

Effect of γ -Irradiation on the Physicochemical Properties of Zein Films

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Abstract

To elucidate the effect of gamma-irradiation on the physicochemical properties of zein films, the molecular and mechanical properties of the films were examined after irradiation at various irradiation doses. Gamma-irradiation of zein solutions caused the disruption of the ordered structure of the zein molecules, as well as degradation, cross-linking, and aggregation of the polypeptide chains based on an SDS-PAGE study. Gamma-irradiation increased the solubility of zein and decreased the viscosity due to cleavage of the polypeptide chains. Protein solubility of the zein films in urea/2-mercaptoethanol also increased with increasing irradiation doses. Alterations of the zein molecules by irradiation decreased water vapor permeability by 12% and increased the elongation of zein films. However, mean tensile strength of the zein films was decreased by gamma-irradiation treatment. Measurement of Hunter color values indicated that irradiation caused a destructive effect on yellow pigments, resulting in a significant decrease in Hunter b values. The microstructure as observed by scanning electron microscopy showed that irradiated zein film had a smoother and glossier surface than the non-irradiated films.

Key words: zein film, irradiation, physicochemical properties

INTRODUCTION

Protein films as biodegradable packaging offer environmental compatibility while simultaneously increasing the quality and shelf life of foods (1-3). Protein films can also improve mechanical properties of foods and minimize the loss of volatile flavors and aromas (4). Wheat gluten, corn zein, egg albumin, whey protein, soy protein isolate, and casein have been utilized for their film forming abilities (5). Protein films are good oxygen and carbon dioxide barriers, but are inferior water vapor barriers compared with plastic films (2), since protein films are highly hydrophilic and have a tendency to absorb water (6). To improve functional properties of protein films, cross-linking agents or ionizing radiation have been tried (3,7,8).

Gamma-irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupture of covalent bonds, and formation of protein free radicals (9). 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 (10-12). The hydroxyl and superoxide anion radicals that are generated by radiation of film forming solution can modify the molecular prop-

erties of the proteins, which can result in alteration of the protein films by covalent cross-linkages formed in the protein solution after irradiation (13).

The objectives of this study were to elucidate the effect of γ -irradiation on the physicochemical properties of zein films and to reduce the water vapor permeability and improve the color of the films.

MATERIALS AND METHODS

Materials

Corn zein and standard marker proteins for SDS-PAGE were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Zein was used without further purification.

Sample irradiation

Fifty mL solutions of zein (10%) in 95% ethanol were irradiated at 0, 4, 16, 32, and 50 kGy at room temperature under air using a ⁶⁰Co gamma irradiator (Type IR-79, MDS Nordion International Inc., Ontario, Canada) at Korea Atomic Energy Research Institute. The ⁶⁰Co exposure was varied from 6 to 189 cm in order to achieve total doses of 4~50 kGy. Dose was determined using a ceric-cerous dosimeter; the dose rate was 6.3 kGy/h.

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Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli (14). Equal amounts of the protein samples were loaded on each lane for comparison, resolved on a 12.5% separation gel, and stained with Coomassie Brilliant Blue (Sigma Chemical Co., St. Louis, MO, USA). The following molecular weight markers were used: myosin (203 kDa), β -galactosidase (120 kDa), bovine serum albumin (90 kDa), ovalbumin (51.7 kDa), carbonic anhydrase (34.1 kDa), soybean trypsin inhibitor (28 kDa), lysozyme (20 kDa), and aprotinin (6.4 kDa).

Measurement of solubility

Solubility of zein irradiated at various radiation doses was measured using a Bradford dye-binding assay (15). After evaporating ethanol from the irradiated zein samples, excess zein powders were dissolved in the distilled water at 25°C. The solutions were then centrifuged at $5,000 \times g$ for 20 min and the amount of the dissolved protein was determined from the supernatants.

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 Labs Inc., Stoughton, MA, USA). A No. 0 spindle at 100 rpm was used and ten readings were recorded for each sample and averaged.

