Analysis of gamma ray–induced radicals and radical-scavenging activities in soybean seeds by using electron spin resonance spectroscopy

Hiromi Kameya*, Kimie Saito, Setsuko Todoriki

National Food Research Institute, National Agriculture Research Organization, Japan

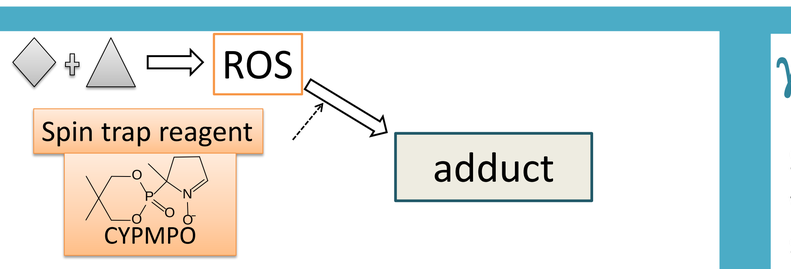
INTRODUCTION

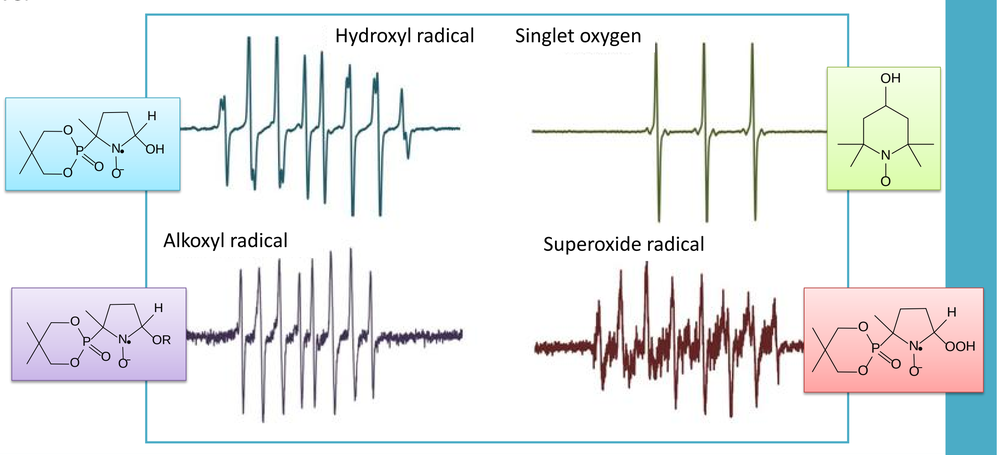
Irradiation is to eliminate harmful microorganisms, insects, fungi, and other pests in food and may reduce the need for using hazardous pesticides, fumigants, and preservatives. Soybeans and their processed products have been acclaimed as health foods due to their high levels of protein and essential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

Gamma-ray irradiation processing at 1 kGy has been recommended for quarantine treatment of legumes, including soybeans. Gamma-ray irradiation causes oxidative stress and affects physiological activity by causing conformational changes, oxidation, covalent bond rupture, and free radical formation. Functional properties of soybeans such as radical-scavenging activities should also be affected, although previous reports on irradiated soybean have been contradictory in this regard.

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

ESR SPIN TRAPPING





γ-RAY IRRADIATION

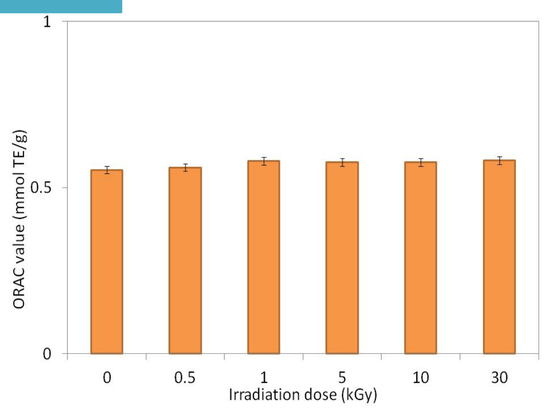
Soybeans in aluminum-sealed polyethylene bags were irradiated with gamma rays from a cobalt 60 source (Gammacell 220; MDS Nordion International Co. Ltd., Ottawa, Ontario, Canada) at the National Food Research Institute of Japan.

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.).

RESULT and DISCUSSION

A multiple comparison test (Tukey's test) was used to analyze all the results. Differences of $p > 0.05$ were deemed insignificant.

