Preparation and Characterization of High-Molecular-Weight Sericin by γ Irradiation

Nak-Yun Sung,[1] Eui-Baek Byun,[2] Sun-Kyu Kwon,[1] Young-Choon Yoo,[3] Pil-Mun Jung,[1] Jae-Hun Kim,[1] Jong-Il Choi,[1] Mee-Ree Kim,[4] Ju-Woon Lee[1]*

[1]*Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, Republic of Korea*
[2]*Division of Bioresources and Biosciences, Faculty of Agriculture, Graduate School of Kyushu University, 6-10-1 Hakozaki, Fukuoka 812-8581, Japan*
[3]*Department of Microbiology, College of Medicine, Kon-Yang University, Deajon 302-718, Republic of Korea*
[4]*Department of Food and Nutrition, Chungnam National University, Daejeon 305-764, Republic of Korea*

Received 3 April 2008; accepted 26 August 2010
DOI 10.1002/app.33332
Published online 8 December 2010 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: We conducted this study to examine the changes in the molecular structure and physiological activities of silk sericin after γ irradiation. Sericin from *Bombyx mori* was extracted with an Na_2CO_3 solution. The molecular weight distribution of sericin increased in the gel permeation chromatography and sodium dodecyl sulfate/polyacrylamide gel electrophoresis results as the irradiation dose increased. Circular dichroism data also revealed that the α-helix contents decreased with the irradiation dose. Ultraviolet absorption was shown a different pattern between the irradiated and unirradiated sericin. However, the Fourier transform infrared spectrum was not changed in all of the groups. Furthermore, the irradiated sericin was significantly increased in 2,2-diphenyl-1-picryl-hydrazil radical scavenging, and the tyrosinase inhibitory activities increased with irradiation dose. Therefore, γ irradiation was an effective method for producing high-molecular-weight sericin and for developing functional foods and cosmetics.
 J Appl Polym Sci 120: 2034–2040, 2011

Key words: crosslinking; molecular weight distribution/molar mass distribution; radiation

INTRODUCTION

Silk derived from the silkworm, *Bombyx mori*, is a natural protein that is mainly made of sericin and fibroin proteins.[1] Until recently, only fibroin was used for silk clothing, but Masahiro et al.[1] reported that the consumption of sericin enhanced the bioavailability of zinc, iron, magnesium, and calcium in rats, and it was also suggested that sericin is a valuable natural ingredient that could be used in the food industry.[2,3] Sericin is a macromolecular protein made of 18 amino acids, whose molecular weights range from approximately 1 to 100 kDa.[4,5] Recently, the biochemical characteristics of sericin were reported in an examination of whether sericin contributed to the toughness and resistance against environmental stresses for silkworms.[6] Despite its physiological activities, such as its antioxidant effects and tyrosinase inhibition, sericin is mostly discarded during silk production processing[7] because of its low physiological activity.

γ irradiation has been used for the improvement of food storage and hygiene of food, and it has also been found to have importance in the medical and beauty care industries.[8] γ irradiation causes structural changes in biomolecules. In the case of proteins, the structures are altered to by fragmentation, crosslinking, and aggregation by oxygen radicals.[9,10] Even the physicochemical and physiological properties of proteins are altered.[11] Recently, radiation technology was reported to alter characteristic properties by causing a change in the structure of biomaterials, such as ovalbumin,[12] β-glucan,[13] hyaluronic acid,[14] and silk fibroin.[15] Through irradiation, the molecular structures of these biomaterials were changed, and their physiological properties were enhanced. However, there has been no study on the changes in the structural and physiological properties of sericin by irradiation. Therefore, in this study, sericin was irradiated, and changes in its molecular structure and physiological activities were investigated.

EXPERIMENTAL

Preparation of sericin

Sericin was prepared according to a method outlined by Hyun et al.[4] Cocoons were supplied by

Correspondence to: J.-W. Lee (sjwlee@kaeri.re.kr).
Contract grant sponsors: This research was co-supported by the National Research Foundation in the Nuclear Research & Development Program, and by the Basic Research Support Program of Korea Atomic Energy Research Institute.

Nuero, Ltd. (Daegu, Republic of Korea). To obtain sericin, 20 g of cocoon material was cut into small pieces and boiled with 2.5 L of 5% (w/v) Na_2CO_3 solution for 1 h. Because the sericin was not completely dissolved in water, the 5% (w/v) Na_2CO_3 solution was used to extract sericin. The remaining Na_2CO_3 solution with dissolved sericin was separated by filtration, and the salts of Na_2CO_3 were removed through a dialysis membrane with a molecular weight cutoff of 2000 Da (Spectrum Laboratories, Inc., Rancho Dominguez, CA); this process was repeated, and then, the solution was dried with a vacuum freeze dryer to obtain sericin powder.

