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Improved Mechanical Property and Water Resistance of Zein Films by Plasticization with Tributyl Citrate

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ABSTRACT: Pure zein film is intrinsically rigid and brittle and lacks necessary mechanical properties for industrial processing. In addition, pure zein film is sensitive to high relative humidity, which limits its application in food packaging. To improve these properties, tributyl citrate (TBC) was incorporated into zein film to the mass ratios from 10% to 50%. A significant decrease in Young's modulus was observed, from 409.86 MPa in pure zein films to 136.29 MPa in zein films with 50% TBC. Among all films, those containing 10% TBC are most flexible and toughest. Both DSC and microscopy methods suggested that the TBC may be loaded up to 20% to avoid microsized phase separation. Through modeling with experimental data, incorporating 50% TBC reduced the water absorption capacity to 12.94% compared to 31.78% by pure zein film. More importantly, the integrity of zein/TBC film was maintained at high relative humidity and even after immersion in water. However, more than 20% TBC in zein films led to microsized phase separation, which was harmful to mechanical properties.

KEYWORDS: *zein, plasticization, tributyl citrate, mechanical properties, barrier properties, biodegradable films, food packaging, surface morphology*

■ INTRODUCTION

Exploration on biodegradable materials is one of the most important trends in the packaging industry and consumer attitudes today, owing to their sustainability prospects. These materials offer appealing alternatives to petroleum-based plastics because of their abilities to slowly decompose in landfills under appropriate conditions, and be environmentally safe for incineration. An example that is commonly referred to and commercially available is polylactic acid (PLA), which is produced from lactic acid obtained through fermentation of corn starch or other agricultural resources.[1] Similarly, plant proteins, such as wheat gluten, soy protein, and zein, are also renewable and abundant, and could also contribute as alternative biodegradable materials. A distinct advantage in protein-based biomaterial is their nature with large variety in composition and structure, which provides a wide range of functional groups for interactions and modifications.[2,3]

Zein, a prolamine protein enriched in the endosperm of maize, is a main coproduct of the bioethanol industry. Zein is unique in its thermoplasticity and hydrophobicity. It can conveniently form films simply by evaporation of the solvents, and the obtained films are glossy, hydrophobic, greaseproof, and resistant to the growth of food-borne microorganisms.[4] However, solvent-cast zein films are rigid and brittle, and thus, plasticizers are needed to improve their flexibility.

Some polyol compounds, such as glycerol, sorbitol, polypropylene glycol, polyethylene glycol, and monosaccharides, have been blended into zein as plasticizers. Addition of these hydrophilic plasticizers generally decreased the strength and stiffness while increased the flexibility of zein films. However their effects on water vapor barrier properties are controversial and are dependent on how films are prepared. Among the hydrophilic plasticizers, addition of polyols increased the water vapor permeability, as a result of their hydrophilic nature and phase separation due to incompatibility between hydrophilic plasticizers and hydrophobic zein matrix.[5−9] Fatty acids, such as palmitic acid, oleic acid, and others, have been mixing into and/or coating the films prepared from zein resins. They surpassed those hydrophilic plasticizers in maintaining the film strength while significantly improving film flexibility as well as water resistance.[10−12] Citrate esters, derived from citric acid, are a large group of hydrophobic plasticizers commercially used in food packaging and medical products, because they are nontoxic and biodegradable, which allows the packaging material to contact food items and will not harm the biodegradability.[13] Tributyl citrate and acetyl tributyl citrate were used to plasticize poly(lactic acid) film, resulting in significantly increased elongation at break and lowered water vapor permeability at the expense of slightly decreased tensile strength at yield.[14,15]

The objective of the present study was to investigate the effects of tributyl citrate as a hydrophobic plasticizer on the mechanical properties and water resistance of cast zein films. Tensile properties, glass transition temperature, water absorption, and water vapor permeability of the zein films plasticized with TBC were evaluated.

