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Photon emission properties of roasted soybean as related to reactive oxygen scavenging activities

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Abstract

Photon emission, from water extract of roasted soybean (RE) in the presence of hydrogen peroxide, was investigated. The photon intensity of RE was proportional to browning (observed at 420 nm) and fluorescence (Ex. 370 and Em. 440 nm) as a function of roasting time. This photon intensity could be improved by the several conditions, such as roasting time, alkalinity, and the combination of metallo compound. The maximum wavelengths of emission spectra of RE were around 560 nm under various conditions. RE also had reactive oxygen scavenging activities in proportion to the photon intensity. Our work suggests that the photon intensity of RE is closely related to the reactive oxygen-scavenging properties. This fact may be utilized to estimate the function and quality of food, especially roasted food.

Keywords: Soybean; Photon emission; Reactive oxygen scavenger; Maillard reaction; Roasting

1. Introduction

Soybean has been utilized after various kinds of processing, such as soak, germination, fermentation and heating. Thermal treatment, which is indispensable for eating soybean, greatly changes functional properties. Increasing attention has lately been paid to the role of radical scavengers in food materials, for human health. It was reported that naturally occurring antioxidants were significantly lost as a consequence of heat processing, but antioxidant Maillard reaction products were produced (Anese, Manzocco, Nicoli, & Lerici, 1999; Yen & Chung, 1999). Yoshiki et al. (1997) demonstrated that reactive oxygen species/catalytic species/receptive species systems, in which electron translation is thought to occur, constituted new photon emission systems, leading to reactive oxygen scavenging activity. Synergistic effects of receptive species such as acetaldehyde or cytochrome *c* on antioxidative activity and hydroxyl radical-scavenging activity of catalytic species, which are radical scavengers, such as gallic acid, have been observed. From these results, investigation of the photon emission mechanism was thought to be helpful to the research on antioxidative mechanisms. This photon emission was also observed in foods such as rice, soymilk (Akiyama, Kawamura, Yoshiki, & Okubo, 2000), soy sauce (Yoshiki, Kikuchi, Otomo, & Okubo, 2000), soybean sprouts and adzuki bean sprouts (Iida, Kawane, Ashikaga, Yoshiki, & Okubo, 2000). In this report, we have investigated, in detail, the phenomenon of photon emission from heated soybean, and the relationship between the photon emission and several properties, such as colour, fluorescence and reactive oxygen-scavenging activities.

2. Materials and methods

2.1. Materials

Soybean (Glycin max var. Miyagisirome) was cultivated in Miyagi prefecture in 1999 and was cleaned and stored at 4 °C. Horseradish peroxidase (HRP), haemoglobin (Hb), cytochrome *c* (Cyt *c*) and hemin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Gallic acid, titanium (IV) sulfate solution, iron

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(II) chloride tetrahydrate, iron(III)chloride (anhydrous) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Nakarai Co. (Kyoto, Japan). Hydrogen peroxide was purchased from Santoku Chemical industry Co. (Tokyo, Japan). Acetaldehyde (MeCHO) and superoxide dismutase (SOD), from bovine erythrocytes, were from Merck (Darmstadt, Germany). Hypoxanthine was from Kohjin (Tokyo, Japan), xanthine oxidase, from cow milk, was from Boehringer-Mannheim (Tokyo, Japan), and 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) from Labotec (Tokyo, Japan). All the test solutions were freshly prepared before use.

2.2. Sample treatment

Soybean seeds were heated by boiling, autoclaving or roasting. Soybean seeds were boiled at 95 °C or autoclaved at 120 °C. The seeds were ground into fine powder using a mill (Retsch, Ultracentrifugal mill, 0.5-mm mesh screen) after freezez-drying. Soybean seeds were also roasted at 195 °C, with drying machine after mill for homogeneity of roasting. Each powder was extracted with distilled water (25 g/200 ml). Crude extracts were obtained after centrifugation at 10,000 rpm for 30 min and the freeze-drying of supernatants. Before the measurement, distilled water was added to the crude extracts (water extract).

