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Effect of gamma-irradiation on the physicochemical properties of gluten films

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Abstract

To elucidate the effect of gamma-irradiation on the physicochemical properties of gluten films, the molecular and mechanical properties of the films were examined after irradiation at 0, 4, 16, 32, and 50 kGy. Gamma-irradiation of gluten solutions caused disruption of the ordered structure of the gluten molecules, as well as degradation and aggregation of the polypeptide chains, based on a sodium dodecyl sulfate–polyacrylamide gel electrophoresis result. Gamma-irradiation decreased the viscosity of film solution due to the cleavage of the polypeptide chains below 16 kGy and increased it by aggregation of the proteins above 32 kGy. Tensile strength of the gluten films was affected by the gamma-irradiation treatment, resulting in 1.5-fold increase at 50 kGy. The elongation of gluten films was decreased. Alteration of the gluten molecules by gamma-irradiation reduced water vapour permeability by 29%.

Keywords: Gamma-irradiation; Gluten film; Physicochemical properties

1. Introduction

Protein films offer environmental compatibility as well as higher quality and longer shelf life of foods than non-biodegradable packaging (Chen, 1995; Krochta & De Mulder-Johnston, 1997; Vachon et al., 2000). Protein films can also improve mechanical properties of foods and minimize the loss of volatile flavours and aromas (McHugh & Krochta, 1994). Wheat gluten, corn zein, egg albumin, whey protein, soy protein isolate, and casein have been utilized for their film-forming abilities (Esen, 1990). Protein films are good oxygen and carbon dioxide barriers, yet inferior water vapour barriers compared to plastic films (Krochta & De Mulder-Johnston, 1997), since protein films are highly hydrophilic without modification (Fu & Weller, 1999).

Wheat gluten protein has high potential as a raw material for technical applications such as protein films (De Graaf, Kolster, & Vereijken, 1997). The obtained transparent and flexible films can be used in the food industry. Wheat gluten films may have unique cohesive and elastic properties (Graveland, 1998), but their very high water sensitivity and permeability constitute a hurdle to commercial application (Gontard, Duchez, Cuq, & Guilbert, 1994).

Protein films usually have poor WVP. Gluten films have been reported to be ineffective moisture barriers due to their hydrophilic nature (Gennadios, Brandenburg, Weller, & Testin, 1993). Therefore, to improve water-resistance properties of gluten films, cross-linking agents or ionizing radiation have been tried (Rhim, Gennadios, Fu, Weller, & Hanna, 1999; Vachon et al., 2000; Yamada, Takahahi, & Noguchi, 1995). As ionizing radiation, gamma-irradiation affects

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proteins by causing conformational changes, oxidation of amino acids, rupture of covalent bonds, and formation of protein free radicals (Cheftel, Cuq, & Lorient, 1985). Chemical changes in the proteins caused by gamma-irradiation include fragmentation, cross-linking, aggregation and oxidation by oxygen radicals that are generated in the radiolysis of water (Cho & Song, 2000; Filali-Mouhim et al., 1997; Schuessler & Schilling, 1984). 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 (Garrison, 1987).

There is a considerable interest in edible gluten films as a potential novel form of packaging. Therefore, the objective of this study was to elucidate the effect of gamma-irradiation on the physicochemical properties of gluten films.

2. Materials and methods

2.1. Materials

Wheat gluten was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Glycerol was purchased from Aldrich chemical Co. (St. Louis, MO, USA). Standard marker proteins for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) were obtained from Bio-Rad Inc. (Richmond, CA, USA).