■ MATERIALS AND METHODS

Materials. α-Zein of biochemical-grade purity was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Tributyl citrate (TBC, ≥97%) and potassium sulfate (>99%) were obtained from Aldrich Chemical Co. Glacial acetic acid was obtained from Fisher Scientific, Inc. (Pittsburgh, PA). Calcium chloride (anhydrous, 8 mesh, Spectrum Chemical Corp., New Brunswick, NJ) was obtained from VWR Corp. (Radnor, PA).

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Zein Film Preparation. Zein and TBC solutions were prepared by dissolving the corresponding amount of TBC and zein in 70 °C glacial acetic acid to the concentration of 2 g of total substances per 10 mL of acetic acid. In the total substances, the mass ratio of TBC was 10%, 20%, 30%, 40%, and 50%. To make zein films, the solutions were magnetically stirred at 70 °C for 30 min, and then 20 mL of solution was cast on diameter 60 mm Teflon Petri dishes and dried in an oven set at 25 inchHg and 40 °C for 48 h. Transparent zein films were obtained for each solution. Films were stored at ambient conditions at 23 ± 2 °C and relative humidity 40−50%.

Differential Scanning Calorimetry (DSC). Differential scanning calorimetry analysis was performed on a DSC 823E Thermal Analyzer (Mettler-Toledo Inc., Columbus, OH) supplied with liquid nitrogen and compressed nitrogen gas. Approximately 5 mg of film sample, packed into aluminum crucibles with lid, were heated from −120 to 210 °C at a rate of 10 °C/min. The glass transition temperature (T_g, the midpoint of the glass transition) and the melting temperature (T_m, the lowest point of melting peak) were automatically recognized by the STARe software (Mettler-Toledo Inc., Columbus, OH) from at least two thermal curves.

Mechanical Property Measurement. Zein film strips (40 × 10 × 0.47 ± 0.12 mm) were prepared as specified in ASTM D882-10 and stored in 50% relative humidity desiccators under 23 ± 2 °C for one month to reach equilibrium before tensile test. The moisture content of zein films was measured using Denver moisture analyzer IR-200 (Denver Instrument, Bohemia, NY). Mechanical properties were measured on a TA.XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY, US) at a test speed of 6 mm/min and initial distance between grips of 18 mm. At least three replications were used to compare the ultimate tensile strength (UTS, the maximum loading stress), elongation to break (ETB, the strain at the moment the sample breaks), Young's modulus (E, the slope of extended initial stress−strain curve), and the toughness (T, the area under the stress−strain curve before breaking point).

Water Absorption. Water absorption was determined by a modification of the ASTM D870[16] and similar to that previously described.[11] Films that were preconditioned at 25 °C and 50% relative humidity were cut into three 30 × 30 mm specimens, which were then conditioned at 25 °C and 50% relative humidity for two days to reach equilibrium. Beakers were predried at 60 °C for 24 h and weighed. The specimens were submerged in distilled water in a beaker at 25 °C for up to two hours, during which the specimens were removed at 15 min intervals and weighed after the surface water was wiped off. Water absorption was calculated as a percentage ratio of water absorbed to initial dry weight. Loss of soluble matter from the sheets after two hours of immersion was monitored by evaporating the soaking water in the beaker at 60 °C for 24 h and then weighing the change of the beaker.

Water Vapor Permeability (WVP). Water vapor permeability was determined at 25 ± 2 °C according to the ASTM E96/E96M-10 method.[17] The inside of aluminum cells was filled with anhydrous calcium chloride. The cells were sealed with the zein films and placed in desiccators containing a saturated potassium sulfate solution (97% relative humidity at 25 °C). At least three specimens for each sample were tested by weighing, twice every day for a period of 10 days. Controls were set up similarly except that the cells contained no desiccant. Data were recorded in a weight gain vs time graph. The slope of the straight line during steady state of permeation, obtained by linear regression, was determined, and the WVP was calculated by

$$\text{WVP} = \frac{G/t}{AP_S(\text{RH}_1 - \text{RH}_2)} \times \text{thickness}$$

where G/t = the slope of the linear regression, thickness = average thickness of zein films, A = permeation area, P_S = the saturated water vapor pressure at 25 °C, and $\text{RH}_1 - \text{RH}_2$ = difference of relative humidity between the two sides of zein films.

