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Effect of gamma-irradiation on the physicochemical properties of porcine and bovine blood plasma proteins

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Abstract

To elucidate the effect of γ-irradiation on the physicochemical properties of blood plasma proteins, bovine and porcine blood, released from a slaughterhouse, was collected and plasma proteins were prepared. Physicochemical properties of blood plasma protein powders and solutions, such as molecular weight distribution, secondary structure, solubility, and viscosity, were examined after γ-irradiation at 1, 5, 7, and 10 kGy. Oxygen radicals, such as hydroxyl and superoxide anion radicals, generated by γ-irradiation, affected the physicochemical properties of the blood plasma protein solutions, but not the plasma protein powders. Circular dichroism and sodium dodecyl sulphate–polyacrylamide gel electrophoresis studies showed that an increase of γ-irradiation decreased the ordered structure of plasma protein solutions and caused initial fragmentation of the polypeptide chains and subsequent aggregation. However, solubility and viscosity of the irradiated plasma protein powders, as well as secondary structure and molecular weight profile, were not changed significantly with radiation dose.

Keywords: Circular dichroism; Irradiation; Physicochemical properties; Plasma proteins

1. Introduction

Radiation treatments of biological materials have been applied to food production as a non-thermal process for reducing microbial contamination and extending the shelf life of food products (Foley, Dufour, Rodriguez, Caporaso, & Prakash, 2002; Mahrour, Lacroix, Nketsa-Tabiri, Calderon, & Gagnon, 1998). However, irradiation also induces chemical changes in biopolymers, such as proteins, including: fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals generated by the radiolysis of water (Davies & Delsignore, 1987; Filali-Mouhim et al., 1997; Garrison, 1987; Schuessler & Schilling, 1984). Hydroxyl and superoxide anion radicals generated by γ-irradiation, could modify primary structure of proteins, resulting in distortions of secondary and tertiary structures (Davies & Delsignore, 1987). The effect of γ-irradiation on protein conformation appears to depend on several factors, such as protein concentration, the presence of oxygen and the quaternary structure of proteins.

In general, radiation causes irreversible changes at the molecular level by breakage of covalent bonds of polypeptide chains. Exposure of proteins to oxygen radicals results in both non-random and random fragmentation (Kempner, 1993). In Schuessler and Schilling's model (Schuessler & Schilling, 1984), bovine serum albumin (BSA) is cleaved by oxidative destruction of proline residues, yielding specific protein fragments. Also, there have been reports of aggregation and cross-linking of proteins caused by irradiation (Filali-Mouhim et al., 1997; Garrison, 1987; Kume & Matsuda, 1995; Puchala & Schuessler, 1993).

Blood released from slaughterhouses is not efficiently utilized and is mostly discarded without proper disposal treatment, which may cause pollution of drinking water in developing countries. In Korea, bovine blood is processed as Sunji or Soondae, which are added and mixed with soup or snacks. However, its processing is not well controlled in terms of sanitation. Therefore, to prevent microbial contamination of bovine and porcine blood, γ-irradiation treatment could be used to extend the shelf life of dried blood plasma protein products (Hayashi,

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Biagio, Saito, Todoroko, & Tajima, 1991). Also, considering that the plasma proteins are nutritionally excellent and have good functional properties, such as emulsifying capacity (Hayashi et al., 1991), their use in food processing has many merits. Also, preparation of plasma protein hydrolysates could be a good way to utilize them as food ingredients. Therefore, the objectives of this study were to examine the gamma-irradiation processing of plasma proteins as a sanitizing method and to investigate its effect on the physicochemical properties of the plasma proteins.

2. Materials and methods

2.1. Materials

2.1.1. Sample preparation

After collecting the bovine and porcine blood from a slaughterhouse, ethylenediaminetetraacetic acid (EDTA, 2 g/l) was immediately added to prevent coagulation and the mixture centrifuged at 11 590×*g* for 30 min to remove blood cells. To the supernatant, 2% trichloroacetic acid (TCA) was added to precipitate the plasma protein. The plasma protein was then freeze-dried.

2.1.2. Sample irradiation

Bovine and porcine plasma protein powder samples (50 g) wrapped with polyethylene films and solutions of proteins (0.5%) in 20 mM phosphate buffer (pH 7.0) in borosilicate glass vials (16×125 mm) were irradiated at room temperature using a ^{60}Co gamma ray irradiator (Type IR-79, Nordion International Inc., Ontario, Canada) with 1, 5, 7, and 10 kGy. ^{60}Co exposure was varied from 6 to 189 cm in order to achieve total doses of 1–10 kGy and the dose rates were 1, 5, 7, and 10 kGy/h.

