

## Evaluation and Improvement of Biodegradable Zein Based Film

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### Abstract

Zein is a hydrophobic protein of maize. Biodegradable zein films both with and without plasticizer were made using various controlled drying conditions for preparation. The zein films were transparent. Physical mechanical properties, tensile strength, contact angle, transition temperature ( $T_1$ ), gas permeability and water vapor permeability (WVP) were measured, and atomic force microscopy (AFM) was used to obtain an image of the surface structure of the film. We tried to improve the physical mechanical properties of the film as affected by composition of polyethylene glycol (PEG; MW: 400) and lauric acid. The tensile strength of the film was between 7 and 30 MPa. The lowest WVP of a zein film was  $0.012 [\times 10^{-9} \text{g} \cdot \text{m}/\text{m}^2 \cdot \text{s} \cdot \text{Pa}]$ .  $T_1$  was between 167.0 and 172.7°C. The surface morphology was found to depend upon the drying conditions. We found a correlation between the surface structure and the contact angle. The films with projections smaller than 200 nm in base diameter on the surface had a high contact angle ( $>70^\circ$ ). Physical mechanical properties were also affected by increasing the proportion of PEG. Water vapor permeability values increased with increasing PEG while values for oxygen permeability were more erratic. It was expected that, using these controlled drying condition, we would make zein film with various useful physical mechanical properties.

### Introduction

In recent years, there has been an interest in biodegradable materials as part of a solution to environmental problems. Biodegradable materials made of natural resources, especially those based on starch, have attracted worldwide interest and a desire to expand their non-food use, partially due to their low cost. In spite of receiving much interest, starch based materials have failed to gain widespread use because they deteriorate very easily when subjected to a high moisture environment. Therefore, zein or water insoluble corn prolamin (1) provides an alternative to starch as a base for the production of biodegradable materials. This natural protein is contained in gluten meal, and is produced as a by-product of the wet-milling starch making process. Zein can be transformed into a transparent, flexible and water-resistant film by dissolving it into a hydrate organic solvent such as ethylalcohol (ethanol) or acetone, and then drying. However, it has been found that films prepared using aqueous ethanol disintegrate when they come into contact with water whereas films produced using aqueous acetone have good water resistance (2). L.C.Swallen suggested that the zein was one of the first

proponents for the use of zein as an industrial protein (3). A good film should be flexibility, be moldable, odorless, moisture resistance, thermoplasticity and transparency. Proteins generally present excellent barriers to the passage of gas and moisture, and are also moldable and transparent when cast as a film sheet. Biodegradable materials produced from zein have been studied by Yamada et al. and Lai et al. (4). Yamada et al. reported on the manufacture of zein film using an aqueous acetone solution and the physical properties of the resulting film. They found that zein film produced using an aqueous acetone solution had water-resistant properties. Lai et al. measured the tensile properties, water absorption and surface microstructure of zein film and the effects of adding plasticizers to the film. However, they did not accurately control the drying conditions. In this study, we produced the zein film, both with and without plasticizer, and used a variety of controlled drying conditions and production methods. Then, some physical mechanical properties, the tensile strength, the contact angle and gas permeability were measured, and an atomic force microscope (5) was used to obtain an image of the surface structure of the film. Secondly, we tried to improve the physical mechanical properties of the zein film as affected by composition of polyethylene glycol and lauric acid.

## **Materials and Methods**

**Reagents.** The purified  $\alpha$ -zein (Showa Zein™; 96.3% protein, 3.4% water) was supplied by Showa Sangyo Co. Ltd., Tokyo, Japan. All reagents, experimental grade, were obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan.

**Preparation of cast zein film.** One gram of zein powder was dissolved in 10 mL ethanol with 20%(v/v) water or acetone with 30%(v/v) water and heated at 50°C for 10 minutes. On the other hand, the preparation of zein film with plasticizer was as follows; plasticizers, polyethylene glycol (PEG; Molecule weight is 400) or lauric acid (LA), (10 - 40% w/w of zein) were dissolved in 10 mL of 80% ethanol. The mixture was stirred and heated simultaneously at 65°C for 15 minutes. The film was cast by pouring the 10 mL of the zein solution onto a level 200 cm<sup>2</sup> smooth polyethylene sheet using an auto casting machine (Automatic Applicator type A; Toyoseki Co. Ltd., Tokyo, Japan), and allowed to dry for 5 hours at temperatures ranging from 30 to 45°C and between 5 and 90% relative humidity in an controlled environmental chamber. The resultant film could be peeled intact from the casting surface. After drying, the films were kept at the same relative humidity used during the film preparation and at room temperature. In this study, denotation (e.g. A-10-35-05) was used to describe the preparation conditions of the zein film type. The first character, A or E denotes whether acetone or ethanol solution was used. The first number is the initial concentration of the zein. The second number is the drying temperature. The last number is the drying relative humidity. A-10-35-05 was prepared using acetone and 10% zein solution at 35°C and 5% relative humidity.

