

Effect of Gamma Irradiation on Soybean Allergen Levels

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The levels of food allergens in gamma-irradiated soybean (0, 2.5, 5, 7.5, 10, 20, and 30 kGy) were investigated by immunoblotting and ELISA, using allergen-specific antibodies and patient serum. After 3 months of storage, Coomassie brilliant blue (CBB) staining indicated similar total protein profiles among the treatments, but that some proteins were degraded by irradiation at high doses. Immunoblotting with specific antibodies for major soybean allergens (β -conglycinin, Gly m Bd 30 K, soybean trypsin inhibitor, and Gly m 4) resulted in apparent band profiles and intensities that were not significantly changed by irradiation. Competitive inhibition ELISA analyses suggested that there were no significant changes in the allergen contents, except for a decrease in the soybean trypsin inhibitor. The patient IgE binding allergenic protein patterns were not changed by irradiation up to 30 kGy. ELISA using patient serum also revealed that the IgE reactivity to the irradiated soybean extract did not increase from the level of the control, but that the reactivity to some patient serum IgE was significantly decreased by irradiation.

Key words: gamma irradiation; allergenicity; soybean; allergen

Food legumes, including soybeans, are susceptible to attack by a variety of insects and microorganisms during storage. Gamma irradiation is a physical treatment used for food preservation. Irradiation at 1 kGy has been proposed for insect disinfestation of grain,¹⁾ and doses of 2.5–5 kGy have been recommended for complete disinfestation of dry legumes and to increase storage life.²⁾ Gamma irradiation may have a significant influence on the physical, chemical, and biological properties of the molecules in a treated sample. In some cases, proteins in treated samples may be degraded by irradiation.^{3,4)} A Joint Expert Committee on the Wholesomeness of Irradiated Food that was convened by FAO, IAEA, and WHO stated that irradiation of any food commodity up to 10 kGy presents no toxicological hazard.⁵⁾ Eval-

uating allergenicity has recently become increasingly important for food safety. Although the safety of radiation has been well characterized, the allergen level or allergenicity of an irradiated product is not yet fully understood.

Ionizing radiation has been reported to modify the antigenicity of the proteins in foods of animal origin.^{6–15)} This suggests a novel application for radiation processing: the antigenicity and IgE-binding ability of irradiated proteins would be altered as a result of the conformational modification that is induced by radiation.

Soybean (*Glycine max* L.) is used in various foods, including traditional Asian foods and Western cuisine. Soybean protein has superior properties for food processing which is why it is widely used as an additive in various processed food preparations. Soybean also has various unique physiological functions, including a serum lipid-lowering effect.^{16–20)}

A variety of allergens in soybeans have already been determined.²¹⁾ The major allergens of soybean are the Kunitz soybean trypsin inhibitor (KSTI),²²⁾ Gly m Bd 30 K,^{23,24)} Gly m Bd 28 K,²⁵⁾ and Gly m Bd 60 K²⁶⁾ which has been identified as the α subunit of 7S globulin (β -conglycinin). The latter three allergens were detected in patients with soybean food allergies. The oral allergy syndrome (OAS) and/or food allergies, including anaphylaxis, that were caused by soybean protein-containing foods have recently been reported in patients with birch pollen allergies. Starvation-associated message 22 (SAM 22: Gly m 4), which has a molecular weight (MW) of 17 kDa, has been reported as a major causative allergen due to this pollen-related food allergy.^{27,28)}

There have been several reports about the effects of irradiation on the allergenic properties of foodstuffs, including plant foodstuffs;^{6–15)} however, the effect of radiation on various soybean allergens has not been well investigated.

The objectives of this study were to evaluate the effect of irradiation on the allergen protein contents and reactivity with specific antibodies and patient serum.

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Abbreviations: CBB, Coomassie brilliant blue; ECL, enhanced chemiluminescence; ELISA, enzyme-linked immunosorbent assay; HRP, horse radish peroxidase; IgG, immunoglobulin G; IgE, immunoglobulin E; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TMB, tetramethylbenzidine

Materials and Methods

Materials. Horseradish peroxidase (HRP)-labeled anti-rabbit or anti-mouse IgG was obtained from Promega (Madison, WI, USA). HRP-labeled anti-human IgE was obtained from Kirkegaard & Perry Laboratories (KPL; Gaithersburg, MD, USA). The ECL Western blotting reagent and Hyperfilm-MP X-ray films were obtained from GE Healthcare (Piscataway, NJ, USA). The PVDF membrane (Immobilon-P) was obtained from Millipore (Billerica, MA, USA).

