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Comparison of the Biological Activity between a Radiation-processed Natural Extract and a Commercial Counterpart for an Industrial Application

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Abstract

Irradiation of natural resources extracted with 70% ethanol improves the sample color and make them desirable for application. Using this technology the green tea leaf extract powder was made and applied for the cosmetic composition to investigate its effect on real industrial application. Electron donating ability, tyrosinase inhibition effect, and superoxide dismutase-like activity were not changed by addition of irradiated green tea leaf extract powder dissolved into butylene glycol. When 1% of the irradiated green tea leaf powder for color improvement were mixed to butylen glycol and 15% of this mixture were added to prepare the cosmetics, a cream lotion, the electron donating ability was sustained. Therefore, the application of irradiation technology to improve quality and physiological activity in industrial cosmetic composition would be beneficial.

Key words : Irradiation, green tea leaf, biological activity, industrial application

Introduction

Polyphenols are known to be major components of natural products in terms of their functional properties. These components have a historical and scientific background for their known functions such as the inhibition of lipid oxidation, cancer, allergy, tooth decay and microbial growth (Jo *et al.*, 2002; Yeo *et al.*, 1995). In addition, several other advantages such as the suppression of a high blood pressure or atherosclerosis, prevention of diabetes, and the reduction of allergenicity, drives a consumer's preference towards natural materials (Sakanaka *et al.*, 1989; Wanasundara and Shahidi, 1998; An *et al.*, 2004). The inhibition effect of green tea polyphenol on lipoid oxidation was higher than that of the synthetic antioxidant, butylated hydroxytoluene (BHT) (Chen *et al.*, 1996). However, natural products with high functions cannot be directly applied to the food or cosmetic industry because of their undesirable dark color.

An irradiation showed advantages in increasing the extraction yields of Korean medicinal herbs (Byun et al., 1999). An irradiation of green tea leaf (Jo *et al.*, 2003a), licorice root (Jo *et al.*, 2002), and persimmon leaf extract (Jo *et al.*, 2003b) revealed an improved color of the extract without any adverse change to their physiological activities including, mainly, the electron donating ability and tyrosinase inhibition effect. Recently, Byun et al. (2004) reported that the xanthine oxidase inhibition and the nitrite scavenging effect of irradiated *Lonicera japonica* were not changed significantly. Furthermore, when the irradiated green tea leaf extract powder was mixed into pork patty, the lipid oxidation was significantly reduced in both the raw and cooked form (Jo *et al.*, 2003b). The international consultative group

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of food irradiation (ICGFI) concluded that an irradiation of food at a dose level of 10 kGy or below was toxicologically safe and nutritionally adequate (WHO, 1981).

The objective of this study was to compare the physiological activity of an extract or a cream lotion containing irradiated green tea leaf extract powder, which was prepared by a previous method (Son *et al.*, 2001) with a commercially available green tea powder commonly used in the cosmetic industry.

Materials and Methods

Sample preparation

The dried green tea leaf was purchased from the Bosung area in Chonnam, Korea, and 200 g was weighed. This sample was transferred to an ethanol solution (70%, 4 L), and extracted overnight. Extraction was performed twice and the extract was transferred into a 2 L container. The commercially available green tea leaf extract powder was obtained from Kolmar Korea Co. Ltd. (Yeongi, Korea).

Irradiation

Samples in tightly capped containers (2 L) were irradiated in a cobalt-60 irradiator (point source, AECL, IR-79, Nordion, Canada) with a 20 kGy absorbed dose. The source strength was approximately 100 kCi with a dose rate of 83 Gy min^{-1} at 15±0.5°C. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer. The actual dose was within ±2% of the target dose. Samples were rotated at 360° continuously with 2.5 turns/min during the irradiation process to achieve uniform target doses.

