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Article

Retention of Biological Activities of the Cosmetics Manufactured with Green Tea Polyphenol and Possible Application of Irradiation Technology

Tae-Soon Park[1], Jin-Young Lee[2], Cheorun Jo[3], and Bong-Jeun An[4]*

[1]*R&D Hanbang Cosmetic Team, Daegu Gyeongbuk Institute for Oriental Medicine Industry, Gyeongbuk 712-260, Republic of Korea*
[2]*Department of Herbal Cosmetic Science, Hoseo University, Chungnam 336-795, Republic of Korea*
[3]*Department of Animal Science & Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea*
[4]*Department of Cosmeceutical Science, Daegu Haany University, Gyeongbuk 712-715, Republic of Korea*

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Ionizing radiation can be used to improve the color of green tea extract to brighter. As a result, the irradiated green tea extract can be applied easier and broader in food or cosmetic industry. To confirm the retention of the biological activities of the cosmetic products added with green tea polyphenols (PPs), the real cosmetic products including a skin lotion (PS) and an essence (PE) cream were manufactured. Irradiation also applied to the manufactured cosmetic products to see their improvement of color and changes of biological activity. The PP showed 72% of electron donating ability (EDA) at a 5 ppm concentration and the PS and PE containing 2% PP showed higher than 60%, which was similar inhibition activity to vitamin C. The inhibition of superoxide dismutase (SOD)-like activity of PP, PS, and PE were higher than 55% at a 500 ppm concentration and the inhibition of xanthine oxidase (XOase) were also higher than 65% at a 200 ppm concentration. The measurement of lipid oxidation by addition of Cu^{2+} and Fe^{2+} as prooxidants showed that PP and PS had higher metal chelating ability for Fe^{2+} than that of PE and the ability increased by increase of polyphenol concentration isolated from green tea. The Cu^{2+} chelating ability of PP and PS showed higher than 90% at a 200 ppm concentration. Therefore, it is concluded that addition of PP in manufacturing PS and PE retains its biological activities including EDA, inhibition of XOase and SOD-like activity, and metal chelating ability in the manufactured cosmetic products. In addition, irradiation of PS and PE improved color of the products containing PP brighter without any adverse changes in biological activity of the products.

Key words: biological activity, essence, green tea polyphenol, irradiation, skin lotion

Introduction

Separation of bioactive compounds from natural resources, which have been consumed safely for a very long time been actively studied [Choi *et al*., 1989]. Green tea has been used daily as a beverage in about 160 countries for decades. Green tea is composed of about 30% of polyphenols such as flavanols, flavandiols, flavonoids, and phenol acids [Valsa *et al*., 1997]. Polyphenols have been well-known to have various excellent biological activities, for example, inhibition of tooth decay [Sakanaka *et al*., 1989], inhibition of allergy [Yeo *et al*., 1995], reduction of blood pressure [An, 1998], prevention of gout [An *et al*., 1996], and inhibition of oxidation. Numerous results have been reported for the effect of green tea polyphenol (PP) itself in different biological activities. However, it is very hard to find the report which demonstrated the biological activity of real cosmetic products containing these valuable natural compounds.

Recently cosmetic industry adapted the concept of functional cosmetics or cosmeceuticals and widely used this concept throughout the world [Kligman, 2000]. In the development of new functional cosmetics, it is prerequisite to develop a raw material which has desirable activity. Among the activities, the strong interests have been focused on the effects of skin-whitening, anti-wrinkle, and UV protection. Researchers have been looking for the natural materials with these activities from various plant sources [Wiedow *et al*., 1990].

Irradiation technology was introduced to improve color of the green tea extracts brighter without any adverse changes in their biological activities [Son *et al*., 2001a]. The international consultative group of irradiation concluded that irradiation of food at a dose level of 10 kGy or below was toxicologically safe and nutritionally adequate. This process is easy and time saving process with economical benefit. Therefore, the study was

*Corresponding author
Phone: +82-53-819-1429; Fax: +82-53-819-1429
E-mail: anbj@dhu.ac.kr

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designed to investigate the retention of the biological activities of the skin lotion (PS) and essence (PE) containing PP after manufactured with real cosmetic composition. Irradiation effect on the biological changes and color of the cosmetic products also investigated.

Materials and Methods

Chemicals. The 1-1-diphenyl-2-picryl-hydrazyl (DPPH), xanthine, XOase, pyrogallol, 2-thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO).