Atomic Force Microscopy (AFM). The images of zein films surface were collected by tapping mode atomic force microscopy (TP-AFM) on a NanoScope IIIA multimode AFM (Veeco Instruments Inc., Santa Barbara, CA) under ambient conditions. The root-mean-square roughness (rms) was calculated by the NanoScope software (Veeco Instruments Inc., Santa Barbara, CA) from three 10 × 10 μm images.

Optical Microscopy. Microscopic images of films were obtained using a Nikon TE-2000 inverted microscope (Nikon Instruments Inc., Melville, NY).

Statistical Analysis. One-way ANOVA analysis and modeling were performed using the SigmaPlot 12 (Systat Software GmbH, Erkrath, Germany) to determine any significant difference between values at a confidence level of 95%.

RESULTS AND DISCUSSION

Transparent light-yellowish films were obtained from pure zein and zein with addition of 10% to 40% TBC, whereas zein films containing 50% TBC was slightly opaque. The surface of zein films containing 30% or more TBC was slightly greasy.

Thermal Properties. Thermograms of zein films measured by differential scanning calorimetry suggested the compatibility between zein and the plasticizer and effectiveness of plasticization. As shown in Figure 1, the thermogram of unplasticized cast zein film revealed two second order transitions at temperature around 110.4 and 180.5 °C, interpreted as glass transition temperature, which were slightly higher than values for unprocessed zein protein, with two glass transitions at 94.4 and 173.6 °C.[2] The changes suggested that, during formation of the zein film, the protein chains established inter- and/or intramolecular interactions that restricted the chain mobility. A first order transition peak was found at −20 °C in the heating scan of pure tributyl citrate which was attributed to its melting process. In the cast plasticized zein films, endothermic peaks related to the melting of tributyl citrate were only observed in plasticization level from 30% to 50%, but not in 10% or 20%. This indicated that the <20% TBC and zein remained a homogeneous mixture after casting. However, it was still possible that the peaks were too small to be detected with the current DSC method, and further analysis such as dynamic mechanical analysis could provide more information on this. If the polymers and plasticizers are immiscible, the mixture would reveal several distinct glass

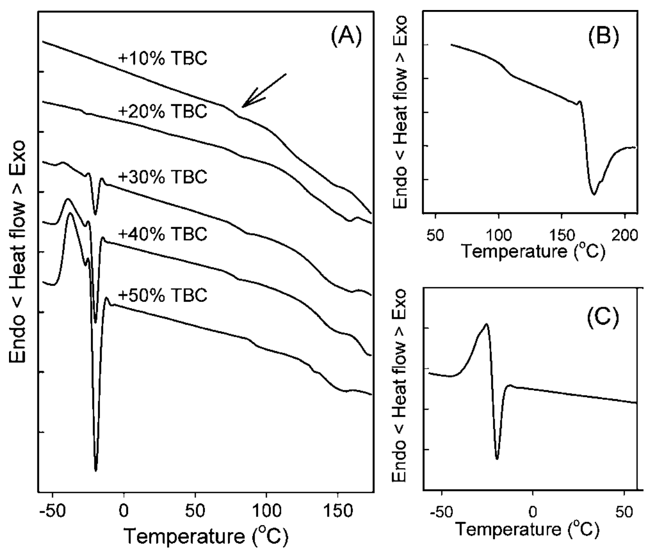

Figure 1. DSC thermograms for (A) zein films plasticized with 10−50% TBC, (B) pure zein films, and (C) TBC. The heat flow between each tick in all three graphs is 0.1 W/g.

