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# Study on improving antioxydant and antibacterial activities of silk fibroin by irradiation treatment

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**Abstract:** The silk fibroin solutions were prepared in solvent system of $CaCl_2$. $CH_3CH_2OH$. $H_2O$ (mole ratio = 1:2:8) followed dialysis against deionized water. The 3% silk fibroin solutions were irradiated under gamma Co-60 source with dose ranging from 0 to 50 kGy at Hanoi Irradiation Centre and bio-activities of the irradiated silk fibroin solutions were investigated with different radiation doses. The results indicated that the antioxidant and antibacterial activities of fibroin were much improved by gamma irradiation. Maximum value of DPPH radical scavenging activity was 70.4% for the solution of silk fibroin irradiated at 10 kGy. Silk fibroin solutions irradiated at doses higher than 10 kGy also exhibited rather high antibacterial activity against *E. coli and S. aureus.* In order to estimate the applicability of our irradiated fibroin, the silk fibroin solutions were lyophilized to obtain a pure fibroin powder, then their bio-activities were compared with those of commercial silk fibroin (Proteines De Soie/ Zijdeproteine, Bioflore, Canada). Our fibroin powder revealed higher antioxidant and antibacterial activities. The amino acid compositions of our irradiated fibroin were also higher than that of the commercial product. Thus, the irradiated silk fibroin can be used for further application in cosmetic and other related fields.

***Key word****: Silk Fibroin, Gamma Irradiation, Antioxidant Activity, Antibacterial Activity*

## I. INTRODUCTION

Silk fiber derived from the silkworm *Bombyx mori* is a natural protein that mainly consists of fibroin and sericin. Fibroin is a major component of silk fiber (75 %), serving as the core. It contains at least two fibroin proteins, light-chain (25 kDa) and heavy-chain (325 kDa). Sericin is a minor component (25 % of the silk fiber), serving as a glue-like protein coated on the two fibroin cores to conceal a unique luster of fibroin. Both fibroin and sericin contain the same 18 amino acids, but in different amounts. Another difference between these two proteins is crystalline repetitive amino acids (-Gly-Ala-Gly-Ala-Gly-Ser-) along its sequence, forming a large number of β-sheet microcrystallines. This reinforcement contributes the strength and stiffness to the silk fiber [1].

From ancient, silk has been used as a textile material because it has excellent natures such as lightness and warmth on wearing, beautiful gloss, etc. Recently, silk fibroin has been also considered as the natural protein with interesting characters and applications in new diversified fields [8]. The silk film and scaffolds are studied for artificial skin and for contact lens, respectively. Silk powder has been already used as food additives, cosmetic or pharmaceutical materials [4]. For practical applications, it is necessary to modify the

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structure of silk fiber in order to obtain desirable properties [11].

In fact, it is difficult to degrade or dissolve silk fibroin in water because of its crystal structure. This limitation influenced on the use of silk fibroin, especially for cosmetic and pharmaceutical applications. The radiation technique has been increasingly used for structural modification of organic compounds. Gamma radiation can cause the macromolecule chains to cross-link, graft and degrade. Recently, some authors reported that the conversion efficiency from fiber to powder of silk fibroin was much improved by EB treatment, that the water solubility of silk fibroin improved while its mechanical properties weakened after treatment with gamma radiation [4, 7, 9]. Despite the mechanisms by which gamma irradiation produce several biological effects on peptide/protein chains are still clearly unknown, but the latest studies showed the bioactivities of silk fibroin such as antioxidant and anti-tumor activities, tyrosinase inhibitory ability, etc. were significantly enhanced by gamma irradiation [2, 3].

This study was undertaken in order to investigate the effectiveness of gamma irradiation for antioxidant and antibacterial activities of silk fibroin which are among important properties of variety of cosmetic and pharmaceutical products. Water-soluble silk fibroin powder was prepared by gamma irradiation and their amino acid amount was also evaluated. In the future, the results of this study may be a prerequisite to promote our radiation technology as well as for further utilization of abundant Vietnam silk fibroin for cosmetic and pharmaceutical applications.