Film casting and drying

Zein film-forming solutions were prepared by heating and stirring a mixture of 10% corn zein, 2% glycerol, and 0.2% soybean oil in 100 mL of 95% ethanol. Film-forming solutions were heated in a water bath at 75°C for 20 min and irradiated at 0, 4, 16, 32, and 50 kGy using a ^{60}Co gamma irradiator. Film forming solutions were then strained through cheesecloth and cast on flat, Teflon-coated glass plates (24 cm \times 30 cm). Uniform film thickness was maintained by casting the same amount (70 mL) of the film-forming solution on each plate. Plates were dried at 25°C for 24 h. Dried films were peeled intact from the casting surface. Specimens were cut for measurements of protein solubility, density, water vapor permeability (2 cm \times 2 cm), tensile strength (2.54 cm \times 10 cm), and color (7 cm \times 7 cm).

Protein solubility of zein films

Protein solubility of zein films in urea/2-mercapthanol was determined. Zein films were conditioned in an environmental chamber at 25°C and 50% relative humidity (RH) for 2 days. Film specimens were then transferred into test tubes containing 10 mL of 4 M urea in 50 mM Tris-HCl (pH 8.0) with 0.2 M 2-mercaptoethanol.

The tubes were then shaken at 23°C for 48 h and filtered with Whatman No. 2 filter paper (Whatman International Ltd., Maidstone, England). Protein concentration in the solvent was measured using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA, USA). Bovine serum albumin was used as the standard for protein quantification.

Determination of film thickness

Film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 2 days. Film thickness was measured with a micrometer (Mitutoyo, Tokyo, Japan) at five random positions and the mean value was used.

Measurement of tensile strength and elongation

Film tensile strength (TS) and elongation at break (E) were determined with an Instron Universal Testing Machine (Model 4484, Instron Corp., Canton, MA, USA) according to ASTM Standard Method D882-91 (16). Film specimens were conditioned in an environmental chamber at 25°C and 50% 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.

Measurement of water vapor permeability

Water vapor permeability (WVP) of zein films was determined according to the modified ASTM E 96-95 method (17) at 25°C and 50% RH using a polymethylacrylate cup (18,19). The cup (22 mL) was filled to 1 cm with distilled water and covered with a film specimen. Film specimens were conditioned in an environmental chamber at 25°C and 50% RH for 2 days. Weight loss of the contents of the cups with time was measured. A linear regression analysis was performed to calculate a slope. $\text{WVP} = (\text{WVTR} \cdot L) / \Delta p$, where water vapor transmission rate (WVTR, $\text{g/m}^2 \text{ s}$) was calculated by dividing the slope by the open area of the cup. L is mean thickness (m), and Δp is corrected partial vapor pressure difference (Pa) across the film specimen.

Color measurements

Color values of zein films were measured using a colorimeter (CR-300 Minolta Chroma Meter, Minolta Camera Co., Osaka, Japan). Film specimens (7 cm \times 7 cm) were placed on a white standard plate and the Hunter Lab color was used to measure color: $L = 0$ (black) to $L = 100$ (white); $a = -80$ (greenness) to $a = 100$ (redness); and $b = -80$ (blueness) to $b = 70$ (yellowness). Total

color difference (ΔE) and yellowness index (YI) were calculated from $\Delta E = [(L_{\text{film}} - L_{\text{standard}})^2 + (a_{\text{film}} - a_{\text{standard}})^2 + (b_{\text{film}} - b_{\text{standard}})^2]^{0.5}$, YI = 142.86 b/L, respectively.

Five measurements were taken at different locations on each specimen.

Scanning electron microscopy

Scanning electron microscopy (SEM) was used to characterize the microstructure of the zein films. Zein films were made conductive by sputter-coating with a gold-palladium alloy coater (Baltec Company, Manchester, NH, USA). The coated films were dried in a desiccator. Samples were then examined using a scanning electron microscope (XL30 ESEM, Phillips, Boston, MA, USA) at an accelerating electron voltage of 15 kV. Micrographs for sample surface were obtained at 500 \times magnifications.

