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Biodegradable Zein-Based Films: Influence of γ-Irradiation on Structural and Functional Properties

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Zein, a predominant corn protein, is an alcohol-soluble protein extracted from corn and is characterized by unique film-forming properties. The characteristic brittleness of zein diminishes its usefulness as a structural material. The objective of this work was to study the effect of γ-irradiation on improving the performance of zein films in packaging applications. This goal has been achieved by irradiating zein film-forming solutions with various doses of γ-rays, namely, 10, 20, 30, and 40 kGy at dose rate of 10.5 kGy/h, using a Co^{60} γ-radiation source. The impact of radiation process on the structural properties has been explored through far-UV CD spectral analysis and scanning electron microscopy. Additionally, viscosity changes that reflect the effect of radiation treatment on degradation and/or cross-linking of protein chains have been measured. However, improvements in the performance of zein films as packaging materials that can be accomplished by radiation treatment have been investigated via monitoring of the color, surface density, roughness, mechanical properties (tensile strength and elongation percentage), water uptake, and water barrier properties. The results indicated that γ-irradiation treatment of the film-forming solution can be used to improve the water barrier properties, as well as color and appearance of the zein films. Moreover, a sterilization effect is considered to be an additional advantage for applying γ- irradiation.

KEYWORDS: Zein; γ-irradiation; viscosity; color; mechanical properties; water vapor permeability

INTRODUCTION

In the light of increased attention to preserve our biosphere, municipal solid waste (MSW) is considered one of the most important environmental problems that face our planet. Petroleum-based synthetic plastic materials are the main culprit due to their nondegradability. The development of biodegradable materials based on renewable resources is the potentially most promising approach to waste management. They have a dual benefit by conversion of waste into valuable ecofriendly materials that also replace synthetic petroleum-based materials and subsequently reduce the solid waste generated by these nondegradable materials (*1*).

Protein-based films are considered to be environmentally friendly materials as well as having potential advantages in packaging applications because of their good oxygen barrier properties. However, they have inferior mechanical properties and water vapor permeability compared to synthetic plastic films. Many attempts have been made to improve the functional properties of protein-based films including chemical (cross-linking, substitution, grafting), physical (plasticization, blend and composite forming, coating, and radiation), and enzymatic methods (cross-linking and hydrolysis). These techniques must be applied using additives that have no negative effect on the biodegradability of produced materials, and thus these additives and their degradation products must have no toxic effect on the environment. This has spurred research into the physical methods including dehydrothermal treatment and ultraviolet and γ irradiation as fabrication and modification approaches for biobased materials (*1*−*3*).

As ionizing radiation, γ-irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupture of covalent bonds, and formation of protein free radicals (*4*). Individual proteins may exhibit different degrees of response to γ-radiation, due to varying amino acid compositions and molecular structures (*5*). Chemical changes in the proteins caused by γ-irradiation include fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals that are generated in the radiolysis of water (*6*−*8*). As an example, the hydroxyl and superoxide anion radicals that are generated by radiation of film-forming solutions can modify the molecular properties of the proteins, which can result in alteration of the protein films by covalent cross-linkages formed in the protein solution after irradiation (*9*). Zein, the predominant protein in corn, has been extensively studied for its potential use as an alternative

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packaging material as edible and biodegradable films. Zein can be cast into films. However, films made of 100% zein are brittle under normal conditions. To make zein films more flexible, plasticizers have to be added to zein prior to casting. Zein is also hygroscopic, meaning it can gain or lose water to reach equilibrium with ambient air. Water can also be a plasticizer for zein. Zein needs some plasticizers to make it flexible at room conditions, but too much plasticizer will make zein films too weak and sticky to be handled and usable (*10*). Indeed, many studies have been done with the aim of improvement of the functional properties of zein films using various physical or chemical modification techniques (*11*−*13*). Little attention has been paid to the application of radiation in improving zein materials. This impelled us to study the effect of γ-radiation on improving the functional properties of zein films to be used in packaging applications.

MATERIALS AND METHODS

Materials. Pure α-zein and other chemicals used in this study were the products of Wako Pure Chemical Industries Ltd. (Osaka, Japan). Polyethylene $glycol_{400}$ was purchased from Sigma Chemicals Co., St. Louis, MO. All materials were used as obtained without further purification.

Preparation of Zein-Based Films. Film-forming solution was prepared by weighing zein powder in a conical flask, followed by adding PEG_{400} (18.5% w/w of zein) and aqueous ethanol (88% w/w in water). The protein concentration was 14% (w/w) with respect to aqueous ethanol. The flask was heated at 70 °C for 10 min under stirring. The film-forming solutions were then irradiated at 0, 10, 20, 30, and 40 kGy at room temperature under air using a Co^{60} γ-irradiator at the Radiation Research Center, Osaka Prefecture University. The dosage rate was 10.5 kGy/h. Then, aliquots (30 mL) were poured into plastic Petri dishes (9 cm diameter), gently swirled, and placed in a ventilated oven at 50 °C for 8 h to evaporate the solvent and produce free-standing films. After drying, the films were conditioned for at least 48 h at 22 °C and 50 ± 5% relative humidity (RH).

