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Reducing the Brittleness of Zein Films through Chemical Modification

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Zein protein is a major coproduct of biofuel from corn. To reduce the brittleness of zein films, a new type of zein-based biomaterial, was synthesized by chemical modification of zein with lauryl chloride through an acylation reaction. The final products were confirmed by ^{1}H NMR, FT-IR analysis, and SDS−PAGE. Thermal analysis detected no microphase separation in the synthesized polymer matrix. As the content of lauryl moiety increased, the glass transition temperatures of modified zein decreased by as large as 25.8 °C due to the plasticization effect of the lauryl moiety. In addition, mechanical and surface properties of cast films from acylated zein were also investigated. The elongation at break of modified zein sheet was increased by about 7-fold at the high modification level with some loss of mechanical strength. The surfaces of modified zein films were as uniform as unmodified zein film but more hydrophobic, further suggesting that no microphase separation happened during the film formation process. This work indicated the potential of these new biomaterials in the development of biodegradable food packaging materials and delivery systems.

KEYWORDS: Zein films; mechanical properties; chemical modification

INTRODUCTION

Zein, an alcohol-soluble protein enriched in the endosperm of corn, is a main coproduct of the bioethanol industry. The commercial α-zein contains two main components with apparent molecular weights of 19 kDa (Z19) and 22 kDa (Z22). Although zein has a long history of being used as a coating material for candies, medical tablets and paper (*1*), other applications of zein are still limited mainly due to the undesirable properties of the zein-based materials (*2−4*). Like many other proteins, zein, without plasticizers, forms brittle films with poor flexibility that cannot withstand industrial processing. Some low molecular weight molecules, such as some polyols and fatty acids, could be used as plasticizers to improve the mechanical properties of zein films (*1*). However, phase separation as a result of immiscibility between zein and these plasticizers is difficult to avoid, which usually leads to instability of the mechanical properties and often heterogeneous surfaces. Another disadvantage of using polyol plasticizers is that they exacerbate the sensitivity of zein-based materials to moisture (*5−7*). Thus, new efforts are needed to improve the water resistance and mechanical properties of zein-based materials.

Chemical modification allows an extensive exploration to the novel functionalities and applications of synthetic polymers. The methods could also be used on biopolymers to generate new biomaterials to substitute synthetic polymers (*8−10*). Cross-linking agents have often been used to improve the properties of protein films. In many reported studies, the addition of cross-linkers, such as formaldehyde, glutaraldehyde, transglutaminase,

multivalent carboxylic acids etc., to zein has been commonly used to increase the mechanical strength and reduce the water permeability. However, such an approach often showed little or even negative effects on the flexibility (*11−17*). Wu et al. (*18*) used polycaprolactone (PCL) to modify zein, where the hydrophobic chain of PCL not only cross-linked the zein molecule but also served as plasticizers and thus significantly increased the elasticity of zein sheets. On the other hand, palmitic chloride was used to acylate the zein in order to improve the thermoprocessability and water resistance. However, the study did not clarify whether the modification improved the intrinsic mechanical properties of zein or the byproduct such as palmitic acid played a role as plasticizer (*19*).

In this paper, we aimed to obtain zein derivatives with improved film properties through chemical modification by lauryl chloride, which would introduce hydrophobic alkyl chains onto zein molecules. The success of the synthesis was confirmed by combined ^{1}H NMR, FT-IR analysis, and SDS−PAGE, and our characterization indicated increased flexibility and hydrophobicity of zein derivative films with no sign of phase separation.

MATERIALS AND METHODS

Materials. The α-zein of biochemical-grade purity was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Deuterated dimethyl sulfoxide (DMSO-d_6) was purchased from Sigma-Aldrich Inc. (St. Louis, MO). Lauryl chloride (99%, Acros Organics), triethylamine (TEA), dimethyl sulfoxide (DMSO), hydrochloric acid, ethanol (95%), and glycerol were obtained from Fisher Scientific, Inc. (Pittsburgh, PA). Pluronic F127 was from BASF Corporation (New Jersey). Milli-Q water was used throughout the experiment.

Modification by Acylation Reactions. Acylation of zein was performed as previously described (*19*) with some modifications.