Statistical analysis

Analysis of variance and Duncan's multiple range tests with $p \geq 0.05$ were performed to analyze the results statistically using a SAS program (1999, SAS Institute, Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Fig. 1 shows the SDS-PAGE profile of zein solutions irradiated at various radiation doses. The major component of corn zein is α -zein, which has molecular weights of about 20 kDa and 24 kDa on SDS-PAGE (Fig. 1). Parris and Dickey (20) reported that zein isolated by extraction with 95% ethanol is primarily composed of α -zein (22 kDa and 24 kDa), and their dimer and tetramer. Our results are in good agreement with their report. SDS-PAGE profiles of the irradiated zein solutions showed that γ -irradiation at low doses causes a slight breakdown of the polypeptide chain with a concurrent decrease of a major band intensity, even with the loading of the same amount of the protein (Fig. 1). Similar results were observed in other studies (12,21). At high dose ranges, 32 and 50 kGy, there were aggregated products of the degraded protein molecules that could not penetrate the running gel. Generally, two types of radiation damage to proteins were observed—fragmen-

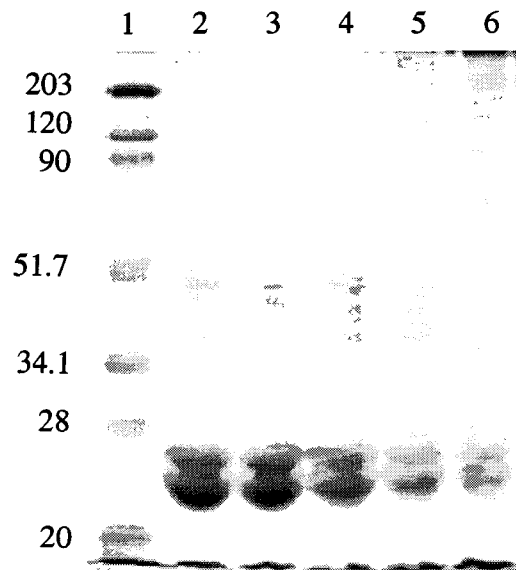


Fig. 1. SDS-PAGE profile of irradiated zein protein solutions. Lane 1, marker proteins; 2, 0 kGy; 3, 4 kGy; 4, 16 kGy; 5, 32 kGy; 6, 50 kGy.

tation and aggregation (12,22). Proteins can be converted to higher molecular weight aggregates, due to the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions, as well as the formation of disulfide bonds (23). Any amino acid radical that is formed within a peptide chain could cross-link with an amino acid radical in another protein. The formation of high molecular weight aggregates was negligible at low-doses, but increased significantly at higher doses.

Gamma-irradiation treatment of zein solutions also affected the solubility of the proteins (Table 1). Irradiation increased solubility significantly due to cleavage of polypeptide chains by oxygen radicals generated in the radiolysis of water (24). Irradiation at 50 kGy increased its solubility by 7.4 times, compared with the control. Gamma-irradiation also decreased the viscosity (Table 1). The increased solubility and decreased viscosity can be explained by increased number of cleavage products of polypeptide chains. Viscosity value was 2.26 cP at 4 kGy, compared with 3.34 cP of the control. However, the viscosity values above 16 kGy were not significantly altered. Protein solubility of zein film in

Table 1. Effect of gamma-irradiation treatment on solubility ($\mu\text{g/mL}$) and viscosity (cP) of zein

	Irradiation dose (kGy)				
	0	4	16	32	50
Solubility in water ¹⁾	13.78 \pm 0.69	22.91 \pm 0.03	28.52 \pm 0.78	86.73 \pm 0.00	102.40 \pm 0.75
Solubility in 4 M urea/2-mercaptoethanol ²⁾	92.17 \pm 0.45	97.07 \pm 0.15	103.58 \pm 1.50	116.05 \pm 0.45	117.44 \pm 2.41
Viscosity ³⁾	3.34 \pm 0.02	2.26 \pm 0.01	2.25 \pm 0.01	2.24 \pm 0.01	2.23 \pm 0.01

^{1),2)}Data are averages of 5 measurements.

³⁾Data are averages of 10 measurements.

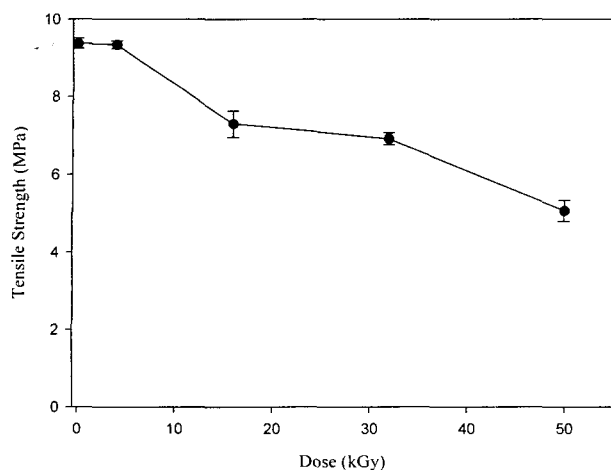


Fig. 2. Effect of gamma-irradiation treatment on tensile strength (MPa) of zein films.

urea/2-mercaptoethanol also increased with increasing irradiation dose (Table 1). Protein solubility values were 92.17 and 117.44 $\mu\text{g}/\text{mL}$ at 0 and 50 kGy, respectively.