2.3. Measurement of photon emission

The photon intensity was measured using a chemiluminescence detector model CLD-110 (Tohoku Electronic Industrial Co.) based on a single photon counting mode, connected to a Waters model 515 pump and recorded as counts per second (cps). The wavelength was set in the range of 300–650 nm. The measurement was conducted under flow conditions using 50 mM phosphate buffer (pH 7.4) as a mobile phase, with a flow rate of 1 ml/min and temperature at 23 °C. The pH-dependence of the photon emission of RE was measured at several pH values ranging from pH 2 to pH 13, by adding either NaOH or HCl to the solution in a 50 mM phosphate buffer. To measure photon intensity of H_2O_2/sample system, 10 μl of H_2O_2 (196 mM) and 10 μl of the sample (20 mg/ml) were directly injected into the injector. For the investigation of the effects of metallo compounds (HRP, Cyt *c*, Hb, hemin, Fe(II)-EDTA complex and Fe(III)-EDTA complex), 10 μl of several concentrations of them were added to the H_2O_2/sample system. All samples were analyzed in triplicate, and the readings were averaged.

2.4. Determination of emission spectra

Photon emission spectra were measured by a simultaneous multiwavelength analyzer, model CLA-SP2 (Tohoku Electronic Industrial Co. Ltd.), equipped with a diffraction grating built into a spectroscope and a two-dimensional photomultiplier tube. The wavelength range of the spectroscope was 400–850 nm. The measurement time was 180 s. Each reagent was dissolved in 50 mM phosphate buffer (pH 2, 7.4, 13). To measure the emission spectra of the H_2O_2/sample system, a reaction mixture, each containing 0.7 ml of H_2O_2 (196 mM), the sample (20 mg/ml) and 50 mM phosphate buffer was poured into a cell (total 2.1 ml). For the H_2O_2/sample/metallo compounds system, a reaction mixture, each containing 0.7 ml of H_2O_2 (196 mM), sample (20 mg/ml) and HRP (5.5 μM) or Hb (19.2 μM) was used.

2.5. Measurement of fluorescence

Fluorescent studies were carried out on a Shimazu model RF-1500 spectrophotometer. The scanning-range of the excitation light and the emission light was 240–800 nm. Fluorescence was measured in 0.2 mg/ml sample solution. The solution was measured at an excitation wavelength of 370 nm and emission wavelength of 440 nm.

2.6. Measurement of scavenging activity of reactive oxygen species and DPPH radical

ESR spectra were recorded on a JEOL JES-RE1X spectrometer. The procedure of measurement for DPPH radical-scavenging activity was as described by Nanjo, Goto, Seto, Suzuki, Sakai, and Hara (1996) with some minor modification. That is, 60 μl of each sample (2 mg/ml), dissolved with distilled water or distilled water itself as control, were added to 60 μl of DPPH radical (60 μM) in ethanol solution. Superoxide radical scavenging activity was measured as described by Mitsuta, Mizuta, Kohno, Hiramatsu, and Mori (1990) with some minor modification. Sample and each of the reagents were dissolved in 50 mM phosphate buffer solution (pH 7.4). Fifty microlitres of sample solution (2 mg/ml) were added to the mixture, consisting of hypoxanthine (2 mM, 50 μl), xanthine oxidase (0.4 unit/ml, 50 μl) and DMPO (9.2 M, 20 μl). Hydroxyl radical-scavenging activity was measured using the ESR spin-trapping method. Hydroxyl radical, generated by Fenton reaction. H_2O_2 (196 mM, 50 μl), was added to the mixture, consisting of sample solution (2 mg/ml, 50 μl), $FeCl_2$ (64 μM. 50 μl) and DMPO (92 mM, 50 μl). The hydroxyl radical-scavenging activity was calculated from the DMPO-OH signal. Hydrogen peroxide-scavenging activity was measured using the method described by Christine, Rodolfo, and Anthony (1985) with slight modification. Sample solution (10 mg/ml), H_2O_2 (4 mM) and HRP (0.55 μM), were dissolved in 50 mM phosphate buffer solution (pH 7.4). Each 0.54 ml

solution was mixed and incubated at 37 °C for 20 min. The solution was reacted with 0.5 ml of 20% H_2SO_4 and 0.3 ml of 1 M $Ti(SO_4)_2$. After centrifuging at 3000 rpm for 10 min, A_{408} was recorded.