**Thermal elongation analysis.** A Dynamic Load Thermomechanical Analyzer (TMA/SS150C; Seiko Instruments Inc. Chiba, Japan) was used to test the thermal elongation. Thermal elongation of the zein films was measured between room temperature and 200°C with the temperature increasing constantly at 2°C per minute while the load constant rate of the film was held at the smallest possible force of 0.098N.

**AFM Image of the surface structure.** A SPM-9500 (Shimadzu Co. Ltd., Kyoto, Japan) was used for most of the imaging, and a D3000 (Digital Instruments, Santa Barbara, CA, USA) was occasionally used. The images were observed by the method in our previous study (6).

**Mechanical properties.** Tensile strength of the films was carried out on a Rheometer NRM-3002D (Fudoh Kogyo Co., Ltd, Tokyo, Japan). Samples were cut from the films using a standard sample cutter (JIS Z1702, Rheotech, Tokyo, Japan). The contact angle means the inside angle between the surface of the zein film and the tangent to the surface of water (see Fig. 4). A FACE CA-Z (Auto Contact Angle Meter, Kyowa Interface Science Co. Ltd., Saitama, Japan) was used to measure the contact angle using 1  $\mu$ L of distilled water.

**Water vapor permeability.** The Water vapor permeability of the films was measured using standard method, JIS (Z208).

Gas permeability. Oxygen and carbon dioxide permeability were measured using the gas permeability tester (Gasperm-100, JASCO Corporation, Tokyo, Japan).

## Results and Discussion

Thermal elongation analysis. The thermal elongation of the films is shown in Figure 1. E-10-35-05 was most elongated before breakage which came at 15.2%. By comparison A-35-35-05 was elongated by only 7.4% before breaking. The difference of the elongation ratio of the different films was due to the initial zein concentration. We found that there was no correlation between the elongation and the drying rate. The films made from high concentration zein solutions had the lowest elongation. The transition temperature,  $T_i$  of the films differed with drying conditions. For example,  $T_i$  of A-35-35-05 was lowest (167.0°C) and  $T_i$  of A-10-45-05 highest (173.5°C). There was no difference in  $T_i$  between the higher and lower humidity drying conditions during film preparation, but the zein concentration caused a significant difference. Some of this may be due to the molecular structure of the film. We assume that the difference of elongation of the films arose due to differences in molecular interactions in the film.

Surface structure of the films. AFM images of the surface structure of the films are shown in Figures 2. The depressions had a steeper edge on the air side of A-10-35-05 than the basal side as shown in Fig. 2. When the samples were dried at high humidity, the number of large depressions and projections increased. The surface structure of the film depends on the evaporation rate of the solution, and the surface structure especially depends on the humidity during drying.

Surface of the stretched zein film. There were small projections about 25 nm in diameter at the base. The surface structure appeared to have a pattern of projections which were repeated every 25 nm. On the stretched zein film, the zein molecules were denatured during heating and when the film was stretched, the molecules rearranged themselves. This indicates that the zein film will become stretched such as in chemical polymer film made using an extruder, because the extrusion method is pressurization, heating in the machine and stretching out on an extruder.

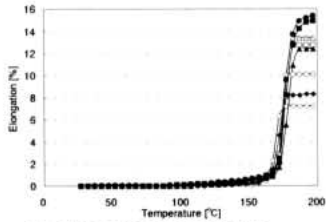
Mechanical properties. The tensile strengths of the films are shown in Figure 3. The acetone films had about 21 MPa of tensile strength. Different drying conditions had no effect. The E-10-45-05 had a maximum of 30.3 MPa of tensile strength, and the E-10-35-90 had a minimum of 7.1 MPa of tensile strength. The tensile strength of the ethanol film depended on drying conditions. The tensile strength of the ethanol films increased with increasing drying rate. Although various films had various tensile strengths, there was no variation in elongation. The contact angle of the films is shown in Figure 4. The air side and the basal side of the zein films differ remarkably from each other. The contact angle of the basal side of the films was higher than the air side. In a film with a projection diameter less than 200 nm of a particular structure, steep projections in the microstructure have a higher contact angle ( $>70^\circ$ ). When the film is used for food packaging, choosing the side with a high contact angle or low contact angle should be considered.

Water Vapor Permeability. There was no large difference in the WVP between the acetone film and the ethanol film. The WVP of both films did depend on the drying conditions. But there was an effective difference in the WVP between the two surfaces of both these films. When the air side of the film was turned upward, the film had a higher WVP than when the basal side was turned upward. Therefore, we can deduce that the film with a high contact angle has a low WVP.

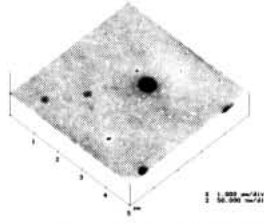
Gas permeability. The results are shown in Figure 5. In synthetic polymers, carbon dioxide permeability is higher oxygen permeability. However, the zein films such as A-10-35-05 and A-10-35-90 have a higher Oxygen permeability than carbon dioxide permeability. The films have a selective gas permeability depend on drying conditions, especially, relative humidity.

