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Effect of Storage Temperature and Antioxidant Additives on the Color and Physiological Activity of Gamma Irradiated Green Tea Leaf Extract

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Abstract

Gamma irradiation was used as part of a new processing method to produce a brighter-colored and mild-flavored green tea leaf extract that retained all of its physiological activities. Dried green tea leaf was extracted with 70% ethanol and gamma irradiated at 0, 5, 10, 20 kGy. Hunter color L*- and a*-values were increased with irradiation in a dose-dependent manner, which was a color range from dark brown to bright yellow. However, the irradiation effect gradually disappeared during 3 weeks of storage, with color reverting to that of untreated samples. There was no difference in the radical scavenging and tyrosinase inhibition effect by irradiation. Among antioxidants used, ascorbic acid was the most effective against color reversion. In contrast, cysteine was shown to protect the effect of color change with irradiation. Results indicated that enhanced color of irradiated green tea leaf extract can be effectively controlled by additives such as ascorbic acid and a low storage temperature.

Key words: irradiation, green tea leaf, color, physiological activity, additive

INTRODUCTION

Tea catechins have been reported to inhibit lipid oxidation in edible oils such as lard (1), canola oil (2), rapeseed oil (3), and marine oils (4). Wanasundara and Shahidi (4) reported that tea catechins have a greater antioxidative effect than butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butyl hydroquinone (TBHQ), and natural antioxidant vitamin E. These all depend on which of the various types of tea catechin is used as well as the concentration. In addition, the antimutagenic activity (5,6), which is the ability to suppress the formation of chromosome aberrations (7), and the protective effects against arteriosclerosis (8) were also investigated. Furthermore, components from the green tea leaves have a scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl radical (9). Green tea also has antimicrobial activities in water or ethanol extracts (10).

Despite its successful use in food and cosmetics, green tea leaf has been used mainly for drinking as a tea. This is probably because of its deep dark color and off-flavor, which makes it very difficult to use efficacious amounts in cosmetics, medicines, or foods without adversely affecting sensory qualities. Irradiation may be one solution (11) because it can change the color of green tea leaf extract from dark red to slightly bright yellow. The lyophyllized green tea leaf extract can be stored for several months without undesirable color change. Jo et al. (12) also applied the lyophyllized green tea leaf extract in a real meat system and successfully demonstrated its antioxidative effect. However, in the extract state, there could be a possibility of reversing its color from bright to dark (13). Because such a change would be a serious disadvantage, the effects of additives on color reversion were also investigated (14).

In the present study, the storage stability of gamma irradiated green tea leaf extract under different storage temperatures, with or without the addition antioxidants alone or combined was studied.

MATERIALS AND METHODS

Sample preparation

Dried green tea leaf was purchased from the Bosung area in Chonnam, Korea. A 200 g sample was transferred to an ethanol solution (70%, 4 L), and extracted overnight. Extraction was performed twice and the extract was divided into 4 groups by irradiation doses of 0, 5, 10, and 20 kGy in 2 L containers. Antioxidant solutions were prepared by dissolving ascorbic acid and cysteine in deionized distilled water (DDW) and vitamin E in ethanol to make 0.2% solutions. Then the solutions were added to the sam-

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ple extract as 9 : 1 ratio for 200 ppm final concentrations. For the combination treatments, exactly same amount of each individual additive (0.2% solution) was added so that the final concentration was 200 ppm for each. The additives were added immediately after gamma irradiation.

Irradiation

Samples in tightly capped containers (2 L) were irradiated in a cobalt-60 irradiator (point source, AECL, IR-79, Nordion, Canada) with 0, 5, 10, and 20 kGy-absorbed doses. The reason for using extracts instead of dried green tea leaf or dried extract powder was that the effects of irradiation are much higher in solutions than in a dried state. The source strength was approximately 100 kCi with a dose rate of 70 Gy min^{-1} at $15\pm0.5^{\circ}C$. 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 $\pm2\%$ of the target dose. Samples were continuously rotated 360° at 2.5 turns/min during the irradiation process, to achieve an uniform target dose. The non-irradiated control was placed outside of the irradiation chamber to assure the same environmental temperature effect as the irradiated samples. After irradiation, the samples were transferred and stored refrigerated ($4^{\circ}C$) or at room ($25^{\circ}C$) temperatures. The samples were stored for 4 weeks prior to analyses.

Color measurement

Samples (9 mL) were transferred 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 to 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 the computerized system using Spectra Magic software (version 2.11, Minolta Cyberchrom Inc. Osaka, Japan).