3. Results and discussion

Photon counts of water extracts of soybean after boiling, autoclaving, or roasting for up to 60 min were measured in the presence of hydrogen peroxide. The photon intensities of boiled or autoclaved sample were not observed after 5 minutes of heat treatment (Fig. 1). On the other hand, after an initial decrease of the photon intensity of roasted sample, a recovery of the photon emission was observed by prolonging heating time. These results showed that the roasting process was the most effective of three heating methods for the induction of photon emissive substances in soybean.

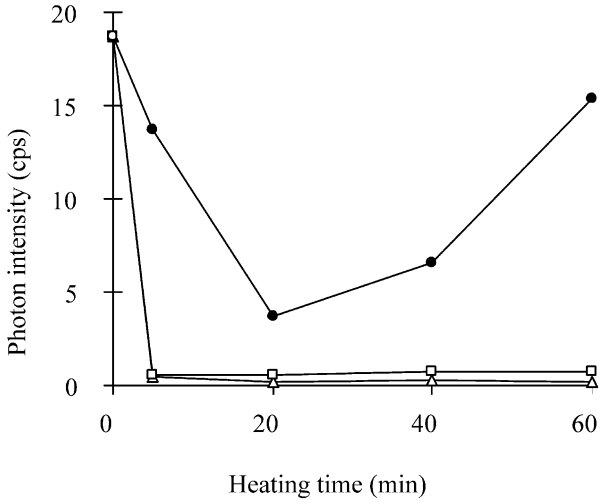

Fig. 1. Effect of the heat treatments of soybean seed on photon intensity in the presence of hydrogen peroxide. (•), roasting; (□), autoclaving; (△), boiling.

The photon intensity from RE is compared with browning (observed at 420 nm) and fluorescence (Ex. 370 nm, Em. 440 nm) in Fig. 2. The values of these factors could be linearly approximated and each R^2 value (coefficient determination) was 0.99. The browning and the photon intensity were increased in proportion to roasting time and their correlation coefficient was 0.99. The fluorescence and the photon intensity were also increased in proportion to roasting time and their correlation coefficient was 0.99. From these facts, it seems that the photon emissive substances occurring in the course of roasting process were quantitatively and progressively increased as roasting time was increased, and that these substances were related to Maillard reaction products (in the advanced stage) with coloured and fluorescent properties.

Photon emission from hydrogen peroxide and polyphenol is enhanced in the alkaline region, since OH^- ions contribute to an initial oxidation step of polyphenol (Slawinska & Slawinski, 1975). A range of pH is thus important for the detection and for the investigation of the mechanism of photon emission. The effect of a wide pH range (pH 2–13) on the photon emission of RE (140 min roasting) in the presence of hydrogen peroxide is shown in Fig. 3. The photon intensity is

