Analysis of radical-scavenging activity in gamma-irradiated soybean seeds by electron spin resonance spectroscopy

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電子スピン共鳴分光法によるガンマ線照射大豆のラジカル消去活性分析
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Summary

Soybeans are an inexpensive and nutritious food with strong radical-scavenging activity, believed to reduce the risk of developing diseases associated with the generation of reactive oxygen species and inhibit the progression of such diseases. Although gamma irradiation of soybeans improves their physical characteristics without adversely affecting flavor, the specific radical-scavenging activity of irradiated soybeans has not been well studied. Here, the scavenging activity of soybean water extracts for individual radical species was investigated by electron spin resonance (ESR) spin trapping. Singlet oxygen-scavenging activity increased, whereas superoxide radical-scavenging activity decreased with increasing radiation dose; however, the hydroxyl and alkoxyl radical-scavenging activity of irradiated and non-irradiated soybeans did not differ significantly. The oxygen-radical absorbance capacity of irradiated and non-irradiated soybeans also did not differ significantly. Our findings demonstrate that gamma-irradiation affects the superoxide radical and singlet oxygen-scavenging activity of soybean water extracts.

Key words: soybean (大豆), gamma-irradiation (ガンマ照射), ESR spin trapping (ESRスピントラップ), radical-scavenging activity (ラジカル消去活性)

1. Introduction

Irradiation in food is performed to eliminate harmful microorganisms, insects, fungi, and other pests and may reduce the need to use hazardous pesticides, fumigants, and preservatives. Soybeans and their processed products have been acclaimed as health foods owing to their high levels of protein and essential amino acids, omega-3 fatty acids, fat-soluble vitamins, polysaccharides, and insoluble fibers. Soybeans also contain isoflavones that may prevent cancer, cardiovascular diseases, osteoporosis, and menopausal symptoms. Therefore, soybeans are widely used to produce healthy food items.

Gamma irradiation up to 1 kGy has been recommended for quarantine treatment of legumes, including soybeans. Gamma irradiation causes oxidative stress and affects physiological activity by inducing conformational changes, oxidation, rupture of covalent bonds, and formation of free radicals. Consequently, soybean functions such as radical-scavenging activity should also be affected, although previous re-
ports on irradiated soybeans have been contradictory. Some studies reported an increase in DPPH radical-scavenging activity \(^6-^7,^10\) whereas others reported a decrease in the DPPH radical-scavenging activity \(^1^1\), ferric-reducing antioxidant power (FRAP) \(^1\), and Trolox-equivalent antioxidant capacity (TEAC) \(^1^2\). Few studies reported no change \(^1^3\) in the radical-scavenging activity.

The electron spin resonance (ESR) spin trap method can be applied to measure the scavenging activity of specific types of ROS in vivo. We applied this method to evaluate the scavenging activity of gamma-irradiated soybeans against hydroxyl, alkoxyl, superoxide radicals, and singlet oxygen.

2. Experimental

2.1 Sample and reagents

Soybeans [\textit{Glycine max} (L.) Merrill cv. Fukuyutaka] commonly consumed in Japan were analyzed. Fluorescein and Trolox \(^8\) were purchased from Aldrich (Milwaukee, WI, USA). The spin-trapping reagent, CYPMPO, was obtained from Radical Research (Hino, Japan) \(^1^0\). All other chemicals were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan) and were of the highest grade available.

2.2 Gamma irradiation

Soybeans in aluminum-sealed polyethylene bags were irradiated with gamma rays from a cobalt 60 source (Gammacell 220; MDS Nordion International Co. Ltd., Ottawa, Ontario, Canada) at the National Food Research Institute of Japan. The dose rate was 4 kGy/h. The soybeans were irradiated at doses of 0.5–30 kGy at room temperature. An alanine pellet dosimeter (Bruker Biospin Ltd., Rheinstetten, Germany) was attached to the surface of each sample and the absorbed dose was determined using an ESR spectrometer (Bruker EMX; Bruker Biospin Ltd.).

2.3 ESR spin trapping method

2.3.1 ESR measurement apparatus

ESR measurements were performed with an X-band ESR spectrometer (EMX-Plus, Bruker BioSpin, Karlsruhe, Germany) with 100 kHz field modulation.