γ irradiation

Sericin was dissolved to a concentration of 5 mg/mL (w/v) in deionized distilled water (DDW). Sericin solution was irradiated at 5, 10, 50, 100, 150, and 200 kGy, respectively, under a Co-60 irradiator (IR-221, Nordion International, Ltd.) with an 11.1-PBq source strength and was operated at a dose rate of 10 kGy/h. The γ-irradiated sericin solutions were stored 4°C for the subsequent experiment. The γ irradiation dose absorbed by the sample was measured with alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), which were 5 mm diameter. The dosimeters were calibrated with international standards of the International Atomic Energy Agency (Vienna, Austria).

Gel permeation chromatography (GPC)

The distribution of the molecular weight of sericin was determined by GPC (Waters, Milford, MA, USA). GPC measurements were performed with a Waters GPC system equipped with a Waters 515 pump (Milford, MA, USA), a 2 × PLaqagel OH Mixed (7.8 × 300 mm) column, and a Waters 2410 refractive-index detector (Milford, MA, USA). The column was operated at 40°C and eluted with distilled water at a flow rate of 1 mL/min. The retention time of the samples was calibrated with a dextran standard at a concentration of 0.1% (w/w).

Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS–PAGE)

The protein concentration of sericin was assayed with the bicinchoninic acid (BCA) method,[16] and the contents of the loaded protein per lane were adjusted to 28.3 μg. Electrophoresis was carried out with precast 4–20% NuPAGE Bis–Tris gels (Invitrogen, San Diego, CA) at 100 V for 1 h in a NuPAGE 2-(N-morpholino)ethanesulfonic acid (MES) sodium dodecyl sulfate running buffer system (Invitrogen) according to the manufacturer's instructions. A SeeBlue Plus2 prestained standard protein marker (Invitrogen) was used to determine the molecular masses of the protein bands. The gel was then stained with Commassie Brilliant Blue R-250 for visualization.

Circular dichroism (CD)

CD spectra were obtained with a Jasco J-715 spectropolarimeter (Jasco, Tokyo, Japan) fitted with a 150-W xenon lamp. Far-ultraviolet (far-UV) spectra were registered in the range 160–280 nm. The sample (0.2 mg/mL) was analyzed in DDW. A sample compartment was purged with nitrogen gas, and a 1-mm-pathlength quartz cuvette was used. Triplicate scans of the CD spectra were averaged, and the spectrum for the DDW background was subtracted.[6] CD spectra were represented in terms of a mean residue ellipticity (degrees × square centimeters per decimole).

Ultraviolet (UV) absorption

UV spectra of irradiated sericin solution were taken on a spectrophotometer (UV-1601PC, Shimadzu, Tokyo, Japan). Irradiated and unirradiated sericin solutions (3 mL, 0.1 mg/mL) were transferred to the quartz cuvette with a 1-mm pathlength and scanned with an ultraviolet–visible spectrophotometer (UV-1601PC, Japan). The scanning range was recorded between 180 and 500 nm.

Fourier transform infrared (FTIR)

The FTIR spectra of the irradiated sericin samples were recorded (4000–400 cm^{-1}) on a Bruker Spectrometer VERTEX 70 FTIR system (Bruker, Ettlingen, Germany) with the KBr pellet technique. To make the sample for FTIR spectroscopy, irradiated sericin solution was lyophilized to form a powder. The pellets were prepared by the mixture of one part of the sericin powder with 49 parts (1/50, w/w) of KBr, and this powder was then compressed under a pressure of 100 kg/cm^2 for 8 min of pressing. The acquired spectra were the results of 24 scans at a spectrophotometer resolution of 8 cm^{-1}.

2,2-Diphenyl-1-picryl-hydrazil (DPPH) radical scavenging activity

The free-radical scavenging activity with the DPPH reagent was measured with the method prescribed by Amarowicz et al.[17] To 0.5 mL of sericin dissolved in DDW at a concentration of 0.2 mg of sericin/mL, the same volume of freshly prepared DPPH reagent in methanol (0.1 mmol) was added and vortexed. After a 25-min reaction at room temperature (25°C) in the dark, the reaction mixtures were centrifuged at 6400 rpm for 5 min. The decolorization of the supernatant was assayed at 517 nm (absorbance at 517 nm) by the spectrophotometer (UV-1601PC,

Shimadzu) and was compared with a blank control containing a sericin solution and pure methanol instead of DPPH. A blind control containing DPPH and distilled water instead of a sericin solution was also assayed. Equation (1) was used for the calculation of the free-radical scavenging activity:

$$\text{Scavenging activity } (\%) = \left(1 - A_{517}^{\text{sample}}/A_{517}^{\text{blind}}\right) \times 100 \quad (1)$$

where the scavenging activity is the free-radical scavenging percentage, A_{510}^{sample} is the absorbance of the sample at 475 nm, and A_{510}^{blind} is the absorbance of the blind control at 475 nm.