2.2. Methods

2.2.1. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed according to the method of Laemmli (1970). Protein samples for SDS–PAGE were prepared by mixing with sample buffer (60 mM Tris–HCl, 2% SDS, 14.4 mM β-mercaptoethanol, 25% glycerol, 0.1% bromophenol blue, pH 6.8). Proteins were resolved on a 7.5% separation gel and stained with Coomassie Brilliant Blue. The following standard marker proteins were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used: rabbit muscle myosin (205 kDa), *E. coli* β-galactosidase (116 kDa), rabbit muscle phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa), and bovine erythrocyte carbonic anhydrase (29 kDa).

2.2.2. Circular dichroism (CD) measurements

CD spectroscopy was performed at 25 °C with a JASCO-720 spectropolarimeter according to the method reported previously (Cho & Song, 1997; Lee & Song, 1997). A 1-mm-pathlength cell was used. Sensitivity of 10 mdeg was used. The reported CD spectra were averages of 5 scans, and were smoothed using a polynomial curve-fitting programme. CD data were expressed as molar ellipticities in deg cm^2 dmol^{-1}.

2.2.3. Measurement of solubility

Solubility of plasma protein powder, irradiated at various radiation doses, was measured by determining protein concentration using the Bradford (1976) dye-binding assay. Excess bovine and porcine blood plasma protein powders were dissolved in 30 ml of phosphate buffer (pH 7.0) at 25 °C. The solutions were then centrifuged at 890×*g* for 40 min and supernatants were collected and the amount of dissolved protein was measured according to Bradford's assay.

2.2.4. Measurement of viscosity

Viscosity of solutions of plasma protein powders, irradiated at various radiation doses, was measured using a Brookfield viscometer. Bovine and porcine blood plasma protein powders (1%) were dissolved in 30ml of phosphate buffer (pH 7.0) at 4 °C. Viscosity was measured at 4 °C using a Brookfield viscometer (Model DV-1, Brookfield Engineering Labs Inc., Stoughton, USA). No. 0 spindle at 100 rpm was used and ten readings were recorded for each sample.

3. Results and discussion

3.1. Effect of γ-irradiation on the molecular weight distribution of plasma proteins

SDS–PAGE shows the molecular weight profile of the proteins. The major component of bovine and porcine plasma proteins is albumin (66 kDa) on SDS–PAGE (Fig. 1). It has been known that blood plasma protein mainly consists of albumin and globulin fractions (Park et al., 1996; Raeker & Johnson, 1995) and their ratio is dependent on species and other conditions, such as age and health condition of animals. Bovine and porcine plasma proteins had very similar SDS–PAGE patterns (Fig. 1). There were two types of patterns of the effect of γ-irradiation on SDS–PAGE profile of plasma proteins observed. For plasma protein powders irradiated, there was no significant change in terms of molecular weight profile pattern (Fig. 1), indicating that there was not much degradation or aggregation of protein molecules in the solid form. This can be explained by lack of oxygen radicals generated by radiolysis of water. This was also observed in our previous studies for soy protein

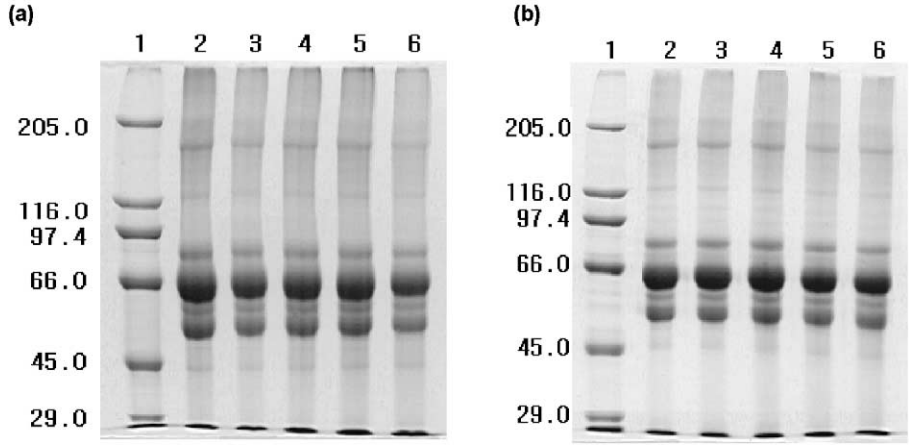

Fig. 1. SDS–PAGE profile of bovine (a) and porcine (b) irradiated plasma protein powders. Lane 1, marker proteins; 2, 0 kGy; 3, 1 kGy; 4, 5 kGy; 5, 7 kGy; 6, 10 kGy.