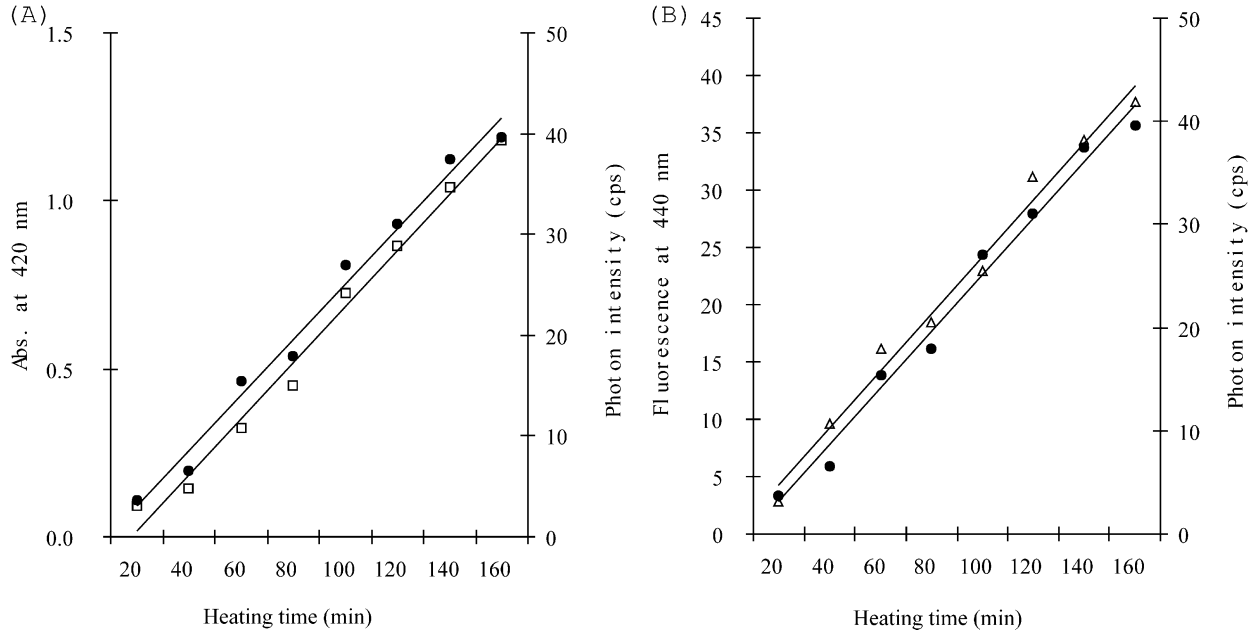

Fig. 2. The proportional relationships among the browning, the fluorescence, and the photon intensity of RE. (A) The relation between the browning and the photon intensity in the presence of hydrogen peroxide; □, browning (at 420 nm); •, photon intensity. (B) The relation between the fluorescence and the photon intensity in the presence of hydrogen peroxide: (△), fluorescence (Ex. 370 nm, Em. 440 nm); •, photon intensity.

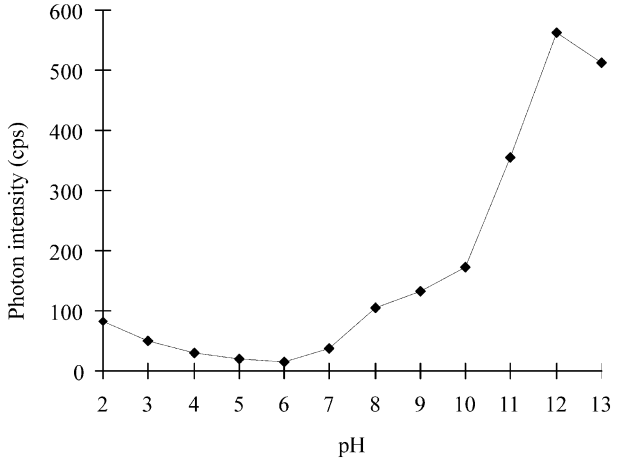

Fig. 3. pH-dependence of the photon intensity of RE in the presence of hydrogen peroxide.

strongly dependent on pH value. Measurement in the alkaline region exhibited a high photon intensity, and pH 12 was an especially optimum condition for the observation of the photon emission, whereas the photon intensity, which was low in an acid or neutral region, was a minimum at pH6.

The increase of the photon emission at pH12, is useful for the measurement of emission spectra using a diffraction grating. In the measurement at pH12, the maximum wavelengths of RE (60, 100, and 140 min roasting) in the presence of hydrogen peroxide appeared at around 560 nm (Fig. 4). From these similarities of the maximum wavelength with different roasting times, it was deduced that the chemical structure which related to the light-producing mechanism in a photon-emissive substance once formed in soybean was qualitatively hard to change with more heating.