Scavenging effects of 1,1-diphenyl-2-picrylhydrazyl (˙DPPH) radical

Both non-irradiated and irradiated extracts were diluted 1,200 times with deionized distilled water (DDW). Free radical scavenging effect was estimated according to the method of Blois (15). 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

Non-irradiated and irradiated green tea leaf extracts were diluted 10 times with DDW and the diluent was 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, 1/15 M sodium phosphate buffer (pH 6.8) and mushroom tyrosinase (100 unit/mL, Sigma Co., Ltd). The reaction mixture was incubated at $25^{\circ}C$ for 15 min. The amount of dopachrome produced in the reaction mixture was spectrophotometrically determined at 475 nm (16).

Statistical analysis

One-way Analysis of Variance was performed using SAS (SAS Institute, Cary, NC, USA) software (17) and the Student-Newman-Keul's multiple range test which was used to compare the differences among the mean values. Mean values and pooled standard errors of the mean (SEM) were reported and the significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Color changes by irradiation

Changes in the Hunter color L*-value of green tea extract from 70% ethanol solution after gamma irradiation are shown in Table 1. The L*-values were increased with increasing irradiation doses at both storage temperatures. During storage at $4^{\circ}C$, the L*-value slowly decreased and the irradiation dose effect was reversed, with the samples receiving 5 or 10 kGy irradiation having higher L*-values than the 20 kGy irradiated sample (Table 1). Byun et al. (18) indicated that irradiation breaks down the chlorophyll in a model system and lipid oxidation can be inhibited by oxygen removal in the irradiated materials by using nitrogen gas flush. The enhancing color of the plant extracts was confirmed by using different materials such as the root or stolon of the Licorice (*Glycyrrhiza uralensis* Fischer) extract (19) and persimmon (*Diospyros kaki* L. folium) leaf extract (20).

The color change progressed more rapidly in the sample stored at $25^{\circ}C$ than at $4^{\circ}C$. As a result, the sample with 5 kGy irradiation had the highest value among all the samples after 2 weeks. Decreases in Hunter color L*-values may indicate deterioration in the quality of the extract. Results also indicated that the storage temperature is important for maintaining the proper color from the adverse changes. Our previous results showed that when the decolored green tea leaf extract was freeze-dried after irradiation, the color of the sample did not change after long-term storage (13).

Hunter color a*-value did not change much after storage for 3 weeks at $4^{\circ}C$ in the non-irradiated sample (Table 2). However, irradiation significantly decreased the a*-value. The sample with 20 kGy irradiation increased its a*-value during storage. Hunter color a*-value may be one of the most important color characteristics for evaluating the

Table 1. Hunter color L*-value of green tea extract in a 70% ethanol solution after gamma irradiation

Storage temperature	Irradiation (kGy)	Storage (week)				SEM[3]
		0	1	2	3	
4°C	0	75.37$^{dz1),2)}$	75.65^{az}	76.21^{bz}	75.57^{cz}	0.005
	5	90.83^{ay}	88.35^{by}	87.50^{cy}	86.28^{dx}	0.013
	10	93.63^{ax}	90.14^{bx}	88.70^{cw}	87.12^{dw}	0.021
	20	96.22^{aw}	91.19^{bw}	88.19^{cx}	86.04^{dy}	0.008
	SEM[3]	0.010	0.010	0.005	0.022	
25°C	0	74.82^{az}	73.27^{bz}	72.98^{cz}	71.99^{cz}	0.007
	5	87.33^{ay}	81.88^{bx}	80.21^{cw}	80.21^{cw}	0.011
	10	89.23^{ax}	82.16^{bw}	79.73^{cx}	79.23^{cx}	0.016
	20	89.98^{aw}	80.87^{by}	77.55^{cy}	76.51^{cy}	0.007
	SEM[3]	0.008	0.012	0.015	0.015	

[1]$^{a-d}$Different letters within the same row differ significantly ($p<0.05$).
[2]$^{w-z}$Different letters within the same column with the same storage temperature differ significantly ($p<0.05$).
[3]Standard errors of the mean (n = 12).

