Nutraceuticals & Food
Vol. 8, p. 85~88 (2003)

Nutraceuticals and Food

Effect of Gamma Irradiation on the Biological Activities and Color Changes of Ethanol Extracts *Lonicera japonica*

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Abstract

Effects of irradiation on color removal, tyrosinase inhibition, xanthine oxidase inhibition and nitrite scavenging capacity of *Lonicera japonica* extracts were evaluated. *Lonicera japonica* extracts were irradiated at 10, 20, and 30 kGy. Hunter color L*- and a*-values increased but b*-values decreased dose-dependently following irradiation. The extracts were potent inhibitors of tyrosinase and xanthine oxidase. Tyrosinase inhibition was higher in the irradiated sample than non-irradiated, and subsequently increased with increasing irradiation doses. The extracts had a higher inhibitory effect against xanthine oxidase, and the effect was not changed by irradiation. Nitrite scavenging capacity was the highest in the extract at pH 1.2, and was not significantly affected by irradiation. These results indicate that gamma irradiation may not influence the biological activities of *Lonicera japonica* extracts when irradiated up to 30 kGy. Furthermore, color of the extracts can be improved to have improved applicability for the food and cosmetic industries without any adverse change in biological functions.

Key words: *Lonicera japonica*, irradiation, biological activities, color

INTRODUCTION

Lonicera japonica, known as *indong* in Korea and as Japanese honeysuckle in America, is widely distributed in Korea, China and Japan. The stem and flower of *Lonicera japonica* has a long history of use in traditional medicine, and is valued for its strong cold-resistant characteristic (1). *Indong* has been traditionally used as an antiflammatory and for the treatment of hyperuricemia and gout. The anti-inflammatory and analgesic properties of *indong* have been scientifically evaluated by Lee et al. (2).

Son et al. (3,4) fractionized and separated flavonoids and two new triterpenolid saponins from the extracts of *Lonicera japonica*. Chang et al. (5) also isolated caffeoylquinates and other tannins, and reported their inhibition effect on human immuno-deficiency virus-1 reverse transcriptase activity. However, the use of *Lonicera japonica* has been very limited except as a prescription in oriental medicinal for composition. The functional benefits of *Lonicera japonica* have potential that may only be realized when some of the undesirable properties such as the dark color of the extract can be avoided, since it is generally accepted that best additives are colorless and odorless.

Gamma irradiation is a type of ionizing radiation which has sufficient penetration ability to ionize the atoms or molecules in food. Gamma irradiation has been used for various purposes such as inhibiting sprouting, disinfestation, sanitation and sterilization of foods, food additives, cosmetics, and medical products (6). Recently, gamma irradiation technology has been studied for the enhancement of food processing procedures, improvement of biological functionality, and the reduction of toxic or undesirable compounds (7-9).

Therefore, the objective of this study is to investigate the effect of gamma irradiation on changes in color and biological activities in *Lonicera japonica* extracts.

MATERIALS AND METHODS

Materials

Korean grown *Lonicera japonica* stems were purchased from Kyungdong Market, Seoul, Korea.

Extraction

Fifty grams of *Lonicera japonica* were weighed and added to an ethanol solution (70%, 500 mL), and extracted overnight with several agitations according to the method of Son et al. (8). After extraction, the extracts were filtered through SiO_2 as preparation for irradiation.

Irradiation

Sample solutions in tightly capped containers (each 2

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L) were irradiated in a cobalt-60 irradiator (point source, AECL, IR-79, Nordion, Canada) with doses of 0, 10, 20 and 30 kGy. The source strength was approximately 100 kCi with a dose rate of 83.3 Gy/min at 13±0.5°C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and free-radical signals were measured using a Bruker EMS 104 EPR Analyzer. The actual dose was within ±2% of the target dose. Samples were turned 360° continuously during irradiation to achieve a uniform dose. The non-irradiated control was placed outside of the irradiation chamber to maintain consistent temperatures with the irradiated sample. The irradiated samples were concentrated to 1/9 of the original volume using a rotatory evaporator at 30°C and stored in a refrigerator (4°C) until analyzed.