Analytical Methods. *Analytical Methods on Zein and Film-Forming Solutions. (a) Circular Dichroism (CD) Measurements.* CD spectroscopy was performed at 25 °C with a JASCO J-720 spectropolarimeter according to the method reported previously by Cho and Song (*6*). A 1 mm path length cell was used. The protein solutions were diluted with 70% ethanol to achieve the desired protein concentration. The protein concentration was 0.5 mg/mL. The reported CD spectra were the average of five scans, and these were smoothed by the method of Chang et al. (*14*). CD data were expressed as molar ellipticity in deg cm^2 $dmol^{-1}$.

(b) Measurement of Fourier Transform Infrared Spectroscopy (FTIR). Infrared spectra were recorded in a Jasco FT/IR-480 Plus (Jasco Co., Tokyo, Japan) with a Golden Gate diamond horizontal attenuated total reflectance (ATR) system. Non-irradiated and irradiated zein samples (2 mg) were spread on the ATR crystal to cover the crystal surface area. The sample was gently squeezed by a screw to promote contact with the crystal. The spectra (128 scans at 2 cm^{-1} resolution) were collected within the frequency range of 4000−800 cm^{-1}. The angle of incidence for the ATR crystal was 45°. The empty crystal was used as background. The FTIR spectra were Fourier-deconvoluted with a resolution enhancement factor of 1.5 and a bandwidth of 15 cm^{-1}. Deconvolution and curve fitting of the infrared spectra were performed using Jasco Spectra Manager software (Window 95/NT version) and according to the method of Kauppinen et al. (*15*). The band assignment to secondary structural components was carried out for amide region I (1600−1700 cm^{-1}).

(c) Measurement of Viscosity. Viscosity of zein solutions irradiated at various radiation doses was determined at 25 °C using a Brookfield viscometer (model DV-1, Brookfield Engineering Laboratories Inc., Stoughton, MA). A spindle no. 0 at 10 rpm was used, and 10 replicates were performed for each sample.

Analytical Methods on Zein-Based Films. (a) Hunter Color Value Measurement. Hunter color of films was measured with a portable colorimeter (CR-300 Minolta Chroma Meter, Minolta Camera Co., Osaka, Japan) according to the method of Gennadios et al. (*16*). Sample specimens were placed on the surface of a white standard plate as a background for color measurements, and Hunter *L*, *a*, and *b* color values were measured. The ranges of the three color coordinates were from $L = 0$ (black) to $L = 100$ (white), from $-a$ (greenness) to $+a$ (redness), and from $-b$ (blueness) to $+b$ (yellowness). Standard values refer to the white calibration plate ($L = 97.75$, $a = 0.49$, $b = 1.96$).

(b) Measurement of Light Transmission. Light transmission was determined using a modified standard procedure of the BSI (*17*). Samples of films were cut into a rectangle and placed on the internal side of a spectrophotometer cell. The light absorbance values between 400 and 800 at 10 nm intervals were recorded for each sample using a UV−vis recording spectrophotometer UV-160A (Shimadzu Scientific Instrument Corp., Columbia, MD).

(c) Determination of Thickness. Film thickness was measured using a Tri-Circle 25 hand-held micrometer (China). Four film specimens (5 × 5 cm^2) were cut from four separately cast films and then placed within the micrometer gap, which was gradually reduced until first indication of contact was noted. Thickness measurements were taken at five different locations for each film specimen, one at the center and the rest around the perimeter. A total of 20 thickness measurements were recorded for each film, and the average was calculated.

(d) Measurement of Surface Density. Sixteen specimens (5 × 5 cm^2) of each film were weighed to the nearest 1 mg. Average weight value was divided by the area of the sample (25 cm^2) to calculate the surface density (mg/cm^2).

(e) Measurement of Tensile Strength and Percentage of Elongation. Film tensile strengths (TS) and elongations at break (*E*) were determined with an Instron Universal Testing Machine (model 4484, Instron Corp., Canton, MA) according to ASTM Standard Method D 882-91 (*18*). Film specimens (2.5−8.0 cm) were conditioned in an environmental chamber at 25 ± 1 °C and 50 ± 4% RH for 2 days. Initial grip distance of 5 cm and crosshead speed of 50 cm/min were used. TS was calculated by dividing the maximum load by initial cross-sectional area of a specimen, and elongation was expressed as a percentage of change of initial gauge length of a specimen at the point of sample failure. Five replicates of each film were tested.