Tyrosinase inhibitory activity

A sample of the irradiated sericin solution (0.2 mL) was added to a reaction mixture containing a 10 m*M* l-3,4-dihydroxyphenylalanine (Sigma Chemical Co., St. Louis, MO) solution, a 67 m*M* sodium phosphate buffer (pH 6.8), and mushroom tyrosinase (final concentration = 100 unit/mL, Sigma Chemical Co.). The reaction mixture was incubated at 25°C for 15 min. The amount of dopachrome produced in the reaction mixture was determined to be 475 nm (absorbance at 475 nm) by the spectrophotometer (UV-1601PC, Shimadzu). Equation (2) was used for the calculation of the inhibitory effect on the tyrosinase (%).

$$\text{Inhibition } (\%) = \left(1 - A_{475}^{\text{sample}}/A_{475}^{\text{control}}\right) \times 100 \quad (2)$$

where A_{475}^{sample} is the absorbance of the sample at 475 nm and A_{475}^{control} is the absorbance at 475 nm in DDW instead of the sericin solution.

Statistical analysis

The data were analyzed by the Statistical Package for the Social Science program (SPSS, Inc., Chicago, IL). Differences among the mean values were obtained by Duncan's multiple comparison tests at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Changes in the molecular weight distribution of sericin after γ irradiation

The effect of γ irradiation on the molecular weight distribution of sericin was investigated with GPC and SDS–PAGE. Figure 1 represents the average molecular weight of sericin as an expression of the radiation effect for different irradiation doses by GPC. The result shows that the average molecular weight of sericin was increased as the irradiation dose increased. The average molecular weight of the unirradiated sericin was found to be 1.8 kDa, whereas those of the sericin irradiated at 50, 100, 150, and 200 kGy were approximately 8, 30, 47, and 135 kDa, respectively.

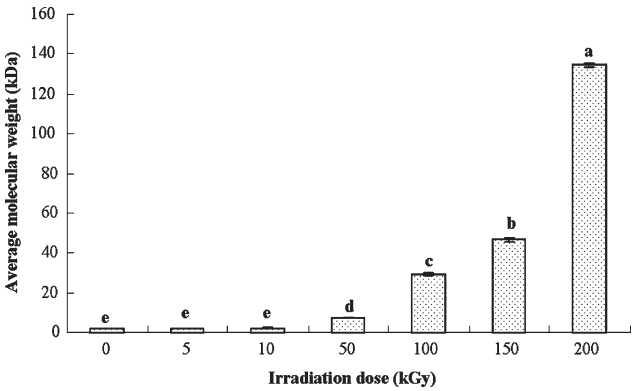

Figure 1 GPC analysis of the average molecular weights of sericin samples irradiated at different doses. The bars represent the means and standard deviations. The letters indicate a statistically significant difference versus the control ($P < 0.05$).

The gel electrophoretic patterns of the sericin irradiated at 5–200 kGy are shown in Figure 2. The SDS–PAGE results show that the molecular weight distribution of sericin was modified by γ irradiation. The unirradiated sericin showed broad smear protein bands with low molecular weight, but in irradiated sericin, the protein with low molecular weight gradually disappeared with γ irradiation. This result indicates that γ irradiation induced a change in the molecular distribution of the sericin protein, and this pattern increased as the irradiation dose increased.

There have been reports on the phenomenon of crosslinking and on the hydrophobic interaction between proteins due to γ irradiation.[10,18] γ irradiation exerts its effect through the radiolysis of water, which results in the production of free radicals, such as hydrated electrons, hydroxyl radicals, and hydrogen atoms.[19] Radiation-induced reactions in proteins are strongly influenced by their complex structure, that is, the folding of the peptide chains, disulfide bonds between the chains, secondary binding forces, such as hydrogen bonds, hydrophobic bonds, or ionic bonds. The splitting reaction of disulfide bonds can cause degradation to smaller proteins, and crosslinking among polypeptide chains via intermolecular bonding can be attributed to the aggregation of proteins.[19,20] It has also been reported that the irradiation of proteins at a high dose induces crosslinking and hydrophobic interactions between the proteins.[15,21,22] Therefore, these data indicated that silk sericin might have been aggregated into higher molecular weight proteins with γ irradiation.