Preparation of green tea polyphenol. The schematic diagram of the sample preparation is shown in Fig. 1 [Cho *et al*., 1993a]. Dried green tea (10 kg) purchased at Boseong was extracted using 60% acetone (50 L) in an extractor for 24 h at room temperature. The extract was centrifuged (Hitachi, CR21, Tokyo, Japan) at 8,000×g for 30 min and upper layer was collected. Then, 60% acetone (50 L) was added to the pellet and the extraction process was repeated three times. The collected upper layer was evaporated (N-N SERIES, Eyela, Tokyo, Japan) and filtered (No. 2, Advantec, Tokyo, Japan) to remove the chlorophyll. The remainder was used for sample separation. The sample was loaded into a Sephadex LH-20 column (Pharmacia biotech, Stokholm, Sweden) and progressed and separated by using the ratio of methanol and acetone at 0 to 1 through 1 to 0. Each fraction of the polyphenol was developed using a silica gel thin layer chromatography (TLC), collected, and lyophilized (FD5510, Ilshin Lab. Co., Yangju, Korea). The content of the polyphenol was quantified by comparing the lyophilized sample to the (+)-cathechin standard (Sigma Co. Ltd., St. Louis, MO). The sample was defined as having 60% polyphenol content and used for the experiment.

Prescription and manufacturing method of skin lotion and essence containing green tea polyphenols. The PS containing PPs were manufactured as followed by a prescription shown in Table 1. Glycerine and 1,3-butylene glycol as a water retention reagent and antiseptics were added into water phase. A polyethylene glycol (PEG)-40 hydrogenated castor oil was added in the ethanol phase and the water and ethanol phases were mixed together by a hand mixer. After mixing, citric acid and sodium citrate were added as a pH regulator and 2% of the PP were added, mixed, and used for experiments.

The essence containing PPs were manufactured as followed by a prescription shown in Table 2. Glycerine, betaine, and PEG-1500 as a water retention reagent and antiseptics were added in water phase. It was heated to 80ºC for 30 min and emulsified for 2 min using a homo mixer (T. K. Homo Mixer Mark II, Tokushu kika kogyo Co. Ltd, Osaka, Japan) at 3,000 rpm. Then a pre-dispersed polyglycerylmethacrylate/propylene and carboxyvinylpolymer were added and emulsified again for 2 min. After emulsification for 3 min when triethanolamine (TEA) was added, a PEG-40 hydrogenated castor oil was added in the ethanol phase and mixed 1 min. Then, 2% of the PP was added and emulsified for 2 min, cooled to the temperature of 30ºC, degassed, and used for experiments.

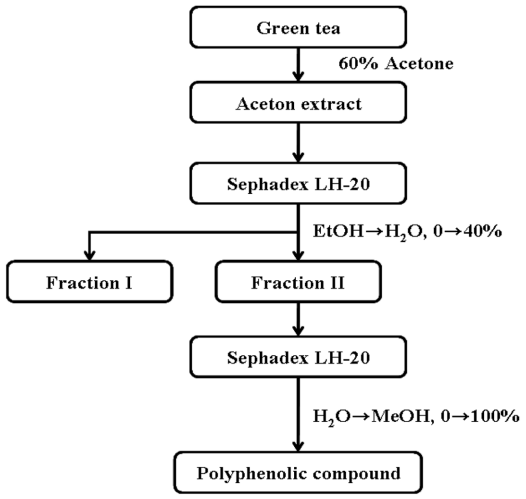

Fig. 1. Extraction and fractionation procedure of polyphenol from green tea.

Table 1. The experimental formulation of the PS containing polyphenol

No.	Technical Name	INCI Name	Contents (%)
1	D.I. water	Deionized Water	to 100
2	Glycerin	Glycerin	2.00
3	1,3-B.G	1,3-Butylene Glycol	3.00
4	Ethanol	Alcohol	5.00
5	Cremophor RH 40	PEG-40 Hydrogenated Castor Oil	0.80
6	Citric Acid	Citric Acid	0.04
7	Sodium Citrate	Sodium Citrate	0.10
8	Polyphenols	-	2.00

Table 2. The experimental formulation of the PE containing polyphenol

No.	Technical Name	INCI Name	Contents (%)
1	D.I. water	Deionized Water	to 100
2	Glycerin	Glycerin	5.00
3	Aminocoat	Betain	7.00
4	PEG-1500	Polyethylene Glycol 1500	2.00
5	Lubrajel DV	Polyglycerylmethacrylate/propylen	3.00
6	Carbopol 940	Carboxyvinylpolymer	0.30
7	T.E.A	Triethanolamine	0.30
8	Ethanol	Alcohol	4.00
9	Cremophor RH 40	PEG-40 Hydrogenated Castor Oil	0.50
10	Polyphenols	-	2.00

Irradiation. The manufactured PS and essence in tightly capped containers (each 100 g) was irradiated in a cobalt-60 irradiator (point source, AECL, IR-79, Nordion, Canada) with 0,

5, 10, and 20 kGy absorbed doses. The source strength was approximately 100 kCi with a dose rate of 10 kGy/h at 13±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 turned 360° continuously during the irradiation process to achieve uniform target dose and the non-irradiated control was placed outside of the irradiation chamber to have the same temperature effect as the irradiating sample.