2.2. Preparation of film-forming solution and gamma-irradiation

A wheat gluten film-forming solution was prepared according to the method of Gontard, Guilbert, and Cuq (1992). For 100 ml of film-forming solution, 10 g wheat gluten, 45 ml ethanol, 3 g glycerol, and the pH 4.0 adjusted with acetic acid were used. Gluten powder was mixed with 30 mg sodium sulfite as a reducing agent, and ethanol before addition of water. As a plasticizer, glycerol was then added. Film-forming solutions were conditioned in a water bath at 70 °C for 20 min and centrifuged at 540*g* for 30 min. Centrifugation removed insoluble gluten, resulting in improvement of film clarity. The film-forming solutions were then irradiated at 0, 4, 16, 32, and 50 kGy at room temperature under air using a ^{60}Co gamma irradiator Type IR-79 (MDS Nordion, Kanata, ON, Canada). The treatment of ^{60}Co 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.

2.3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed according to the method of Laemmli (1970). 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. 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).

2.4. Measurement of viscosity

Viscosity of gluten 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). No. 0 spindle at 10 rpm was used and 10 replicates were performed for each sample.

2.5. Film casting and drying

After irradiation, film-forming solutions were strained through 4-fold cheese cloth and cast on flat, Teflon-coated glass plates (24 cm × 30 cm). Uniform film thickness was maintained by casting the same amount (70 ml) of film-forming solution on each plate. Plates were dried at 25 °C for 24 h. Dried films were peeled intact from the casting surface.

2.6. Determination of film thickness

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

2.7. Measurement of tensile strength and elongation

Film tensile strengths (TS) and elongations at break (*E*) were determined with an Instron Universal Testing Machine (Model 4484, Instron Corp., Canton, MA, USA) according to ASTM Standard Method D 882–91 (1995). Film specimens (2.54 cm × 10 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.

2.8. Measurement of water vapour permeability

Water vapour permeability (WVP) of gluten films was determined according to the modified ASTM E 96–95 method (1995) at 25 ± 1 °C and 50 ± 4% RH using polymethylacrylate cup (Park & Manjeet, 1995; Ryu, Rhim, Roh, & Kim, 2002). The cup was filled to 1 cm with distilled water and covered with a film specimen. Film specimens (2 cm × 2 cm) were conditioned in an environmental chamber at 25 ± 1 °C and 50 ± 4% RH for 2 days. Weight loss of cups with time was measured. A linear regression analysis was performed to calculate a slope. WVP (ng m/m^{2}s Pa) values were then calculated from

$$\mathrm{WVP} = (\mathrm{WVTR} \times L)/\Delta p,$$

where water vapour transmission rate (WVTR, g/m^{2}s) was calculated by dividing the slope by the open area of the cup, L is mean thickness (m), and Δp is corrected partial vapour pressure difference (Pa) across the film specimen.

2.9. Statistical analysis

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

3. Results and discussion

Fig. 1 shows the SDS–PAGE profile of gluten film-forming solutions irradiated at various radiation doses. Wheat gluten had gliadin bands similar to those reported by Lookhart and Albers (1988). Gliadin is assumed to be the major contributor to the gluten film formation. SDS–PAGE profiles of the irradiated gluten solutions showed that gamma-irradiation, at low dose ranges, caused a slight breakdown of the polypeptide chain with a concurrent decrease of a major band intensity under loading of the same amount of the protein (Fig. 1). Similar results were observed in other studies (Cho & Song, 2000; Le Maire et al., 1990). At high dose ranges, above 16 kGy, there were cross-linked products of the degraded protein molecules that could not penetrate the running gel. Generally, two types of radiation damage to proteins were observed – fragmentation and aggregation (Cho & Song, 2000; Lee & Song, 2002). Proteins can be converted to higher molecular weight aggregates, due to the generation of inter-protein