(f) Measurement of Water Uptake. Sample strips (40 × 20 mm) were dried in a desiccator until their weights became constant (W_0). These samples were then placed in a moisture-saturated atmosphere for 7 days. Afterward, the samples were weighed (W_1). Moisture absorption (%*M*) can be calculated from the following equation:

$$\%M = (W_1 - W_0)/W_0 \times 100$$

(g) Measurement of Contact Angle. Contact angle measurements were carried out using a goniometer (Kruss G10, Germany). To perform these measurements, a 4 μL drop of deionized water was placed on the surface of the film with an automatic piston syringe and photographed. An image analyzer was used to measure the angle formed between the base, constituted of the surface of the film in contact with the drop of water, and the tangent to the drop of water. As usual, high contact angle values are indicative of high hydrophobicity for protein films.

(h) Measurement of Water Vapor Permeability (WVP). WVP was determined gravimetrically according to ASTM standard method E96-95 (*19*) by a water vapor permeability tester (L80-5000, Switzerland). The films were inserted between the lower chamber containing distilled deionized water and the upper chamber containing a sensor for controlling the temperature and relative humidity (23 °C and 15% RH). Air velocities were approximately 750 m/min across the films. Silica gel (desiccant) was used for the absorption of air water. The moisture adsorbed by the silica gel was detected by weighing periodically until a stationary state was reached. For each measurement, at least five repetitions were made. The final measurement was done after incubation for 1 day. The slope of the weight versus time plot was divided by the effective film area to obtain the water vapor transmission rate (WVTR). This was multiplied by the thickness of the film and divided by the pressure difference to obtain the WVP.

$$\text{WVTR} = \text{slope/film area}$$

$$\text{WVP} = \text{WVTR} \times \text{thickness}/\Delta\text{vapor pressure}$$

$$\text{WVP}_C = \frac{\Delta W}{A\Delta T}\frac{X}{\Delta p}$$

Where, ΔW = weight gain by desiccant (g), X = film thickness (mm), A = area of the exposed film surface (m^2), ΔT = incubation period (h), and Δp = difference of partial pressure (kPa).

(i) Measurement of Roughness. The roughness of the surface was determined by measuring roughness parameters (R_{rms}). R_{rms} is defined as the root-mean-square (rms) average of the height (z) taken from the mean data plane, expressed as the average roughness measured using surface roughness tester SJ- 201P. Samples were mounted onto a glass slide with double-sided tape. Minimum sample dimensions were 25 × 25 mm. Scans were performed on both sides of the membrane. All results are the average of triplicate samples.

$$R_{rms} = \sqrt{\frac{1}{N}\sum_{i=1}^{N} Z_1^2}$$

Where, N = evaluation length and z = height.

(j) Microstructure Examination. The surface characteristic of zein films was examined by a JEOL 6360LA scanning electron microscope (JEOL Ltd., Tokyo, Japan) operated at an acceleration voltage of 15 kV. Film specimens were mounted on stainless steel stubs with double-sided tape, and a 10−20 nm thick layer of gold was sputtered on the samples by a JFC-1100E sputter (JEOL Ltd.).

Statistical Analysis. Analysis of variance and Duncan's multiple-range tests with $P \leq 0.05$ were performed to analyze the results statistically using a SAS program (SAS Institute, Inc., Cary, NC). For viscosity measurement, 10 replicates were tested, and 5 replicates were assayed for tensile strength and WVP measurements.

RESULTS AND DISCUSSION

Effect of Radiation Treatment on Protein Structure and Rheological Properties. Shown in **Figure 1** are the far-UV CD spectra (200−250 nm) of zein in aqueous ethanol 90% (0.5 mg/mL) obtained after thermal denaturation at 70 °C and cooling at 25 °C (non-irradiated sample) and for γ-irradiated samples that subjected to radiation at 10, 20, 30, and 40 kGy.

Corresponding spectra for both samples show two negative maxima around 207−208 and 222−224 nm that indicate the helical structure content. The β-sheet content exhibited a strong negative band at 207−208 nm and a positive one around 191 nm, typical of proteins with α- and β-structure, in which the α-helix band intensity is stronger than the β-sheet structure. The effect of γ-irradiation on the protein structure was noted from the obtained spectra. The CD spectra of irradiated samples showed a noticeable change in peak intensities. A significant diminution of the two negative maxima was observed when γ-irradiation treatment was applied, but no shift in CD bands was observed. The analysis of CD spectra also pointed to a decrease of helical content and an increase of β-sheet content with increasing irradiation dosage. The conformation changes were more pronounced at 10 and 30 kGy. Notwithstanding, the effect of irradiation process on zein conformation structure was unnoticeable between those irradiated at 10 and 20 kGy or between those irradiated at 30 and 40 kGy. Furthermore, the changes occurring for the samples irradiated at 10 and 30 kGy were more pronounced than that occurring at 10 kGy compared with non-irradiated ones. This can indicate that a radiation dose of 20 kGy represents an inversion point in zein structure. The typical CD spectra for non-irradiated and irradiated samples propose that the structural changes occur mainly at a level of tertiary structure of zein that is related to variation in aggregate size and formation. However, changes in the secondary structure