Electron donating ability (EDA). EDA was measured by the Blois [1958] method. The extract (1 mL) and DPPH solution (1 mL, 2×10^{-4} M) was vigorously vortexed and placed for 30 min at room temperature and measured using a spectrophotometer (AAS, Hitachi Z-6000, Tokyo, Japan) at 517 nm. The EDA was calculated as;

EDA (%)=[1−(absorbance value of testing solution/ absorbance value of control solution)]×100

Superoxide dismutase (SOD)-like inhibition activity. SOD-like activity was assayed by the method of 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 placed 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

Xanthine oxidase inhibition activity. XOase inhibitory effect was assayed by the method of Stirpe and Della Corte [1969]. The reaction mixture containing 0.2 mL substrate (2 mM xanthine) in a 0.1 M potassium phosphate buffer (pH 7.5), 0.1 mL (0.2 U/mL) of an enzyme solution, and 0.1 mL of the extract solution was reacted at 37°C for 5 min. The control solution was prepared by adding 0.1 mL of distilled water instead of the extracts. The reaction was stopped by adding 1 mL of 1 N HCl. The formation of uric acid by the reaction was measured at 292 nm using a spectrophotometer. The inhibition of the XOase activity was expressed as;

Inhibitory effect (%)=[1−(uric acid of the reaction/ uric acid of the control)]×100

Lipid oxidation. To assess the development of the lipid oxidation, oil emulsion was prepared just before experiment. After adding 0.1 M maleic acid buffer (8 mL), Tween-20 (50 mL), and fish oil (0.5 mL), the mixture was homogenized for 15 min. KOH (2 g) was added into the mixture and the pH was adjusted to 6.5 by adding 2 N HCl.

The lipid oxidation inhibition effect by chelating Fe^{2+} and Cu^{2+}, a prooxidant of lipid oxidation, was measured by the method of Buege and Aust [1978]. Fe^{2+} or Cu^{2+} (each 50 ppm) were added into the reaction mixture prepared for the TBARS method and incubated at 37°C for 1 h. After vortexing the reaction mixture, a 20 mM TBA/T CA (2 mL) solution was added and heated in boiling water for 15 min. Tap water was used to cool the reaction mixture for 10 min and it was centrifuged (VS-5500, Vision Scientific, Co., Seoul, Korea) at 2,000×g for 15 min. The upper layer was read by the spectrophotometer (AAS, Hitachi Z-6000, Tokyo, Japan) at 531 nm.

Chelating effect (%)=[1−(absorbance of metal added-testing solution/absorbance of control solution)]×100

Statistical analysis. The data was collected and calculated by a Statistical Package of the Social Science (SPSS Inc., 10.0, 2000, Chicago, Illinois) program. Differences among the mean values were obtained by the Duncan's multiple range tests after conducting Analysis of the Variance with confidence level at $p<0.05$.

Results and Discussion

Polyphenols contents and color. A silica TLC plate was developed to identify the polyphenol separated from green tea (Fig. 2). After coloring of the TLC by anisaldehyde-H_2SO_4 and a 1% $FeCl_3$-methanol solution, each was presented with orange and blue. Cho *et al*. [1993b] revealed the result of flavone-3-ols isolated from Korean green tea by using TLC analysis. According to the presented color the separated polyphenol were estimated as flavone-3-ols. As a representative functional group, the flavone-3-ols, in green tea, it is proposed as catechin family including (+)-catechin, (-)-epicatechin, (-)-epigalocatechin gallate, (+)-gallocathechin [Ohmori *et al*., 1995; Jean, 2002].

The polyphenol fraction separated from green tea showed 94% or higher total phenol content (Data not shown). In the respect of the fact that the amount of polyphenol contains in

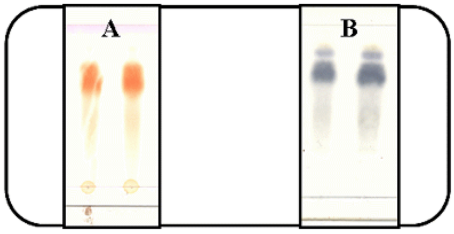

Fig. 2. Thin layer chromatogram of the polyphenols contained in green tea. Developing solvent: benzene:ethylformate:formic acid=2:7:1. Colorization reagent: A, anisaldehyde-H_2SO_4; B, $FeCl_3$-MeOH. Polyphenol contents: A, 1 mg/mL; B, 1 mg/mL

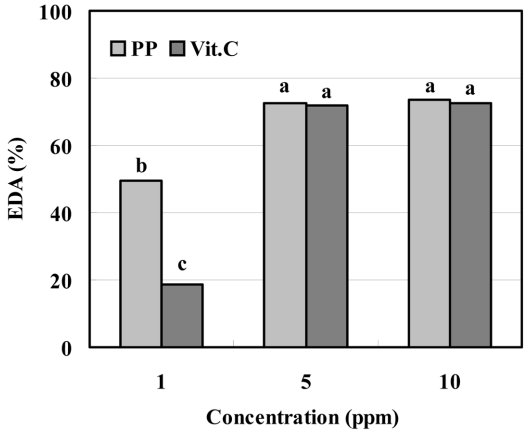

Fig. 3. EDA of polyphenol isolated from green tea and vitamin C. PP: polyphenol, Vit. C: vitamin C. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

plants can be an indirect indicator of biological activities, the polyphenol fraction separated from green tea may have strong biological activities.

