected by the national origin protection list [45]. The content of iron yam starch is up to 28%, which can be used as the base material for the development of edible film [46]. Corn zein contains more hydrophobic amino acids and is a good material for preparing waterproof film [47].

In this study, a bilayer film with an outer iron yam–pea starch layer and an inner oil and water-resistant corn zein layer was developed and stored under normal temperature (25 °C; RH: 43%, 54%, 65%), refrigeration (4 °C) and freezing (−17 °C) for 150 days. The physicochemical properties and microstructures were analyzed every 30 days. The effects of temperature, humidity and time on the mechanical properties, color, transmittance, water content, water resistance, and oxygen resistance were studied. On this basis, the cross-sectional microstructures of the bilayer films were analyzed by SEM, and the effects of the microstructures of the bilayer films on their chemical properties were studied.

2. Materials and Methods

2.1. Materials

Iron yam (*Dioscorea opposita Thunb*) starch was purchased from Hangzhou Mei Yan Trade (Hangzhou, China). Pea starch was purchased from Shanghai Luyuan Starch (Shanghai, China) and corn zein (CZ, 91.1% protein content) was purchased from Dulai Biotechnology (Nanjing, China). Absolute ethyl alcohol (≥99.7%), D-Sorbitol (≥98.0%), polyethylene glycol-400 (≥99.0%), citric acid (≥99.5%), and glycerol (≥99.0%) were purchased from Tianjing Chemical Reagent (Luoyang, China). All other chemicals were analytical grade.

2.2. Film Preparation and Storage

The bilayer films were prepared following Song and Wang [46]. Our previous study found that IYP10/CZ3 (10 mL iron yam–pea starch forming solution with 3 mL corn zein solution) bilayers have good mechanical and barrier properties [46]. Therefore, IYP10/CZ3 bilayers were selected as the research object and stored for 150 days at five different conditions: normal temperature (25 °C) and 43% RH, 54% RH, 65% RH, refrigeration (4 °C), freezing (−17 °C), respectively. For five storage conditions the film characterizations were performed on days: 0, 30, 60, 90, 120 and 150.

2.3. Color

The measurement of color was carried out by a Lab-Scan II colorimeter (Hunter Lab, Inc., Reston, VA, USA) according to the method of Zuo et al. [44]. The color was presented in terms of $L^*$, $a^*$ and $b^*$ values. The whiteness index (WI) of film was calculated using the following equation.

$$WI = 100 - \left[\left(100 - L^*\right)^2 + a^*2 + b^*2\right]^{0.5}$$  

(1)

2.4. Transmittance and Haze

The measurements of transmittance and haze were carried out by a transmittance haze meter (WGT-S, Shanghai Yanhe Scientific Instrument Co., Ltd., Shanghai, China) according to the method of Zuo et al. [44]. These were made six times for each test.

2.5. Water Content

The measurements of water content (WC) were carried out according to the method of Zuo et al. [44] and calculated as the following equation:

$$WC = \frac{(M_0 - M)}{M_0}$$  

(2)
where \( M_0 \)—the initial mass (g), \( M \)—the bone-dry mass (g). WC was expressed as g H\(_2\)O/g dry solids. Measurements were carried out three times.

### 2.6. Water Vapor Permeability

The measurements of water vapor permeability (WVP) were carried out according to the modified method of Song et al. [48]. The measurements were carried out five times.

### 2.7. Oxygen Permeability

The peroxide value (POV) of soybean oil was expressed by oxygen permeability (OP) [47]. We put 20 g of soybean oil into a 250 mL conical flask, sealed the bilayer film (\( D = 9 \) cm) at the mouth of the conical flask, and stored it at 60 \( ^\circ \)C for 10 days for measurement. The measurement was to be made in triplicate.

### 2.8. Mechanical Properties

The measurements of tensile properties were carried out by a TA-XT2i Texture Analyzer (Stable Microsystems, Godalming, UK) [48]. Each sample was measured nine times.

### 2.9. Scanning Electron Microscope (SEM)

A field emission scanning electron microscope (Nova NanoSEM400, FEI company, Hillsboro, OR, USA) was used to analyze the microstructure of the film, and the working voltage was 3 kV. We exposed the samples to P\(_2\)O\(_5\) at 25 \( ^\circ \)C and 0% RH for 2 weeks and soaked the fragments (50 \( \times \) 60 mm\(^2\)) in liquid nitrogen, then installed them on the metal grid, and gold plated them under a vacuum.

### 2.10. Statistical Analysis

The ANOVA program of SPSS software (version 16.0 (IBM, Chicago, IL, USA) was used for analysis of variance. LSD test was used to test the difference of mean value, and \( p < 0.05 \) was considered statistically significant.