transition temperatures corresponding to the individual glass transition of each component. The glass transition temperature for pure tributyl citrate was detected at −90.2 °C, which was consistent with previous study.[1] For plasticized zein films, several glass transitions in the range between 70 and 180 °C were observed, taken as evidence of plasticization. In the case of film containing 10% tributyl citrate, the lowest glass transition was observed at 72.1 °C. According to the Gordon and Taylor equation,[18]

$$T_g = \frac{x_1 T_{g_1} + k_{zein} x_2 T_{g_2}}{x_1 + k_{zein} x_2}$$

where x is the mass percentage and subscripts 1 and 2 refer to zein and tributyl citrate respectively. If T_{g_1} uses the value of 180.5 °C as measured, T_{g_2} uses the value of −90.2 °C, and k_{zein} uses the value of 6.24,[19] the calculation for films containing 10% tributyl citrate suggests a glass transition temperature of 69.7 °C, which is close to observed result, indicating a good miscibility at this plasticization level. However, the equation did not work for films containing a higher level of plasticizer: the glass transition temperatures did not decrease to a point lower than 72 °C. The calculation implies that, when a casting method is used, the tributyl citrate can be mixed homogenously into zein to a possibly maximum level of about 10% and further addition of tributyl citrate would not contribute to the plasticization effect. In contrast to mixing with hydrophilic plasticizers, films plasticized with TBC did not reveal any peak at temperature range around 0 °C, which was suggested as the melting of residue water observed with zein film plasticized with hydrophilic plasticizers.[20] This supported the expectation of a very hydrophobic nature of this film with negligible amount of water.

Tensile Properties. The mechanical properties of zein films are influenced by the plasticizers and the preparation methods. A plasticizer is a substance incorporated into a polymer to increase its flexibility, workability, or distensibility. A plasticizer works by spacing the chains of polymer, allowing the chains to move more flexibly and thus making the plastics softer.

A good method to evaluate mechanical properties of films is to study the stress−strain curves from the tensile test. Typical tensile curves of pure and plasticized zein films are included in Figure 2. Without addition of TBC, the zein film (black solid curve) was brittle, suggested by a linear relationship between the loading stress and the strain curves during a small elastic deformation. With addition of TBC, the stress−strain curves revealed a curving toward plastic behavior at the end of the proportional region. The films with 10% TBC experienced a larger elastic extension. With films containing 30% to 50% TBC, yielding points could be observed.

A useful parameter to characterize a polymeric material is the Young's modulus, which measures the normalized force necessary to make an initial elastic deformation to polymers. As summarized in Table 1, compared to the pure zein film, the Young's modulus of plasticized zein films was first increased and reached the highest value when 10% TBC was incorporated. It was then dramatically decreased as the mass ratio of TBC increased from 25% to 50%, suggesting that addition of TBC at a low level (10%) made the film even more rigid, whereas at higher level (≥20%) plasticizer increased the motility of zein molecules. The observation was possibly a result from the anti-plasticization effects. On the other hand, similar trends have been observed with other hydrophobic plasticizers such as palmitic acid and stearic acid, where the reason was suggested as the higher moisture contents in the pure zein films than plasticized films.[11] It was previously known that water was a good plasticizer for zein films.[21] Incorporation of hydrophobic plasticizers diminished the water content from 6.52% in pure zein film to 3.79% in zein film with 10% TBC, thus increasing the rigidity of the films. TBC at percentage of 20% to 50% seemed to be as effective in reducing the rigidity of zein films as other hydrophobic plasticizers, such as oleic acid.[12]

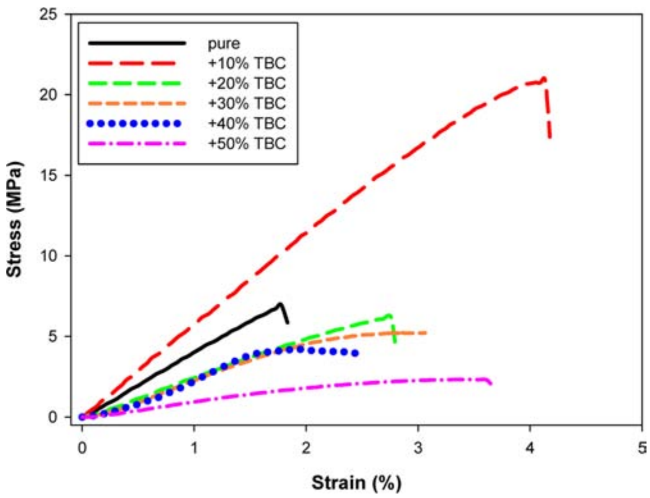

Figure 2. Representative stress−strain curves for pure zein sheets and zein sheets plasticized with TBC.

