

Physical Evaluation of Pure Zein Films by Atomic Force Microscopy and Thermal Mechanical Analysis

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ABSTRACT: Biodegradable edible films can be made from corn protein, α -zein. Pure zein films are cast from an organic solution of α -zein. This report outlines the surface conditions of such pure zein films. First, the transition temperature, T_t , of the pure zein film was measured with a thermomechanical analyzer. T_t was between 167.0 and 172.7°C. The thermal elongation of the films depended on the drying conditions used during film preparation. Second, the surface microstructure of pure zein films, produced under several different drying conditions, was observed by atomic force microscopy. The surface had a morphology that showed depressions either with acutely (90–120°) or obtusely (121–180°) angled features, depending upon the drying conditions. On the other hand, the surface microstructure after thermal elongation analysis appeared to have a pattern of projections that was repeated every 25 nm. Third, we measured the contact angle of the pure zein films. We found a correlation between surface microstructure and contact angle. Pure zein films with projections smaller than 200 nm in base diameter on the surface had a high contact angle (>70°).

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KEY WORDS: Atomic force microscopy (AFM), biodegradable film, contact angle, thermal mechanical analysis, zein film.

As concern for the environment mounts, the importance of using biodegradable materials instead of synthetic polymers, which do not decompose naturally, has increased (1,2). The recent proliferation of published articles on the production of biodegradable materials indicates that numerous researchers are actively studying and developing biodegradable materials from natural resources (3).

Biodegradable materials made from natural resources, especially those based on starch, have attracted worldwide interest and a desire to expand their nonfood 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 too easily when subjected to a high-moisture environment.

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Zein, or water-insoluble corn prolamin (4,5), 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 process. Zein can be transformed into a transparent, flexible, and water-resistant film by dissolving it in a hydrated organic solvent, such as ethanol or acetone, and then drying. However, zein films prepared from aqueous ethanol disintegrate when they come in contact with water, whereas films produced from aqueous acetone have good water-resistant properties (6). Additionally, zein molecules in aqueous acetone form basic small aggregates relatively rapidly, which then form into large aggregates (7–9) and form the film structure more rapidly than zein in aqueous ethanol.

Swallen (10) was one of the first proponents of the use of zein as an industrial protein. He suggested that it had a variety of applications in food packaging and could form the basis of films for wrapping food as well as for a number of nonfood uses. One of its earliest applications was as an ingredient in quick-drying printing inks (11,12). Since then, over the last 20 yr, research into zein has been conducted in a number of different research fields, including structural chemistry (7–9), pharmaceutical science (13), and biodegradable materials (14).

Biodegradable materials produced from zein have been studied by Yamada *et al.* (6,15) and Lai *et al.* (16–18). Yamada *et al.* reported on the manufacture of zein film from an aqueous acetone solution and the physical properties of the resulting film. They found that zein film produced from 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. The films made by Lai *et al.* and Yamada *et al.* contained plasticizers and cross-linking reagents, and drying conditions were not accurately controlled.

In this study we produced pure zein film, without additional reagents, and used a variety of controlled drying conditions and production methods. Then, thermal elongation and the contact angle were measured, and an atomic force microscope (19) was used to obtain an image of the surface microstructure of the biodegradable film.

EXPERIMENTAL PROCEDURES

Reagents. The purified α -zein (Showa Zein™; 96.3% protein, 3.4% water) was supplied by Showa Sangyo Co. Ltd. (Tokyo, Japan). Molecular weight of α -zein (Z19) is 19,000. Ethyl alcohol (ethanol) and acetone, both 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% (vol/vol) water or acetone with 30% (vol/vol) water and heated at 50°C for 10 min. The film was cast by pouring 10 mL of the zein solution onto a level 200 cm² smooth polyethylene sheet in an auto casting machine (Automatic Applicator type A; Toyoseki Co. Ltd., Tokyo, Japan). Zein films were prepared from ethanol or acetone solution. The solution was spread evenly and allowed to dry for several hours (4 or 5 h) at temperatures ranging from 30 to 45°C and between 5 and 90% relative humidity in a controlled environmental chamber. The resultant film could be peeled intact from the casting surface. After drying, the films were kept at room temperature at the same relative humidity used during film preparation. Then, prior to the various experiments, the specimens of pure zein films were stored in a desiccator with silica gel for 24 h at room temperature.