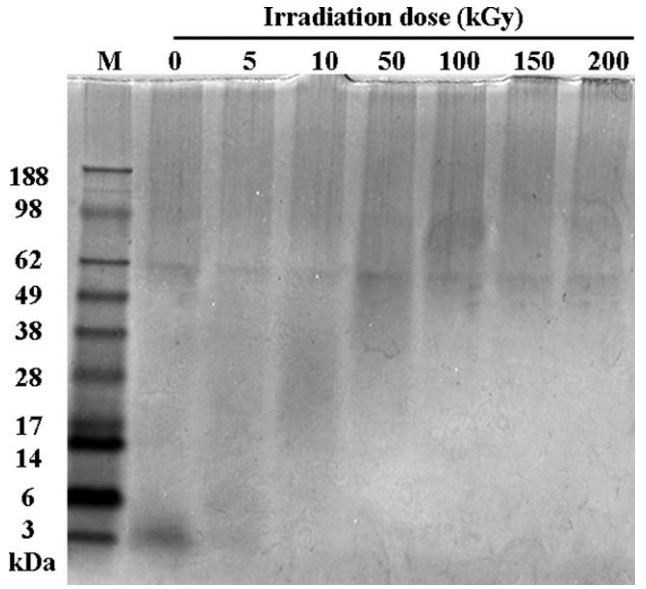

Figure 2 SDS–PAGE analysis of sericin samples γ-irradiated at 0, 5, 10, 50, 100, 150, or 200 kGy. M indicates standard markers, and the numbers on the left side indicate the molecular weights of the standard markers.

Changes in the CD spectrum of sericin with γ irradiation

CD spectroscopy measures the differences in the absorption of a left-handed polarized light versus a right-handed polarized light and can be used to determine a protein's secondary structure in the far-UV spectral region (190–250 nm). At these wavelengths, a chromophore is a peptide bond, and a signal arises when it is located in a regular, folded environment, α helix, β sheet, and random-coil structures that provide the characteristic shape and magnitude of a CD spectrum. It is commonly known that two negative peaks at 208 and 220 nm are characteristic of an α helix of proteins and that a negative peak at 214 nm is characteristic of a β sheet of proteins.[23,24]

Figure 3 shows the change in the CD spectrum of sericin caused by γ irradiation. When the radiation dose was increased, the ratio of α helixes in sericin decreased. On the other hand, the ratio of β sheets and random coils increased with the alteration in the α-helix structure.

Several researchers reported that the oxygen radicals, because of the radiolysis of water, subsequently destabilized the α-helix structure of the proteins, and the changes in CD spectra by γ irradiation were mainly due to a cleavage of the covalent bonds of the proteins and a formation of aggregated products.[24–26] In this study, the CD results clearly support the fact that oxygen radicals, generated by γ irradiation in solution, disrupted the ordered structure of sericin and caused a change in the molecular properties of sericin.

Changes in the UV spectrum of sericin with γ irradiation

UV absorption spectra are frequently used to analyze the structural changes of the side chains in aromatic amino acids on the protein's surface. Three aromatic amino acids (phenylalanine, tyrosine, and tryptophan) have strong absorptions at 280 nm.[27,28] The UV spectra of sericin irradiated at various irradiation doses are shown in Figure 4. The data showed that the absorption intensities at 280 nm of sericin increased after γ irradiation. There was a negligible shift in the absorption wavelength that depended on the irradiation dose. In general, UV absorbance spectroscopy responds to changes in the environment of the tryptophan and tyrosine residues