## II. EXPERIMENTAL

### A. Materials

Silk fibroin fiber was purchased from My Duc Commune, Ha Noi. The medium for the cultivation of microorganism such as Nutrient Broth and Nutrient Agar was supplied by Difco, USA. The chemicals such as $Na_2CO_3$, $CaCl_2$, DPPH (2, 2-diphenyl-1-picrylhydrazin), $CH_3OH$, ascorbic acid at analytical grade were supplied by Merck, Germany, while $C_2H_5OH$ was bought from a domestic company.

### B. Sample preparation of silk fibroin and irradiation

The raw silk fibers were subjected to removal of the sericin by the method described by Byun et al. [2]. In brief, 10 g of silk fibroin was dissolved in 100 ml of the calcium chloride solution ($CaCl_2/C_2H_5OH/H_2O$=1:2:8 in mole ratio) at 70 ± 2 $^oC$ for 1 h. The mixed solution was dialyzed with distilled water by using the dialysis tubing membrane of MWCO 12-14 kDa (molecular weight cutoff) for 72 h. The final concentration of the silk fibroin aqueous solution was modified to 3 % (w/v).

The silk fibroin solution after dialyzed was irradiated under gamma Co-60 source of Hanoi Irradiation Center at dose range of 0÷50 kGy.

### C. Bioactivities tests

*Antioxidant activity*

To evaluate the antioxidant of silk fibroin, a radical oxygen species (ROS) scavenging method (DPPH) was used, according to Lucconi [6]. In particular, the silk fibroin solution was tested at different concentrations (0.005, 0.0075 and 0.01 mg/ml). 1 ml of the solution was mixed with 2 ml of methanol solution containing DPPH (10 μg/ml) for sampling. The samples were incubated in dark for over 30 min at 25 $^oC$ and their absorbencies were measured at 517 nm with UV-VIS spectrophotometer AV-2450,

Shimadzu, Japan. Reaction mixture without fibroin was used as negative control, while ascorbic acid was used as positive control at the same concentration of fibroin samples.

Radical scavenging activity was calculated by the following formula:

$$\% \text{ Activity} = (A-B)/A \times 100$$

Where A is the absorbance of negative control and B is the absorbance of tested solution. The analyses were performed in three replicates.

*Antibacterial activity*

Three kinds of bacteria strains *E. coli, B. subtillis and S. aureus* were used for antibacterial activity testing of silk fibroin. Briefly, the antimicrobial activities of irradiated fibroin solutions were investigated and compared with antimicrobial activities of non-irradiated fibroin solution as control. The procedure for testing was performed as the modified methods of agar disk diffusion and agar dilution methods (AOAC 2000).

D. Determination amino acid composition of irradiated silk fibroin

The amino acid components existing in the irradiated silk fibroin was determined using a high performance liquid chromatography (HPLC, National Institute for Food Control). The results were also compared to a commercial product in order to estimate the applicability of irradiated silk fibroin in practice.

## III. RESULTS AND DISCUSSION

### A. Effects of irradiation doses on antioxidant activity of silk fibroin

**Table I.** Antioxidant activity of silk fibroin solution with dose

| Irradiation dose (kGy) | ROS- radical scavenging activity (%) | | |
|---|---|---|---|
| | **0.005 mg/ml** | **0.0075 mg/ml** | **0.01 mg/ml** |
| **0** | 5.55 ± 0.18 | 8.43 ± 0.26 | 12.05 ± 0.18 |
| **5** | 7.95 ± 0.21 | 8.65 ± 0.25 | 32.20 ± 0.63 |
| **10** | 23.80 ± 0.38 | 27.8 ± 0.66 | 70.04 ± 0.18 |
| **20** | 14.45 ± 0.18 | 21.40 ± 0.56 | 53.60 ± 0.79 |
| **30** | 11.30 ± 0.44 | 15.38 ± 0.41 | 48.05 ± 0.74 |
| **40** | 8.30 ± 0.26 | 12.20 ± 0.45 | 35.80 ± 0.62 |
| **50** | 5.60 ± 0.17 | 10.10 ± 0.12 | 32.20 ± 0.65 |
| **Commercialized Fibroin** | 6.20 ± 0.21 | 7.60 ± 0.35 | 12.76 ± 0.41 |
| **Ascorbic acid** | 91.07± 0.42 | - | - |

(-) No investigated

The 3 % solution of silk fibroin was irradiated at dose range from 0 to 50 kGy and its DPPH radical scavenging activity was evaluated and compared with that of ascorbic acid and the commercialized fibroin powder. Table I shows the antioxidant activity of the fibroin solutions irradiated at different doses and Fig. 1 presences the UV spectra of the mixed solutions of the irradiated fibroin in methanol containing DPPH.