2.3.2 Radical generation and trapping reaction

Soybean powder sample (1 g) was soaked in 30 mL extraction solution (pure water) and incubated at 4°C for 24 h. After filtration with filter paper, the volume of the extract was adjusted to 50 mL with water. A mixture containing 50 μL extract, 50 μL precursor/sensitizer, and 20 μL CYPMPO (10 mmol/L) in 80 μL sodium phosphate buffer was added to an ESR flat cell (Radical Research, Hino, Japan). Radicals were produced by illumination with the light source in the ESR cavity, followed by immediate measurement of the ESR spectrum. Table 1 summarizes the measurement conditions for each precursor/synthesizer reagent for the generation of hydroxyls, alkoxyls, superoxide radicals, and singlet oxygen.

| Radical       | Precursor/sensitizer (concentration) | Light source | Filter | Wavelength range (nm) | Illumination period (s) | Spin trap |
|---------------|-------------------------------------|--------------|--------|------------------------|------------------------|-----------|
| Hydroxyl      | Hydrogen peroxide (100 mmol/L)      | LC-8         | None   | 300–400                | 5                      | CYPMPO    |
| Alkoxyl       | AAPH (5 mmol/L)                     | LC-8         | None   | 300–400                | 5                      | CYPMPO    |
| Superoxide    | Riboflavin (30 μmol/L)              | LC-8         | Band path | 500–600            | 60                     | CYPMPO    |
| Singlet oxygen| Pterine (100 μmol/L)                | LC-8         | None   | 300–400                | 5                      | TMPD      |
2.3.3 ESR measurements

Spectrometer settings were as follows: resonance field, 3522.2 G; field modulation width, 1.0 G; and microwave power, 6 mW. ESR spectra were accumulated at room temperature. The UV light source for photolysis was a 200-W medium pressure mercury/xenon arc lamp (LC-8; Hamamatsu Photonics K.K., Hamamatsu, Japan), where UV and visible light was guided through a quartz light guide into the ESR sample cavity. We used a G-533 band-pass filter (HOYA, Tokyo, Japan) for visible light. The illuminator was equipped with a computer-controlled mechanical shutter and the illumination period was set in the range 0.1–100 s with a precision of 0.01 s. UV illumination intensity was 2.78 mW/cm², as measured by a UV intensity meter (Cole-Parmer International, Vernon Hills, IL, USA).

2.3.4 Kinetics of ESR measurements

Data analysis was performed as described 10. The results can be expressed in terms of standard scavenger concentration. Mannitol, trolox, and glutathione (GSH) were selected as the standard scavengers of hydroxyl radical, alkoxyl radical, superoxide radical, and singlet oxygen scavenging activity, respectively. Scavenging capacities were converted to unit equivalents of standard scavenger per weight (g) soybean seed by the kinetics method.

2.4 H-ORAC

Soybean seeds were extracted for use in the ORAC method as described 10. The H-ORAC value for each sample was calculated from the Trolox standard curve and expressed in terms of moles of Trolox equivalent (TE).

2.5 Statistical analysis

Statistical analyses were performed using Microsoft Office Standard 2007 Excel software (Microsoft Corporation, Redmond, WA, USA). The multiple comparison by Tukey’s test was performed to analyze all results. Differences of p > 0.05 were deemed insignificant.

3. Results

Table 2 shows the H-ORAC values of soybean extracts. The H-ORAC values did not change significantly (p > 0.05) and did not correlate well with the irradiation dose. Table 3 shows the radical-scavenging activity of soybean extracts determined by ESR spin trapping. The superoxide radical-scavenging activity dose-dependently decreased from 0.5 to 30 kGy, with a curvilinear regression of \( y = 0.0014x^2 - 0.0651x + 0.9613 \) \( (r^2 = 0.99) \), where x and y equal to irradiation dose and scavenging activity respectively. In addition, the singlet oxygen-scavenging activity dose-dependently increased from 0.5 to 30 kGy \( (p < 0.05) \), with a curvilinear regression of \( y = -0.0048x^2 + 0.2098x + 0.8577 \) \( (r^2 = 0.98) \). Conversely, hydroxyl and alkoxyl radical-scavenging activity did not change significantly \( (p > 0.05) \) and did not correlate well with the irradiation dose.

4. Discussion

4.1 ORAC values

ORAC is a useful method for assessing food extracts that contain various antioxidants 10. ORAC analysis revealed no significant difference between irradiated and non-irradiated soybeans. Therefore, the scavenging activity of soybean extracts for individual radical species was investigated by ESR spin

| Table 2. H-ORAC values of soybean extracts determined by ORAC method |
|---------------------------------|-----------------|
| Irradiation dose (kGy)          | ORAC method     |
|                                 | H-ORAC (mmol of TE/g) |
| 0                               | 0.554 ± 0.001 *   |
| 0.5                             | 0.558 ± 0.002 *   |
| 1                               | 0.581 ± 0.003 *   |
| 5                               | 0.577 ± 0.002 *   |
| 10                              | 0.577 ± 0.007 *   |
| 30                              | 0.581 ± 0.001 *   |

Mean value ± standard deviation.
* Different letters within the same column differ significantly \( (p < 0.05) \).
trapping.