The other two parameters that can be extracted from the tensile curves are the ultimate tensile strength (UTS) and the elongation to break (ETB). Incorporation of TBC at least retained the UTS while slightly increased the ETB. Among various level of plasticization, 10% was found to be optimal in that it increased the UTS to 17.8 MPa, which was about 3-fold compared to pure zein films. The extensibility (ETB) of zein films with 10% TBC was 4.53%, which doubled the extensibility of pure zein films. In fact, tensile strength is influenced by intermolecular association in polymer matrix, which is often weakened by incorporation of plasticizer. However, some hydrophobic plasticizers, such as palmitic acid and stearic acid, showed exceptional beneficial effects on the strength of zein films,[11] which was explained by strong interactions between the polymer and certain plasticizers.[12] With enhancement on both UTS and ETB, the zein films with 10% TBC were six times tougher than pure zein films, suggested by the toughness calculated as the area under the curve. In the films containing 30% or higher TBC, micro phase separation may exist as the DSC result suggested. This disturbed the molecular structure and led to loss of toughness compared to films containing 10% TBC.

Water Absorption. To investigate the effects of TBC on the water absorption of zein films, samples were immersed in deionized water for two hours and the weight gains were recorded at intervals of 15 min. After two-hour soaking, pure zein films and zein films with 10% TBC turned to slightly opaque because of excessive absorption of water. The films became much softer and stickier on the surface; whereas the films containing 20% TBC or more generally maintained their yellowish color and integrity.

The water absorption was calculated as the percentage ratio of weight gain to original weight of films and plotted against the

Table 1. Effect of Tributyl Citrate (TBC) on the Tensile Properties of Zein Films[a]

	modulus (MPa)	UTS (MPa)	ETB (%)	toughness (MPa)
pure zein	409.86 ± 7.62 b	6.70 ± 0.37 b	1.96 ± 0.18 c	0.071 ± 0.007 b
+ 10% TBC	556.29 ± 29.42 a	17.80 ± 4.26 a	4.53 ± 0.54 a	0.414 ± 0.079 a
+ 20% TBC	255.28 ± 27.36 c	5.47 ± 0.64 b	3.03 ± 0.20 b	0.092 ± 0.004 b
+ 30% TBC	256.64 ± 33.57 c	5.36 ± 1.33 b	3.26 ± 1.01 b	0.112 ± 0.072 b
+ 40% TBC	268.03 ± 48.20 c	4.34 ± 0.19 b	2.44 ± 0.12 bc	0.061 ± 0.005 b
+ 50% TBC	136.29 ± 32.05 d	3.25 ± 0.94 b	3.53 ± 0.17 b	0.066 ± 0.017 b

[a]Values with no letters in common are significantly different, $p < 0.05$, using the Holm−Sidak multiple-comparison method.

duration of immersion in Figure 3. No significant loss of soluble composition into water (averagely 0.6%) was observed. When

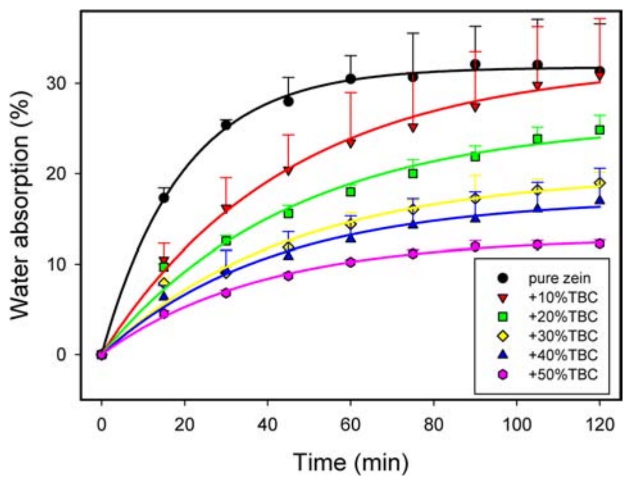

Figure 3. Effect of incorporated TBC on the water absorption of zein sheets.

the mass ratio of TBC increased from 10% to 50%, the amount of absorbed water decreased. A previous study adopted the concepts from drying operations and developed the below equation to describe the kinetics of water absorption by zein films:[11]

$$y(t) = M_S(1 - e^{-t/T})$$

where $y(t)$ = percentage of weight gain by water absorption (a percentage ratio of water absorbed to initial dry weight) and t = time.