Scavenging effects of 1,1-diphenyl-2-picryl-hydrazyl (•DPPH) radical

The irradiated extracts and the commercial counterpart were diluted 400 and 800 times with ethanol and butylenes glycol (BG). Chemical additives industrially available such as butylated hydroxytoluene, ascorbic acid, and sodium metasulfate were also compared. Free radical scavenging effect was estimated according to the method of Blois (1958). A sample (1 ml) was added to the 0.2 mM DPPH radical in methanol (1 ml) and the mixture was shaken and held for 30 min at room temperature. Absorbance was then measured at 517 nm with a spectrophotometer (UV 1600 PC, Shimadzu, Tokyo, Japan).

Tyrosinase inhibition effect

The irradiated green tea leaf extracts and the commercial counterparts as well as the additives were diluted 1 and 5 times with ethanol or butylene glycol and the diluents were used for analysis. A sample (0.2 ml) was added to the reaction mixture containing 10 mM L-3,4-dihydroxyphenyl-alanine (L-DOPA, Sigma Co., Ltd, St. Louis, USA) solution, a 1/15 M sodium phosphate buffer (pH 6.8) and mushroom tyrosinase (100 unit/mL, Sigma Co., Ltd). The reaction mixture was incubated at 25°C for 15 min. The amount of dopachrome produced in the reaction mixture was spectrophotometrically determined at 475 nm (Masamoto *et al.*, 1980).

Superoxide dismutase (SOD)-like activity

SOD-like activity was assayed by the method of

Table 1. Biological activities (%) of the laboratory scale irradiated green tea leaf extract powder (1%) dissolved in butylene glycol and a commercial counterpart for a cosmetic composition.

Physiological activity (%)	Dilution factor	Laboratory made[1)]	Commercial[2)]
Superoxide dismutase-like	1	48.0±0.8	25.1±0.4
DPPH radical scavenging	400	78.0±0.9	78.0±0.6
	800	51.7±0.6	53.7±0.9
Tyrosinase inhibition	1	81.1±1.5	82.5±0.9
	5	44.0±0.8	29.0±0.3

[1)]Dried green tea leaf was extracted, irradiated at 20 kGy, and freeze-dried in laboratory.
[2)]Commercially available green tea leaf extract powder for a cosmetic composition obtained from the industry.

Marklund and Marklund (1974). The reaction mixture was prepared by 0.2 mL of the sample solution, 2.6 mL of the tris-HCl buffer (50 mM TRIZMA + 10 mM EDTA, pH 8.5), 0.2 mL of 7.2 mM pyrogallol and held at 25°C for 10 min. The oxidized pyrogallol was measured at 420 nm using a spectrophotometer (AAS, Hitachi Z-6000, Tokyo, Japan) after stopping the reaction by adding 0.1 mL of 1.0 N HCl. The SOD-like activity was expressed as the reduction rate of the absorbance.

SOD-like activity (%) = [1 - (absorbance value of testing solution/absorbance value of control solution)] × 100

Statistical analysis

One-way Analysis of the Variance was performed using SAS (SAS Institute, Cary, NC, USA) software (SAS, 1989) and the mean values and standard deviation were reported.

Results and Discussion

Superoxide dismutase (SOD) is a type of protein or enzyme, which oxidizes a superoxide radical into oxygen *in vivo*. This is an important self-defense mechanism of a body cell against oxidative damage (Ji, 1993). SOD-like activity of 1% irradiated green tea leaf extract powder dissolved in butylenes glycol showed 48.0% but that of a commercial product in the cosmetic industry only showed 25.1%. When the polyphenols isolated from green tea leaf was investigated, the SOD-like activity was increased as concentration of green tea polyphenols increased in both irradiated (40 kGy) or non-irradiated control (An *et al.*, 2004), but no difference was found in between.

DPPH is the free radical used for measuring the electron donating ability. Jo *et al.* (2003) indicated that the electron donating ability of irradiated green tea leaf extract did not differ from the non-irradiated control. When 1% of the irradiated green tea leaf extract powder was diluted for 400 and 800 times, the DPPH radical scavenging effect showed 78.0 and 51.7%, respectively. The DPPH radical scavenging effect of the irradiated, freeze-dried green tea leaf powder was not different when compared to the commercial product. At 40 kGy of irradiation did not affect on the DPPH radical scavenging activity of green tea polyphenols compared with non-irradiated control (An *et al.*, 2004). Recently, Huang and Mau (2005) reported that the DPPH radical scavenging effect of the methanolic extracts of Brazilian mushroom (*Agaricus blazei* Murrill) did not show any difference by irradiation. However, there was no report published so far comparing the activities using a real cosmetic composition.