Color measurement

Samples (9 mL) were put into a glass cell (CM A-98, 10 mm in width) and measured with a Color Difference Meter (spectrophotometer CM-3500d, Minolta Co., Ltd. Osaka, Japan). The instrument was calibrated with standard black and white tiles before measurement. The measurements were made in triplicate using a large size aperture. The Hunter color L-, a-, and b- values were reported through a computerized system using Spectra Magic software (version 2.11, Minolta Cyberchrom Inc. Osaka, Japan).

Measurement of tyrosinase inhibition

An aliquot of each sample (0.2 mL) was added to the reaction mixture containing 10 mM L-3,4-dihydroxyphenylalanine (L-DOPA, Sigma Co., Ltd, St. Louis, MO USA) solution, 1/15 M potassium phosphate buffer (pH 6.5) and mushroom tyrosinase (220 unit/mL, Sigma Co., Ltd.). The reaction mixture was incubated at 37°C for 20 min. The amount of dopachrome produced in the reaction mixture was spectrophotometrically (UV 1600 PC, Shimadzu, Tokyo, Japan) determined at 475 nm (10). Tyrosinase inhibition was calculated as follows:

$$\text{Inhibition effect (\%)} = \{1 - (\frac{S_{Abs} - B_{Abs}}{C_{Abs}})\} \times 100$$

where S_{Abs} was the absorbance of the sample, B_{Abs} was the absorbance of the solution with 0.1 mL of distilled water instead of enzyme solution, and C_{Abs} was the absorbance of the solution with 0.2 mL of distilled water instead of *Lonicera japonica* extracts.

Measurement of xanthine oxidase (XOase) inhibition

XOase inhibition was assayed spectrophotometrically at 292 nm as described by Moon and Lee. (11). The reaction mixture was prepared with 0.1 M potassium phosphate buffer (pH 7.5), 2 mM xanthine and 0.2 unit xanthine oxidase. Xanthine oxidase activity was expressed as percent inhibition of xanthine oxidase, calculated as $(1 - B/A) \times 100$, where A is the change in absorbance without sample and B is the change in absorbance with the sample.

Measurement of nitrite scavenging

Nitrite scavenging capacity was determined by the method of Gray et al. (12) with some modifications. An aliquot of each sample (0.6 mL) was added to a nitrite solution (1 mL) made up to 10 mL at pH 1.2 using 0.1 N HCl, or at 4.2 and 6.0 using a 0.2 M citric acid buffer. The reaction mixture was incubated in a 37°C water bath for 1 h. Then, 1 mL of sample with 5 mL of 2% acetic acid and 0.4 mL of Griess reagent (mixed solution at 1 : 1 ration with 1% of sulfanilic acid in 30% acetic acid and 1% of naphthylamine in 30% acetic acid) was mixed and held at room temperature for 15 min. Spectrophotometric analysis was performed at 520 nm for residual nitrite determination. Nitrite scavenging capacity was calculated as follows:

$$\text{Nitrite scavenging effect (\%)} = (1 - \frac{A - C}{B}) \times 100$$

A : Absorbance after reaction of added extracts in 1 mM $NaNO_2$ for 1 hr.
B : Absorbance of $NaNO_2$ solution.
C : Absorbance of extracts.

Statistical analysis

Two-way analysis of variance was performed using SAS (SAS Institute, Cary, NC USA) software (13). Duncan's multiple range test was used to compare the differences among mean values. Mean values with pooled standard errors of the mean (SEM) were reported, and statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

Color changes

The Hunter color L* values of *Lonicera japonica* extracts were 91.99 in the non-irradiated control but those of irradiated extracts increased to 101.08 by irradiation of 30 kGy (Table 1). The Hunter color a* value, which represents the redness of the sample, increased from -6.01 in the non-irradiated control to -0.87 in the sample irradiated at 30 kGy. Hunter color b*-value decreased with irradiation. The overall color difference from the original was calculated as ΔE, which also differed significantly with radiation ($p<0.05$). These results agreed well with those of Son et al. (14) who reported that the destruction of color components, such as chlorophyll and flavonoids, by gamma irradiation made green tea extracts brighter than