4.2 Evaluation of radical-scavenging activity by ESR spin trapping method

Hydroxyl and alkoxyl radical-scavenging activities were unaffected by gamma irradiation; however, superoxide radical- and singlet oxygen-scavenging activities were clearly influenced by gamma irradiation.

In this study, we measured the radical-scavenging activity of soybean water extracts. Byun et al. investigated the effects of gamma irradiation in Korean soybean fermented foods, and reported no correlation to the DPPH radical-scavenging activity of water-extracted soybean and enzymatic (xanthine oxidase) inhibition. Štajner et al. investigated the effects of different gamma irradiation doses (100–200 Gy) on the antioxidant properties of water-extracted soybean seeds and attributed the decrease in ferric reducing antioxidant power (FRAP) to non-enzymatic antioxidants (vitamins C and E and polyphenol constituents) rather than to the glutathione, malondialdehyde (MDA) quantities, and soluble protein content.

We hypothesized that non-enzymatic antioxidants (vitamins and phenolic constituents) are responsible for the radical-scavenging activity of water-extracted soybeans. Soybean seeds contain isoflavones as their major phenolic constituents and antioxidant components. Vairyan et al. assessed isoflavone contents in irradiated and non-irradiated soybeans and found a decrease in total isoflavone and increase in aglycon content with increasing irradiation dose (0.5–5 kGy). Flavonoid compounds mainly scavenge superoxide radicals. Therefore, we considered that the decrease in superoxide radical-scavenging activity was due to the reduction in total isoflavone content with increasing irradiation dose. We observed that singlet oxygen-scavenging activity increased with increasing irradiation dose. Ichiyamagi et al. studied the activity of aglycones against hydroxyl radicals and singlet oxygen by capillary zone electrophoresis. They found that aglycone is the singlet oxygen scavenger, with structure-dependent variability. Based on these reports, we hypothesize that the increase in singlet oxygen-scavenging activity was due to an increase in aglycon content with increasing irradiation dose.

We evaluated the anti-oxidant capacity of water-extracted soybeans by ORAC and ESR-spin trapping methods for radicals found in biological systems. The soybeans also contain fat-soluble antioxidant components such as lutein and tocopherol. Water-soluble antioxidant components can be measured by the ESR spin trapping method; however, fat-soluble components cannot be measured by this approach. In the future, we aim to develop a method to measure the antioxidant capacity of fat-soluble components by the ESR spin trapping method.

5. Conclusion

H-ORAC values, commonly used to evaluate scavenging activity, did not differ significantly be-
between irradiated and non-irradiated soybean extracts. We analyzed the specific radical-scavenging activity of gamma irradiated soybeans by ESR spectroscopy. Singlet oxygen-scavenging activity increased and superoxide radical-scavenging activity decreased with increasing radiation dose. In contrast, the hydroxyl and alkoxy radical-scavenging activities of irradiated soybeans did not change significantly from those of non-irradiated soybeans. We performed ESR spin trapping to evaluate the effect of gamma irradiation to determine the dose dependency of individual radical-scavenging activities in soybeans.

要 旨
大豆は、強いラジカル消去活性を有する安価で栄養価の高い食品で、活性酸素種に関連する疾患を発症する危険性を低減、もしくは疾患の進行を阻害すると考えられている。大豆へのガンマ線照射は、風味に影響を及ぼすことなく、特性を向上させるが、照射された大豆のラジカル消去活性について詳細な研究されていない。そこで、本研究では、電子スピノーレス（ESR）スピントラップにより、各ラジカル種に対する大豆水抽出物のラジカル消去活性について調べた。その結果、大豆に照射する線量が増加すると、一重項酸素消去活性は増加、スーパーオキシドラジカル消去活性は減少した。しかし、ヒドロキシルラジカル、アルコキシラジカル消去活性は、照射と非照射大豆で有意差は認められなかった。さらに、照射と非照射大豆は、ORACでも有意差は認められなかった。本研究結果により、ガンマ線照射は、大豆の水抽出物が有するスーパーオキシドラジカルおよび一重項酸素消去活性に影響を与えることが示された。

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