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TEA (3, 9, or 15 mmol) and lauryl chloride (1, 3, or 5 mmol) per gram of zein were added to a 5% w/v solution of zein in DMSO dropwise at ambient temperature while stirring. The resultant solution was heated to 65 °C under stirring for 3 h. Subsequently, the reaction mixture was poured into water, acidified to pH 5−6 using HCl in order to neutralize the extra TEA and to facilitate its removal through dialysis. The product, acyl zein, was separated by centrifugation, and washed with water three times before lyophilization. The powder obtained was dissolved in 80% ethanol and dialyzed against 80% ethanol for one week, and then dialyzed against water for another 24 h, followed by lyophilization. The lauryl-substituted zein molecules were named zein-C12-1, zein-C12-3 and zein-C12-5, respectively, according to the amount of lauryl chloride per gram of zein. If the average molecular weight of zein was assumed to be 20 kDa, the initial molar ratio of lauryl chloride to zein was 20:1, 60:1, and 100:1, respectively.

¹H NMR Spectroscopy. The ^1H NMR spectra were acquired using a Varian VNMRS 500 MHz spectrometer. The ^1H solution spectra of zein and modified zein were recorded in DMSO-d_6 at 25 °C.

ATR-FTIR Spectroscopy. The attenuated total reflection Fourier transform infrared spectra were collected under ambient conditions, using a Thermal Nicolet Nexus 670 FT-IR spectrometer (Thermo Electron Corp., Madison, WI) with a Smart MIRacle horizontal attenuated total reflectance Ge crystal accessory. Each spectrum was averaged by 128 scans with 4 cm^{-1} resolution.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS−PAGE). Each sample was dissolved in sodium dodecyl sulfate/nonreducing sample buffer to 1 mg/mL solution, and then heated at 95 °C for 5 min. Each sample solution (5 μL) or protein marker was loaded onto sodium dodecyl sulfate polyacrylamide gel with 5% stacking gel and 15% separating gel. The gel was run in a BioRad Mini-Protean II system under 90 V for about 2 h. The gel was then stained with Coomassie blue (20) and photographed by a monochrome gel documentation system.

Differential Scanning Calorimetry (DSC). DSC analyses were performed on a DSC 823E thermal analyzer (Mettler-Toledo Inc., Columbus, OH) supplied with liquid nitrogen and compressed nitrogen gas. Approximately 5 mg of each sample was packed into an aluminum crucible with lid and heated from 32 to 140 °C at a rate of 10 °C/min. The sample was then cooled to −120 °C at a rate of 30 °C/min in order to remove any prior thermal history within the sample. Subsequently, the thermogram was obtained by heating the sample to 210 °C at a rate of 10 °C/min. Glass transition temperature (T_g, the midpoint of the glass transition) and the melting temperature (T_m, the lowest point of each melting peak) were automatically analyzed from the thermogram by the STARe software accompanied with the instrument. Triplicate readings were taken.

Film Surface Properties. Zein films were prepared by a spin-casting method as described previously with some modifications (21). Sample solutions were prepared by dissolving zein or modified zein into glacial acetic acid (0.1% and 1%, w/v), and then heating at 70 °C for 30 min. Zein films were prepared by spin coating the solutions onto precleaned silicon (Si) wafers, using a Laurell model WS-400A-6NPP/LITE spin coater (Laurell Technologies Corp., North Wales, PA) at 4000 rpm for 80 s. Clear and homogeneous zein films were obtained and dried at 50 °C in a vacuum oven overnight to remove the residual solvent. The zein films prepared here were denoted as ZF*m-n*, where *m* indicated the level of lauryl chloride added in the reaction, and *n* indicated the concentration of protein solution used for film preparation. For example, the film ZF5-0.1 was prepared from 0.1% (w/v) zein-C12-5 in acetic acid solution.

The surface morphology images of zein films 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 surface hydrophilicity was represented by the static water contact angle measured on a VCA Optima XE Dynamic Contact Angle Analyzer (AST Products Inc., Billerica, MA) at ambient conditions. The images were recorded by a CCD camera immediately after the water droplets were deposited onto the zein film surface. The water contact angles were determined using the associated software provided by the manufacturer. At least six measurements were averaged for each sample.