Table 1. Effect of gamma irradiation on color changes of *Lonicera japonica* extract from 70% ethanol[1)]

Hunter color value	Treatment				SEM[2)]
	Control	10 kGy	20 kGy	30 kGy	
L	91.99[c]	99.84[b]	100.95[a]	101.08[a]	0.2450
a	-6.01[d]	-2.15[c]	-1.22[b]	-0.87[a]	0.0267
b	36.21[a]	9.65[b]	5.21[c]	3.99[d]	0.0171
ΔE[3)]	0.00	27.96	32.62	33.87	

[1)]Different letters ([a-d]) within the same row differ significantly ($p<0.05$).
[2)]Standard errors of the mean (n = 12).
[3)]Overall color difference ($\sqrt{(\Delta L)^2+(\Delta a)^2+(\Delta b)^2}$).

the non-irradiated control. Furthermore, Song et al. (15) indicated that browning in gamma irradiated *kangjang*, a traditional Korean fermented soybean sauce, was significantly changed. Byun et al. (16) also reported that the chlorophyll in soybean oil was degraded by irradiation at 2.5 kGy or above. Therefore, gamma irradiation of *Lonicera japonica* extract, significantly improves color characteristics making it more acceptable for applications in the food and cosmetic industries.

Tyrosinase inhibition effect

The melanin pigment in human skin is a major defense mechanism against ultraviolet light damage to the skin, but darkened skin color, which is the result of increased and redistributed epidermal melanin, can be a serious aesthetic problem (17). Tyrosinase is primarily responsible for melanin biosynthesis (melanogenesis) in animals and enzymatic browning (melanosis) in plants. Inhibition of tyrosinase by the *Lonicera japonica* extract was tested for possible application as a functional natural additive for skin whitening in cosmetics (Fig. 1). The *Lonicera japonica* extract showed about 10% tyrosinase inhibition in the non-irradiated control, but following irradiation the inhibition increased dose dependently up to 75%. Son et al. (4) reported that the *Lonicera japonica* extract was composed of flavonoids, saponins, and lignan. According to Jung et al. (18), green and black teas showed 89 and 80% tyrosinase inhibition effect, respectively. When Kwak et al. (19) separated hexane and chloroform layers from ethanol extraction of fresh mugwort, the tyrosinase inhibition effects were 96.7 and 98.9%, respectively. However, until now there have been no reports of increased tyrosinase inhibition following gamma irradiation. Likewise none of our previous studies found differences in tyrosinase inhibition between non-irradiated and irradadiated extracts of green tea (8) or licorice root or stolon (20). This phenomenon cannot currently be explained, thus, further study is needed to elucidate the effects of radicals or other compounds uniquely present in *Lonicera japonica*.

Xanthine oxidase inhibition effect

Xanthine oxidase catalyses the metabolism of hypoxanthine and xanthine to uric acid. The overproduction and/or underexcretion of uric acid results in hyperuricemia which causes gout (21).

Lonicera japonica extract inhibited xanthine oxidase by up to 90% in the non-irradiated control and 72, 68 and 76% in the irradiated at 10, 20, and 30 kGy, respectively (Fig. 2). Yeo et al. (22) reported that the xanthine oxidase inhibiton of green tea, oolong tea and black tea extracts were 89.2~93.2%, 88.8%, and 78.7%, respectively. The authors also reported that the crude catechin fraction, which is composed of polyphenols, showed the highest inhibitory effect. When the concentration of the fraction increased the inhibitory effect also increased. However, this study found that irradiation of *Lonicera japonica* extract significantly decreases the xanthine oxidase inhibition.