Film thickness was measured to be 121 μm using a micrometer. Film thickness values were not significantly different among treatment groups since the same amount of film forming solution was used. Mean tensile strength (TS) of zein films was decreased by gamma-irradiation treatment (Fig. 2). TS value was 5.06 MPa at 50 kGy, compared with 9.37 MPa of the control. It is apparent that the decrease of TS is caused by the increase in cleavage products of polypeptide chains under the experimental condition in this study. These results are in good comparison with the results of other investigators who also reported increases in tensile strength of irradiated protein films (7,25). However, % elongation increased with increasing irradiation dose (Fig. 3). Percent elongation at 50 kGy was 15.43, which was an 81% increase compared with the 8.53 of the control. In general,

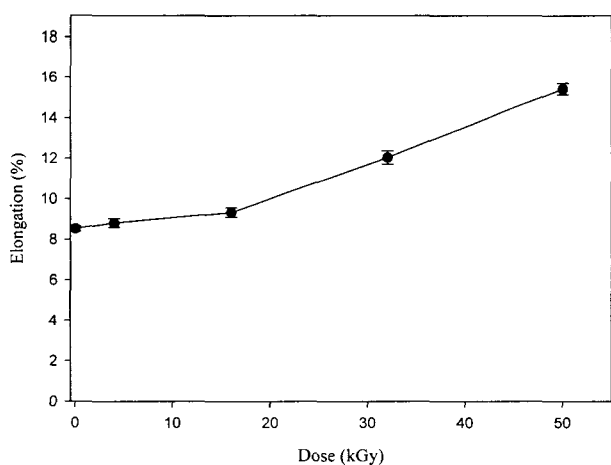


Fig. 3. Effect of gamma-irradiation treatment on % elongation of zein films.

decreases in tensile strength accompany increases in the percentage of elongation.

Protein films usually have poor WVP. Therefore, to improve water-resistance properties of zein films, cross-linking agents have been used (7). Gamma-irradiation treatment was used as a cross-linking method in this study. Water vapor permeability of zein films was significantly decreased when irradiated (Fig. 4). At 50 kGy, it was decreased by 12%, compared with non-irradiated film. These results show that treatment with gamma-irradiation reduces the WVP. It can be assumed that the formation of high molecular weight protein aggregates from cleaved polypeptide chains generated by gamma-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 (16). Ouattara et al. (26) reported that gamma-irradiation cross-linking of milk protein films improved the WVP and induced an increase of high molecular weight protein components in film forming solutions. However, UV-treatment did not affect the WVP of zein films (8).

Corn zein films have a distinct yellowish color, which is one of their drawbacks. Measurements of Hunter L, a, and b color values of gamma-irradiated zein films were compared (Table 2). Increase of irradiation increased Hunter a color values. However, gamma-irradiation had a destructive effect on yellow pigments, resulting in a significant decrease in Hunter b values. Hunter b value at 50 kGy was 10.598, compared with 27.516 of the non-irradiated film. It is evident that gamma-irradiation treatment decreased the yellow color of zein film. Furthermore, color difference (ΔE) and yellow index (YI) were significantly decreased with increasing doses of irradiation, which is in good agreement with the results of UV-irradiated zein films reported by others (8).

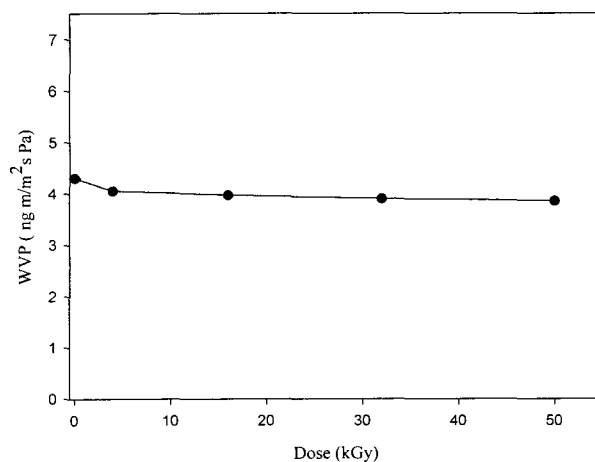


Fig. 4. Effect of gamma-irradiation treatment on water vapor permeability of zein films.