### 3. Results and Discussion

#### 3.1. Color

The color stability of the edible film is essential for practical application. It is of great significance to study the color change of the bilayer film during storage to determine the shelf life of convenient food packaging materials [49,50]. As shown in Table 1, the changes of \( L^* \), \( a^* \), \( b^* \) and WI values of bilayer films during storage were analyzed. It can be seen that with the prolongation of storage time, the \( L^* \) value of bilayer film did not change obviously on the whole, and a small decrease occurred after 120 days. Moreover, the change of temperature and humidity did not cause a big change of \( L^* \) value of bilayer film during the same storage period, which indicated that the brightness of bilayer film was well-preserved [4]. The \( a^* \) value decreased slightly with time but increased significantly with the increase of temperature and relative humidity. At 25 \( ^\circ \)C and 65% of relative humidity, the \( a^* \) value reached the maximum values of \(-1.50 \) (30 d), \(-1.66 \) (60 d), \(-1.81 \) (90 d), \(-1.69 \) (120 d) and \(-2.05 \) (150 d), respectively. Indicating that the green of the bilayer film was gradually weakening. In addition, with the increase of temperature and relative humidity, the \( b^* \) value of bilayer films decreased significantly, and the yellowness of bilayer films decreased gradually. The increase of \( a^* \) value and decrease of \( b^* \) value of bilayer film might be due to the loosening of the internal structure of bilayer film with the increase of storage temperature and relative humidity [18,29]. Some corn zein entered the iron yam–pea starch blend film. The variation of WI value of bilayer film was consistent with that of \( L^* \), \( a^* \) and \( b^* \) value. Therefore, the following conclusions could be drawn from the experiments: different storage environments affected the appearance color of bilayer film to a certain extent [19]. However, under this experimental condition, with the storage time prolonged, the same storage conditions did not lead to significant changes in the color of the bilayer film for 150 days’ worth of storage periods.
Table 1. Change of color of bilayer film during storage.

| Storage Conditions | L*  | Storage Time | a*  | Storage Time | b*  | Storage Time |
|--------------------|-----|--------------|-----|--------------|-----|--------------|
|                    | 30 d | 60 d         | 90 d | 120 d        | 150 d | 180 d |
| −18 °C             | 94.47 ± 0.32 b,a | 94.31 ± 0.24 a,b | 94.28 ± 0.31 a,b | 94.28 ± 0.54 a,b | 93.77 ± 1.0 a,b |
| −5 °C              | 94.62 ± 0.14 b,a | 94.53 ± 0.40 a,b | 94.64 ± 0.22 b,a | 94.46 ± 0.56 b,a | 94.44 ± 0.54 a,b |
| 25 °C, RH 43%      | 94.67 ± 0.33 a,a | 94.47 ± 0.07 a,a | 94.22 ± 0.31 a,b | 94.35 ± 0.59 a,b | 94.17 ± 0.31 a,b |
| 25 °C, RH 54%      | 94.75 ± 0.30 a,a | 94.33 ± 0.22 a,a | 94.33 ± 0.28 a,b | 94.37 ± 0.12 a,b | 93.75 ± 0.41 a,b |
| 25 °C, RH 65%      | 94.44 ± 0.16 b,a | 94.34 ± 0.38 a,b | 94.29 ± 0.24 b,a | 94.06 ± 0.24 a,b | 94.08 ± 0.29 a,b |
| −18 °C             | −2.90 ± 0.12 a,b | −3.11 ± 0.26 a,b | −3.15 ± 0.04 a,b | −3.17 ± 0.15 a,b | −3.19 ± 0.19 a,b |
| −5 °C              | −2.70 ± 0.10 a,b | −2.76 ± 0.03 a,b | −2.88 ± 0.20 b,a | −3.02 ± 0.16 a,b | −3.16 ± 0.13 a,b |
| 25 °C, RH 43%      | −2.63 ± 0.08 b,b | −2.66 ± 0.10 a,b | −2.70 ± 0.17 b,a | −2.72 ± 0.14 a,b | −2.72 ± 0.16 b,a |
| 25 °C, RH 54%      | −2.14 ± 0.07 b,c | −2.31 ± 0.13 a,c | −2.30 ± 0.16 c,b | −2.42 ± 0.13 a,c | −2.63 ± 0.33 a,b |
| 25 °C, RH 65%      | −1.50 ± 0.18 a,b | −1.66 ± 0.15 b,c,d | −1.69 ± 0.09 b,c,d | −1.82 ± 0.15 c,d | −2.05 ± 0.14 a,c |

Note: Data were mean ± standard deviation. The different lowercase letters indicate significant differences (p < 0.05) in the color with varying storage temperature and RH; the different capital letters indicate significant differences (p < 0.05) in the color on different storage time.