In this study, a special notation (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 that either 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 from acetone at 10% zein solution at 35°C and 5% relative humidity.

Thermal elongation analysis. The zein film was cut into 50 × 3 mm rectangles. Both ends of the sample were held by chucks on the sample holder. 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/min while the load constant rate of the film was held at the smallest possible force of 0.098 N.

Atomic force microscopy (AFM) image of the surface microstructure of zein films. A SPM-9500 (Shimadzu Co. Ltd., Kyoto, Japan) was used for most of the AFM imaging, and a D3000 (Digital Instruments, Santa Barbara, CA) was occasionally used. The zein films were cut to 25 mm² and pasted onto the sample stage with double-sided tape.

The images were recorded in both contact mode and tapping mode at atmospheric conditions. We used a 120 μ m scanner (Digital Instruments) and an etched silicon probe with around 270 kHz of resonant frequency (Model TESP; Digital Instruments). The scan rate ranged from 0.5 to 1 Hz, indicating that the imaging time of each frame was between 4 and 10 min. The set point voltage was adjusted to the lowest possible voltage, between 1.6 and 2.5 V, so as not to damage the biodegradable films.

Measurement of the contact angle of zein films. 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). The zein films were cut into 5 × 2 cm rectangles and pasted onto the sample stage with double-sided tape. A FACE CA-Z (Auto Contact Angle Meter, Kyowa Interface Science Co. Ltd., Saitama, Japan) was used to measure the contact angle with 1 μ L of distilled water.

Analysis of data. A minimum of five samples was prepared for each measurement. Mean values for the thermal elongation and contact angle of the pure zein films were determined. The thermal elongation of the zein films was corrected to remove the influence of the variability of thickness between films. The equation used is as follows:

$$\text{elongation (\%)} = \frac{\text{measured elongation value (mm)}}{\text{cubic volume of sample (mm}^3\text{)}} \times 100 \quad [1]$$

RESULTS AND DISCUSSION

Thermal elongation analysis. The thermal elongation of the pure zein films is shown in Figure 1. E-10-35-05 showed the greatest elongation before breakage at 15.2%. By comparison, A-35-35-05 could be elongated by only 7.4%. The difference in the elongation ratio of the different pure zein films was due to the initial zein concentration. We found that there was no correlation between elongation and drying rate. The pure zein films made from high-concentration zein solutions had the lowest elongation.

The transition temperatures, T_i , of the pure zein films are shown in Table 1. T_i 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 was 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 zein film. We assume that the difference in elongation of the pure zein films arose due to differences in molecular interactions in the film. This assumption is based on the fact that high-concentration organic solu-

TABLE 1
Transition Temperatures of Pure Zein Films^a

Film code	Temperature (°C)
A-10-35-05	171.0
A-10-35-90	172.4
A-10-45-05	173.5
A-35-35-05	167.0
E-10-35-05	172.0
E-10-35-90	172.2
E-10-45-05	172.7
E-35-35-05	168.5

^aThe thermal elongation of pure zein films was measured from room temperature to 200°C with a constant rate of increase of 2°C/min, and the load constant rate was held at the smallest possible force of 0.098 N. For codes, A is acetone, E, ethanol; first number is initial zein concentration; second is drying temperature (°C); last number is the drying relative humidity.