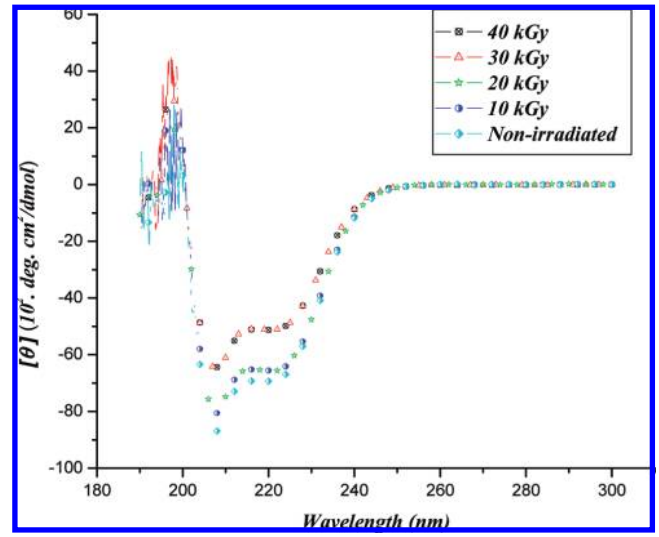

Figure 1. Influence of γ-irradiation treatment on CD spectra of zein.

of zein protein owing to radiation process are less pronounced. This explanation can be indicated from the secondary structural content results obtained from FT-IR analysis. The amide I region (1600−1700 cm^{-1}) in spectra was analyzed according to the methods described under Materials and Methods, and the results of band assignment are shown in **Table 1**. As shown from these results, the secondary structural contents have been altered by increasing the radiation dosage because, in agreement with the results obtained by CD spectra analysis, the content of the bands around 1690 cm^{-1}, which was assigned to be antiparallel intermolecular β-sheets, was diminished by increasing irradiation dosages. γ-Irradiation treatment also slightly decreased the α-helix content by increasing the applied dosage. In contrast, the contents of β-turn (around 1670 cm^{-1}) and random coil (around 1640 cm^{-1}) were increased with increasing irradiation dosage. Moreover, these changes were highly noticeable at a radiation dosage of 20 kGy. Destabilizing the configuration of the helical segments by radiation process can be attributed to degradation of the natural carotenoid, lutein, that is found to fit into the core of the triple-helical segments and help stabilize the configuration (*20*). Corresponding to the reduction in intermolecular β-sheets, it was apparent that by γ-irradiation, the aggregation ability of the molecules in the zein solution was weakened. The increase in β-turn and random coil suggested the changing of irradiated zein into a more flexible or extended form.

Viscosity is considered to be one of the most important properties reflecting the changes in polymer−polymer and polymer−solvent interactions that are mainly correlated with changes occurring in the structure and molecular weight of protein. From the viscosity measurements that are presented in **Figure 2**, it was observed that viscosity of the zein film-forming solution decreased significantly by the action of γ-radiation at

Table 1. Secondary Structure Contents in Non-irradiated and Irradiated Zein Films

	secondary structures (%)			
irradiation dosage (kGy)	α-helix	β-sheet	β-turn	others
0	42	28	15	15
10	40	23	21	16
20	38	21	24	17
30	36	20	27	17
40	35	18	28	19

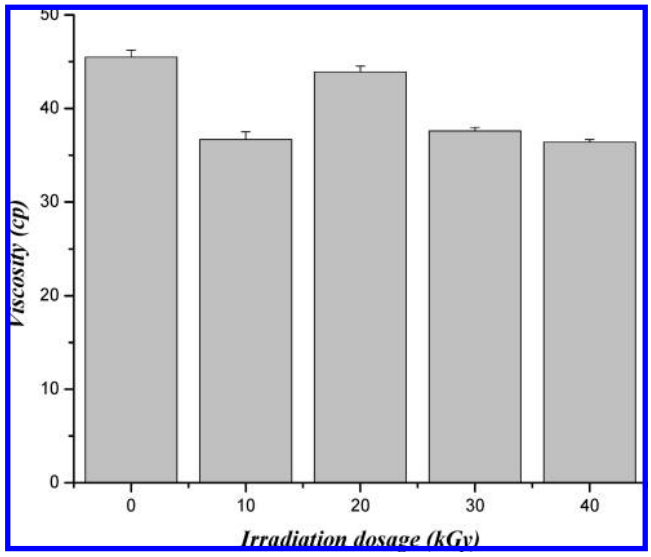

Figure 2. Influence of γ-irradiation treatment on viscosity of zein film-forming solutions.

the lowest applied dose (10 kGy), whereas the zein solution viscosity increased with increasing radiation dose up to 20 kGy and, afterward, it decreased again with a further increase for radiation dosage up to 40 kGy. These results can be explained on the basis of the ability of applied radiation dosage to cause change in aggregate formation and unfolding, scission, or cross-linking of the polypeptide chains of zein. The decline of viscosity at 10 kGy can be interpreted by the formation of smaller agglomerates instead of a large one because of the surface charges of particles by the action of radiation that repel each other. The large aggregates might be attributed to the higher carotenoid content of zein, which promoted hydrophobic interactions and formation of large particles. However, the increase of viscosity at 20 kGy can be probably due to melting of the zein agglomerates and formation of cross-linking bonds between them and between the polypeptide chains, whereas the decline of viscosity at higher radiation doses can be due to breaking of the formed cross-linking bonds and scission of zein molecules. In other words, it can be assumed that the changes in solvent system occurring by γ-irradiation may be responsible for the variation of viscosity at dosages under 20 kGy, but at higher doses, the radiation impact can be mainly upon protein molecules. The structural inversion of zein molecules or aggregates had been suggested by Yamada et al. (*21*) when they observed an odd behavior of zein when films were prepared from two different types of solvent systems.