When the PS and PE was manufactured with PP, the color of the products was darker (lower L*-value) than those without addition of PP (Data not shown). The PS and PE without PP did not show any difference by irradiation treatment, however, those with PP (2%) showed increase of L*-value with increase of irradiation dose. The a*- and b*-value of the PS and PE were decreased with increase of irradiation doses, indicating that the color of PS and PE went brighter by irradiation.

Electron donating ability. The EDA of PP, PS, and PE containing the PP are shown in Figs. 3-5. The PP (1 ppm) showed higher EDA (49%) than vitamin C (19%). The PS and PE containing a 5 ppm of polyphenol showed 60% or higher EDA while those without polyphenol showed less than 10%. Irradiation of the PS and PE products did not affect on EDA. This result agreed well with Son *et al.* [2001b] that irradiation of 20 kGy did not affect on the EDA of the ethanol extract of green tea leaf. Irradiation of PP at 40 kGy [An *et al*., 2004] and irradiation of *Oryza sativa*, Rice Bran, and Barley Bran [Bae *et al*., 2002] at 5, 10, 15 and 20 kGy did not show any difference on EDA. Choi *et al*. [2003] investigated the EDA in *Eucommia ulmoides*, *Paeonia suffruticosa* Andrews, *Paeonia japonica*, and *Rubus coreanus* using different extraction solvent including *n*-hexane, chloroform, and ethyl acetate and reported that the EDA of ethyl acetate fraction had higher than 95% at 1,000 ppm concentration. Kim *et al*. [2000] reported that the EDA of methanol fraction of *Coix lachrymajobi* var. were 50% at a 795 ppm level. Koh *et al*. [2005] studied the anti-oxidative property of the various extracts of *Pomegranate Seed* and reported that hot water, ethanol, and *Pomegranate Seed* oil contained 18.8, 28.5, and 9.7% of EDA at a 1,000 ppm level. From the results and references, both the PP tested for present

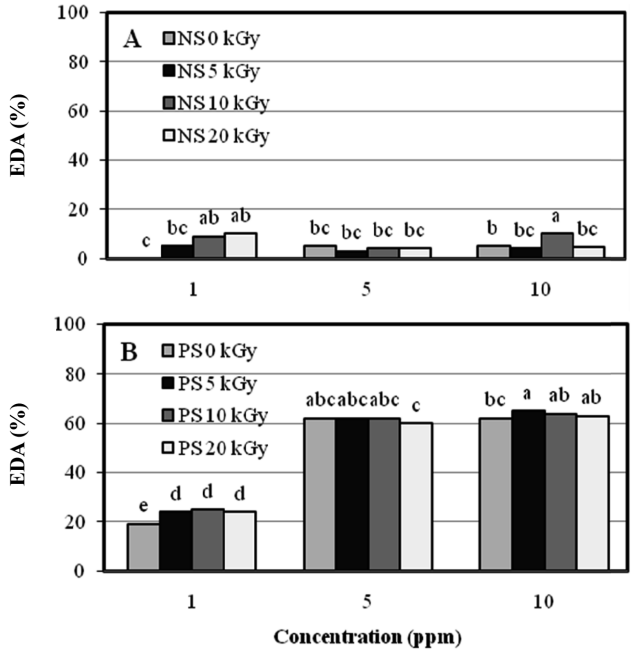

Fig. 4. EDA of irradiated PS manufactured without and with PP. A, PS without PP; B, PS containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

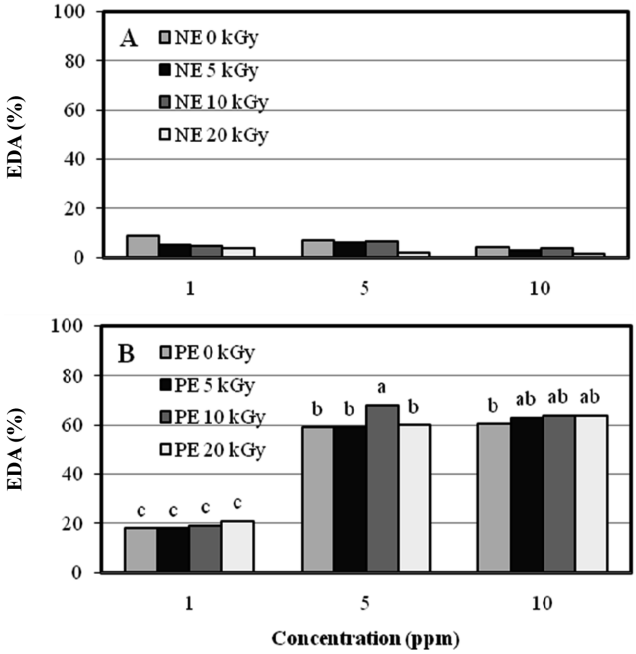

Fig. 5. EDA of irradiated PE manufactured without and with PP. A, PE without PP; B, PE containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

study and the PS and PE which were manufactured by addition of the PP contain strong EDA compared to other plant-derived extracts.