Irradiation of the soybeans. Soybeans (*Glycine max* (L.) Merrill cv. Fukuyutaka and Enrei) were obtained from a retailer and used within one year after harvesting. The dry soybeans were put in aluminum-sealed polyethylene bags. The sample bags containing soybeans (100 g) were put in a Gamma Cell-220 irradiation chamber (Nordion International, Kanata, Ontario, Canada), and irradiated at a dose of 2.5, 5, 7.5, 10, 20, or 30 kGy with gamma rays from cobalt-60. The dose rate was 0.65 kGy/h and the temperature during irradiation was $21 \pm 2^\circ\text{C}$. An alanine pellet dosimeter (Bruker Biospin, Rheinstetten, Germany) was attached to the surface of each sample bag, and the absorbed dose was determined with a Bruker EMX electron spin paramagnetic spectrophotometer (Bruker Biospin).

Preparation of the soybean extract and protein analysis. The dry soybean samples were stored at room temperature for 3 months after irradiation, and the protein was then extracted. Approximately 5 g of gamma-irradiated soybeans were immersed in distilled water for 2 h. The soaked soybeans were then homogenized for 30 s in 25 mL of distilled water with a commercial food mixer. The homogenate was filtered through three layers of gauze, and the filtrate was used for determining the protein concentration, this being followed by SDS-PAGE or ELISA after dilution. The protein concentration of the extracted soybeans was determined by the Bradford method²⁹ with a commercial kit (Bio-Rad, USA). The extracted protein samples were either immediately analyzed or stored at -20°C until needed for analysis.

Antibodies against the major soybean allergens. The mouse monoclonal antibody against Gly m Bd 30K was a kindly presented by Dr. Tadashi Ogawa of Kansai Welfare University, Osaka, Japan. The rabbit polyclonal antibodies against 7S globulin (β -conglycinin; α' , α , and β subunits) were obtained as previously described.³⁰ The rabbit polyclonal antibodies against Gly m 4 were obtained by immunizing recombinant Gly m 4 in our laboratory (unpublished data). The rabbit antibody against the soybean trypsin inhibitor was obtained from Rockland (Gilbertsville, PA, USA). The soybean allergens used in this study are listed in Table 1.

Electrophoresis and immunoblotting. The extracted protein from soybeans was subjected to SDS-PAGE.³¹ Proteins on the gel were stained with CBB R-350 (GE Healthcare) in order to detect the total protein patterns. The immunoblotting analysis was conducted by transferring the SDS-PAGE gel on to an Immobilon-PTM PVDF membrane (Millipore) by using a semi-dry blotting method.³² The

membrane was incubated in 10 mM PBS (pH = 7.5) containing 0.1% Tween-20 (PBST) and 5% skim milk for blocking. The membrane was then incubated for 1 h at room temperature in a blocking buffer containing the allergen-specific antibodies. After washing the membranes four times with PBST for 10 min, the bound primary antibodies were detected by using HRP-conjugated goat anti-rabbit or anti-mouse IgG (Promega) and an ECL western blotting kit (GE Healthcare). The resultant chemiluminescent signals were detected on X-ray film (Hyperfilm MP, GE Healthcare). The blocking buffer used for immunoblotting with patient serum was the same as that for soybean protein immunoblotting. Diluted serum (20-fold) in the same blocking buffer was used as the primary antibody. After blocking, HRP-labeled anti-human IgE (KPL) was used as a secondary antibody. The enhanced chemiluminescent (ECL) signals were recorded on X-ray film. Commercially available serum (serum A–D) from soybean allergy patients was purchased from Kokusai Bio (Tokyo, Japan).

Competitive inhibition ELISA using allergen-specific antibodies. Competitive inhibition ELISA was used to evaluate the allergen levels of irradiation-treated soybean samples. Briefly, the control soybean (0 kGy) extract was coated on to an ELISA plate. After coating with the sample and blocking, allergen specific antibodies and various concentrations of the irradiated soybean extract (competitors) were co-incubated and placed into the wells. After reacting for 1 h at room temperature and washing with PBST four times, HRP-labeled second antibodies were added to the wells. The bound HRP-labeled second antibodies were detected by reacting with a tetramethylbenzidine (TMB) peroxidase substrate (KPL, Gaithersburg, MD, USA) for 5 min. The reaction was stopped by adding 100 μL of 1 M phosphoric acid to amplify the signal. Absorption was measured at 450 nm by using an ARVosx-1 1420 multilabel counter (PerkinElmer Life Sciences, Boston, MA, USA). Measurements were performed twice and the mean values were plotted.