This equation was applied to fit the data points in Figure 3, shown as the solid lines. The values of parameter and the correlation coefficient, R, for all zein films are included in Table 2, where the M_S reflects the water absorption capacity, and M_S/T reflects the initial rate of water absorption. The R values for all groups are higher than 0.989, indicating that this equation can provide a valid fitting for the current experimental data. The M_S value suggested that an initial addition of TBC up to the level of 10% did not decrease the capacity of zein films on water absorption, because statistical analysis indicated no significant difference between M_S values of pure zein films and those of zein films with 10% TBC. However, 10% TBC slowed down the initial water absorption rate, suggested by a smaller M_S/T values. With further increase of TBC content, both the water absorption capacity and the initial absorption rate of zein films were reduced. Statistical analysis suggested that TBC has dramatic effects on limiting the water absorption by zein films, which may be due to the hydrophobic nature of TBC. Also as described previously, those films containing 30% or more TBC bear a slightly greasy surface, which prevented the film from absorbing water.[11]

Hydrophilic plasticizers are shown to be especially effective in improving the extensibility and reducing the rigidity of zein films at ambient conditions because those hydrophilic plasticizers absorb water which adds to the plasticization. However, in an environment where excessive free water is available, such as in food where the water activity is normally high, these zein films plasticized with hydrophilic plasticizers absorbed too much water and became weak.[5,22] In contrast, hydrophobic plasticizers better maintained the film strength even at high relative humidity.[22] TBC is a molecule containing hydrophobic butyl chains. As expected, TBC could maintain the film integrity even after immersion in water. This is especially important for biopolymer-based films to be applied in food packaging.

Water Vapor Barrier Properties. The barrier properties of zein films were measured as the water vapor permeability with the results included in Table 3. The water vapor permeability of polymer is usually affected by the hydrophilic/hydrophobic nature of the polymer and the homogeneity of the polymer matrix, especially the surface hydrophobicity and homogeneity. Statistical analysis suggested that addition of TBC lowered the water vapor permeability of zein films at a high relative

Table 2. Kinetic Parameters of Water Absorption TBC-Plasticized Zein Films

	M_S (g)	M_S/T (g/min)	R
pure zein	31.78 ± 0.24 a	1.65	0.999
+ 10% TBC	32.29 ± 0.95 a	0.72	0.997
+ 20% TBC	25.89 ± 1.43 b	0.56	0.991
+ 30% TBC	20.00 ± 1.17 c	0.44	0.989
+ 40% TBC	17.26 ± 0.65 d	0.42	0.995
+ 50% TBC	12.94 ± 0.20 e	0.34	0.999

[a]Values with no letters in common are significantly different, $p < 0.05$, using the Holm−Sidak multiple-comparison method.

Table 3. Effect of Tributyl Citrate (TBC) on the Water Vapor Barrier Property of Zein Films

	WVP (g·mm/(h·m^2·mmHg))
pure zein	0.0225 ± 0.0099 a
+ 10% TBC	0.0220 ± 0.0049 a
+ 20% TBC	0.0086 ± 0.0021 a
+ 30% TBC	0.0109 ± 0.0042 a
+ 40% TBC	0.0133 ± 0.0031 a
+ 50% TBC	0.0199 ± 0.0020 a

[*]Values with no letters in common are significantly different $p < 0.05$ using the Holm-Sidak multiple-comparison method.