It was proposed that some combinations of three chemical compounds, such as H_2O_2/gallic acid/heme compounds, H_2O_2/anthocyanins/MeCHO and H_2O_2/

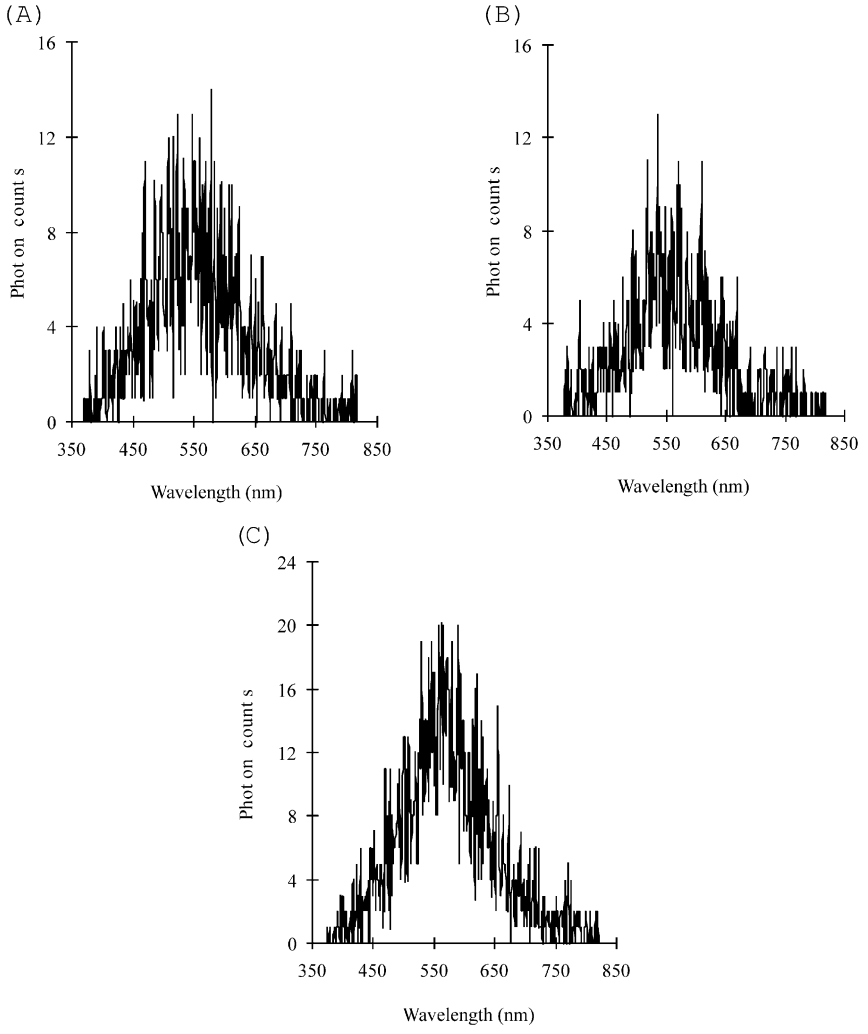

Fig. 4. Emission spectra of RE in the presence of hydrogen peroxide at pH 12. (A) Roasting for 60 min; (B) roasting for 100 min; (C) roasting for 140 min.

catechins/MeCHO, promoted the efficiency of photon emission in comparison with the combinations of two chemical compounds (Yoshiki, Igarashi, & Okubo, 1995; Yoshiki, Kahara, Igarashi, Yotsuhashi, & Okubo, 1996; Yoshiki et al., 1997, 1998). It was hence thought that RE also needed the combination of other chemical compounds for efficient photon emission, which led to the efficient elimination of reactive oxygen species in a neutral region. The experiment of the combination of H_2O_2/RE (20 mg/ml)/phenol compounds (gallic acid or catechin, 0.01–50 mM), or H_2O_2/RE (20 mg/ml)/MeCHO (0.36 mM–3.6 M) did not show a great effect in comparison to the original photon intensity of H_2O_2/RE (data not shown). However, metallo compounds had appreciable effects on the photon emission of RE in the order HRP≫Hb = Hemin = Cyt*c*≫Fe(II)-EDTA complex > Fe(III)-EDTA complex (Fig. 5). The maximum wavelengths of the emission spectra of H_2O_2/RE, H_2O_2/RE/HRP, and H_2O_2/RE/Hb were the same (around 560 nm) (Fig. 6). From the spectral results of Figs. 4–6, increasing the photon intensity of H_2O_2/RE by heme compounds, such as HRP and Hb, presumably