3.2. Transmittance and Haze

The change of the transmittance of the bilayer film with time under different storage conditions is shown in Figure 1. It can be seen from Figure 1A that the transmittance did not change significantly during the storage period, except that at 60th and 150th day, the transmittance increased, indicating that the water content increased with the extension of storage time, reaching 13.19% (−17 °C), 8.84% (4 °C), 7.41% (25 °C, RH 43%), 9.29% (25 °C, RH 54%) and 14.39% (25 °C, RH 65%) after 150 days, which increased the roughness of the film surface, and might also be one of the reasons for the increase of haze.

3.3. Water Content

Water is a good plasticizer for packaging materials based on biological macromolecules, especially for edible polysaccharide films. The change of water content in the film indirectly affects its barrier and mechanical properties [30]. Under different storage conditions, the change of water content of the bilayer film with time was shown in Figure 2. The water content decreased significantly from 0 to 30 days (p < 0.05), probably due to the migration of PEG-400 during storage, which led to the decrease of water holding capacity of the bilayer film. Compared with other storage environments, the film water content was relatively low at 40% RH and 4 °C, indicating that the storage environment had a greater impact on the water content of edible biomacromolecule-based film, which was also one of the main factors limiting its practical application. [32]. However, the water content generally increased with the extension of storage time, reaching 13.19% (−17 °C), 8.84% (4 °C), 7.41% (25 °C, RH 43%), 9.29% (25 °C, RH 54%) and 14.39% (25 °C, RH 65%) after 150 days, which...
might be due to the oxidation of protein film and the regeneration of starch film during storage, and the increase of cross-linking between water molecules and crystalline structure in films [40].

![Graph A: Change of transmittance](image)

![Graph B: Haze](image)

**Figure 1.** Change of transmittance (A) and haze (B) of double-layer film during storage. A (−17 °C), b (4 °C), c (25 °C, RH 43%), d (25 °C, RH 54%), e (25 °C, RH 65%).

### 3.4. Barrier Properties

The change of humidity in storage environment has a great influence on the properties of starch-based film matrix with strong hydrophilicity, especially its water resistance. In different storage environments, the variation of WVP with storage time is shown in Figure 3A. At normal temperature (25 °C) and high relative humidity (RH 65%), the water resistance of bilayer films became worse after 150 days of storage because its WVP reached 11.88 × 10⁻¹¹ g/m·s·Pa. This was mainly because water played a certain role in plasticizing the bilayer film [46]. Excessive water made the internal structure of the film became loose, and water molecules were easier to penetrate through the film matrix. Under freezing (−17 °C) and refrigeration (4 °C) conditions, the WVP value reached 13.80 × 10⁻¹¹ g/m·s·Pa and 12.11 × 10⁻¹¹ g/m·s·Pa, respectively, after 150 days of storage, which were higher than that of WVP at normal temperature. This was mainly due to the closer bonding between polymers and the smaller pore size, which hindered the transfer of water molecules [44]. The WVP of bilayer film reached 10.08 × 10⁻¹¹ g/m·s·Pa after 120 days of storage at 25 °C and 54% RH, indicating that the bilayer film has good water resistance.
Figure 2. Water content of double-layer membrane during storage. A (−17 °C), b (4 °C), c (25 °C, RH 43%), d (25 °C, RH 54%), e (25 °C, RH 65%).

Figure 3. Water vapor permeability (WVP, A) and peroxide values (POV, B) of bilayer films during storage. A (−18 °C), b (4 °C), c (25 °C, RH 43%), d (25 °C, RH 54%), e (25 °C, RH 65%).
As shown in Figure 3B, the POV value of the contents of bilayer film packaging increased significantly after aging for 10 days ($p < 0.05$). It showed that the oxygen resistance tends to be worse in different storage environments. POV reached 140.47 mmol/kg and 158.97 mmol/kg after 150 days aging at normal temperature (25 °C) and RH was 43% and 65%, respectively. It can be clearly seen that the bilayer film had good oxygen resistance in low humidity storage environment, but poor oxygen resistance in high humidity conditions. The possible reason is that in the low humidity environment, the humidity difference between the bilayer film and external environment led to the loss of some water in the bilayer film, the internal structure of the film became compact, and the oxidation resistance was improved [33,48]. In the high humidity environment, the swelling phenomenon inside the bilayer film made its internal structure loose and the oxygen permeability increased. Oxygen permeability of film matrix usually increases with the increase of temperature [46]. Under the experimental conditions, the oxygen permeability at the early stage of storage conformed to this change rule, but at the later stage, the change rule was contrary. This might be because the process of oxygen molecules passing through films could be divided into two aspects: diffusion and dissolution. The change of water content resulted in the above phenomena.