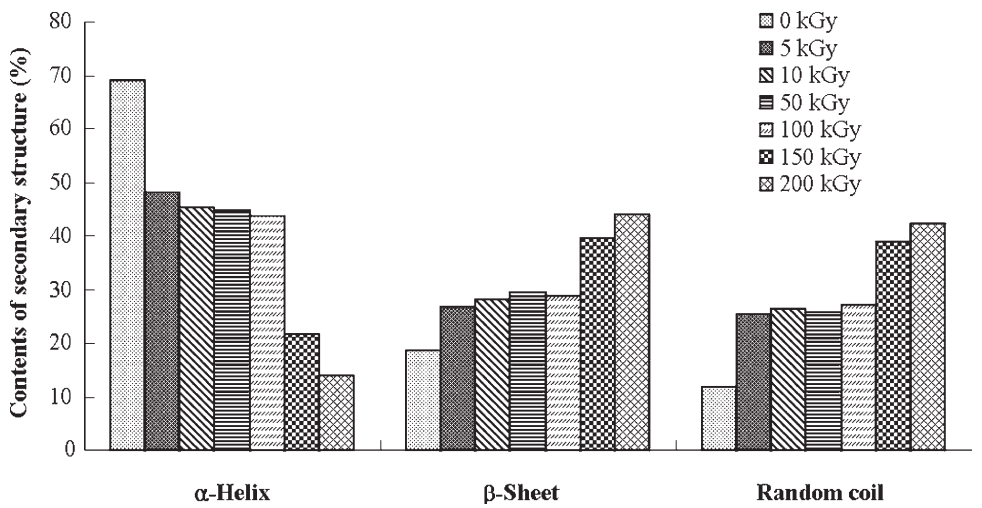

Figure 3 Contents of the secondary structural conformations from the far-UV CD spectra of sericin samples γ-irradiated at 0, 5, 10, 50, 100, 150, or 200 kGy.

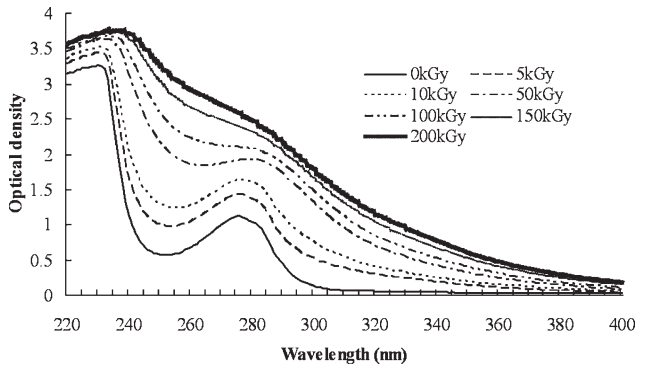

Figure 4 UV absorption spectra of sericin samples γ-irradiated at 0, 5, 10, 50, 100, 150, or 200 kGy.

and, hence, to changes in tertiary structure. Therefore, the change in the absorption intensities at 280 nm indicated that the aromatic amino acid residues were more unfolded by γ irradiation.

Changes in the FTIR spectrum of sericin after γ irradiation

The FTIR spectrum of the irradiated sericin is presented in Figure 5. In general, the peptide group of the proteins shows nine characteristic bands called amides A, B, I, II, III, IV, V, VI, and VII in the FTIR spectrum.[29] It was reported that amide I (1600–1700 cm^{-1}) is the most intense absorption band of proteins and is primarily governed by the stretching vibration of the C=O (70–85%) and C—N (10–20%) groups. Amide II (1510–1580 cm^{-1}) is mainly derived from in-plane N—H bending. The rest of the potential energy arises from the C—N and C—C stretching vibrations. The peaks in the region 3000–3500 cm^{-1} are amide A and amide B bands associated with N—H stretching vibrations.[30] In these results, the peaks of sericin found in the regions 1600–1700, 1510–1580, and 3000–3500 cm^{-1} confirmed the presence of the amide I, II, A, and B bands. The other peaks were also found in the fingerprint region of 1500–400 cm^{-1}. The FTIR peaks of the sericin prepared in this study were very similar to those of sericin obtained by other researchers.[31,32] Also, Kojthung et al.[33] reported that γ irradiation did not change the FTIR peak of fibroin in the range 100–1000 kGy because the γ irradiation did not alter the primary structure of the polypeptide arrangement. In this study, irradiated sericin also had a pattern in the FTIR spectra similar to that of the unirradiated sericin. Therefore, we concluded that the γ radiation did not affect the primary structure of the polypeptide arrangement on the sericin protein.