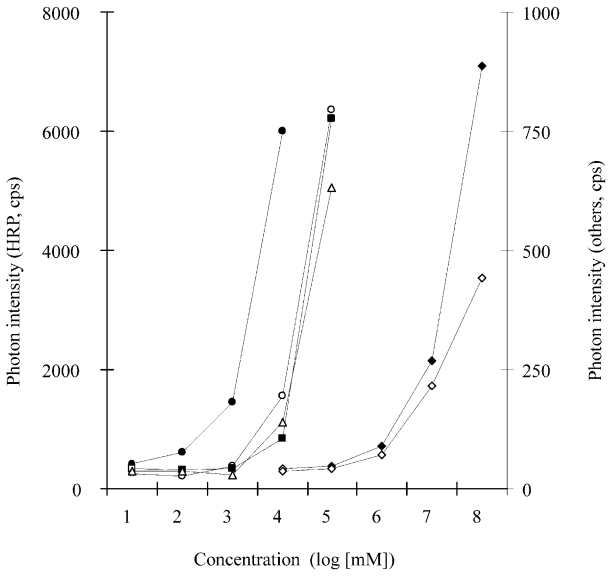

Fig. 5. Effects of metallo compounds on the photon intensity of RE in the presence of hydrogen peroxide. •, HRP; ○, Hb; ■, Cyt *c*; △, Hemin; ◆, Fe(II)-EDTA complex; ◇, Fe(III)-EDTA complex.