isolate and whey protein concentrate (Cho & Song, 1999). However, in the case of plasma protein solution, significant change was observed (Fig. 2). This is easily explained, since hydroxyl and superoxide anion radicals, generated by radiolysis of water, could modify the primary structure of proteins. Two types of radiation damage to proteins can be observed: fragmentation and aggregation (Filali-Mouhim et al., 1997). SDS–PAGE profiles of plasma proteins show that γ-irradiation, at a low dose of 1 kGy, causes breakdown of the polypeptide chain and, as a result, decrease of intensity of an albumin band under the same amount of loading of the protein (Fig. 2). Similar results were observed in other studies (Cho, Yang, & Song, 1999; Le Maire, Thauvette, De Foresta, Viel, Beauregard, & Potier, 1990; Schuessler & Schilling, 1984). Schuessler and Schilling (1984) proposed that proline residues were the targets for chain scission caused by radiation. Wolff, Garner, and Dean (1986) reported that the peptide bond could be cleaved as a result of direct oxidation of proline residues. However, on the SDS–PAGE gel, at above 5 kGy of dose, there were only smeared degraded patterns with aggregated protein molecules which could not penetrate the separating gel when the same amount of the protein was loaded on each lane (Fig. 2). Proteins may be converted to higher-molecular-weight aggregates due to the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions (Cho et al., 1999; Davies & Delsignore, 1987; Le Maire et al., 1990). Any amino acid radical formed within a peptide chain could cross-link with an amino acid radical in another protein. At high doses, proteins exposed to radiation underwent

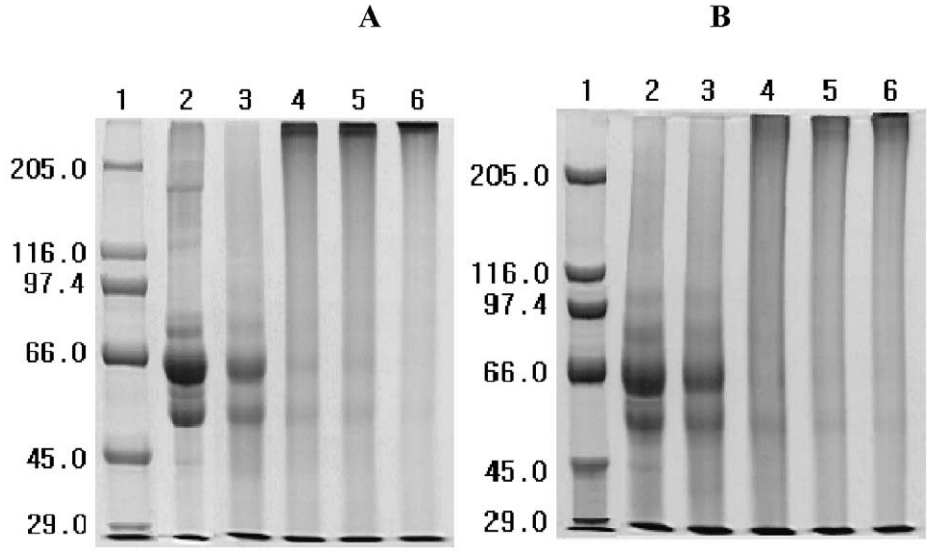

Fig. 2. SDS–PAGE profile of bovine (A) and porcine (B) irradiated plasma protein solutions. Lane 1, marker proteins; 2, 0 kGy; 3, 1 kGy; 4, 5 kGy; 5, 7 kGy; 6, 10 kGy.

covalent cross-linking. The formation of the high molecular weight aggregates was negligible at low doses, but increased significantly with higher doses (Fig. 2). Similar results were previously reported for egg white lysozyme and BSA (Schuessler & Schilling, 1984; Stevens, Sauberlich, & Bergstrom, 1967). To avoid aggregation of proteins at high doses, oxygen radical scavengers, such as ascorbic acid, can be used (Moon & Song, 2001).