3.5. Mechanical Properties

The shelf life of bilayer film as packaging material is directly determined by the change of mechanical properties during storage [18]. Figure 4 shows the changes of TS (Figure 4A) and E (Figure 4B) of bilayer films under different storage conditions. It can be seen that at normal temperature (25 °C) and RH of 43%, 54% and 65% for 30 days, TS increased to 10.22 Mpa, 11.04 Mpa and 11.07 Mpa, respectively, while E decreased significantly ($p < 0.05$). This might be due to the decrease of internal voidage and the compactness of internal structure of bilayer films during the early storage period. With the prolongation of storage time, TS of bilayer films decreased significantly ($p < 0.05$), E increased significantly ($p < 0.05$). After 150 days of storage, TS of bilayer films decreased to 5.56 Mpa, but the lowest E value could still reach 40.92%. This might be due to the migration of plasticizers in different degrees during storage, which improved the plasticizing effect [51]. It can also be seen from Figure 4 that under different storage conditions, the variation of TS and E of bilayer films was different in the same storage period. Under the conditions of normal temperature (25 °C) and high humidity (RH 65%), the bilayer films had relatively good tensile strength. Under the conditions of low temperature (−17 °C and 4 °C) and high humidity (RH 65%), the bilayer films had relatively good toughness. After 150 days of storage, the mechanical properties of bilayer film were still better than those of Ciannamea et al. [52].

3.6. Scanning Electron Microscope (SEM)

The changes of barrier and mechanical properties of bilayer film reflect the change of its internal microstructure to a certain extent [46]. The scanning electron micrographs of IYP-CZ bilayer films obtained on days 0, 30 and 150, for different storage environments, are shown in Figure 5. From the micrographs obtained on day 30, we can see that the internal network structure of bilayer films no longer existed and became denser under the conditions of freezing and normal temperature, and there was no obvious boundary between bilayer films. However, the cross section of the bilayer film was relatively rough in the freezing environment (−17 °C). In the cold storage environment (4 °C), there were still some network structures in the cross section of the bilayer films, and the transition between the bilayer films was natural. Figure 5 also shows that after 150 days of storage, network structure appeared again in the cross section of bilayer films, but the two phases of starch film and protein film became more easily distinguished. At the same time, the 2000-fold cross-section SEM analysis showed that some cracks appeared in the bilayer film after 150 days of storage, which also explained the reason why the barrier performance of the bilayer films became worse even though its internal structure became more compact.
at the later stage of storage [52]. At the same time, the changes in bilayer films structure observed by SEM also verified the changes of water content and mechanical properties [43].

![Graph](A)

![Graph](B)

**Figure 4.** Tensile strength (A) and fracture elongation (B) of bilayer films during storage. A (−17 °C), b (4 °C), c (25 °C, RH 43%), d (25 °C, RH 54%), e (25 °C, RH 65%).
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Figure 5. Cont.
1 day: Control; Storage for 30 days: (A1) −17 °C, (B1) 4 °C, (C1) 25 °C, RH 43%, (D1) 25 °C, RH 54%, (E1) 25 °C, RH 65%; Storage for 150 days: (A2) −17 °C, (B2) 4 °C, (C2) 25 °C, RH 43%, (D2) 25 °C, RH 54%, (E2) 25 °C, RH 65%.

4. Conclusions

The disadvantages of single-layer starch film and single-layer corn zein film in practical application were overcome, and the formation mechanism of iron yam–pea starch blend and corn zein bilayer film (IYP/CZ) was explored by using the respective advantages of iron yam–pea starch and corn zein as film materials. The bilayer film IYP/CZ was stored for 150 days under the conditions of freezing, cold storage and normal temperature. Every 30 days, the physicochemical properties and microstructure were analyzed to study the effects of different temperatures, humidity and time on the appearance color, light transmittance, haze, moisture content, water resistance, oxygen resistance and mechanical properties of the bilayer film. On this basis, the microstructure of the cross section was analyzed by SEM to study the effects of the microstructure of the bilayer film on its physicochemical properties. These results revealed that we developed the iron yam–pea starch blend/corn zein bilayer films with satisfactory barrier, mechanical properties and
storage properties. This work provides a new method for developing inner packaging materials for convenient food seasoning.

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