Changes in the DPPH radical scavenging capacity of sericin with γ irradiation

DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical

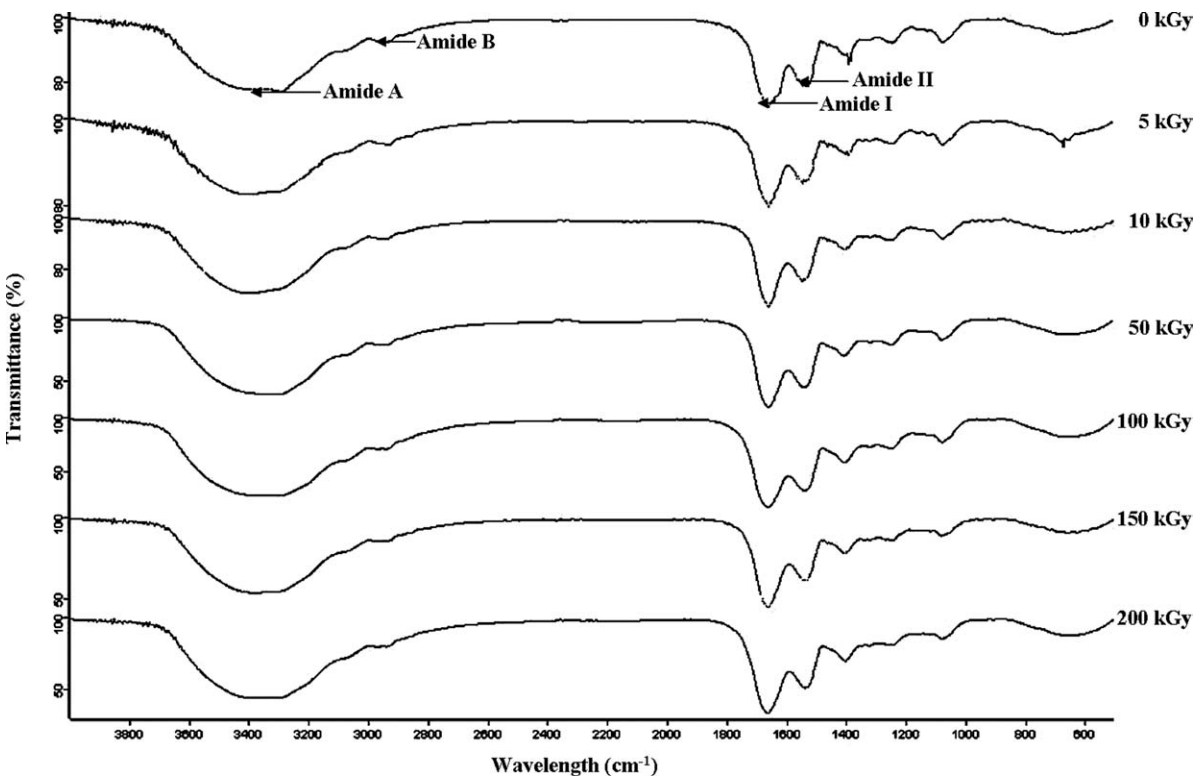

Figure 5 FTIR spectra of sericin samples γ-irradiated at 0, 5, 10, 50, 100, 150, or 200 kGy.

scavenging effects of sericin. In previous studies, Kato et al.[34] reported that sericin completely inhibited lipid peroxidation, and Wu et al.[7] also provided evidence of the antioxidant activity of sericin by DPPH assay. Therefore, the antioxidant activity of high-molecular-weight sericin was investigated by irradiation. The DPPH radical scavenging capacity of the γ-irradiated sericin is shown in Figure 6. The DPPH radical scavenging capacity of sericin increased and was dependent on the irradiation dose, but the sericin irradiated at 200 kGy showed a lower antioxidant activity compared to that at 150 kGy. When the sericin solution was irradiated at a dose of 150 kGy, the DPPH scavenging capacity was increased from 34.7 to 89.9%. Although the effect of irradiation on the antioxidant activity of proteins has not been studied, irradiation could increase the antioxidant properties of various foods and natural extracts, such as green tea leaf extract,[35] phytic acid,[36] and soybean.[37] In this study, γ irradiation also increased the DPPH radical scavenging capacity of sericin through structural change, as shown in the CD results and UV spectra.

Changes in the tyrosinase inhibitory activity of sericin with γ irradiation

Tyrosinase is responsible for the biosynthesis of melanin in human skin, and tyrosinase inhibitors have important roles in the cosmetics industry because of their skin-whitening effects.[38,39] The inhibitory effect of γ-irradiated sericin on tyrosinase is shown in Figure 7. Although unirradiated sericin had a tyrosinase inhibition effect, the γ-irradiated sericin showed a much stronger inhibitory activity on tyrosinase than the unirradiated sericin. The inhibitory effect on tyrosinase increased and was dependent on the irradiation dose. When the sericin solution was irradiated at a dose of 200 kGy, the tyrosinase inhibitory activity was increased from 10.6 to 31.4%.