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humidity of 97% (P = 0.020). However the difference on the WVP between pure zein films and films with 20% was insignificant (P = 0.053). Usually, moisture absorption at high relative humidity made polymer films soft and swollen. The absorbed water serves as a plasticizer for the polymer films and increased the flowability of polymer chains.[22] This less dense structure as well as more flexible polymer chains in the matrix allows the water molecules to diffuse quickly, resulting in high water vapor permeability.[23] It was expected that incorporation of TBC could reduce the WVP of zein films due to its hydrophobic nature limiting the water absorption. However, with the present result, it is hard to determine the effects of TBC on WVP, and further research is needed to clarify this.

Surface Microstructure. The surface of pure zein films bears many cracks, voids, and wrinkles shown in the optical microscopic images and AFM images (Figure 4). It probably resulted from the high surface tension during the solvent casting of pure zein films. There could also be cracks and voids inside the films which caused the pure zein films to be brittle and less tough. When the TBC was added to the mass ratio of 20%, the surface tension may be released and the film surface was much smoother under the examination of optical microscopy. Root-mean-square (rms) roughness calculated from AFM images indicated a decrease of roughness on zein films from 66.3 nm on pure zein films to 24.1 nm on films containing 20% TBC (Table 4). However, the roughness was again increased to 45.5 nm at the level of 50% incorporated TBC. From the optical images, a micro phase separation was obvious in films containing 50% TBC but not in films with 20% TBC. AFM images also revealed small soft spherical structure which could be a separate domain formed by TBC. As the mass ratio of TBC increased from 0 to 20%, the film surface appeared to be much smoother. The cracks and voids on the surface of pure zein films could be the site for water binding during moisture absorption, which explained the slightly higher water vapor permeability. However, when the loading of TBC reached the limitation, possibly about 20%, TBC started to form phase separation from the zein matrix. Therefore the heterogeneous films with 50% TBC were again less effective as a moisture barrier.

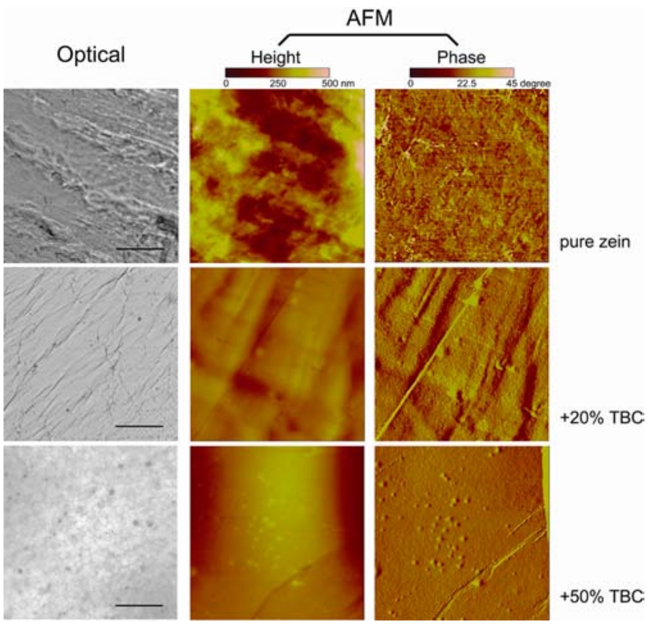

Figure 4. Optical microscopy and AFM of pure zein films and TBC-plasticized zein films. Bar in optical microscopy = 50 μm; AFM image size: 10 × 10 μm.

Table 4. Effects of TBC on the Surface Roughness of Zein Films Analyzed by AFM

	rms (nm)
pure zein	66.3 ± 2.2
+ 20% TBC	24.1 ± 1.2
+ 50% TBC	45.5 ± 3.3

In summary, the effects of TBC on the mechanical properties, water resistance, water vapor barrier properties, and surface microstructure were investigated. Films incorporated with 10% TBC showed optimally improved toughness and flexibility. The plasticized films were also more resistant to water shown as lowered water absorption capacity and/or rate. In our following study entitled "Developing zein films with ice nucleation function and its application in frozen dough", zein film plasticized with TBC was adopted for the application on frozen food. Incorporating TBC allowed the cast zein films to be flexible enough for wrapping, and water-resistant enough to retain integrity through freezing storage.

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Notes

The authors declare no competing financial interest.

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