IgE-ELISA using patient serum. The patient serum IgE binding capacity of the irradiated soybean extracts was determined by using IgE-ELISA. Briefly, a protein extract (100 μL at 10 $\mu\text{g}/\text{mL}$ in PBS) from irradiated soybeans was coated on an ELISA plate. After coating the sample overnight at 4°C and blocking for 1 h, diluted patient serum was added to the wells and incubated for 2 h. After washing the wells with PBST, the biotin-labeled anti-human IgE antibody (KPL) was added to the wells, and the samples were incubated for a further 1 h. After washing the wells, HRP-labeled streptavidin (GE Healthcare) was added to the wells and they were incubated for another 1 h. Finally, the bound IgE-biotinated second antibody-streptavidin HRP complex was detected by reacting with a TMB peroxidase substrate (KPL) for 5 min. The reaction was stopped and the signal amplified by adding 100 μL of 1 M phosphoric acid. The absorption at 450 nm was measured as already described. Measurements were performed three times ($n = 3$).

Statistical analysis. Statistical tests were performed by using Microsoft Office Standard 2007 Excel software (Microsoft Corporation, Redmond, WA, USA). Results are expressed as the mean \pm

Table 1. List of Major Soybean Allergens Analyzed in This Study

| Allergen name | Reported Functions | Molecular mass (kDa) | References |
|-----------------------------------|--|----------------------|------------|
| β -conglycinin ¹ | Storage glycoproteins | | 26, 36, 37 |
| α' -subunit | | 72 | |
| α -subunit | | 68 | |
| β -subunit | | 50 | |
| Gly m Bd 30K | Papain superfamily (no activity) ² , Receptor for syringolide elicitors | 34 (30) ³ | 23, 24, 38 |
| Soybean Trypsin inhibitor | Trypsin inhibitor (Kunitz type) | 18 | 22 |
| Gly m 4 | PR-10 family | 16–17 | 27, 28, 39 |

¹Alternative name: 7S globulin, Gly m 6.

²The catalytic amino acid residue is displaced.

³The theoretical molecular mass of Gly m Bd 30K from cDNA is approximately 30 kDa, but the position determined *via* SDS-PAGE is reported to be 34 kDa.

standard deviation (SD). Data were analyzed by Student's *t*-test with Excel Statistics software (SSRI Co., Tokyo, Japan). $p < 0.05$ between groups was accepted as significantly different.

Results

Effect of gamma irradiation on the total protein pattern of soybeans

Figure 1 shows similar overall protein profiles for the irradiated and control soybeans. A faint band around 20 kDa appeared in the soybeans of the Enrei and Fukuyutaka cultivars that had both been treated by high-dose irradiation (20 or 30 kGy). The molecular weight bands around 90 kDa were obviously decreased in Fukuyutaka with irradiation doses greater than 10 kGy. It therefore appears as though the proteins with molecular weight bands of approximately 90 kDa had been partially degraded into lower molecular weight protein fragments by irradiation at high doses. The molecular weight bands of approximately 90 kDa were not obviously diminished in the Enrei cultivar samples.

Effect of gamma irradiation on the levels of major soybean allergens, as detected by immunoblotting using specific antibodies

Figure 2 shows that the allergen levels were almost the same irrespective of irradiation dose tested for the Enrei and Fukuyutaka soybean cultivars. However, Enrei contained less soybean trypsin inhibitor than Fukuyutaka.