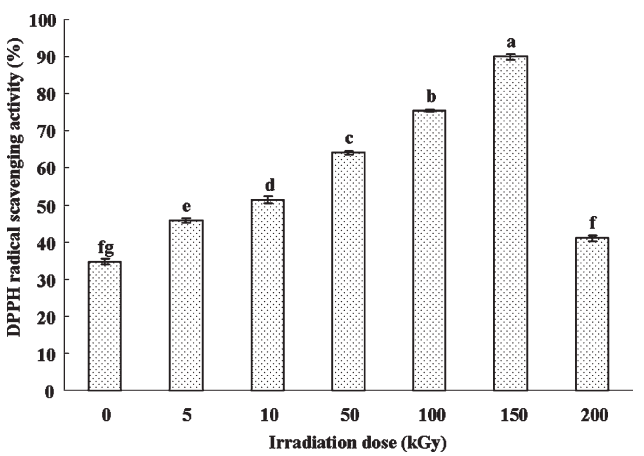

Figure 6 Radical scavenging activity of sericin samples γ-irradiated at 0, 5, 10, 50, 100, 150, or 200 kGy. The bars represent the means and standard deviations. The letters indicate a statistically significant difference versus the control ($P < 0.05$).

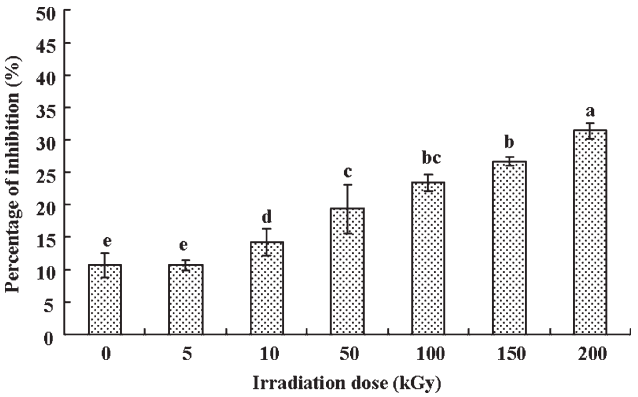

Figure 7 Tyrosinase inhibitory activity of sericin samples γ-irradiated at 0, 5, 10, 50, 100, 150, or 200 kGy. The bars represent the means and standard deviations. The letters indicate a statistically significant difference versus the control ($P < 0.05$).

Amino acids, peptides, and proteins can inhibit tyrosinase activity by means of more complicated mechanisms, including reactions with intermediates and quinines[39] and the formation of stable Cu^{2+} chelates,[40,41] and they have different pathways because of their structure, composition, and molecular weight.[42] It has also been reported that silk sericin inhibited tyrosinase.[33] From our results, we concluded that γ irradiation changed the molecular structure of sericin, which resulted in an increase in its physiological activities.

CONCLUSIONS

Sericin was prepared from a boiled solution of silk cocoons and was irradiated with a Co-60 γ irradiator to produce a high-molecular-weight sericin. The structural and physiological properties of the irradiated sericin were characterized and evaluated. The GPC and SDS–PAGE results showed that the molecular weight of sericin increased and were dependent on the irradiation dose. The CD analysis showed that the ratio of α helixes decreased, but the ratio of β sheets and random coils increased as the irradiation dose increased. The FTIR spectrum of the irradiated sericin was similar to that of the unirradiated sericin, without any notable changes in the functional group status. Also, the biological activities of sericin, such as antioxidant and tyrosinase inhibitory activities, increased with γ irradiation. From these results, we believe that irradiation was an effective method for producing high-molecular-weight sericin and could be used to develop functional foods and cosmetics. However, further studies should be carried out to elucidate the relationship between the

molecular structure and physiological activities of sericin.