From the these results, we found that 70.04 % is maximum value of DPPH radical scavenging activity obtained with the

fibroin solution irradiated at dose of 10 kGy. The antioxidant activity of the irradiated fibroin solutions also reduced gradually to 32.2 % when doses increased continuously to 50 kGy.

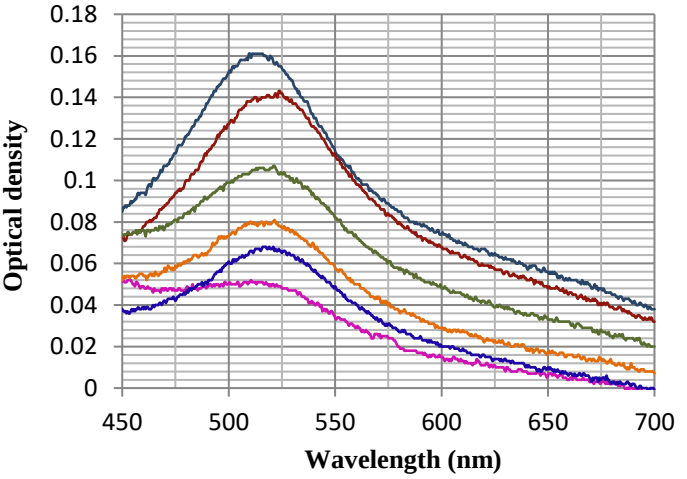


**Fig.1.** UV absorbency of fibroin (0.01 mg/ml) derived from 3% irradiated fibroin solutions in DPPH solution (— Negative control, — 0 kGy, — 5 kGy, —10 kGy, — 20 kGy and — 30 kGy)

Similar effect also was observed by some Korean authors when they studied on some physiological activities of fibroin solution (1 mg/ml). They reported that DPPH radical scavenging activity of fibroin solution increased to more 80 % at irradiation dose of 5 kGy compared with 9% of unirradiated fibroin solution. DPPH radical scavenging activity slightly reduced to about 53 % at dose of 50 kGy. The authors suggested that the changes of molecular weight of fibroin by irradiation treatment induced the enhancement of its antioxidant activity [3].

### B. Effects of irradiation doses on antibacterial activity of silk fibroin

The antibacterial activity against 3 tested bacteria strains *(E. coli, S. aureus , B. subtillis)* were investigated through the parameters as width of clear zone of growth inhibition (Table 2 and Fig. 2) and minimum inhibitory concentration (MIC) (Table III).

**Table II.** Width of clear zone of growth inhibition of the irradiated fibroin solution for 3 tested bacteria strains

| Irradiation dose (kGy) | Width of clear zone of growth inhibition (mm) | | |
|---|---|---|---|
| | *E.coli* | *S. aureus* | *B. subtillis* |
| **0** | – | – | – |
| **5** | – | – | – |
| **10** | 5.4 ± 0.09 | 3.6 ± 0.10 | – |
| **20** | 5.6 ± 0.25 | 3.6 ± 0.12 | – |
| **40** | 5.4 ± 0.11 | 3.5 ± 0.08 | – |

**Concentration of fibroin solution using for tested was 1%*
(–) No observed

The results show that, antibacterial activity of fibroin solution had been improved by gamma irradiation. However, there were no significant differences in

antibacterial activity against *E. coli* and *S. aureus* of all samples in dose ranges from 10 to 40 kGy. The 30 mg/ml solution of fibroin was not enough to inhibit growth of *B. subtillis* for all investigated doses.