Superoxide dismutase-like activity. SOD-like activity of the PP, which is the important self-defense mechanism of body cell against oxidative damage [Pryor, 1986] is shown in Figs.

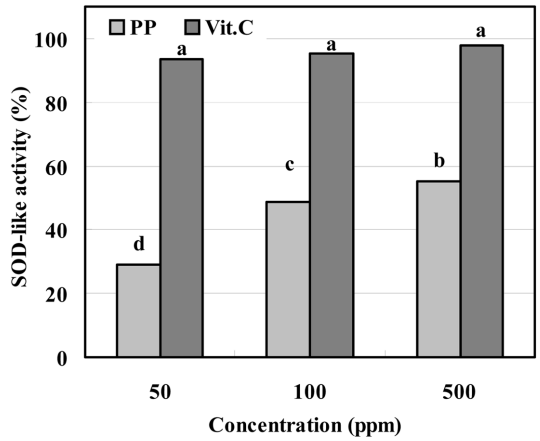

Fig. 6. SOD-like activity of polyphenol isolated from green tea and vitamin C. PP: polyphenol, Vit. C: vitamin C. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

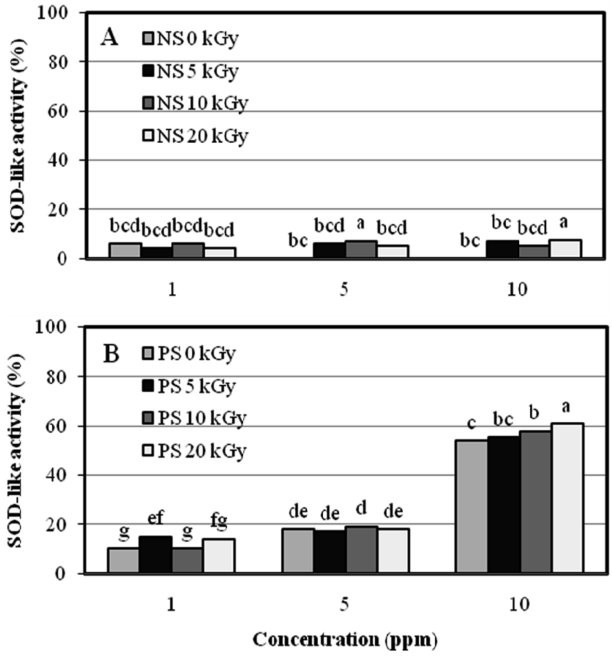

Fig. 7. SOD-like activity of irradiated PS manufactured without and with PP. A, PS without PP; B, PS containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

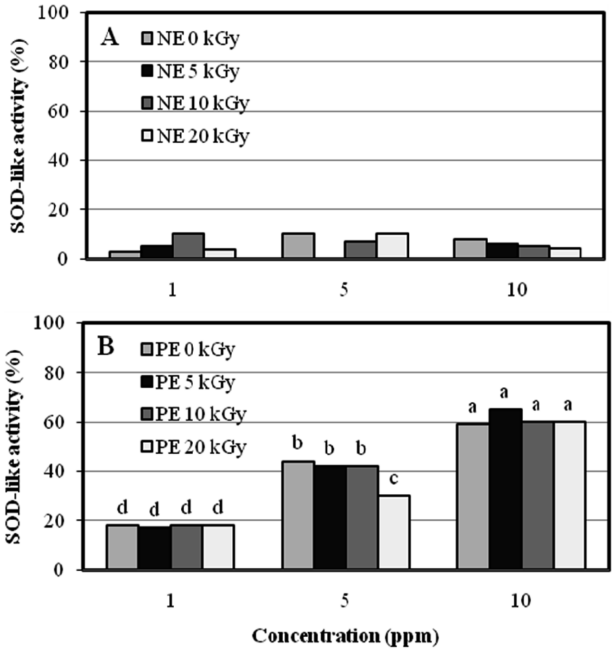

Fig. 8. SOD-like activity of irradiated PE manufactured without and with PP. A, PE without PP; B, PE containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