Mechanical Properties Measurement. Zein sheets were prepared by solvent casting in Teflon Petri dishes using solutions consisted of 20% zein, zein-C12-1, zein-C12-3, or zein-C12-5 in acetic acid (HAc) plasticized by
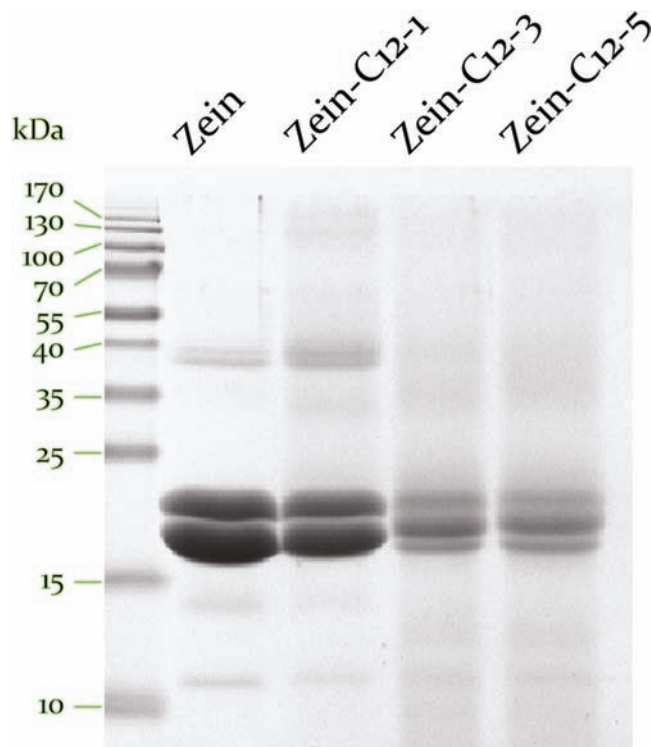

Figure 1. SDS−PAGE of pure zein and modified zein. The protein marker was run in the first lane, with the molecular weights labeled.

25% Pluronic F127 and 12.5% glycerol based on the weight of zein or modified zein and then dried in a vacuum oven at 50 °C for 48 h. Yellow and transparent sheets were obtained from each sample. The zein sheets were denoted as ZS, ZS1, ZS3 or ZS5, where ZS stands for zein sheet, and ZS1, -3, and -5 stand for sheets made from different amount of acylated zein. Dumbbell-shaped specimens with dimensions of 40 × 10 × (0.05−0.30) mm^3 were cut from the central region of the sheets and stored in 50% relative humidity desiccators under ambient temperature for more than 30 days before conducting tensile tests. Mechanical properties were measured on a TA.XT2 texture analyzer (Texture Technologies Corp., New York) with an initial grip distance of 20 mm and a test speed of 6 mm/min.

RESULTS AND DISCUSSION

Preparation and Structure of Acylated Zein. According to Gianazza et al. (22), about 8.2% of the amino acids of zein were either serine or threonine, while 26.6% amino acids were any of glutamic acid/glutamine/aspartic acid/asparagine. The hydroxyl group and the amino group could potentially serve as the sites to form ester or amide bonds in the acylation reaction. However, due to the limitation of protein conformation, some of the hydroxyl and amino groups may be buried inside the protein and, thus, are not accessible to reactions. In the present study, acylated zein molecules were synthesized with three different amounts of lauryl chloride. To ensure complete removal of unreacted lauryl chloride (or its hydrate laurylate acid), the products underwent extensive dialysis.