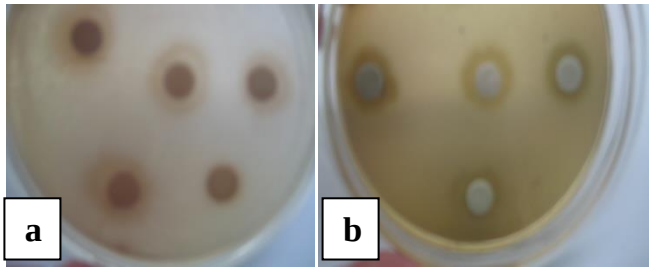


**Fig.2.** Clear zone of growth inhibition of the irradiated fibroin solution at different concentrations treated at dose of 10 kGy for (a) – *E. coli* and (b) – *S. aureus*

**Table III.** Minimum inhibitory concentration of irradiated silk fibroin solution at diffident doses

| **Irradiation dose (kGy)** | **MIC (mg/ml)** | | |
|---|---|---|---|
| | *E. coli* | *S. aureus* | *B. subtillis* |
| **0** | 30 | 30 | >30 |
| **10** | 4.2 | 6.5 | >30 |
| **20** | 4.5 | 6.5 | >30 |
| **40** | 4.5 | 7.0 | >30 |

**Table IV.** Amino acid profile of irradiated fibroin powder and commercial product

| **Amino acid** | **Irradiated fibroin (mg/g)** | **Commercial fibroin - bioflore (mg/g)** |
|---|---|---|
| Aspartic | 9.99 | No observed |
| Serine | 104.96 | No observed |
| Glutamic | No observed | No observed |
| Glycine | 247.24 | 0.19 |
| Histidine | No observed | No observed |
| Threonine | 3.23 | 0.70 |
| Arginine | No observed | No observed |
| Alanine | No observed | No observed |
| Proline | No observed | No observed |
| Cystine | 1.40 | 0.37 |
| Tyrosine | 75.06 | 4.03 |
| Valine | 42.47 | 7.55 |
| Methionine | No observed | 3.72 |
| Lysine | 2.13 | 10.61 |
| Isoleucine | 6.47 | 7.49 |
| Leucine | 5.19 | 11.65 |
| Phenyalanine | 7.71 | 8.46 |

The antibacterial activity of fibroin powder treated at dose of 500 kGy was also reported by Jindaron [5]. The authors found that the MICs of fibroin powder for *E. coli B/r a*nd *S. aureus K* were 2.4 mg/l and this concentration was also not enough to inhibit growth of *Bacillus subtillis.* In this study, similar activity was obtained for silk fibroin solution irradiated at doses from 10 to 40 kGy.

**C. Amino acid composition of irradiated silk fibroin**

From the results above, it is evident that the water-soluble yellowish silk fibroin powder can be prepared by lyophilization of the irradiated silk fibroin solution. The amino acid components of the irradiated silk fibroin were also analyzed and presented in Table 4. As one can see, the amounts of some amino acid exsisting in the irradiated fibroin such as serine, glycine, tyrosine and valine were much higher than those in the commercial product. This might be because the amino acid compositions of raw materials for silk fibroin production were effected by species of silk. In addition, the silk protein may be degraded into amino acid during gamma irradiation. The result was consistent with the experiment done by Vaithanomsat [10] that the chemical compositions of silk protein could be influenced by silk species and feed and this, therefore would be able to indicate the amino acid pattern of silk products.

## IV. CONCLUSIONS

The silk fibroin solutions were obtained by dialysis method, irradiated under gamma source, then their bio-activities and amino acid components were analyzed. Our results showed the bio-activities of fibroin solution had been improved by gamma irradiation. The maximum antioxidant activity was 70.4% obtained for the fibroin solution that irradiated at dose of 10 kGy. These irradiated fibroin solution also shown antibacterial activity against the tested bacteria strains *(E. coli* and *S. aureus)*.

The water-soluble silk fibroin powder can be prepared by lyophilization of irradiated silk fibroin solution. The irradiated silk fibroin powder revealed the higher antioxidant and antibacterial activities in comparision with a commercial silk fibroin (Proteines De Soie/ Zijdeproteine, Bioflore, Canada). The amino acid components of the irradiated fibroin were also higher than those of commercial product. Thus, the irradiated silk fibroin may potential be used for cosmetic and other related applications.

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