Effect of Radiation Treatment on Functional Properties of Zein Films. *Optical Properties. (a) Color.* Irradiation treatment of zein film-forming solution with γ-rays exhibited a great effect on the appearance and color of the resultant films, which can be indicated in the presented photographs (**Figure 3**). It appears that the resultant films become smoother and glossier by irradiation. Moreover, the irradiated films had a lighter color in comparison with non-irradiated ones. This can also be indicated from the Hunter color values measurements. Hunter *L*, *a*, and *b* color values of control and irradiated films were compared (**Table 2**). Noticeable effects have been observed for γ-radiation treatment on the color of zein films, which had an intense yellow color but appeared less yellow after radiation treatment (at 10 kGy) as evidenced by lower $+b$ values. It is possible that γ-radiation had a destructive effect on pigments which impart the yellow color to zein. Also, irradiated films had greater *L* values (lighter) and lower $-a$ values (less green) but, again, the differences were small and not visually perceivable at radiation doses above 10 kGy. These results were in good agreement with the previous report that yellowness of films was decreased by UV radiation (*22*).

(b) Light Transmission. As illustrated from the data presented in **Figure 4**, transmission of light through the zein-based film was significantly increased with irradiation at 10 kGy, but at higher irradiation dosage, the effect of irradiation was less pronounced. This can be explained by high destruction of yellow pigments at low applied irradiation dosage (10 kGy).

Mechanical Properties. Results of thickness and surface density reported in **Table 3** indicated that the thickness of the zein resultant film (142 μm) decreased with increasing irradiation dosage, whereas the surface density increased with increasing dosage of irradiation. These changes can be attributed to

Table 2. Influence of γ-Irradiation Treatment on Color of Zein Films[a]

	Hunter color values		
irradiation dosage (kGy)	*L*	*a*	*b*
0	90.43 ± 0.16b	−6.36 ± 0.04a	50.56 ± 0.67a
10	92.96 ± 0.22a	−5.62 ± 0.11b	38.28 ± 1.06b
20	93.08 ± 0.09a	−5.16 ± 0.07c	36.20 ± 0.56b
30	92.84 ± 0.18a	−5.33 ± 0.05bc	37.09 ± 0.43b
40	92.68 ± 0.32a	−5.45 ± 0.03b	36.98 ± 0.78b

[a] Reported values are means of replications ± standard deviation. Different letters in the same column indicate significant ($P \leq 0.05$) difference between property means.

Table 3. Influence of γ-Irradiation Treatment on Physical and Mechanical Properties of Zein Films[a]

irradiation dosage (kGy)	thickness (μm)	surface density (mg/cm^2)	tensile strength (MPa)	elongation (%)
0	142 ± 11a	32.54 ± 0.65c	11.22 ± 0.64a	3.1 ± 0.09b
10	125 ± 19b	39.46 ± 0.43ab	7.73 ± 0.47bc	3.6 ± 0.10a
20	110 ± 14bc	41.76 ± 0.56a	8.86 ± 0.20b	2.5 ± 0.21c
30	107 ± 18c	42.22 ± 0.34a	6.32 ± 0.32c	3.2 ± 0.28b
40	105 ± 15c	42.43 ± 0.49a	5.60 ± 0.37cd	3.1 ± 0.16b

[a] Reported values for each property are means of replications ± standard deviation. Different letters in the same column indicate significant ($P \leq 0.05$) difference between property means.

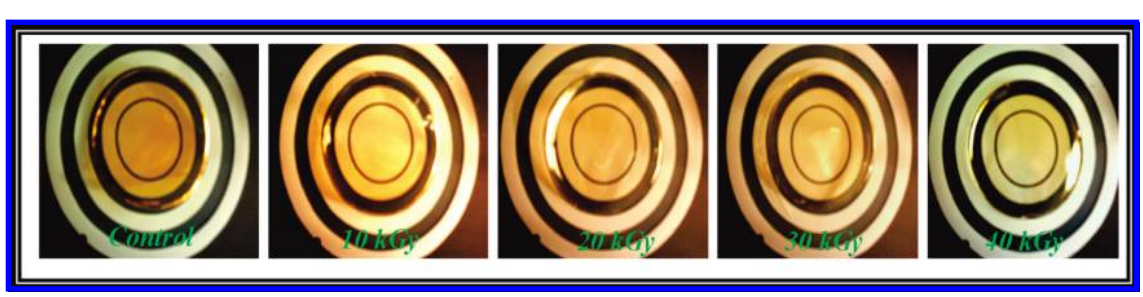

Figure 3. Photographs of non-irradiated and irradiated zein films.