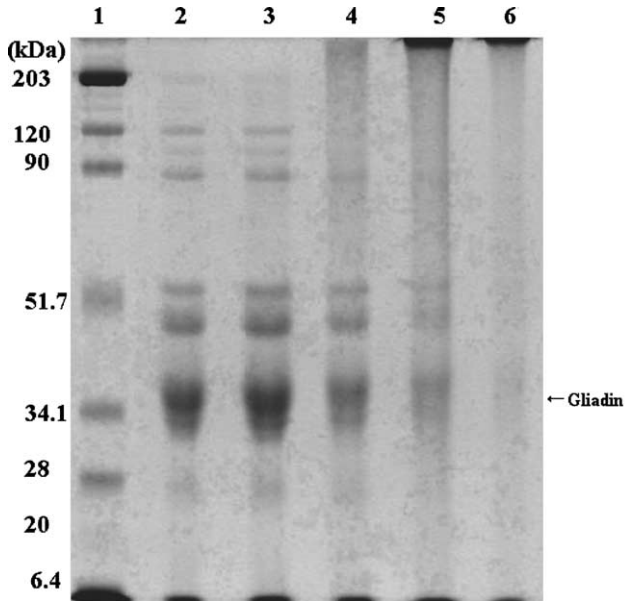

Fig. 1. SDS–PAGE profile of irradiated gluten film-forming solutions. Lane 1, marker proteins; 2, 0 kGy; 3, 4 kGy; 4, 16 kGy; 5, 32 kGy; 6, 50 kGy.

cross-linking reactions, hydrophobic and electrostatic interactions, as well as the formation of disulfide bonds (Davies & Delsignore, 1987). 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-dose range, but increased significantly at higher doses.

The gamma-irradiation treatment of gluten solutions also affected the viscosity of the film-forming solutions (Fig. 2). The viscosity of irradiated gluten solution decreased upto 16 kGy but increased over 32 kGy. The change in viscosity was in good agreement with

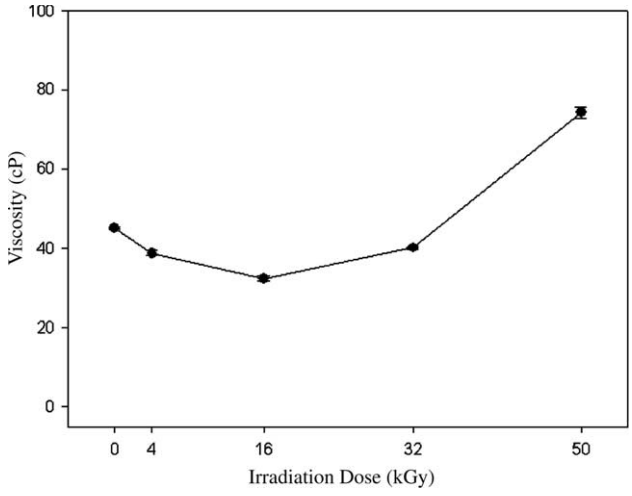

Fig. 2. Effect of gamma-irradiation treatment on viscosity of gluten film-forming solutions.

SDS–PAGE results, implying that cross-linked proteins increased above 32 kGy.

Film thickness was determined to be 92 μm by using a micrometer. Film thickness values were not significantly different among treatments.

Mean tensile strengths (TS) of gluten films were increased by gamma-irradiation treatment (Fig. 3). TS value was 3.99 MPa at 50 kGy, compared with 2.68 MPa of the control. Increased TS of gluten films suggests that cross-linking occurred as a result of gamma-irradiation treatment. Increase of TS was possibly caused by the increase of aggregation of polypeptide chains under the experimental condition in this study. It is in good agreement with the finding that there were increases in tensile strength of irradiated protein films (Parris & Coffin, 1997; Yamada et al., 1995).

Percent elongation decreased with increase of irradiation dose (Fig. 4). Percent elongation (E) at 50 kGy was

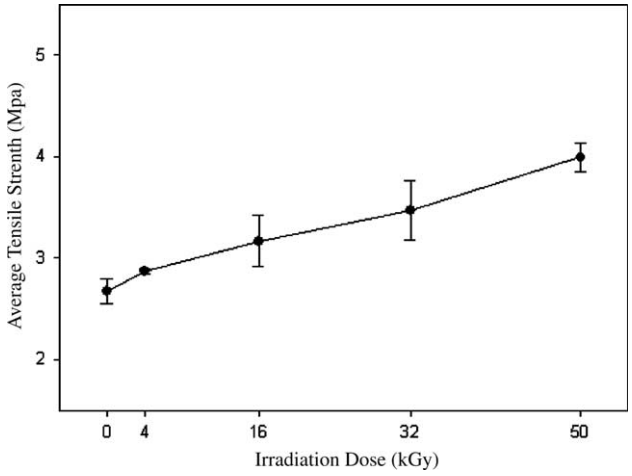

Fig. 3. Effect of gamma-irradiation treatment on tensile strength of gluten films.