References

1. Masahiro, S.; Hideyuki, Y.; Norihisa, K. Nutr Res 2000, 20, 1505.
2. Sasaki, M.; Yamada, H.; Kato, N. Nutr Res 2000, 20, 1505.
3. Takahashi, M.; Tsujimoto, K.; Yamada, H.; Tahagi, H.; Nakamori, S. Biotechnol Lett 2003, 25, 1805.
4. Hyun, C. K.; Kim, I. Y.; Frost, S. C. J Nutr 2004, 134, 3257.
5. Wei, T.; Li, M. Z.; Xie, R. J. Macromol Mater Eng 2005, 290, 188.
6. Dash, R.; Mukherjee, S.; Kundu, S. C. Int J Biol Macromol 2006, 38, 255.
7. Wu, J. H.; Zhang, W.; Xu, S. Y. Food Chem 2007, 103, 1255.
8. Byun, M. W. Radioisotope News 1994, 9, 32.
9. Davies, K. J. J Biol Chem 1987, 262, 9895.
10. Garrison, W. M. Chem Rev 1987, 87, 381.
11. Dogbevi, M. K.; Vachon, C.; Lacroix, M. Meat Sci 1999, 51, 349.
12. Seo, J. H.; Lee, J. W.; Kim, J. H.; Byun, E. B.; Lee, S. Y.; Kang, I. J.; Byun, M. W. Radiat Phys Chem 2007, 76, 1855.
13. Byun, E. H.; Kim, J. H.; Sung, N. Y.; Choi, J.; Lim, S. T.; Kim, K. H.; Yook, H. S.; Byun, M. W.; Lee, J. W. Radiat Phys Chem 2008, 77, 781.
14. Kim, J. K.; Srinivasan, P.; Kim, J. H.; Choi, J.; Park, H. J.; Byun, M. W.; Lee, J. W. Food Chem 2008, 109, 763.
15. Sung, N. Y.; Byun, E. B.; Kwon, S. K.; Kim, J. H.; Song, B. S.; Choi, J. I.; Kim, J. K.; Yoon, Y.; Byun, M. W.; Kim, M. R.; Yook, H. S.; Lee, J. W. Food Sci Biotechnol 2009, 18, 228.
16. Krieg, R. C.; Yan, D.; Schwamborn, K.; Knuechel, R. J Biochem Biopharm Methods 2005, 65, 13.
17. Amarowicz, R.; Pegg, R. B.; Rahimi, M. P.; Barl, B. J.; Weil, A. Food Chem 2004, 84, 551.
18. Kume, T.; Matsuda, T. Radiat Phys Chem 1995, 46, 225.
19. Feldberg, R. S.; Carew, J. A. Int J Radiat Biol Relat Stud Phys Chem Med 1981, 40, 11.
20. Stevens, C. O.; Sauberlich, H. E.; Bergstrom, G. R. Biochemistry 1967, 242, 1821.
21. Diehl, J. F. Safety of Irradiated Foods; Marcel Decker: New York, 1995; Chapter 3.
22. Masuda, T.; Koseki, S. Y.; Yasumoto, K.; Kitabatake, N. J Agric Food Chem 2000, 48, 2670.
23. Chang, C. T.; Wu, S. C.; Venaminov, S. Y.; Yang, J. T. Anal Biochem 1978, 91, 13.
24. Mayer, L. J Phycol 1998, 274, 77.
25. Cho, Y.; Song, K. B. Biosci Biotechnol Biochem 1997, 61, 1194.
26. Moon, S.; Song, K. B. Food Chem 2001, 74, 479.
27. Wetlaufer, D. B. Adv Protein Chem 1962, 17, 303.
28. Mathews, C. K.; Von, K. E.; Ahern, K. G. Biochemistry 1999, 5, 126.
29. Jabs, A. Image Library of Biological Macromolecules. http://www.imb-jena.de/imglibdoc/ftir/image_ftir.html (accessed April 2006).
30. Kongdee, A.; Bechtold, T.; Teufel, L. J Appl Polym Sci 2005, 96, 1421.
31. Zhaorigetu, S.; Yanaka, N.; Sasaki, M.; Watanabe, H.; Kato, N. J Photochem Photobiol B 2003, 71, 11.
32. Gulrajani, M. L.; Brahma, K. P.; Senthil Kumar, P.; Purwar, R. J Appl Polym Sci 2008, 109, 314.
33. Kojthung, A.; Meesilpa, P.; Sudatis, B.; Treeratanapiboon, L.; Udomsangpetch, R.; Oonkhanond, B. Int Biodeterior Biodegrad 2008, 62, 487.
34. Kato, N.; Sato, S.; Yamanaka, A.; Yamada, H.; Fuwa, N.; Nomura, M. Biosci Biotechnol Biochem 1998, 62, 145.
35. Jo, C.; Son, J. H.; Lee, H. J.; Byun, M. W. Radiat Phys Chem 2003, 66, 179.
36. Ahn, H. J.; Kim, J. H.; Yook, H. S.; Byun, M. W. J Food Sci 2003, 68, 2221.
37. Variyar, P. S.; Limaye, A.; Sharma, A. J Agric Food Chem 2004, 52, 3385.
38. Iskender, A.; Ahmet, Y. Food Chem 2007, 103, 301.
39. No, J. K.; Soung, D. Y.; Kim, Y. J.; Shim, K. H. Life Sci 1999, 65, 241.
40. Synge, R. L. M. Plant Food Hum Nutr 1975, 24, 337.
41. Eichhorn, G. L. Inorganic Chemistry; Elsevier: New York, 1975.
42. Viola, R. E.; Hartzell, C. R.; Villafranca, J. J. J Inorg Biochem 1979, 10, 293.