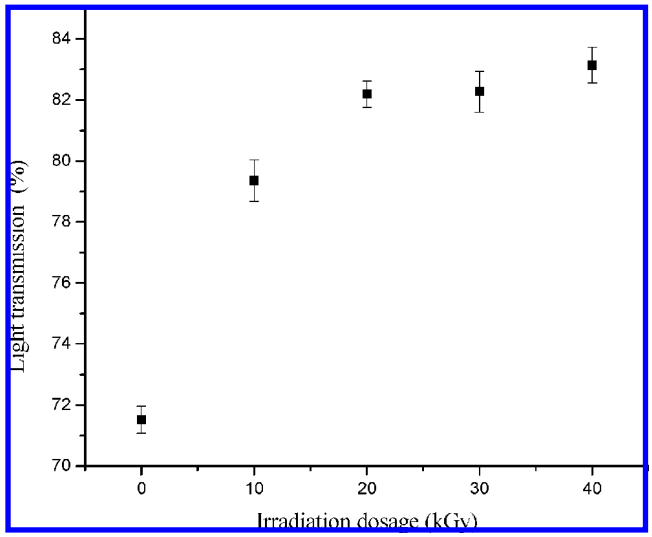

Figure 4. Influence of γ-irradiation treatment on light transmission of zein films.

the changes occurring in aggregates and protein conformation that can be represented in disaggregation and melting of protein particles. Moreover, from the mechanical properties measurements (**Table 3**), it was noted that γ-radiation treatment can led to decreasing tensile strength (TS). However, at a radiation dosage of 20 kGy, a slight increase in TS was observed. In contrast, the elongation of the film increased by about 18% at 10 kGy. Afterward, elongation was slightly decreased at 20 kGy, but a noticeable increase at higher dosages was observed. This is not in agreement with the results showing increases in TS and decreases in *E* of irradiated protein films (*13*, *22*, *23*). Interpretation of the effects of γ-radiation on the mechanical and barrier properties is considered to be an extremely complicated issue because the fundamental interactions occurring under the process and environmental conditions are numerous and interlaced. These interactions can include solution/solid (casting) substrate, air/protein interface, and protein−protein and protein−solvent interactions. All of these interactions variably affect the structure of the resultant films as well as their functional properties. However, the variation of tensile strength of zein films can be simply explained on the basis that decrease of TS is caused by disaggregation (at 10 kGy) and degradation (at >20 kGy) of polypeptide molecules under the experimental condition in this study, whereas at 20 kGy, an increase of TS can be explained on the basis of formation of cross-links between the partially unfolded polypeptide molecules. On the other hand, increase of elongation of films prepared at 10 kGy may be attributed to disaggregation of protein molecules, whereas, increases in *E* at >20 kGy can be a result of the degradation of the polypeptide chains and the breaking of cross-links that can be formed within the film matrix at lower dosage.

(a) Water Uptake. γ-Irradiation treatment of zein film-forming solution exhibited a great effect on water uptake for the resultant films (**Figure 5**). This figure indicates that water uptake values were increased with increasing irradiation dosage except that obtained at 20 kGy, which decreased to be nearly comparable to that of non-irradiated (control) film. These increases in water uptake can be attributed to changes in the protein configuration and loss of water and plasticizer content with increasing irradiation dosage that led to decreased water activity of the resultant films and subsequently increased ability to absorb more water from the surrounding environment. However, decrease of water uptake for the obtained films prepared using 20 kGy can be explained on the basis of cross-linking of polypeptide chains that can cause a

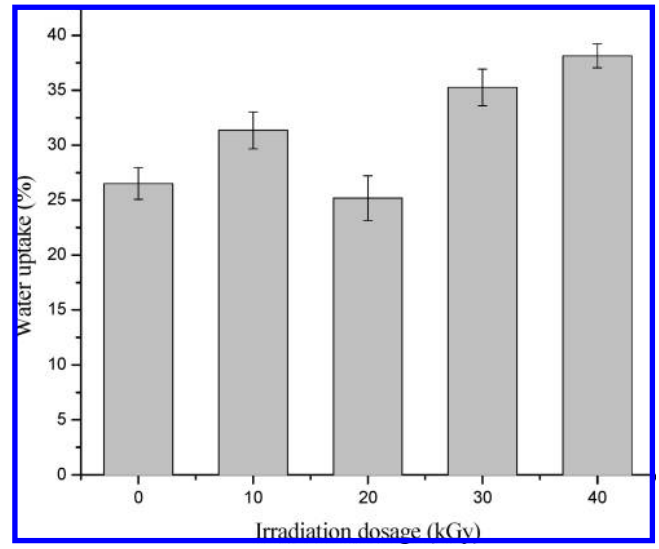

Figure 5. Influence of γ-irradiation treatment on water uptake of zein films.

decrease of hydrophilic functional groups and therefore lower water-binding capacity.