Effect of gamma irradiation on the levels of major soybean allergens, as determined by competitive inhibition ELISA

Competitive inhibition ELISA allows the allergen levels in each sample to be compared by measuring the absorbance after an antibody-linked enzyme reaction. Figure 3 indicates the results for Gly m Bd 30 K and 7S globulin. The inhibitory curves for samples of both cultivars that were exposed to various doses of radiation were almost same. The inhibition curves for the soybean trypsin inhibitor were shifted to the high-dosage area (Fig. 4A). This suggests that the soybean trypsin inhibitor level in irradiated soybeans might have been

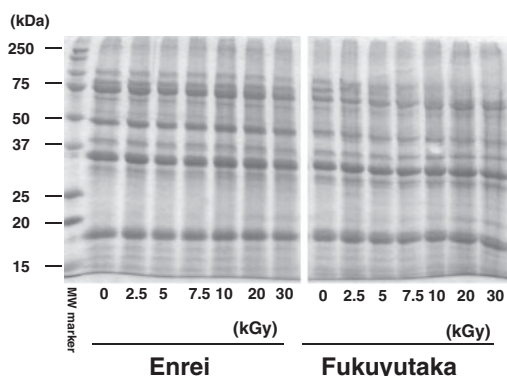


Fig. 1. Protein Profiles of Gamma-Irradiated Soybeans Detected by CBB Staining.

Total proteins were extracted from soybeans (Enrei and Fukuyutaka) treated with various doses of gamma-rays and subjected to SDS-PAGE (50 µg/lane). Total proteins were stained with CBB G-350 as described in the Materials and Methods section.

lower than that in non-irradiated soybeans. The inhibitory curves for Gly m 4 were similar for all doses of irradiation, indicating that the Gly m 4 level was unchanged by irradiation (Fig. 4B).

Effect of gamma irradiation on the patient IgE binding protein profiles of soybeans.

The sera from four soybean allergy patients were used to determine the IgE binding protein profiles for the soybean allergy patients (Fig. 5). Although the immunoblot patterns differed among the patients, there were several protein bands found for IgE in the sera from more than one patient. Protein bands of 50, 24, 22, 18, and 17 kDa were detected in both cultivars when serum A was used; protein bands of 50 and 30 kDa were detected in both cultivars when serum B was used; protein bands of 100, 75, 35, 24, and 22 kDa were detected in both cultivars when serum C was used; and smear bands of 75–50 kDa and an intense 18 kDa band were detected when serum D was used. All of these detected IgE-binding allergenic protein bands were similar for the non-treated and irradiated soybean extracts. In some cases (for Enrei, 100, 75, and 35 kDa detected by serum C and the 75–50 kDa smear bands detected by serum D), the detected IgE-binding allergenic bands of irradiated soybeans were weaker than those of the control soybeans. In addition, no novel allergenic proteins appeared in the irradiated soybeans that were not present in the control soybeans. On the basis of molecular weight, the 75 kDa bands might have been subunits of 7S globulin, the 50 kDa bands might have been subunits of 7S globulin, the 30 kDa bands might have been Gly m Bd 30 K, and the 18 kDa bands might have been the soybean trypsin inhibitor.

ELISA analysis of the effect of gamma irradiation on the patient IgE binding reactivity of soybeans.

The patient IgE binding reactivity toward gamma-irradiated soybeans of both cultivars was similar to, or slightly lower than the reactivity level toward the controls (Table 2). The absorbance value for the reaction between 30 kGy-treated Enrei soybeans and serum C was significantly lower than that for 0 kGy-

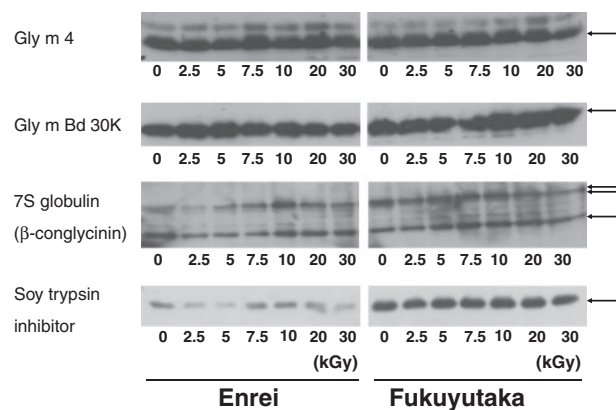
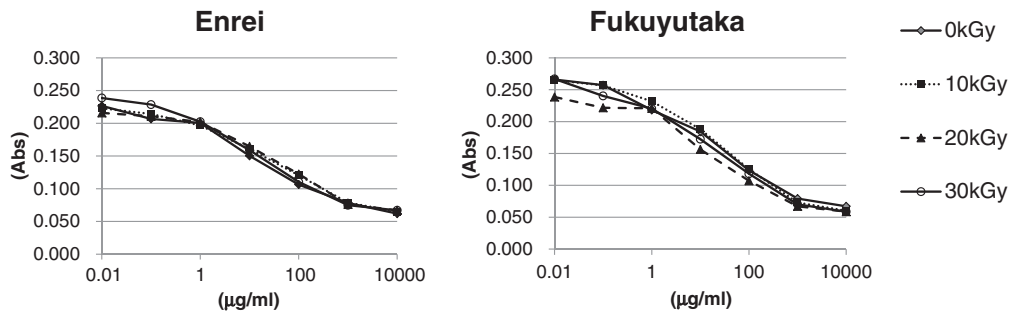


Fig. 2. Immunoblotting of Gamma-Irradiated Soybean Proteins Detected with Allergen-Specific Antibodies.