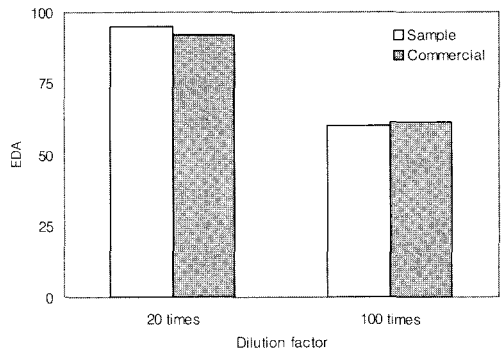

Fig. 1. Comparison of the DPPH radical scavenging activity (%) of the cream lotion mixed with 15% of the irradiated green tea leaf extract powder solution (1% dissolved in butylene glycol) and a commercially available counterpart.

Tyrosinase is primarily responsible for melanin biosynthesis (melanogenesis) in animals and enzymatic browning (melanosis) in plants. Abnormal pigmentations such as melasma, freckles, senile lentigines, and other forms of hyperpigmentation are often considered as undesirable (Byun *et al.*, 2004). The tyrosinase inhibition

Table 2. Biological activities (%) of irradiated and freeze-dried green tea leaf extract powder (1%) dissolved in ethanol and a comparison of other additives for a cosmetic composition used in the industry.

Physiological activity (%)	Laboratory made[1)]	BHT[2)]	AA[2)]	SMS[2)]
DPPH radical scavenging	53.0±0.9	20.0±0.7	76.8±1.6	12.0±0.4
Tyrosinase inhibition	45.7±0.8	-	98.7±2.0	97.4±0.6

[1)]Sample, Dried green tea leaf was extracted, irradiated at 20 kGy, and freeze-dried
[2)]Abbreviation : BHT, butylated hydroxytoluene; AA, ascorbic acid; SMS, sodium metasulfate.

effect of the irradiated green tea leaf extract powder at dilution factors of 1 and 5 were 81.1 and 44.0% and those of the commercial product were 82.5 and 29.0%, respectively. Results showed that 1% of the powder dissolved in butylene glycol for a cosmetic composition was higher than that of the commercial product or it had at least the same physiological activity in the irradiated green tea leaf extract powder. The tyrosinase inhibition effect of *Lonicera japonica* was increased as irradiation dose increased (Byun *et al.*, 2004) but no difference was found in the present study or others (Jo *et al.*, 2002; Jo *et al.*, 2003a). An *et al.* (2005) reported that tyrosinase and xanthin oxidase inhibition effects of irradiated polyphenols separated from persimmon leaf were not different from non-irradiated control. The authors also indicated that the tyrosinase inhibition effect was higher than those of the extracts of radish, garlic, mushrooms, and black tea at the same concentration.

The irradiated green tea leaf extract powder solutions in ethanol showed a higher DPPH radical scavenging effect when compared to those of BHT or sodium metasulfate but lower than ascorbic acid (Table 2). In an ethanol solution, the tyrosinase inhibition effect was higher in the ascorbic acid and sodium metasulfate than in the irradiated green tea leaf extract powder.

After 1% of the irradiated green tea leaf extract powder solution dissolved in buytlene glycol was prepared, the solution was mixed (15%) to a cosmetic composition for manufacturing a cream lotion to conduct a real application study. The DPPH radical scavenging effect of the cream lotion prepared from the solution of the irradiated green tea leaf extract powder did not differ from the cream lotion prepared from the solution of a commercial product (Figure 1).

Conclusion

An irradiation of green tea leaf extract for a color improvement and its freeze-dried powder has a higher or at least the same, physiological activities as the commercial product in a cosmetic composition. Therefore, irradiation technology is one of the effective and economic methods to process naturally functional materials for the food or cosmetic industry.

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