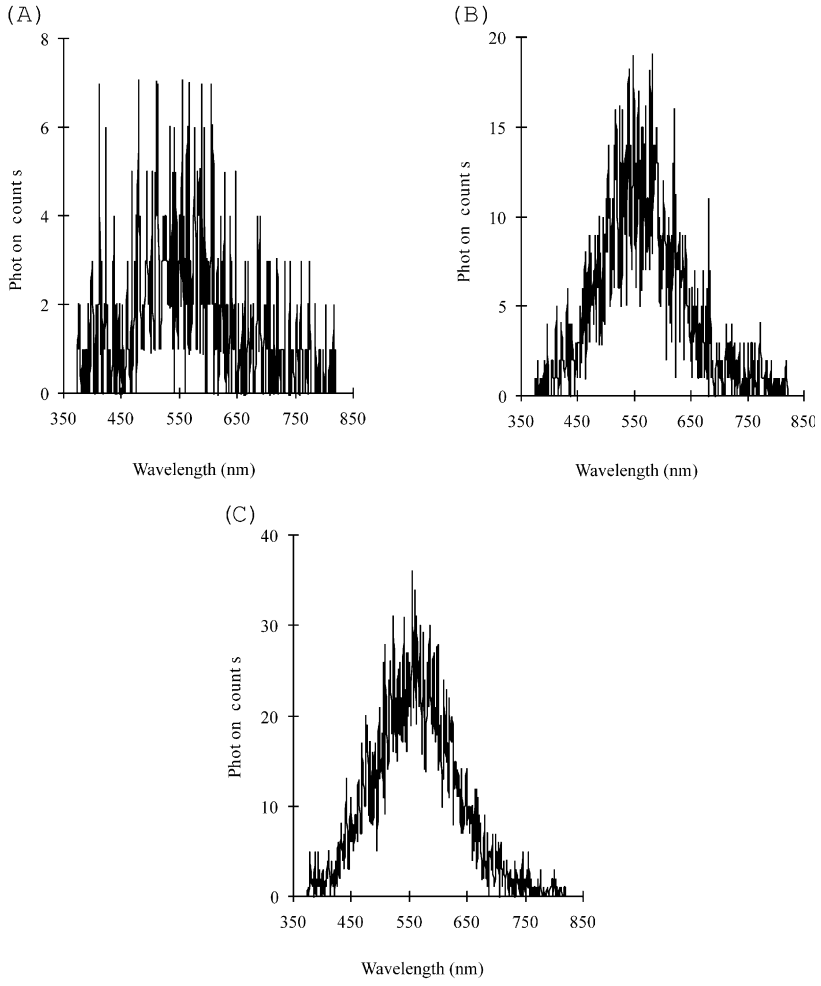

Fig. 6. Effects of metallo compounds on emission spectra of RE in the presence of hydrogen peroxide. (A) No addition; (B) addition of Hb; (C) addition of HRP.