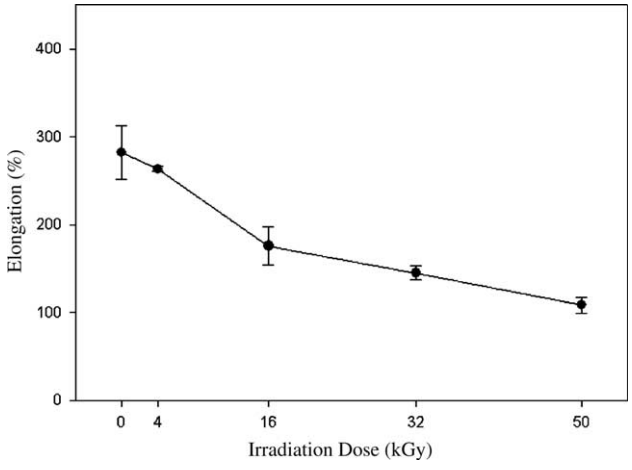

Fig. 4. Effect of gamma-irradiation treatment on elongation of gluten films.

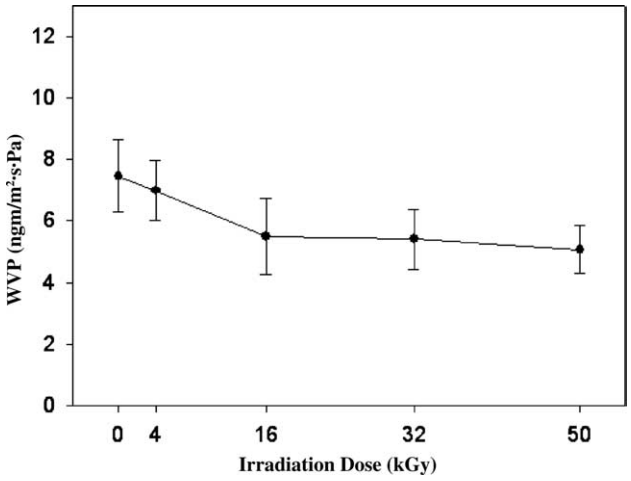

Fig. 5. Effect of gamma-irradiation treatment on water vapour permeability of gluten films.

108, compared to 282 of the control. Increased TS and decreased E have been reported for gluten films cross-linked by UV radiation (Micard, Belamri, Morel, & Guilbert, 2000).

Gluten films have been reported to be ineffective moisture barriers due to their hydrophilic nature (Gennadios et al., 1993). Therefore, to improve water-resistance properties of gluten films, gamma-irradiation treatment was used as a cross-linking agent in this study. Water vapour permeability of gluten films decreased significantly when irradiated (Fig. 5). At 50 kGy, it was decreased by 29%, compared to unirradiated samples. These results showed that gamma-irradiation treatment significantly reduced the WVP. It can be assumed that the formation of high molecular weight proteins, aggregated from cleaved polypeptide chains by gamma-irradiation, may be responsible for the reduction of WVP, by reducing the rate of diffusion through the film (Ouattara, Canh, Vachon, Mateescu, & Lacroix, 2002).

In conclusion, molecular properties of gluten film-forming solutions were altered by gamma-irradiation. The gamma-irradiation treatment of the gluten solutions caused the disruption of ordered structure of the protein molecules, changing TS, percent elongation and WVP. Gamma-irradiation may be a useful tool as a cross-linking agent to improve functional properties of gluten films.

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