3.2. Effect of γ-irradiation on the secondary structure of plasma protein solutions

Far-UV CD spectra show the conformational change of the secondary structure of proteins. Especially if there is a change in the local environment of ordered structure of a polypeptide chain, it is easily differentiated from the native state. Far-UV CD spectra of

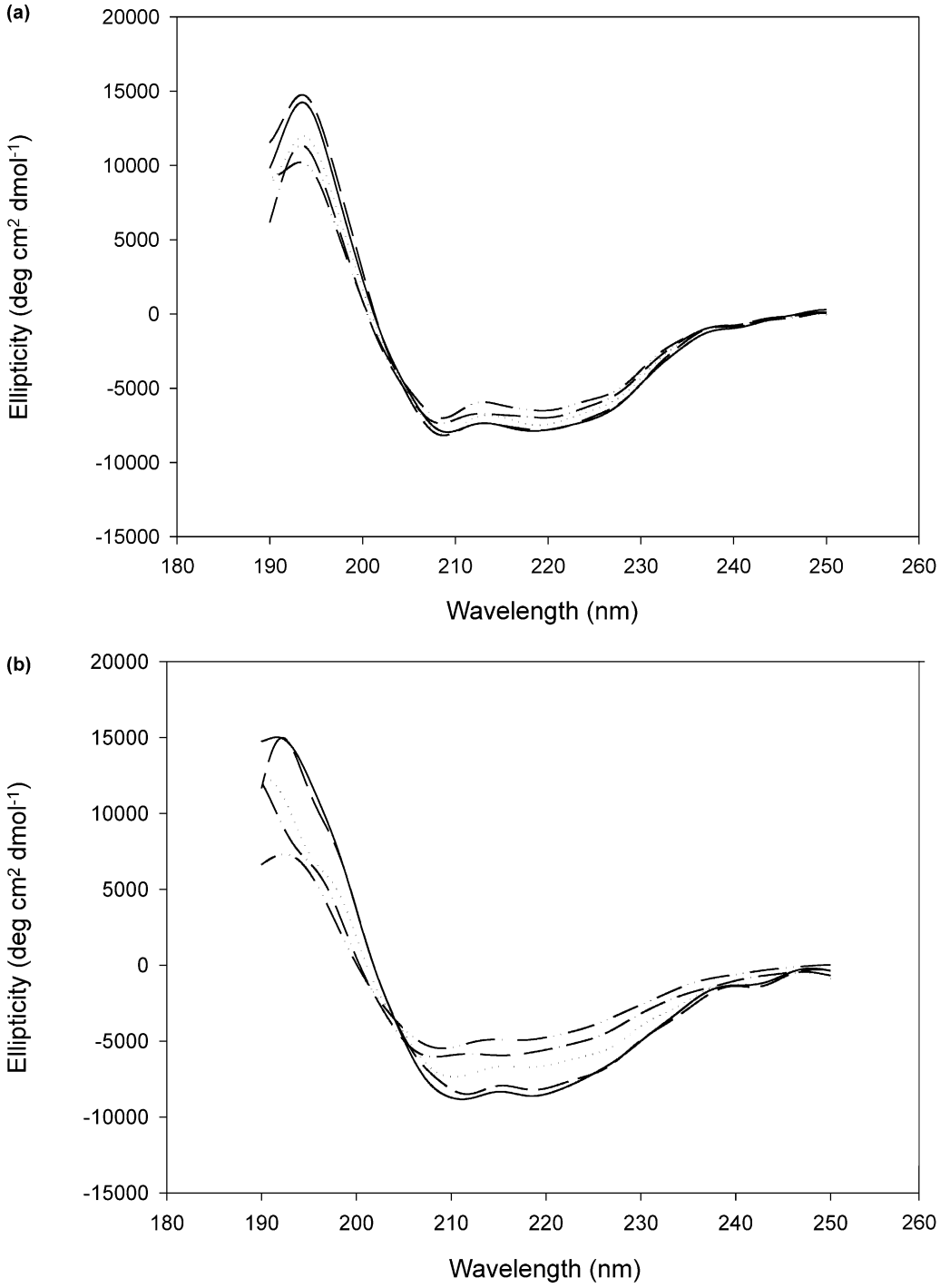

Fig. 3. Far-UV CD spectra of irradiated bovine (A) and porcine (B) plasma protein solution. ———, O kGy; —, 1 kGy; ·····, 5 kGy; -·-·-, 7 kGy; -·····, 10 kGy.