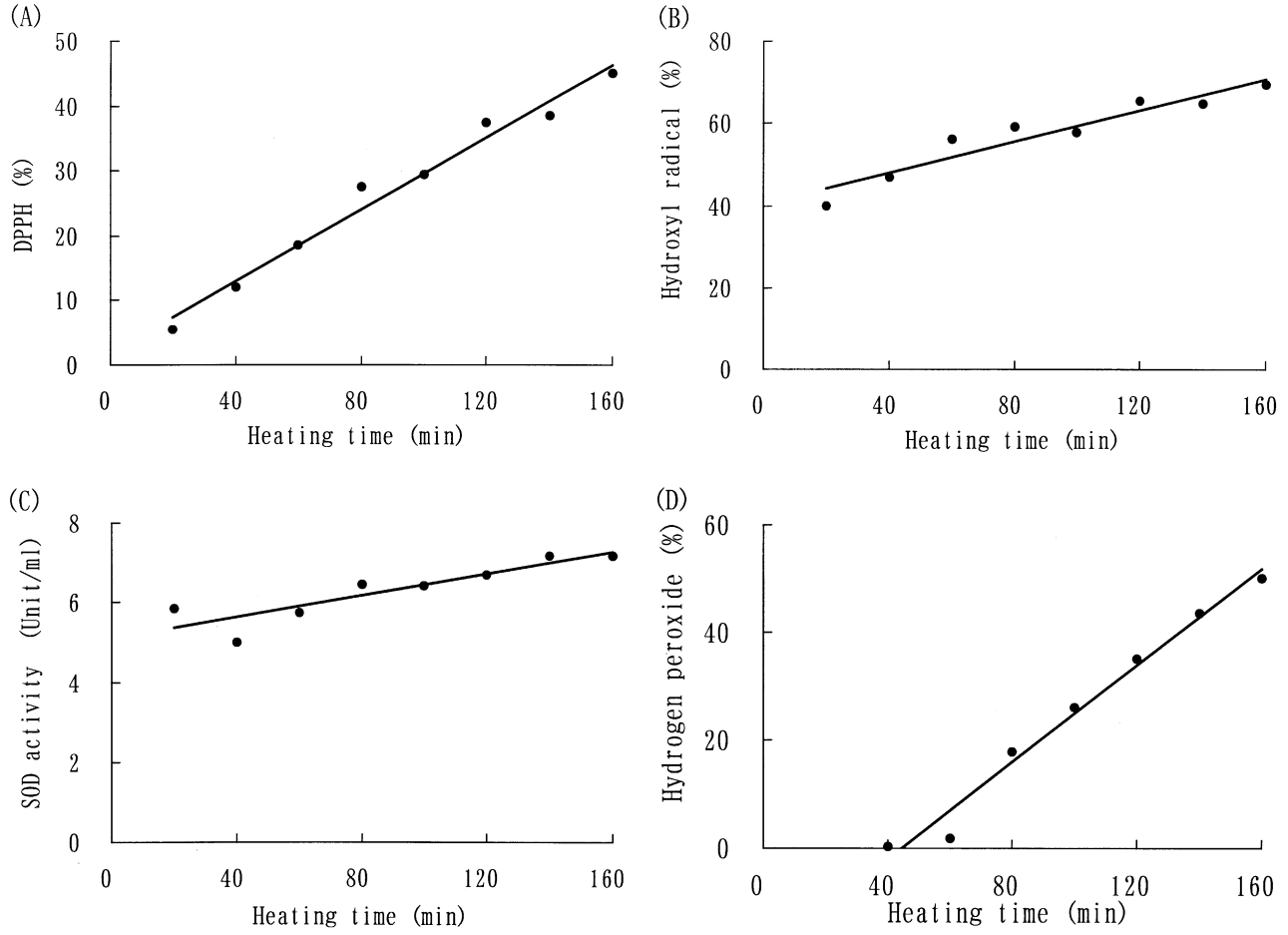

Fig. 7. Reactive oxygen species and free radical scavenging activities of RE. (A) DPPH radical scavenging activity; (B) hydroxyl radical scavenging activity; (C) SOD activity; (D) hydrogen peroxide scavenging activity in the presence of HRP.

occurred without changing the mechanism of the photon emission of H_2O_2/RE. It is well known that peroxidase, which causes oxidation of certain kinds of reducing substances by electron transfer, is particularly apt to catalyze the generation of electronically-excited products (Cilent & Adam, 1995; Yoshiki et al., 1998), and that one of the properties of Maillard reaction products is a strong reducing power. By approximate evaluation of molecular weight, using Sephadex G-25 column chromatography, a low molecular weight fraction in RE was mainly related to the photon emission (data not shown). It therefore appears that the transfer of electrons from low molecular weight Maillard reaction products to hydrogen peroxide is significant for the photon emission, and that metallo compounds promote this electron transfer.

Nicoli, Anese, Manzocco, and Lerici (1997) showed that overall antioxidant properties of coffee beverages were greatly increased with increasing degree of roasting. Moreover, browning and fluorescence of Maillard reaction products were related to antioxidant activity or free radical-scavenging activities (Kirigaya, Kato, & Fujimaki, 1968; Morales & Jiménez-Pérez, 2001). In this way, the heat treatment of food, or its components, is profoundly related to antioxidant capacity or radical-scavenging capacity. To evaluate the reactivity of RE with reactive oxygen species, DPPH radical, hydroxyl radical, superoxide radical and hydrogen peroxide-scavenging activities of RE were studied (Fig. 7). DPPH radical is a stable radical which gives a typical ESR spectrum. Though hydroxyl radical and superoxide radical are short-lived, these radical spins are possible to trap with DMPO. The decrease of these radical signals during reaction, displays the capacity of RE to eliminate radicals with different properties. On the other hand, hydrogen peroxide is not a radical, so a chemical technique, using Ti $(SO_4)_2$ in acid, was utilized for detecting the elimination of hydrogen peroxide. The DPPH radical-, hydroxyl radical-, superoxide radical- and hydrogen-peroxide scavenging activities of RE increased almost linearly with heating time, and individual R^2 values were: 0.98 (DPPH radical), 0.91 (hydroxyl radical), 0.80 (superoxide radical) and 0.98 (hydrogen peroxide). The correlation coefficients between each scavenging

activity and the photon intensity, in the presence of hydrogen peroxide, were: 0.98 (DPPH radical), 0.94 (hydroxyl radical), 0.91 (superoxide radical), 0.97 (hydrogen peroxide). From the results of these highly positive correlations, it was shown that the photon emissive substances, generated in the roasting process, related to reactive oxygen scavenging capacity. This fact indicates that more detailed research on the properties of photon emission would be useful for elucidating reaction mechanisms between reactive oxygen species and roasted food ingredients.

Results reported above showed that it was meaningful to research the antioxidation mechanisms of Maillard reaction products from the perspective of the photon emission, which related to electron transfer among widespread compounds in food materials or living organisms, and to the emission of energy derived from reactive oxygen species. In order to develop the utilization and the processing of soybean for human health, it is important and greatly beneficial for roasted soybean to have a reactive oxygen-scavenging property. For the clarification of the detailed mechanism of photon emission from roasted soybean, which relates to radical-scavenging activity, more comprehensive information and research are needed.

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