Nitrite scavenging effect

Secondary and tertiary amines in protein foods, medicines, and residual pesticides react with nitrite to form car-

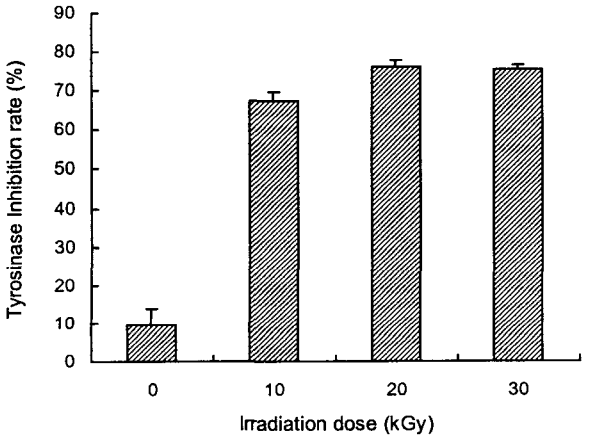

Fig. 1. Tyrosinase inhibition effect of *Lonicera japonica* extract from 70% ethanol solution.

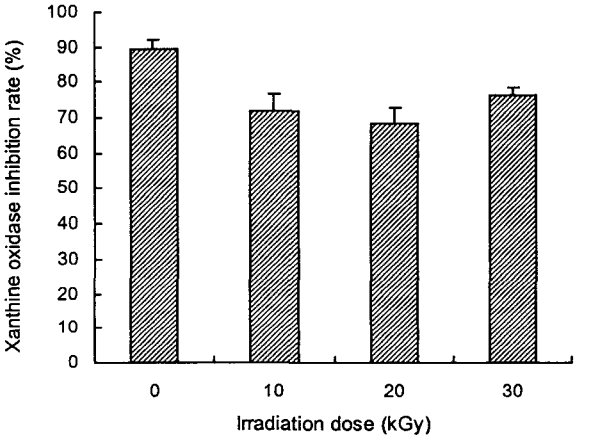

Fig. 2. Xanthine oxidase inhibition effect of *Lonicera japonica* extract from 70% ethanol solution.

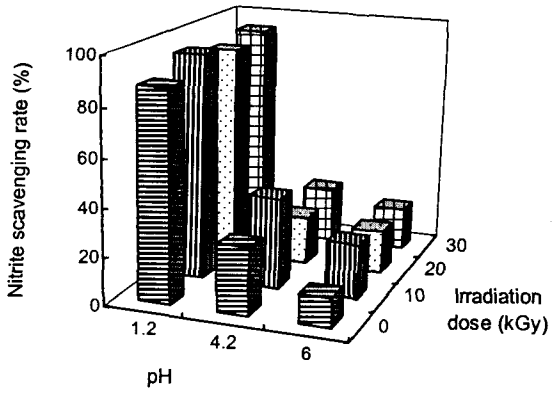

Fig. 3. Nitrite scavenging effect of *Lonicera japonica* extracted from 70% ethanol under different pH conditions after gamma irradiation.

cinogenic nitrosamines. When nitrites are consumed in food, nitrosamine can be formed in the human stomach because of the high acidity (23). Therefore, effective nitrite scavenging in acidic conditions is very important for inhibiting the formation of carcinogenic nitrosamines.

Both irradiated and non-irradiated extracts of *Lonicera japonica* showed between 85 and 90% nitrite scavenging capacity (Fig. 3) at pH 1.2. However, if the pH increased to 4.2 or 6.0, nitrate scavenging in both irradiated and non-irradiated extracts was reduced to less than 40%. According to Kang et al. (24) phenolic compounds have high nitrite scavenging capacity. Our results agree with the results of Noh et al. (25), who reported that nitrite scavenging by phenolic compounds is enhanced in a low pH environment. There were no statistically significant differences between the non-irradiated and irradiated samples.

ACKNOWLEDGEMENTS

The present study was carried out under the Nuclear R & D program of the Ministry of Science and Technology in Korea

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(Received November 28, 2002; Accepted January 2, 2003)