Effect of plasticizer. The highest tensile strength of 33.8 MPa was recorded when 20% PEG was added, while the lowest, 7.5 MPa was noted at 40% PEG. The WVP of the film plasticized with 20% of PEG added alone was  $0.3[\times 10^{-9} \text{g} \cdot \text{m}/\text{m}^2 \cdot \text{s} \cdot \text{Pa}]$ , but it decreased to  $0.2[\times 10^{-9} \text{g} \cdot \text{m}/\text{m}^2 \cdot \text{s} \cdot \text{Pa}]$  when plasticized with a composite of 20%PEG and

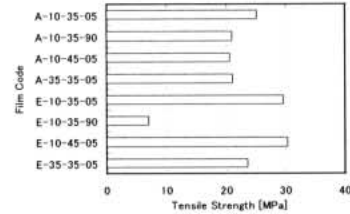
10%LA. LA might have played secondary role related to film matrix building. LA (20%, w/w of zein weight) added alone on the film enormously decreased oxygen permeability (OP) from  $44 [\times 10^{-9} \text{ cm}^3 \cdot \text{cm}/\text{cm}^2 \cdot \text{s} \cdot \text{cmHg}]$  (unplasticized film) to  $0.2 [\times 10^{-9} \text{ cm}^3 \cdot \text{cm}/\text{cm}^2 \cdot \text{s} \cdot \text{cmHg}]$ . OP seems highly dependent on the porousness of the film. As mentioned above, the LA might have filled pores and gaps in the structure of film, thus the film plasticized with LA would have lower OP. On the other hand, high hydrogen bonding between polymers would form an excellent barrier to oxygen.



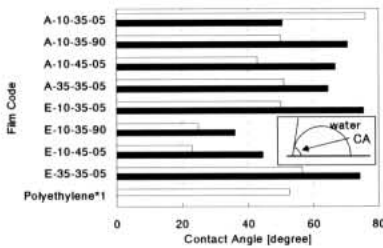
**Fig. 1 Elongation of the zein films.**  
 ○: A-10-35-05, △: A-10-35-90, □: A-10-45-05, ◇: A-35-35-05, ●: E-10-35-05, ▲: E-10-35-90, ■: E-10-45-05, ◆: E-35-35-05



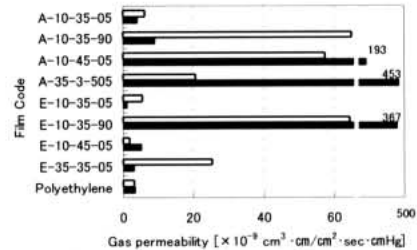
**Fig. 2 AFM image of the surface structure of the zein film.**  
 The scanning area is  $5 \times 5 \mu\text{m}$ . The height range is  $\pm 50\text{nm}$ .



**Fig. 3 Tensile strength of the zein films.**  
 The tensile strength was measured at room temperature.



**Fig. 4 The contact angle of the zein films.**  
 □: The air side of the film, ■: the basal side of the film. The contact angle (CA) means the inside angle between the surface of the zein film and the tangent to the surface of water such as show in the illustration.



**Fig. 5 Gas permeability of the zein films.**  
 □: Oxygen permeability, ■: Carbon dioxide permeability.

## Conclusions

According to the results of this study, the relationship between the physical mechanical properties and preparation methods of zein film are as follows. The transparent zein films were made from corn hydrophobic protein ( $\alpha$ -zein) in organic solutions. We found that the physical mechanical properties of the zein film depended on the drying rate during preparation. Our hypothesis is that the difference in physical mechanical properties of the different zein film was caused by a variation in the internal microstructure of the film. We expect that it will be possible to make zein film with various useful physical mechanical properties by using controlled drying conditions.

## References

- Argos, P. Pedersen, K. Marks, M. D. and Larkins, B. A. (1982). A Structural Model for Maize Zein Proteins. *J. Biol. Chem.*, 257, 9984-9990.
- Yamada, K. Takahashi, H., and Noguchi, A. (1995). Improved Water Resistance in Edible Zein Films and Composites for Biodegradable Food Packaging. *Int. J. Food Sci. and Tech.*, 30, 599-608.
- Swallen, L. C. (1941). Zein - A New Industrial Protein. *Ind. Eng. Chem.*, 33, 394-398.
- Lai, H. M. and Padua, G. W. (1998). Water Vapor Barrier Properties of Zein Films Plasticized with Oleic Acid. *Cereal Chem.*, 75, 194-199.
- Binning, G. Quate, C. F. Gerber, C. H. (1986). Atomic Force Microscope. *Phys. Rev. Lett.*, 56, 930-933.
- Yoshino, T. Isobe, S. Maekawa, T. (2000). Physical Evaluation of Pure Zein Films by Atomic Force Microscopy and Thermal Mechanical Analysis, *JAOCS*, 77, 699-704.