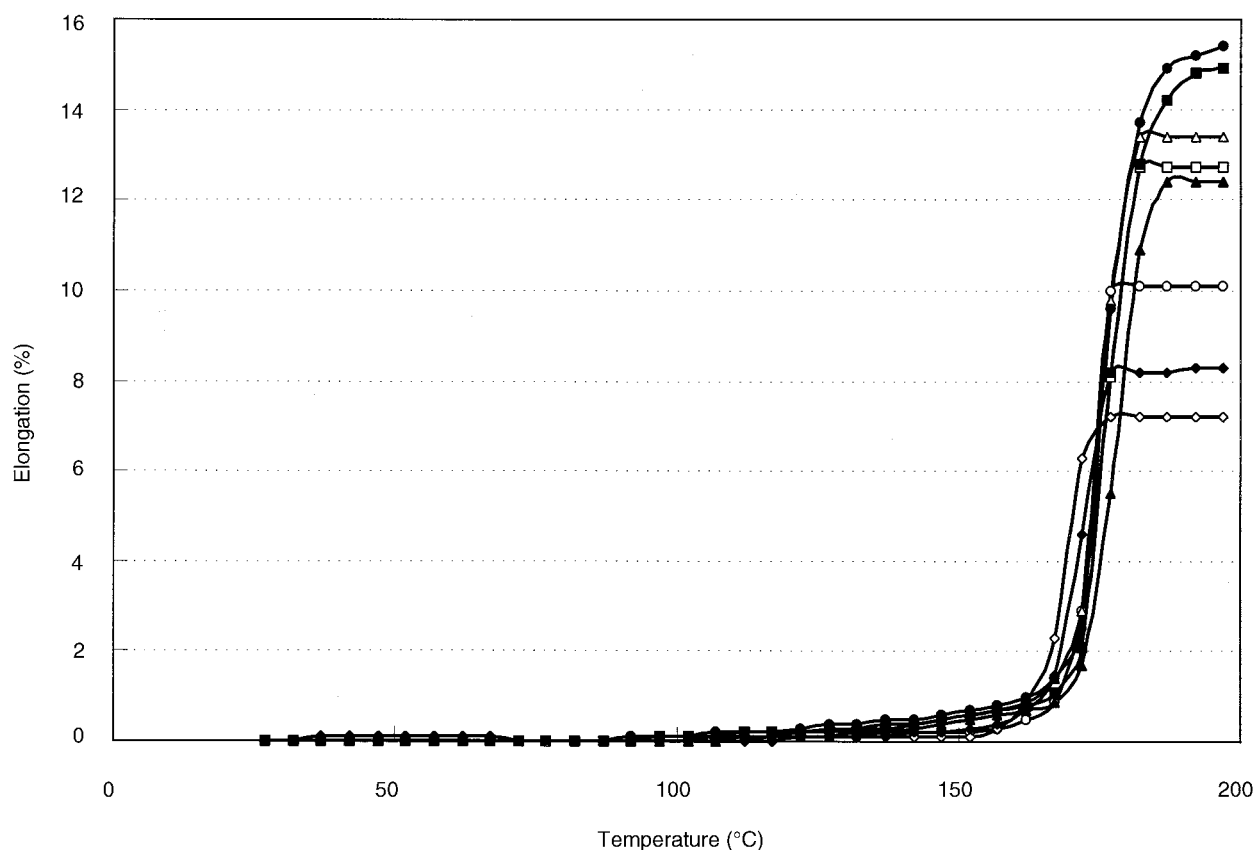


FIG. 1. Elongation of pure zein films. The thermal elongation of pure zein films was measured between room temperature and 200°C, increasing at a constant rate of 2°C/min, and the load constant rate of the film was held at the smallest possible force, 0.098 N. ○: A-10-35-05, △: A-10-35-90, □: A-10-45-05, ◇: A-35-35-05, ●: E-10-35-05, ▲: E-10-35-90, ■: E-10-45-05, ◆: E-35-35-05. See Experimental Procedures section for explanation of sample designations.

tions of zein form smaller zein particles in the organic solution than low-concentration solutions (8,9). Therefore, this indicates that the significant difference in elongation may be due to different aggregated zein particle sizes in the films.

Surface of pure zein films. Data on the surface microstructure of the zein films are outlined in Table 2. AFM images of the surface microstructure of cast zein films are shown in Figure 2A–D. The surface microstructure of the polyethylene used as the base to make the zein films is shown in Figure 2E. The depressions had a steeper edge on the air side of A-10-35-05 than the basal side, shown in Figure 2A,B. There were *ca.* 450 depressions per 10^{-6} m², with the maximum diameter of the depressions being 1,000 nm. The diameter of the projections was constant at 200 nm on the air side of A-10-35-05 (Table 2). The surface microstructures depended on the drying conditions. When the drying conditions included low humidity, the small projections increased in number. Also, the depressions became more rounded on the air side of the zein film (Table 2).

There were *ca.* 900 depressions per 10^{-6} m² with a maximum diameter of 500 nm on the air side of the zein film made from 80% ethanol (E-10-35-05, Fig. 2C). The surface microstructure of the basal side of the films was independent of

the surface microstructure of the polyethylene sheet. We assumed that this is caused by physical interactions (hydrophilic and/or hydrophobic) between zein molecules and polyethylene sheet. Many more projections were found with a diameter of 40 nm per 10^{-6} m² on the basal side of A-10-35-05 (Fig. 2B). When the samples were dried at high humidity, the number of large depressions and projections increased. There were two or three depressions of 500 nm in diameter per 10^{-6} m² on the basal side of the zein film made from 80% ethanol solution (E-10-35-05, Fig. 2D).