plasma proteins irradiated at various doses were obtained (Fig. 3). Similar to the data of SDS–PAGE, the CD spectra of plasma protein powder samples did not change much with degree of γ-irradiation (data not shown). However, for plasma protein solution, radiation affected CD spectra significantly. CD spectra of the native bovine plasma protein and porcine plasma protein solutions indicate that they mostly have α-helical structure, since they have typical negative minimum ellipticity values at 207 and 221 nm. However, γ-irradiation decreased the negative ellipticity values significantly in the range of 210 and 225 nm, resulting in a decrease of ordered structure. This trend was observed in other studies (Cho & Song, 2000; Moon & Song, 2001). The ellipticity value at 221 nm, which is a typical indicator of α-helix content, increased from -7680 for 0 kGy to −6410 for 10 kGy in the case of bovine blood plasma protein. For porcine plasma protein, it increased from −8290 to −4620, indicating that γ-irradiation decreased the α-helix content with a concomitant increase of random coil structure. Oxygen radicals, due to radiolysis of water, subsequently destabilized the α-helical structure of proteins. The changes in CD spectra of these proteins by γ-irradiation were mainly due to the cleavage of covalent bonds of proteins and formation of aggregated products. CD results clearly support the idea that oxygen radicals, generated by γ-irradiation of proteins in solution, break covalent bonds easily and disrupt the ordered structure of proteins, resulting in change of molecular properties of proteins in solution.

Table 1
Solubility change of irradiated bovine and porcine plasma protein powder

Sample	Irradiation dose (kGy)				
	0	1	5	7	10
BBPP	119.0[a]±1.3[b]	123.3±2.9	118.8±1.2	116.3±3.2	114.7±3.9
PBPP	144.6±2.2	149.1±3.4	149.9±3.2	149.1±3.2	144.1±3.2

[a] Solubility value (mg/ml) is the mean of five replicates.
[b] Standard deviation.

Table 2
Viscosity change of irradiated bovine and porcine plasma protein powder

Sample	Irradiation dose (kGy)				
	0	1	5	7	10
BBPP	1.88[a]±0.01[b]	1.88±0.01	1.89±0.01	1.87±0.01	1.86±0.01
PBPP	1.88±0.01	1.87±0.01	1.88±0.0	1.86±0.0	1.85±0.0

[a] Viscosity value (cP) is the mean of ten replicates.
[b] Standard deviation.

3.3. Effect of γ-irradiation on the solubility and viscosity of plasma protein powders

Gamma-irradiation did not significantly affect the solubility and viscosity of plasma protein powders irradiated below 10 kGy. Table 1 shows that γ-irradiation did not influence the solubility values of bovine and porcine plasma protein powders irradiated. This suggests that γ-irradiation should be applied to extend the shelf life of plasma protein powders without affecting the physicochemical properties by elimination of microbial contamination. Destruction of microbial organisms by γ-irradiation is well documented in the literature (Foley et al., 2002; Hayashi et al., 1991; Mahrour et al., 1998). Hayashi et al. (1991) reported that the decimal reduction of microbial count of dehydrated plasma was 0.82 kGy. Our preliminary data indicated that radiation below 10 kGy could eliminate viable pathogenic bacteria completely (data not shown). Also, the effect of γ-irradiation on the viscosity of plasma protein powders was further examined. Table 2 shows that γ-irradiation below 10 kGy did not significantly change the viscosity values of irradiated bovine and porcine plasma protein powders (similar to solubility values). In summary, γ-irradiation did not affect the physicochemical properties of blood plasma protein powders irradiated below 10 kGy.

4. Conclusions

Gamma-irradiation of blood plasma protein powders did not affect the physicochemical properties, while it could eliminate the microbial hazard. γ-irradiation did not influence the solubility and viscosity values of the protein powders or molecular properties, such as secondary structure and molecular weight profile. However, γ-irradiation of blood plasma protein solutions, irradiated below 10 kGy, significantly affected the secondary structure and molecular weight profile of protein solutions by cleaving polypeptide chains, due to the oxygen radicals produced by radiolysis of water.

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References

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254.
Cho, Y., & Song, K. B. (1997). Effect of chaotropic salt on the sec-

ondary structure of pig skin gelatin. *Bioscience, Biotechnology, and Biochemistry*, *61*, 1194–1195.