(b) Surface Hydrophobicity. The contact angles of the non-irradiated and irradiated zein films with water were measured (**Figure 6**). It is well-known that the water contact angle will increase with increasing surface hydrophobicity. Protein films similar to polysaccharide films are known for their hydrophilic character, but the zein films exhibited a relatively high contact angle (54.32) because of their high content of hydrophobic amino acids. According to the obtained results, it appears that the water contact angles of films increase with increasing radiation dosage, indicating films become more hydrophobic, which may be due to the loss of plasticizer (degradation or cross-linking) and moisture content (radiolysis) because the hydrophilicity of the samples is mainly attributable to the hygroscopicity (the water binding capacity) of plasticizer. On the other hand, changes in zein molecule conformation occurring by the action of radiation may make the surface of the film more hydrophobic by exposing and orienting the hydrophobic functional groups toward the air side. The results of these measurements indicate that there is a correlation between contact angle and surface microstructure of pure zein films as observed by

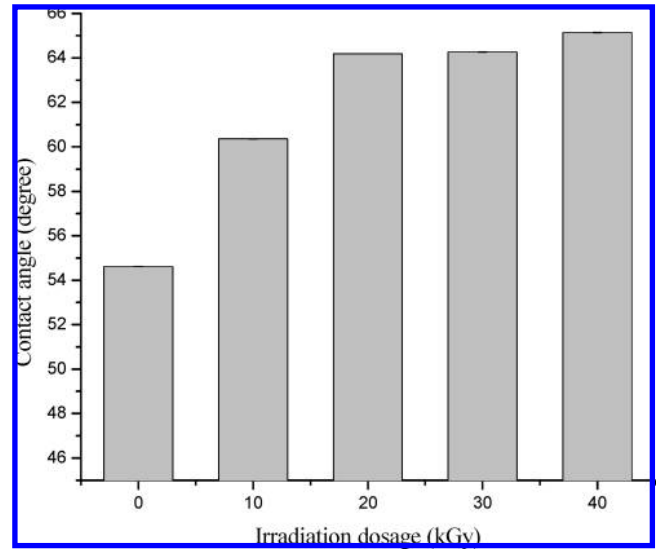

Figure 6. Influence of γ-irradiation treatment on hydrophobicity of zein films.

SEM. In other words, non-irradiated films characterized by uneven surface have a low contact angle (<60°). In irradiated films with a smooth surface, the structure will have a high contact angle.

(c) Water Vapor Permeability. The WVP values of the untreated and γ-irradiated zein films are shown in **Figure 7**. The non-irradiated zein film had the highest WVP compared with films prepared from irradiated solutions. Moreover, the WVP values were decreased with increasing irradiation dosages. This is in good agreement with the results where there was decrease in WVP of irradiated protein films (*13*, *23*). Permeability is influenced by the hydrophobic or hydrophilic nature of the material, by the presence of voids or cracks, and by steric hindrance and tortuosity in the structure. On the other hand, it can be assumed that the formation of high molecular weight proteins aggregated from cleaved polypeptide chains generated by γ-irradiation may be responsible for the reduction of WVP by reducing the absorption of water molecules into the film and the diffusion through the film (*24*). However, this assumption can be interpreted as the reduction of WVP values when it is accompanied with an increase in film strength as reported in the aforementioned studies. Nevertheless, in this study, where the variations in the strength properties of zein film were not in agreement with the results of these studies, the decline of the WVP value with increasing irradiation dosages can be attributed to degradation of plasticizer molecules and lowering moisture content. Water, absorbed by both the zein and its plasticizers, will likely affect the tensile strength and water barrier properties of zein films because water is a very good plasticizer for zein (*10*). Moreover, this decrease may be related to the structural modifications of the protein network (high density), probably due to well chain association and formation of fewer void spaces and cavities in films (less free volume) that can contribute to melting the protein units and forming more flexible and extended forms of protein molecules as indicated before in the study of the structure. When pure zein film is used for food packaging, choosing a treatment that results in a low WVP should be considered.

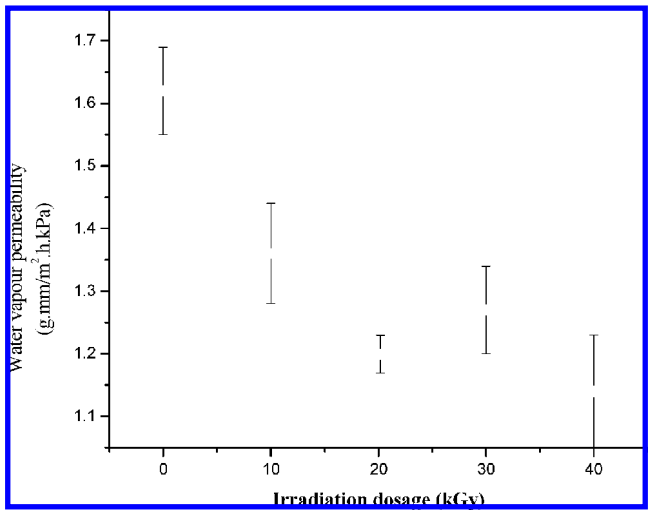

Figure 7. Influence of γ-irradiation treatment on water vapor permeability of zein films.