Table 2. Hunter color a*-value of green tea extract in a 70% ethanol solution after gamma irradiation

Storage temperature	Irradiation (kGy)	Storage (week)				SEM[3]
		0	1	2	3	
4°C	0	9.53$^{cw1),2)}$	9.79^{bw}	9.79^{bw}	10.51^{aw}	0.007
	5	-3.62^{dx}	-0.92^{cz}	0.53^{bz}	2.17^{az}	0.010
	10	-4.29^{dy}	-0.76^{cy}	1.07^{by}	2.89^{ay}	0.007
	20	-5.25^{dz}	0.45^{cx}	2.93^{bx}	5.24^{ax}	0.006
	SEM[3]	0.007	0.006	0.007	0.011	
25°C	0	10.63^{dw}	13.96^{cw}	15.39^{bx}	17.33^{ax}	0.006
	5	0.30^{dy}	7.57^{cz}	10.61^{bz}	13.35^{az}	0.012
	10	-0.01^{dz}	8.58^{cy}	12.33^{by}	15.59^{ay}	0.014
	20	0.47^{dx}	11.08^{cx}	15.84^{bw}	19.27^{aw}	0.009
	SEM[3]	0.016	0.005	0.008	0.010	

[1]$^{a-d}$Different letters within the same row differ significantly ($p<0.05$).
[2]$^{w-z}$Different letters within the same column with the same storage temperature differ significantly ($p<0.05$).
[3]Standard errors of the mean (n = 12).

change in samples, because most tea polyphenols and β-carotene have dark brown- or red-colored pigments. Son et al. (13) reported that the conformational changes of color components by irradiation such as flavonoids, anthocyanins and other coloring materials, which can produce redness, may make the color brighter. As shown in Table 2, the Hunter color a*-value between the non-irradiated and 20 kGy-irradiated samples were similar after 3 weeks of storage at 25°C. In contrast the a*-value was very low following irradiation. This phenomenon can be explained as a consequence of browning and oxidation reactions of components, which may reverse color changes. Therefore, room temperature storage is not a good method to maintain the color of irradiated green tea extract during storage. Irradiation significantly decreased Hunter color b*-value dose dependently and likewise, it was also decreased by storage.

Scavenging effects of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and tyrosinase inhibition effect

Irradiation at 10 and 20 kGy increased the DPPH radical scavenging effect significantly at 4°C (Table 3). The effects of irradiation gradually disappeared after 1 week and, the 5 kGy-irradiated samples had a higher scavenging effect at 3 weeks storage. Generally the effect decreased after storage for 3 weeks regardless of irradiation treatments. When a sample was stored at room temperature, the radical scavenging capacity was unaffected by irradiation treatments, but the storage effect still existed, resulting in a lowering of the radical scavenging activity during storage. Previous results using Korean traditional soybean-based fermented foods (21) or sauce of processed meat products, *bulgogi* (22) as well as a natural plant extract (19,20) support the observation that irradiation does not change the radical scavenging effect.

There was no difference in the tyrosinase inhibition effect, either by gamma irradiation or storage (Table 4) except for the sample that was 5 kGy-irradiated for 1 week-storage at 25°C. Effect of storage temperature also was not seen and the results were similar to Son et al. (13).

Color change of the green tea extracts with different additives

Three natural antioxidants commonly used in the food, pharmaceutical, and cosmetic industries, and their combinations, were added to the green tea extracts and irra-

Table 3. DPPH radical scavenging effect of green tea extract in a 70% ethanol solution after gamma irradiation

Storage temperature	Irradiation (kGy)	Storage (week)				SEM[3)]
		0	1	2	3	
4°C	0	48.74$^{az1),2)}$	30.57^{b}	27.37^{c}	31.66^{by}	2.232
	5	48.35^{az}	33.20^{b}	28.82^{c}	36.95^{bx}	1.078
	10	51.71^{ay}	26.74^{b}	27.73^{b}	30.69^{by}	1.805
	20	53.33^{ax}	26.73^{b}	29.70^{b}	29.98^{by}	3.301
	SEM[3)]	0.363	1.282	1.080	0.818	
25°C	0	39.96^{a}	33.36^{b}	23.83^{c}	25.58^{cy}	0.795
	5	44.85^{a}	33.53^{b}	28.58^{c}	31.08^{cx}	0.758
	10	45.14^{a}	34.85^{b}	27.51^{b}	29.98^{bx}	2.450
	20	45.44^{a}	33.00^{b}	28.57^{b}	33.33^{bx}	1.368
	SEM[3)]	1.087	2.445	0.975	0.982	

[1)a,b]Different letters within the same row differ significantly ($p<0.05$).
[2)w-z]Different letters within the same column with the same storage temperature differ significantly ($p<0.05$).
[3)]Standard errors of the mean (n = 8).

diated (Table 4). Especially for cysteine, human serum (0.5%, v/v), lipoprotein-deficient human serum at an equivalent concentration and the amino acids L-cysteine (25 μM) and L-histidine (25 μM) inhibited effectively the oxidation of LDL by copper at pH 7.4 (23). Richard-Forget et al. (23) reported that cysteine inhibits enzymatic browning catalyzed by apple polyphenol oxidase.