Proteins in the extracts of irradiated soybeans (Enrei and Fukuyutaka) were separated by SDS-PAGE on 10–15% polyacrylamide gel, before immunoblotting, as described in the Materials and Methods section. The major soybean allergens analyzed are listed in Table 1.

A. Glym Bd 30K



B. 7S-globulin

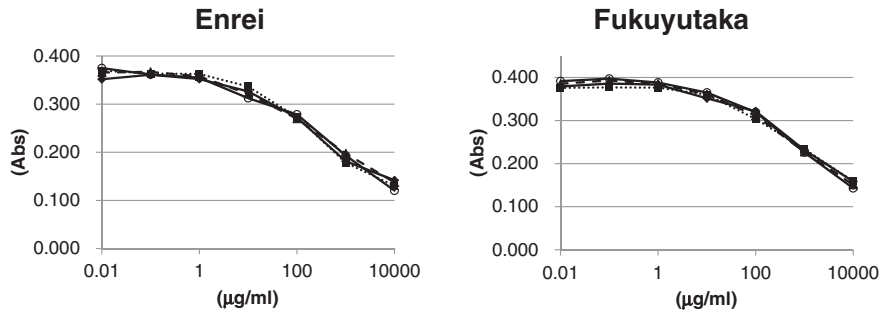
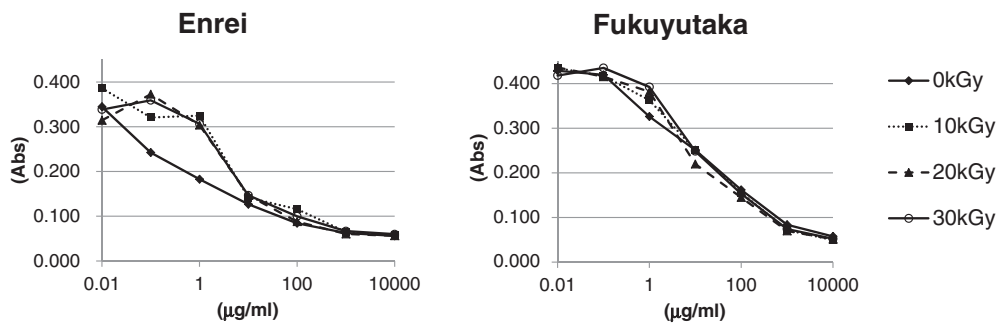


Fig. 3. Competitive Inhibition ELISA Analysis of the Major Allergens in Soybean (A, Gly m Bd 30K; B, 7S globulin). Changes in the allergen levels of irradiated soybeans (Enrei and Fukuyutaka) were quantitatively analyzed by competitive inhibition ELISA as described in the Materials and Methods section.

A. Soybean Trypsin Inhibitor



B. Gly m 4

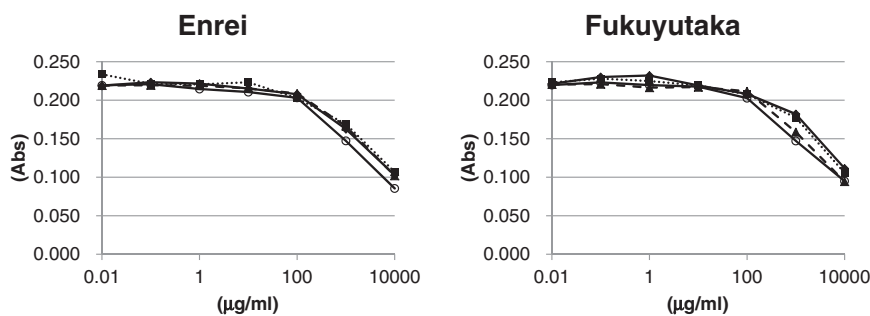


Fig. 4. Competitive Inhibition ELISA Analysis of the Major Allergens in Soybean (A, soybean trypsin inhibitor; B, Gly m 4). Changes in the allergen levels of irradiated soybeans (Enrei and Fukuyutaka) were quantitatively analyzed by competitive inhibition ELISA as described in the Materials and Methods section.

treated Enrei soybeans ($p < 0.05$). Similarly, the absorbance values for 20 and 30kGy-treated Fukuyutaka soybeans after the reaction with sera A and C were significantly lower than those for 0kGy-treated Fukuyutaka soybeans ($p < 0.05$).