SDS−PAGE analysis of the modified zein was used to reveal the modification extent upon adding different amounts of lauryl chloride (**Figure 1**). The two major bands at 19 kDa (Z19) and 22 kDa (Z22) represented the two main components in α-zein (23). Because the samples were run in a nonreducing condition, the bands at around 39 kDa should be the respective dimeric forms of Z19 and Z22 (24). The bands at around 11 kDa and 14 kDa were also suggested to be two β-zein components (25). On the SDS−PAGE gel, modified zein would shift to higher molecular weights. Interestingly, after modification at the two higher levels
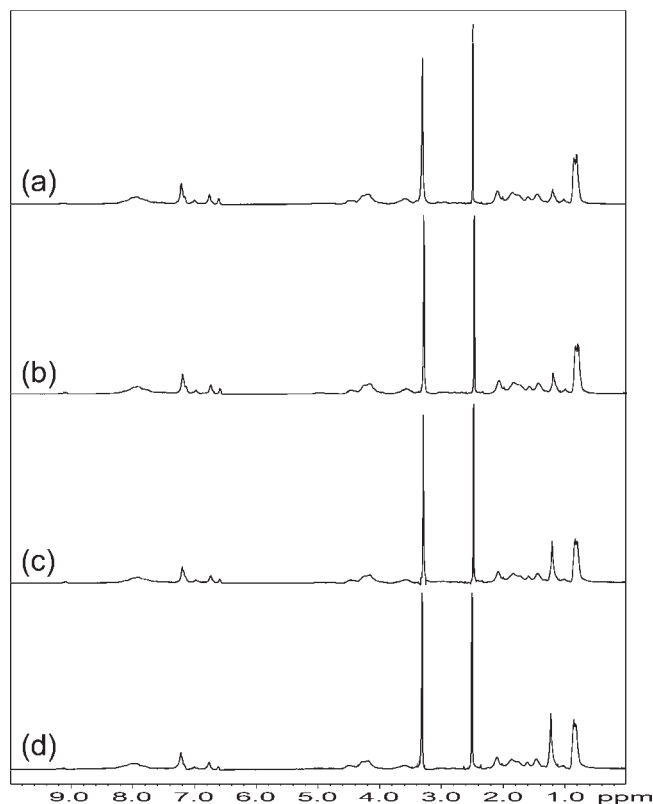
Figure 2. ^{1}H NMR spectra of (**a**) zein, (**b**) zein-C12-1, (**c**) zein-C12-3 and (**d**) zein-C12-5.

Figure 3. FT-IR spectra of (**a**) lauryl chloride, (**b**) zein, (**c**) zein-C12-1, (**d**) zein-C12-3 and (**e**) zein-C12-5.

of lauryl chloride, three bands appeared at the position between 19 kDa and 22 kDa. Without further analysis, it is not clear what forms of zein the three aggregates comprised. In spite of the sequence homology, the reactivity and surface hydrophobicity of the two subunits were found to be different on the basis of reversed-phase HPLC analysis (*24*). The molecular conformations of the two subunits of zein (Z19 and Z22) were also thought to be different (*26*). Thus, it is possible that the lowest band came from the modified Z19 component. According to our previous experience, the Z19 component was more accessible to chemical modification than the Z22. The addition of 12-carbon alkyl chains would lead the denatured protein to adopt different thermodynamic conformation in SDS−PAGE gel compared to the pure protein. In addition, other studies suggested that the hydrophobic interactions, exceeding the down effects from the lost positive charges, enhanced the binding of SDS with protein modified with long alkyl-chain (*27*, *28*). This could result in higher density of positive charge on acylated protein molecules which would migrate faster than a pure protein with the same molecular weight in electrophoresis. Thus, low-degree modified Z19 aggregate may appear to separate from original Z19 bands, even though the molecular weight did not change significantly.

Modified zein binds less staining dye per unit mass than unmodified zein. Therefore, it is not appropriate to use a densitometry method to calculate the unmodified zein contents, which can only be roughly estimated if there is more than 12% modified zein in zein-C12-1 (*18*). This value increases to more than 24% in both zein-C12-3 and zein-C12-5, regardless of the amount of lauryl chloride added.