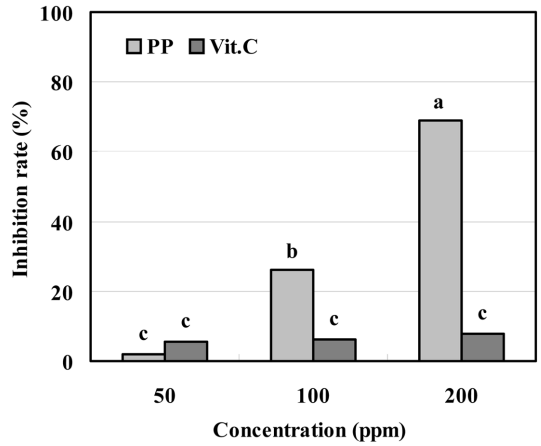

Fig. 9. Inhibition rate of polyphenol isolated from green tea and vitamin C on xanthine oxidase. PP: polyphenol, Vit. C: vitamin C. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

6-8. At a 500 ppm concentration, PP, PS, and PE showed 55, 70, and 60% of inhibition against SOD-like activity. Hong *et al*. [1998] reported that the inhibition rate of SOD-like activity of the physically pressed extract of apple, kale, kiwi, and radish were 14.6, 26.7, 27.6, and 24.1%, respectively. Compared with the previously reported results, it is concluded that the inhibition of SOD-like activity of PP and PS and PE containing the PP is relatively high. Kim *et al*. [2005] studied the antioxidative activity of *Cucurbita maxima* and *Cucurbita spp* and reported that the lyophilized powder of *C. maxima* and *Cucurbita spp* had 60.4 and 12.6% of inhibition against SOD-like activity at 500 ppm. Similarly, Lee *et al*. [2005] reported that the inhibition of SOD-like activity of *Lespedeza bicolor* extract was 20.0, 44.1, and 29.9% in extraction method using hot water, ethanol, and hot water with pressure, respectively, at a 1,000 ppm level.

Xanthine oxidase inhibition activity. XOase activity of vitamin C and the PS and PE without addition of PP showed less than 20% while the activity of PP, PS, and PE showed higher than 65% at 200 ppm (Figs. 9-11). Previous study using a methanol extract of *Ecklonia cava* at 400 ppm showed that the XOase inhibition activity of 53.1% [Kim *et al*., 1996]. The

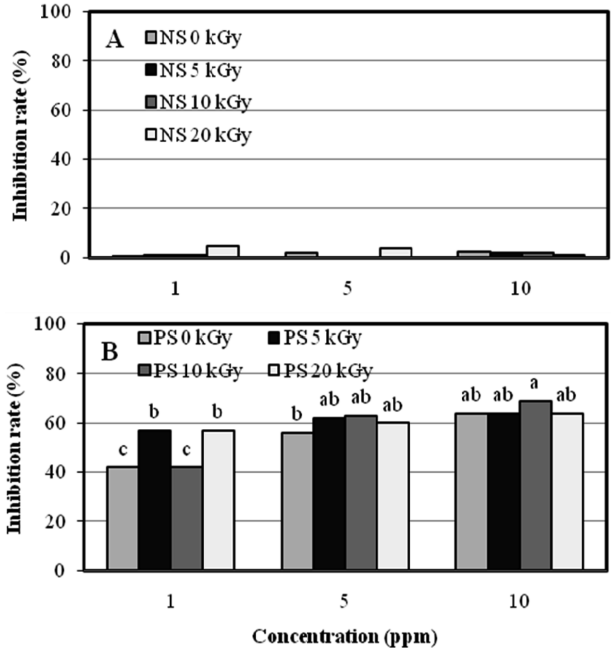

Fig. 10. Inhibition rate of irradiated PS manufactured without and with PP on XOase. A, PS without PP; B, PS containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

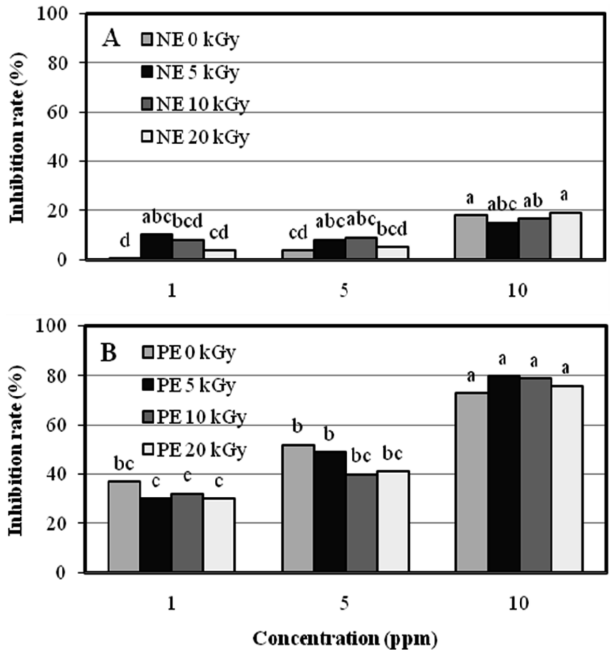

Fig. 11. Inhibition rate of irradiated PE manufactured without and with PP on XOase. A, PE without PP; B, PE containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