Discussion

The overall protein pattern for both cultivars was not different between the untreated and irradiated samples at doses up to 30kGy. However, the high-molecular-

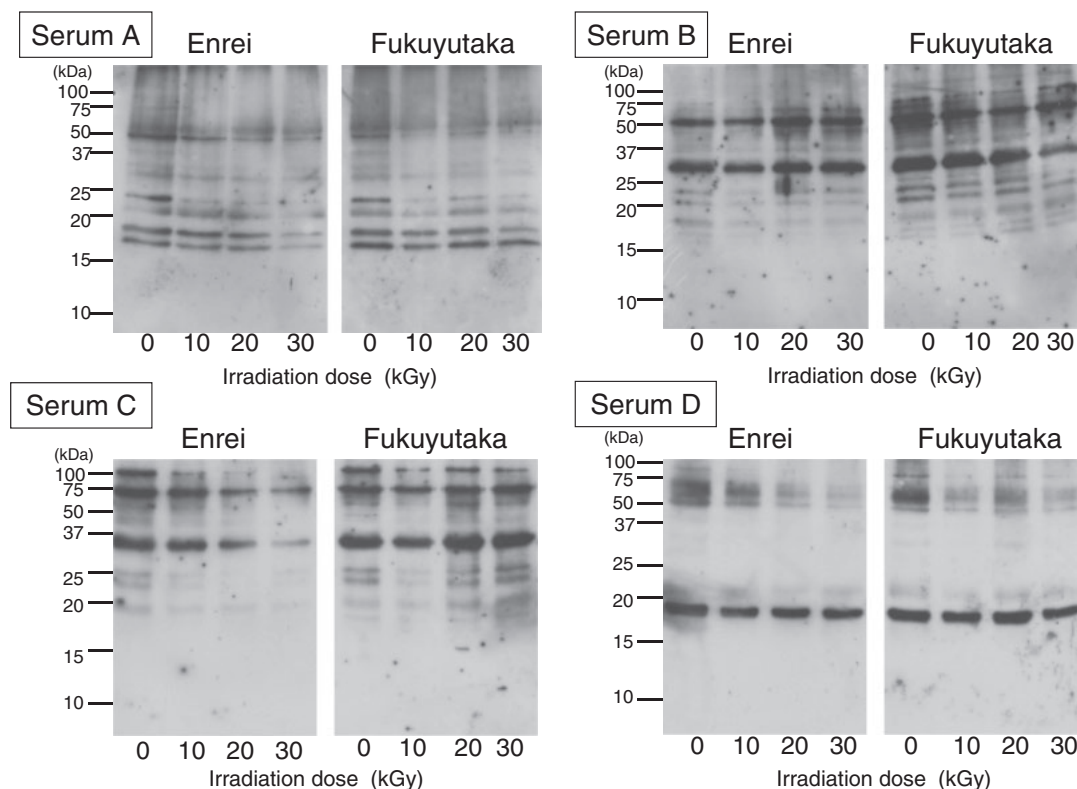


Fig. 5. Immunoblot Analysis of Gamma-Irradiated Soybean Proteins Detected with Patient Serum.

Proteins were extracted from gamma-irradiated soybean (Enrei and Fukuyutaka) and separated by SDS-PAGE on 12.5% polyacrylamide gel, before immunoblotting with soybean allergy patient serum, as described in the Materials and Methods section.