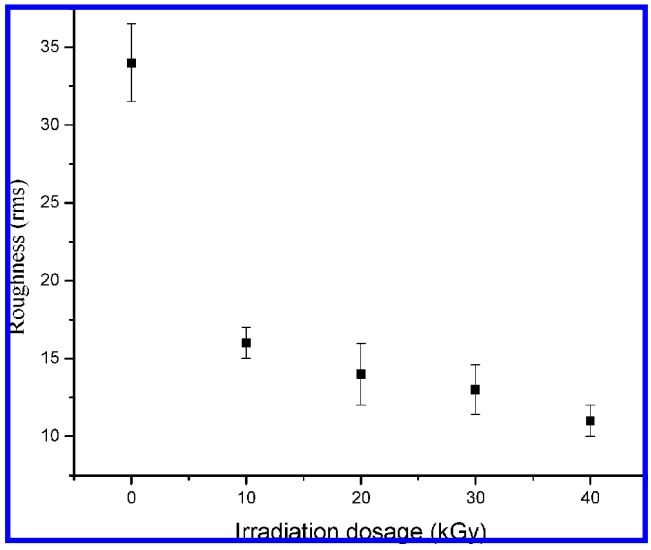

Figure 8. Influence of γ-irradiation treatment on surface roughness of zein films.

Effect of Radiation Treatment on Structural Properties of Zein Films. *Surface Roughness.* The effect of irradiation dosages on surface roughness of zein films is illustrated in **Figure 8**. From this figure, it can be noted that surface roughness of zein films has been greatly reduced with increasing irradiation dosage. However, this effect was more pronounced at irradiation dosage up to 20 kGy than at higher dosages because the roughness of the surface of zein films was decreased by about 65% when irradiation was applied at a dosage of 20 kGy. These results can reflect the changes occurring in protein structure such as aggregate formation and the variance in aggregate size and configuration.

Microstructure. Microstructure observed by scanning electron microscope showed that irradiated soy protein isolate films had a smoother surface than the control film (**Figure 9**). It is in good agreement with Vachon et al. (*3*) that the microstructure of protein films that were cast from irradiated film-forming

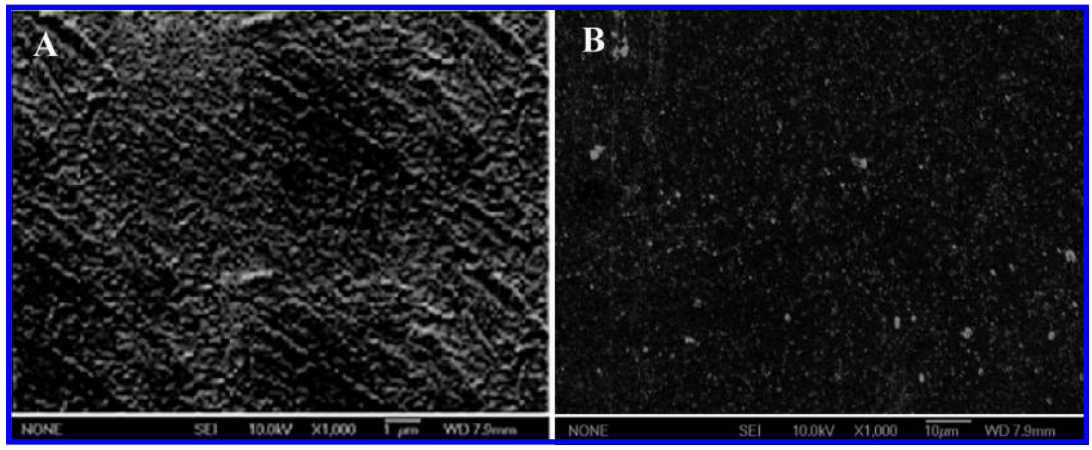

Figure 9. Scanning electron micrographs of non-irradiated (**A**) and irradiated (at 10 kGy) (**B**) zein films.

solutions is smoother and denser than that of the control film. This change in microstructure of the resultant films can be attributed as mentioned before to changes occurring in zein molecule conformation and subsequently led to disaggregation and then melting of the protein particles, forming this dense and smooth structure.

This study showed that γ-irradiation treatment has a potential for modifying the physicochemical properties of zein films, particularly the color, appearance, and water barrier properties. Therefore, γ-irradiation may hold practical significance as a means of improving the functionality of cast zein films. Synergistic effects of γ-treatment with other physical modification methods (plasticization with various plasticizers) on the properties of zein films merit investigation.

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