XOase inhibition activity of the extract of difference sea products such as *Undaria pinnatifida, Sargassum fulvellum, Enteromorpha, Porphyra tenera, Laminaria,* and *Codium fragile* had 10.8, 10.7, 14.8, 8.6, 27.9, and 33.0%, respectively, at a 500 ppm concentration. Therefore, the XOase inhibition

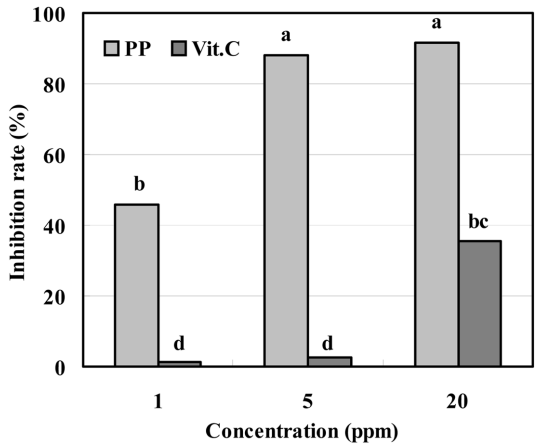

Fig. 12. Effect of polyphenol isolated from green tea and vitamin C on lipid oxidation in the presence of copper ion (Cu^{2+}). PP: polyphenol, Vit. C: vitamin C. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

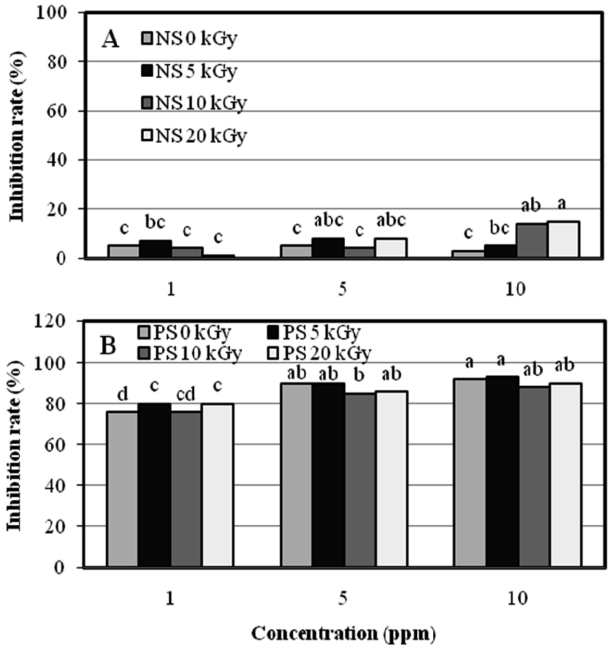

Fig. 13. Effect of irradiated PS manufactured without and with PP on lipid oxidation in the presence of copper ion (Cu^{2+}). A, PS without PP; B, PS containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

activity of prepared PP and even the PS and PE manufactured with the PP is superior to other plant-derived natural extract.

Metal ion chelating ability. To investigate the inhibition effect on lipid oxidation, metal ions including Fe^{2+} and Cu^{2+} were added as prooxidants (Figs. 12-17). The use of Vitamin C (20 ppm) showed less than 35% of metal chelating ability in the addition of both Fe^{2+} and Cu^{2+} however PP and PS showed higher than 80% at 5 ppm in Fe^{2+} and 70% at 20 ppm in Cu^{2+} addition. There was no irradiation effect on the changes of metal chelating ability. PS also showed higher than 60% in both Fe^{2+}

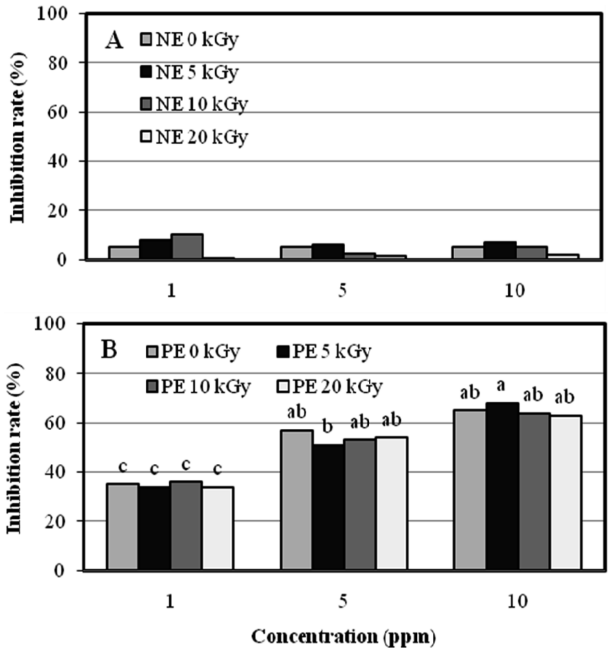

Fig. 14. Effect of irradiated PE manufactured without and with PP on lipid oxidation in the presence of copper ion (Cu^{2+}). A, PE without PP; B, PE containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