Table 2. IgE-ELISA of Irradiated Soybean Extracts Using Serum from Soybean Allergy Patients

| Enrei | (kGy) | | | |
|---------|---------------|---------------|---------------|----------------|
| | 0 | 10 | 20 | 30 |
| Serum A | 0.336 ± 0.017 | 0.320 ± 0.029 | 0.321 ± 0.043 | 0.300 ± 0.039 |
| Serum B | 0.187 ± 0.024 | 0.198 ± 0.026 | 0.278 ± 0.029 | 0.222 ± 0.038 |
| Serum C | 0.467 ± 0.018 | 0.459 ± 0.035 | 0.434 ± 0.012 | 0.420 ± 0.022* |
| Serum D | 0.217 ± 0.015 | 0.209 ± 0.018 | 0.209 ± 0.010 | 0.228 ± 0.021 |

| Fukuyutaka | (kGy) | | | |
|------------|---------------|---------------|----------------|----------------|
| | 0 | 10 | 20 | 30 |
| Serum A | 0.395 ± 0.013 | 0.372 ± 0.019 | 0.349 ± 0.011* | 0.328 ± 0.028 |
| Serum B | 0.253 ± 0.006 | 0.293 ± 0.025 | 0.241 ± 0.010 | 0.245 ± 0.024 |
| Serum C | 0.528 ± 0.042 | 0.480 ± 0.007 | 0.515 ± 0.025 | 0.446 ± 0.006* |
| Serum D | 0.278 ± 0.039 | 0.248 ± 0.014 | 0.261 ± 0.015 | 0.259 ± 0.032 |

*: $p < 0.05$ (vs 0 kGy)

The IgE binding capacities of the irradiated soybean extracts were determined using biotin-labeled anti-human IgE and HRP-labeled streptavidin, as described in the Materials and Methods section. Values are the mean ± SD (n = 3).

weight protein bands of around 90 kDa were degraded in the Fukuyutaka cultivar after irradiation at doses of 20 and 30 kGy. Low-molecular-weight protein bands of around 20 kDa were detected after the high-dose treatment. This protein band appeared to be from protein fragments derived from the degraded high-molecular-weight protein bands of around 90 kDa.

While there were faint fragmented bands (20 kDa) in the Enrei cultivar after the high-dose treatment (20 or 30 kGy), there were no obvious degraded high-molecular-weight protein bands of around 90 kDa. These results suggest that the effect of irradiation may have varied between the cultivars. Such differences may result from differences in the water and/or protein content. Fur-

thermore, it is unknown whether protein degradation (fragmentation) is caused directly by irradiation or by an enzyme reaction of endogenous protease activated during storage by irradiation.

The molecular weight of the degraded protein was around 90 kDa, which suggests that this protein was a lipoxygenase.³³⁾ Soybean lipoxygenases were detectable at around 90 kDa by CBB staining.

All of the protein bands of irradiated peanut butter (a dose of 27.7 kGy) are reportedly visible by SDS-PAGE, and the amino acid profile of peanut butter has shown no significant decrease in the crude protein or total protein content; therefore, the total protein degradation after irradiation is likely to be small.³⁴⁾

Maity *et al.* have also shown that *Oryza sativa*, in comparison with other seeds, lost little of its total soluble protein content at 6 kGy. They have also shown that the soluble protein fraction, containing 14–16 kDa albumins and 22 kDa globulin, was unchanged by irradiation at doses up to 6 kGy.³⁵⁾ They concluded that while the quantity of protein was unchanged by gamma radiation, the quality of the protein might have changed.³⁵⁾ These findings are similar to those of the present study.

Soybean contains several protein allergens²¹⁾ that cause allergenic reactions in humans. One of the major allergens is 7S globulin (β -conglycinin). This allergen is composed of three subunits: α (about 68 kDa), α' (about 72 kDa), and β (about 50 kDa). The 7S globulin α subunit was the first to be identified as an allergen, the other subunits (α' , β) being identified as protein allergens later.³⁶⁾ The structural homology among these three subunits is relatively high (approx. 70–75%). This protein is a soybean storage protein. This protein has recently been reported to be an allergen responsible for food-dependent exercise-induced anaphylaxis (FDEIA) that was caused by the processed soybean food, tofu.³⁷⁾ Immunoblotting and competitive inhibition ELISA in the present study indicated that the soybean 7S globulin levels of both cultivars were not obviously changed by irradiation. These results suggest that the allergenicity of similar plant storage proteins in such other species as sesame, nuts, and other legumes might not be affected by irradiation.

Gly m Bd 30 K is another major allergen.^{23,24)} Gly m Bd 30 K is part of the papain superfamily. This allergen molecule has been reported to be the receptor for syringolide elicitors.³⁸⁾ This allergen was detected at the same level in both cultivars, and immunoblotting and the competitive inhibition ELISA analysis indicated that irradiation did not change the Gly m Bd 30 K content of either cultivar (Figs. 2 and 3).