Cho, Y., & Song, K. B. (1999). Effect of γ-irradiation on the physicochemical properties of soy protein isolate and whey protein concentrate. *Korean Journal of Food Science and Technology*, *31*(6), 1488–1494.

Cho, Y., & Song, K. B. (2000). Effect of γ-irradiation on the molecular properties of BSA and β-lactoglobulin. *Journal of Biochemistry and Molecular Biology*, *33*(2), 133–137.

Cho, Y., Yang, J., & Song, K. B. (1999). Effect of ascorbic acid and protein concentration on the molecular weight profile of BSA and β-lactoglobulin by γ-irradiation. *Food Research International*, *32*(7), 515–519.

Davies, K. J. A., & Delsignore, M. E. (1987). Protein damage and degradation by oxygen radicals III: modification of secondary structure and tertiary structure. *The Journal of Biological Chemistry*, *262*, 9908–9913.

Filali-Mouhim, A., Audette, M., St-Louis, M., Thauvette, L., Denoroy, L., Penin, F., Chen, X., Rouleau, N., Le Caer, J. P., Rossier, J., Potier, M., & Le Maire, M. (1997). Lysozyme fragmentation induced by radiolysis. *International Journal of Radiation Biology*, *72*, 63–70.

Foley, D. M., Dufour, A., Rodriguez, L., Caporaso, F., & Prakash, A. (2002). Reduction of Escherichia coli 0157:H7 in shredded iceberg lettuce by chlorination and Gamma- irradiation. *Radiation Physics and Chemistry*, *63*, 391–396.

Garrison, W. M. (1987). Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. *Chemistry Review*, *87*, 381–398.

Hayashi, T., Biagio, R., Saito, M., Todoriki, S., & Tajima, M. (1991). Effect of ionizing radiation on sterility and functional qualities of dehydrated blood plasma. *Journal of Food Science*, *56*, 168–171.

Kempner, E. S. (1993). Damage to proteins due to the direct action of ionizing radiation. *Quarterly Review of Biophysics*, *26*, 27–48.

Kume, T., & Matsuda, T. (1995). Changes in structural and antigenic properties of proteins by radiation. *Radiation Physics and Chemistry*, *46*, 225–231.

Laemlli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–685.

Le Maire, M., Thauvette, L., De Foresta, B., Viel, A., Beauregard, G., & Potier, M. (1990). Effects of ionizing radiations on proteins. *The Biochemical Journal*, *267*, 431–439.

Lee, M. J., & Song, K. B. (1997). Purification of streptodornase from Streptococcus equisimilis and its DNA-induced conformational change. *Biochemical and Biophysical Research Communications*, *230*, 13–15.

Mahrour, A., Lacroix, M., Nketsa-Tabiri, J., Calderon, N., & Gagnon, M. (1998). Antimicrobial properties of natural substances in irradiated fresh pourtry. *Radiation Physics and Chemistry*, *52*, 81–84.

Moon, S., & Song, K. B. (2001). Effect of gamma-irradiation on the molecular properties of ovalbumin and ovomucoid and protection by ascorbic acid. *Food Chemistry*, *74*(4), 479–483.

Park, E., Lee, H., & Song, K. B. (1996). Characterization of plasma proteins from bloods of slaughtered cow and pig and utilization of the proteins as adhesives. *Agricultural Chemistry and Biotechnology*, *39*(2), 123–126.

Puchala, M., & Schuessler, H. (1993). Oxygen effect in the radiolysis of proteins. *International Journal of Radiation Biology*, *64*, 149–156.

Raeker, M. O., & Johnson, L. A. (1995). Thermal and functional properties of bovine blood plasma and egg white proteins. *Journal of Food Science*, *60*, 685–690.

Schuessler, H., & Schilling, K. (1984). Oxygen effect in the radiolysis of proteins. *International Journal of Radiation Biology*, *45*, 267–281.

Stevens, C. O., Sauberlich, H. E., & Bergstrom, G. R. (1967). Radiation-produced aggregation and inactivation in egg white lysozyme. *The Journal of Biological Chemistry*, *242*, 1821–1826.

Wolff, S. P., Garner, A., & Dean, R. T. (1986). Free radicals, lipids and protein degradation. *Trends in Biochemical Sciences*, *11*, 27–31.