Matsushima *et al.* (8,9) measured zein aggregate size in 70% (vol/vol) aqueous ethanol by small angle x-ray scattering (SAXS) and recognized the presence of aggregated zein particles in solution. They suggested that the high concentration of zein in the aqueous ethanol solution produced a lower radius of gyration of the cross-section than at low zein concentration. Zein molecules are polar and can be dissolved in polar organic solvents. During acetone evaporation from the aqueous acetone solution, the molecules of zein gather to form one large aggregation in the solution. High zein concentration solutions may have a greater chance to aggregate than low zein concentration solutions. Therefore, the surface microstructures of a high zein concentration film (A-35-35-05)

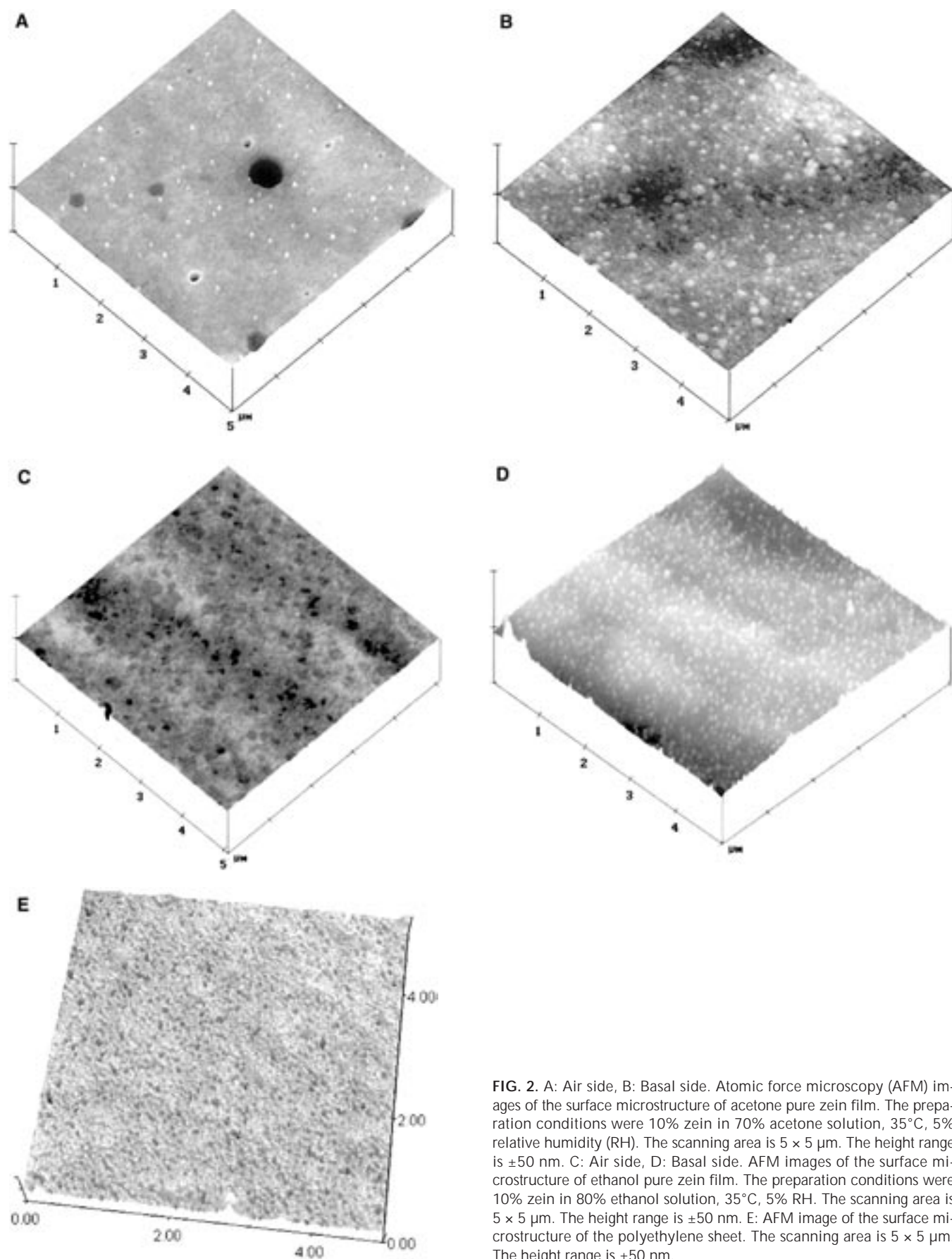


FIG. 2. A: Air side, B: Basal side. Atomic force microscopy (AFM) images of the surface microstructure of acetone pure zein film. The preparation conditions were 10% zein in 70% acetone solution, 35°C, 5% relative humidity (RH). The scanning area is 5 × 5 μm. The height range is ±50 nm. C: Air side, D: Basal side. AFM images of the surface microstructure of ethanol pure zein film. The preparation conditions were 10% zein in 80% ethanol solution, 35°C, 5% RH. The scanning area is 5 × 5 μm. The height range is ±50 nm. E: AFM image of the surface microstructure of the polyethylene sheet. The scanning area is 5 × 5 μm. The height range is ±50 nm.

TABLE 2
Surface Microstructures of Pure Zein Films

Film code ^a	Sharpness ^b	Diameter of major microstructure		Depth/height of depression/projection (nm)