The soybean trypsin inhibitor was reported as a soybean allergen in 1980.²²⁾ The Enrei and Fukuyutaka cultivars differed in the amount of soybean trypsin inhibitor they contained. The Enrei cultivar contained less of this allergen than did the Fukuyutaka cultivar. However, there was no obvious increase in the level of this allergen after irradiation in either cultivar, as shown in Fig. 2. In fact, competitive inhibition ELISA indicated that the quantity of this allergen or its reactivity to the antibody used had decreased after irradiation (Fig. 4). It is not clear why irradiation affected the concentration or reactivity of only this allergen. However, in comparison with the other proteins tested, the soybean trypsin inhibitor may be more susceptible to radiation. The response of the soybean trypsin inhibitor to irradiation was detected only by competitive inhibition ELISA, indicating that this experimental approach might be useful for studying the protein responses to irradiation. The reason why the response of the soybean trypsin inhibitor to irradiation was obvious in the Enrei cultivar also needs to be revealed.

Gly m 4 is a pollen-related soybean allergen.²⁷⁾ This molecule is the Bet v 1 homologous protein which is the major allergen in birch pollen. A follow-up study by Mittag *et al.*²⁸⁾ has confirmed that Gly m 4-specific IgE was positive in 21 of 22 birch pollinosis patients who developed the soybean allergy. Three patients with

alder-birch pollinosis who developed OAS or anaphylaxis after ingesting soymilk have been reported in Japan, and the involvement of Gly m 4 has been suggested.³⁹⁾ Figures 2 and 4 show that the Gly m 4 allergen levels were not changed by the tested doses of irradiation in either soybean cultivar. This was confirmed by immunoblotting and the competitive inhibition ELISA analysis. This allergen is categorized as a pathogenesis-related protein (PR-P). PR-Ps are up-regulated when a plant is stressed by such conditions as starvation, injury, and infestation by pests. Therefore, under the current conditions, irradiation might not have induced the up-regulation of a PR-P such as Gly m 4. These results are important, because many plant-derived food allergens are PR-Ps,^{40–42)} and the lack of Gly m 4 up-regulation suggests irradiation stress to be weaker than such conventional stresses as starvation, injury and infestation.

There have been several reports of irradiation inducing the degradation of allergens in such animal foods as shrimp, milk, and eggs.^{6–15)} In the present study, only some of the allergenic proteins detected by patient serum tended to be decreased by irradiation (Fig. 5). The results obtained from IgE-ELISA also suggest that the IgE reactivity of irradiated soybeans tended to be lower than that of untreated soybeans (Table 2). The difference between plant and animal foods in their susceptibility to degradation by irradiation might have been due to differences in water composition. Compared to a plant foodstuff like the dry soybeans used in the current study, such animal foodstuffs as milk and eggs contain much more water.

Figure 5 shows that no novel IgE binding proteins appeared as a result of irradiation. Our results also suggest that IgE-reactive modifications to soybean proteins may not occur during storage after irradiation.

Leszczynska *et al.* have investigated the influence of gamma irradiation on the immunoreactivity of gliadin and wheat flour. They found that irradiated gliadin samples showed increased allergenicity, as measured by ELISA. Moreover, there was a greater immune response to gliadin extracted from irradiated wheat flour than to pure gliadin that was irradiated with the same dose.¹⁴⁾

We have shown in the present study that the allergen levels and IgE-reactivity of soybeans were not changed, or only slightly decreased, by irradiation. The differences between our results and those obtained by Leszczynska *et al.* might have been due to differences in the protein properties; wheat protein contains a large amount of SH groups, suggesting that the reactivity of these proteins may be easily affected by irradiation.

Zoumpoulakis *et al.* have reported that the allergenicity of storage seed proteins in white sesame seeds was not significantly affected by irradiation up to 10.0 kGy.¹²⁾ Their results are similar to those of the present study.

In conclusion, this study has demonstrated that doses of up to 30 kGy of gamma irradiation might not affect the level and reactivity of the major allergenic proteins in dry soybeans following 3 months of storage. This suggests that the risk of soybean allergenicity is not changed by gamma irradiation at a dose lower than 30 kGy. Further risk assessment of irradiated foodstuffs should be conducted in future studies.

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