ORAC

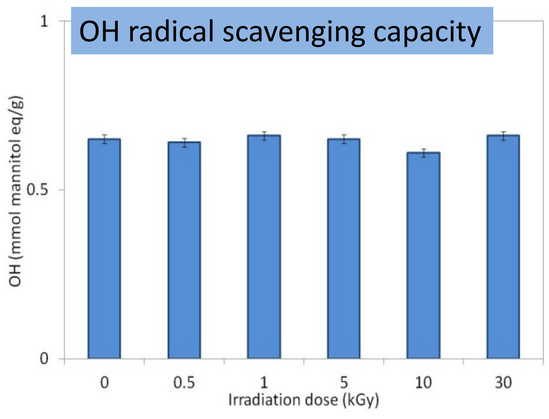


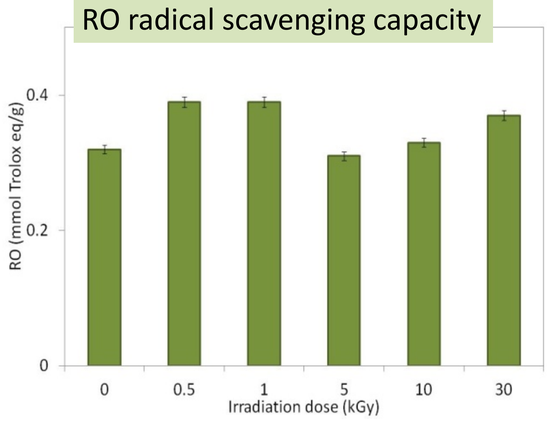
The ORAC assay is useful for assessing the antioxidant capacity of food extracts that contain various antioxidants. The ORAC analysis did not show any significant difference between irradiated and non-irradiated soybeans.

Therefore, the scavenging activity of soybean extracts for individual radical species was investigated by ESR spin trapping.

ORAC (oxygen radical absorbance capacity**)**
Many chemical techniques, including ORAC, were developed in an attempt to measure the antioxidant capacity of foods. The ORAC assay measures the degree of inhibition of peroxy radical–induced oxidation by the compounds of interest in a chemical milieu. It measures the value as Trolox equivalents and includes both inhibition time and the extent of oxidation inhibition. Some newer versions of the ORAC assay use other substrates, and therefore the results of the various ORAC assays are not comparable.

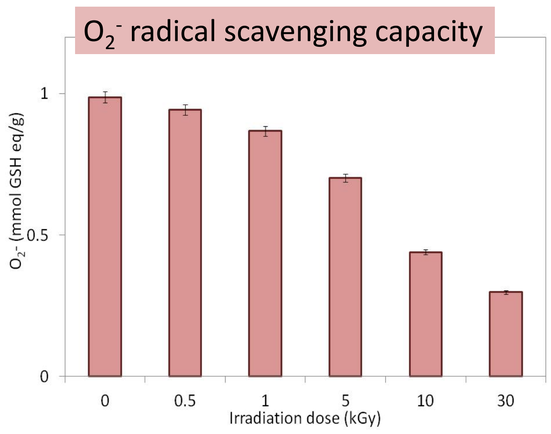
ESR spin trapping

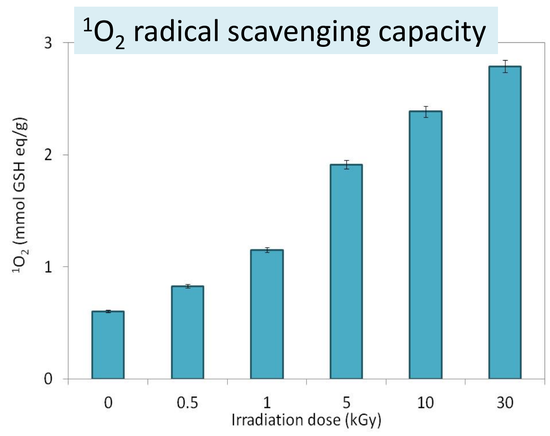




The hydroxyl- and alkoxyl radical–scavenging activities did not change significantly ($p > 0.05$) and did not correlate well with the irradiation dose.

ESR spin trapping





The superoxide radical–scavenging activity linear proportionally damped from 0.5 to 30 kGy, with a linear regression of $y = -0.147x + 1.22$ ($p < 0.05$). x, irradiation dose; y, scavenging activity. In addition, the singlet oxygen–scavenging activity linear proportionally increased from 0.5 to 30 kGy, with a linear regression of $y = 0.468x - 0.0244$ ($p < 0.05$). Thus, singlet oxygen–scavenging activity increased with the irradiation dose.

We hypothesized that nonenzymatic 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. Variyar et al. (2004) assessed the isoflavone content of irradiated and non-irradiated soybeans and found a decrease in total isoflavones and increase in aglycons with increase in the irradiation dose (from 0.5 to 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 increase in the irradiation dose.

Ichiyanagi et al. (2004) found that aglycone is a singlet oxygen scavenger, with structure-dependent variability. On the basis of these reports, we suggest that singlet oxygen–scavenging activity increases with increase in the irradiation dose.

Variyar, P.S., Limaye, A., Sharma, A., 2004. *J. Agric. Food. Chem.* **52**, 3385–3388.

CONCLUSION

H-ORAC values, commonly used to evaluate scavenging activity, did not differ significantly between irradiated and non-irradiated soybean extracts. We analyzed the specific radical-scavenging activity of gamma ray–irradiated soybeans by ESR spectroscopy. Singlet oxygen–scavenging activity increased and superoxide radical–scavenging activity decreased with increase in the radiation dose. In contrast, the hydroxyl- and alkoxyl radical–scavenging activities of irradiated soybeans did not differ significantly from those of non-irradiated seeds. Thus, **we evaluated the effect of gamma irradiation by using ESR spin trapping to determine the dose dependency of individual radical-scavenging activities in water-extracted soybeans.** 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. Therefore, we aim to develop a method for measuring the antioxidant capacity of fat-soluble components by the ESR spin trapping method.