		Depression (nm)	Projection (nm)	
A-10-35-05/A	○	100	200	42.0/36.5
A-10-35-05/B	○	— ^c	200	37.3
A-10-35-90/A	✕	—	1500	31.1
A-10-35-90/B	○	—	150	50.5
A-10-45-05/A	✕	1000	—	10.8
A-10-45-05/B	○	—	50	16.6
A-35-35-05/A	✕	500	—	7.2
A-35-35-05/B	○	—	200	2.9
E-10-35-05/A	○	—	30	25.9
E-10-35-05/B	○	—	50	31.0
E-10-3-590/A	✕	2000	—	26.9
E-10-35-90/B	○	200	—	38.9
E-10-45-05/A	✕	500	—	44.7
E-10-45-05/B	○	200	—	42.5
E-35-35-05/A	○	—	800	11.7
E-35-35-05/B	○	200	—	45.3

^aThe characters A and B indicate the air side and basal side of the pure zein films, respectively. For film code see Table 1.

^b○: steep microstructure (angle of edge range between 90 and 120°); ✕: shallow microstructure (angle of edge range between 121 and 180°).

^cContours of the microstructure were obscured.

had larger dimensions than in a low zein concentration film (A-10-35-05). Aqueous acetone solution has a higher evaporation rate than aqueous ethanol solution during drying at low relative humidity. After the acetone has evaporated from the aqueous solution, molecules of zein do not move within the solution during further drying and, therefore, form depressions and projections on the surface of the film. However, the

evaporation rate of an aqueous ethanol solution varied only slightly between drying conditions, and the aggregate size of zein molecules did not change and was independent of the zein concentration in the aqueous ethanol solution.

The surface microstructure of the zein film depends on the evaporation rate of the solution, and the surface microstructure especially depends on the humidity during drying.