^{1}H NMR spectra of zein and its derivatives also confirmed the successful grafting of lauryl groups onto zein molecule. In comparison with the ^{1}H NMR spectrum of zein (**Figure 2a**), the ratio of the number of H in CH$_2$ (δ 1.21) to that in CH$_3$ (δ 0.83) significantly increased from 0.26:1 to 0.32:1, 0.47:1 and 0.51:1,
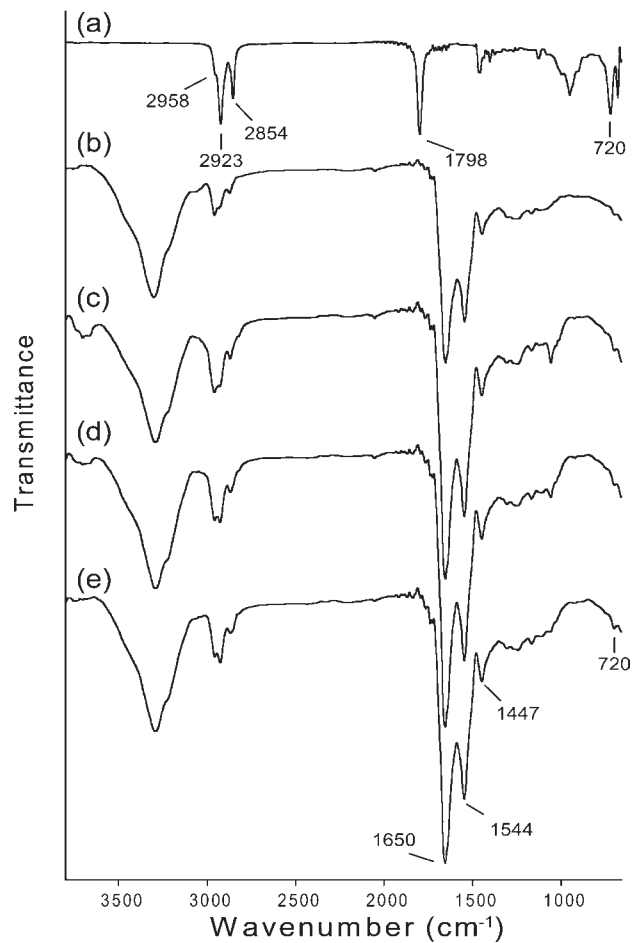
respectively (**Figures 2b−2d**), which suggested more alkyl chains in the molecule of modified zein and further indicated that the lauryl groups were grafted to the molecule of zein as expected.

By combining the results of SDS−PAGE and ^{1}H NMR analysis, we found that, at the initial lauryl chloride to zein molar ratio of 20:1, zein was only slightly acylated, and at the ratio of 60:1 and 100:1, acylation occurred to a similar extent, suggesting the possible saturation of the reaction groups on zein surface.

Moreover, the FTIR spectra of the zein samples also revealed the difference in molecular structures between modified and pure zein. The FTIR spectrum of lauryl chloride (**Figure 3a**) contained a sharp peak at 1798 cm^{-1} which is associated with the C=O stretching in the CO−Cl group (*29*). Another sharp peak at 720 cm^{-1} represented the vibrations of alkyl chain with at least four methylene groups (*30*). The three adjacent peaks at 2958, 2923, and 2854 cm^{-1} were assigned to the vibrations of the methylene group, the symmetric and asymmetric vibrations of the methylene group, respectively (*31*). After modification, the newly formed ester and amide groups intensified the absorption of the amide I band (stretching vibrations of the C=O bond of the amide) at around 1650 cm^{-1} and the amide II band (bending vibrations of the N−H bond) at 1544 cm^{-1}. The vibrations of attached hydrocarbon chains gave rise to those bands in the 2800−3000 cm^{-1} range and the bands at 1447 cm^{-1} and 720 cm^{-1}.

Besides acylating the protein, lauryl chloride could also react with water and form fatty acid. The fatty acid could function as a plasticizer and interfere with other characterizations. The free fatty acid could be detected from the FTIR spectrum as a shoulder peak at 1700−1800 cm^{-1}, as seen in the previous study

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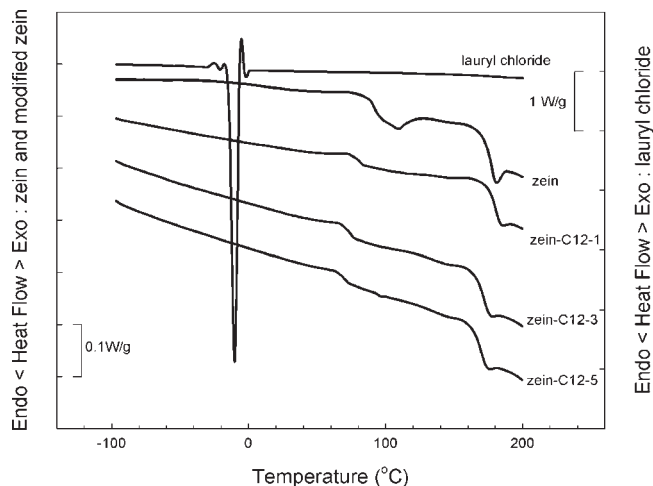*J. Agric. Food Chem.,* Vol. 59, No. 1, 2011 **59**

Figure 4. DSC thermograms for lauryl chloride, zein, and modified zein.