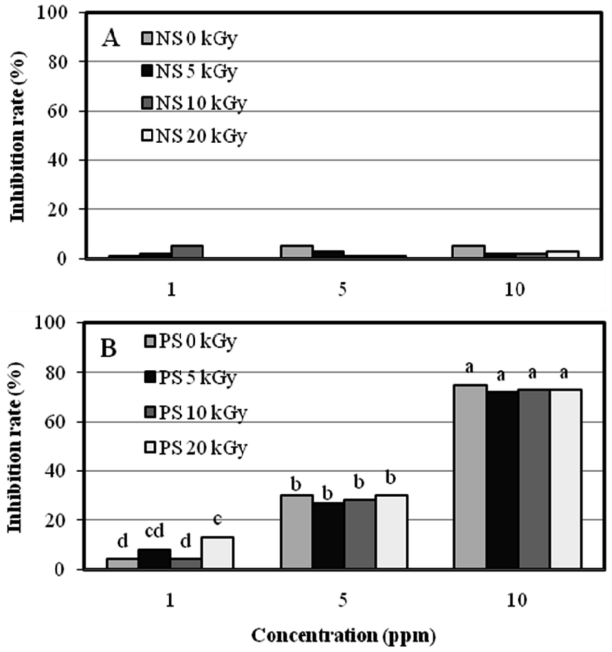

Fig. 16. Effect of irradiated PS manufactured without and with PP on lipid oxidation in the presence of ferrous ion (Fe^{2+}). A, PS without PP; B, PS containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

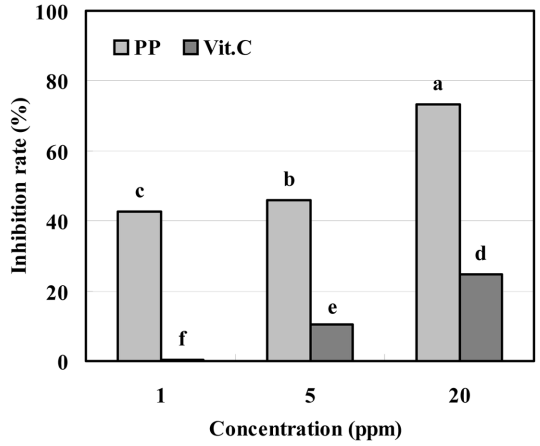

Fig. 15. Effect of polyphenol isolated from green tea and vitamin C on lipid oxidation in the presence of ferrous ion (Fe^{2+}). PP: polyphenol, Vit. C: vitamin C. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

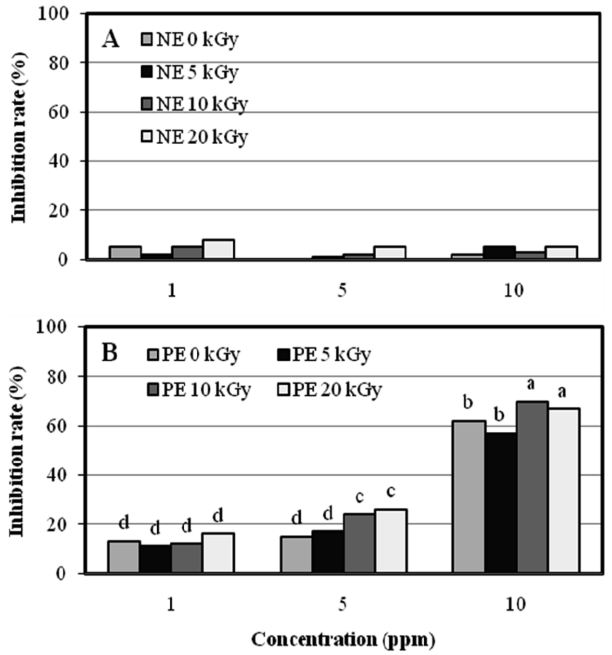

Fig. 17. Effect of irradiated PE manufactured without and with PP on lipid oxidation in the presence of ferrous ion (Fe^{2+}). A, PE without PP; B, PE containing PP. Values are means of 3 replicates and those with different alphabet letters are significantly different at $p<0.05$.

and Cu^{2+} added solution. An *et al*. [2005] reported that the ethanol extract of *Coptidis Rhizoma* showed higher than 80% of metal chelating ability at a 1000 ppm level. In comparison to *Coptidis Rhizoma* extract, the PP, PS, and PE tested in this study showed relatively high activity. Bae *et al*. [2002] also reported that there was no changes occurred by irradiation of the extracts of *Oryza sativa* and rice bran on their metal chelating ability.

From the serious of results it can be concluded that addition of PP in manufacturing PS and PE retains its biological activities including EDA, inhibition of XOase and SOD-like activity, and metal chelating ability in the manufactured cosmetic products. In addition, irradiation of PS and PE improved color of the products containing PP brighter without any adverse changes in biological activity of the products.

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