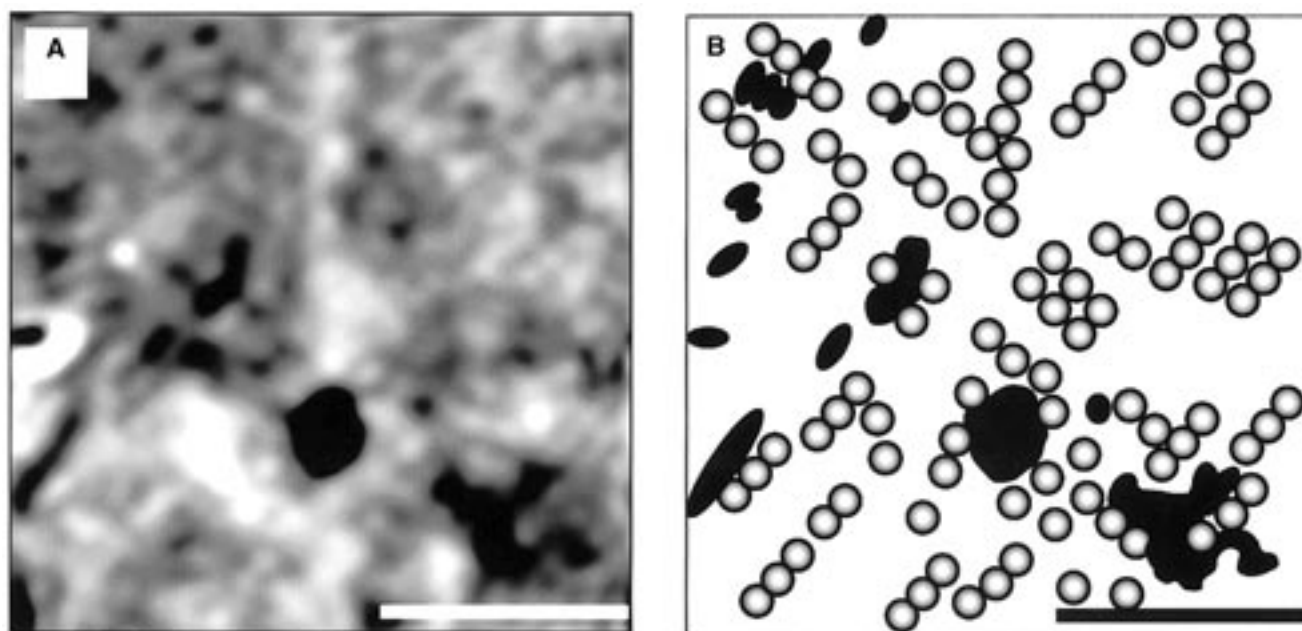


FIG. 3. AFM image of the surface microstructure of stretched pure zein film. (A) The scanning area is 500 × 500 nm. The height range is ±4 nm. Bar is 200 nm. (B) Schematic representation of the surface of a stretched zein film. The diameter of circles is 25 nm. Black areas indicate the depression microstructure. The surface microstructure appeared to have a pattern of projections that were repeated every 25 nm. For abbreviation see Figure 2.

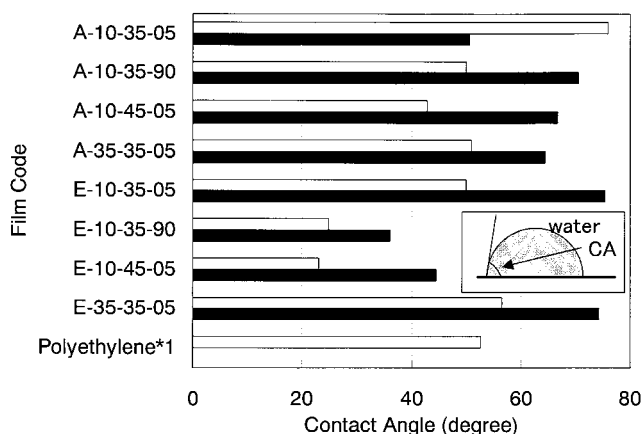


FIG. 4. The contact angle of the pure zein films. □: air side of pure zein film, ■: basal side of pure zein film. *1: measurement on upper side of polyethylene film. The contact angle (CA) means the inside angle between the surface of the zein film and the tangent to the surface of water, such as shown in the illustration.

Surface of the stretched zein film. An AFM image of the surface microstructure of a zein film after measurement of elongation is shown in Figure 3. There were small projections about 25 nm in diameter. The surface microstructure appeared to have a pattern of projections that was repeated every 25 nm (Fig. 3B). Yamada *et al.* (6,15) reported that the size of the aggregated zein molecules in organic solution was between 15 and 50 nm and that the aggregated zein molecules formed a pile structure in the film. On a stretched zein film, the zein molecules were denatured during heating, and when the film was stretched, the molecules rearranged themselves. The treatments of heating and stretching increased pile formation in the film. This indicates that the zein film will become stretched like a chemical polymer film made in an extruder, because the extrusion method also creates pressurization, heating, and stretching.

Contact angles of zein films. The contact angles of zein films are shown in Figure 4. The air side and the basal side of the zein films differ remarkably from each other. The contact angle on the basal side of the films was higher than on the air side. However, the opposite phenomenon was observed for A-10-35-05. The results of these measurements indicate that there is a correlation between contact angle and surface microstructure of pure zein films as observed by AFM. In other words, in a film with a protection diameter less than 200 nm of a particular structure, steep projections in the microstructure have a higher contact angle ($>70^\circ$). In films with shallow projections and depressions, the structure will have a low contact angle. When pure zein film is used for food packaging, choosing between the side with a high contact angle or a low contact angle should be considered.

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