Table 1. Glass Transition Temperatures of Zein and Modified Zein Samples Measured by DSC

sample	glass transition temperature (°C)	
	T_g^1	T_g^2
zein	94.4 ± 2.1	173.6 ± 0.3
zein-C12-1	81.1 ± 2.2	177.2 ± 1.3
zein-C12-3	74.7 ± 1.6	169.8 ± 0.6
zein-C12-5	68.8 ± 1.0	167.7 ± 0.3

on modifying soy proteins with palmitic acid chloride (*19*). In **Figure 3**, the absence of absorption bands at around 1798 cm^{-1} confirmed the effective removal of the fatty acid by the dialysis procedure (*32*).

Thermal Properties. DSC is conventionally used to study the plasticizer compatibility with biopolymer and the effectiveness of plasticization. The DSC thermograms of lauryl chloride, zein and modified zein were shown in **Figure 4**, and the glass transition temperature data were summarized in **Table 1**. Previously, Sessa et al. reported that the Japanese white zein had a lower T_g than Freeman zein, at around 158 °C (*15*, *33*), whereas Wu et al. also performed DSC on Showa zein and found two T_g at 180.1 and 200.1 °C, potentially attributed to the two zein subfractions (*18*). The zein used in this study could be from the same manufacturer as their zein. However, the zein thermograms obtained here were quite different. Two distinct glass transition processes were observed, with T_g at 94.4 and 173.6 °C, which may be attributed to the two components in α-zein, Z19 and Z22, respectively. The differences in glass transition temperatures among zein may be due to the different origins of zein.

Table 1 summarizes the glass transition temperatures of zein and modified zein samples measured by DSC. After modification, the lower T_g decreased from 94.4 to 68.8 °C, by about 25.8 °C, while the higher T_g (i.e., 173.6 °C) only decreased by about 5 °C. This also suggested that the reaction showed certain preference between various components in zein, and was consistent with the SDS−PAGE and ^{1}H NMR studies (see above): compared to Z22, Z19 is more reactive in acylation.

Pure lauryl chloride had a melting point at 10.4 °C. Modified zein did not show any endothermic peak at that temperature, indicating that there was no microcrystal in the samples formed by lauryl chains. The FT-IR spectra also confirmed this, because the absorption in the 2800−3000 cm^{-1} range derived from the vibration of methylene groups in ordered long hydrocarbon
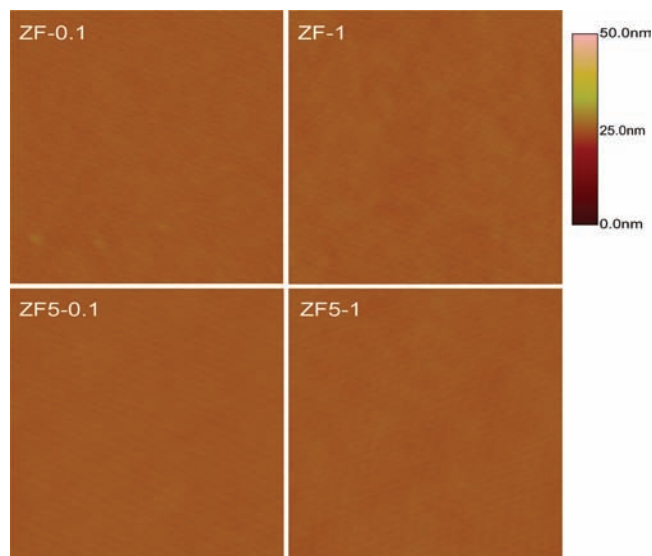

Figure 5. Tapping mode atomic force microscopy (TP-AFM) images of films prepared from zein 0.1% and 1% solutions (ZF-0.1, ZF-1) and from zein-C12-5 0.1% and 1% solutions (ZF5-0.1, ZF5-1). Each image size is 500 nm × 500 nm, and the *z* scale is 50 nm.