Table 2. Hunter color values of irradiated zein films

	Irradiation dose (kGy)				
	0	4	16	32	50
L	93.51 \pm 0.56 ^c	94.22 \pm 0.16 ^b	94.51 \pm 0.10 ^{ab}	94.60 \pm 0.16 ^{ab}	94.65 \pm 0.10 ^a
a	-4.87 \pm 0.09 ^d	-2.58 \pm 0.04 ^c	-1.88 \pm 0.03 ^b	-1.77 \pm 0.02 ^a	-1.75 \pm 0.05 ^a
b	27.52 \pm 0.95 ^a	15.82 \pm 0.24 ^b	11.47 \pm 0.99 ^c	10.63 \pm 0.17 ^d	10.60 \pm 0.10 ^d
ΔE	26.21 \pm 0.89 ^a	14.32 \pm 0.26 ^b	9.94 \pm 0.21 ^c	9.11 \pm 0.14 ^d	9.06 \pm 0.10 ^d
YI	42.03 \pm 1.28 ^a	23.99 \pm 0.40 ^b	17.34 \pm 0.32 ^c	16.05 \pm 0.24 ^d	15.98 \pm 0.15 ^d

^{a-d}Means of five replicates \pm standard deviations. Any figures in the same column with the same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

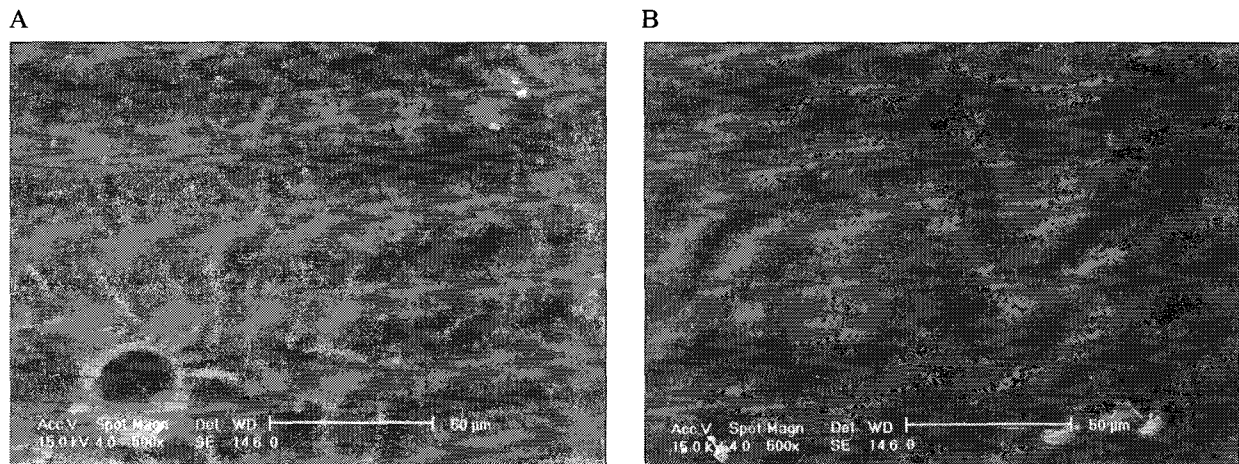


Fig. 5. Microstructure of irradiated zein films (500 \times magnification). A: Non-irradiated zein film, B: Zein film irradiated at 50 kGy.

Microstructure observed by scanning electron microscopy showed that irradiated zein film had a denser, smoother and glossier surface than the non-irradiated film (Fig. 5). These results are in good agreement with earlier reports (3) that the microstructure of protein films cast from irradiated film forming solutions is denser than that of the control.

In conclusion, this study clearly indicates that γ -irradiation of zein film forming solutions can alter the physicochemical properties. Gamma-irradiation treatment of the zein solutions caused the disruption of the ordered structure of the protein molecules, as well as degradation, cross-linking, and aggregation of the polypeptide chains, resulting in change in water vapor permeability as well as decreases in yellowish color.

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