Table 2. Surface Water Contact Angles of Zein and Modified Zein

sample	water contact angle (°)
ZF	75.6 ± 1.1
ZF1	78.5 ± 0.5
ZF3	86.4 ± 0.3
ZF5	86.0 ± 1.0

chains did not shift to a lower wavelength compared to the spectrum obtained for our modified zein (*31*).

Film Surface Properties. The surface properties are believed to be important for the barrier properties of films, where homogeneous and smooth surface is usually preferred. In our previous study, it was observed that the spin-cast zein films from acetic acid solutions bear smooth and featureless surfaces (*21*). However, a heterogeneous surface is often observed on biopolymer films that are simply plasticized with fatty acids, due to the instability of the emulsions formed between fatty acids and biopolymers, resulting in phase separation afterward. For this reason, cast zein films plasticized by fatty acids often show granules on the top of the film surface (*6*, *7*, *34*). AFM tapping mode images shown in **Figure 5** revealed that chemical modification by lauryl chloride did not create any visible aggregates or pattern, and the film surfaces maintained as uniform as the untreated zein.

Water permeability and moisture sensitivity of protein-based materials were also directly affected by surface and overall hydrophobicity (*35*). Water surface contact angle, representing the surface hydrophobicity, was also measured on the zein and modified zein films. **Table 2** contained static water contact angles of films prepared by zein and its derivatives. Films cast from unmodified zein showed relatively large water surface contact angles, due to its hydrophobic nature. Meanwhile, water contact angles of modified zein films were further slightly increased, due to the increased surface hydrophobicity through the acylation reaction, implying possible decreased water permeability and moisture sensitivity.

Mechanical Properties. Plasticized with Pluronic F127 and glycerol, zein and acylated zein were cast into films and their mechanical properties were measured. As shown in **Table 3**, the elongation at break of zein-C12-5 was about 7 times that of the

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Table 3. Mechanical Properties of Cast Films Prepared from Pure Zein and Modified Zein

	Young's modulus (MPa)	TS (MPa)	ETB (%)
ZS	215.5 ± 27.5	3.5 ± 0.6	46.8 ± 20.5
ZS1	96.3 ± 36.0	2.8 ± 0.4	101.9 ± 56.6
ZS3	89.3 ± 41.5	3.0 ± 0.3	163.1 ± 69.3
ZS5	78.7 ± 26.5	3.7 ± 0.5	302.3 ± 23.7

control. Given that the tensile strength was slightly lowered after modification, the modified zein sheets containing higher portions of lauryl moiety revealed higher toughness, which was calculated as the energy required for breaking divided by the volume of specimen tested. At the same time, the Young's modulus was decreased from 215.5 MPa of pure zein films to 78−96 MPa of modified zein sheets but with no obvious difference among each modification level.

Unplasticized zein cast films are usually brittle and exhibit no yielding point before rupture at low strain. Plasticized zein films containing 15% (w/w) free fatty acids, such as stearic acid or palmitic acid, slightly decreased the tensile strength, but showed little effect on the elongation at break and the Young's modulus, whereas the same plasticization level of oleic acid decreased both tensile strength and Young's modulus (*36*,*37*). Additionally some processing during film formation, such as kneading, blowing and/ or extrusion, could strengthen the plasticization effect of free fatty acids in zein films (*37*, *38*). This may suggest that processing may be necessary to input energy to promote the interactions between fatty acid plasticizers and zein molecules. In contrast, covalently attaching acyl chain will allow the plasticizers to evenly distribute in the zein film matrix, as suggested by the previous DSC and AFM results, to allow maximum interactions. Thus, this chemical modification was able to significantly increase the extensibility of zein sheets.

In summary, new zein-based biomaterials were synthesized through a single step acylation reaction with lauryl chloride. By chemically attaching plasticizer molecules on the protein, modified zein showed lower glass transition temperatures. Also, solvent cast sheets from modified zein became more flexible and tougher, which suggested that potential improved processability of acylated zein compared to pure zein. In addition, the solvent cast films from modified zein maintained the uniformity and smooth surface of pure zein films, indicating that the attachment of lauryl chains did not introduce any macro- or microscale phase separation. Furthermore, the hydrophobicity on the surface was further increased because of the attached alkyl chains, which could potentially improve the water barrier properties of these zein-based biomaterials. This study suggests a great potential of acylated zein as food packaging and coating materials with improved properties.

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