Hunter color L*-value increased significantly with irradiation up to 20 kGy and the samples with ascorbic acid and vitamin E alone or in combination showed higher L-values than those with other treatments. The sample with cysteine had the lowest L-value, and seemed to interfere with the L-value changes from the other antioxidants when combined with them. Ascorbic acid and vitamin E are potent radical scavengers and they may affect the irradiation process, with a slightly lower result in the sample with irradiation. The L*-value of the irradiated control without antioxidant was, therefore, higher than those added with ascorbic acid or vitamin E.

Hunter color a*-value also decreased with irradiation regardless of the antioxidants used (Table 5). Similarly to the Hunter color, L*-value, ascorbic acid, vitamin E, or both combined, was the most effective. Cysteine still interrupted the change of sample color and even reversed the change observed, suggesting that the color change can be maintained by cyteine addition if needed. Of course, there are other problems that should be solved such as off-flavor from S-containing compounds produced by irradiation.

After 3 weeks at room temperature, the Hunter color L*-value decreased in all the samples compared to 0 month, and generally, irradiation at 20 kGy adversely affected the sample color, resulting in lower L-value than in samples irradiated at 5 or 10 kGy (Table 6). This trend was continued in the result of the Hunter color a*-value but ascorbic acid addition was the most effective way to maintain the sample color of bright yellow as opposed to dark red.

Effect of additives on radical scavenging and tyrosinase inhibition effects

The capacity to scavenge DPPH radicals was higher in 10 kGy-irradiated samples than the non-irradiated ones when antioxidants were not added (Table 7). The irra-

Table 4. Tyrosinase inhibition effect (%) of green tea extract in a 70% ethanol solution after gamma irradiation

Storage temperature	Irradiation (kGy)	Storage (week)				SEM[2)]
		0	1	2	3	
4°C	0	32.36	35.78	37.44	31.71	0.480
	5	32.12	34.00	37.93	30.39	0.496
	10	33.24	34.91	37.32	31.33	0.472
	20	32.22	34.44	35.81	30.40	0.251
	SEM[2)]	0.612	0.373	0.385	0.316	
25°C	0	32.08	36.15$^{x1)}$	33.86	30.96	0.512
	5	32.89	31.62^{y}	34.59	30.47	0.781
	10	32.74	35.92^{x}	34.50	29.92	0.744
	20	32.05	35.55^{x}	34.66	31.40	0.742
	SEM[2)]	0.492	0.708	0.464	1.596	

[1)x,y]Different letters within the same column with the same storage temperature differ significantly ($p<0.05$).
[2)]Standard errors of the mean (n = 8).

Table 5. Hunter color changes of green tea extract in 70% ethanol with various antioxidants and their combinations at 4°C at 0 week

Hunter	IR	Control	VC	VE	Cys	VC+VE	VC+Cys	VE+Cys	All	SEM[3)]
L*	0	68.6$^{gz1),2)}$	71.4^{fz}	64.9^{hz}	75.4^{aw}	71.5^{ez}	74.2^{bw}	73.7^{dw}	73.8^{cz}	0.014
	5	87.6^{by}	87.8^{ay}	79.0^{dy}	66.6^{hx}	87.5^{cy}	72.1^{fz}	70.0^{gx}	74.8^{ey}	0.012
	10	94.0^{ax}	91.4^{cx}	87.0^{dx}	59.8^{hy}	92.1^{bx}	72.2^{fy}	66.0^{gy}	76.5^{ex}	0.008
	20	96.2^{aw}	93.3^{bw}	91.3^{dw}	53.7^{hz}	93.2^{cw}	73.6^{fx}	64.9^{gz}	82.7^{ew}	0.009
	SEM[4)]	0.009	0.008	0.018	0.009	0.015	0.007	0.010	0.008	
a*	0	15.9^{aw}	14.2^{cw}	14.5^{bw}	9.0^{gz}	14.1^{cw}	10.6^{ew}	10.5^{fz}	11.4^{dw}	0.036
	5	-2.5^{fx}	-3.7^{gx}	1.7^{ex}	13.0^{ay}	-4.0^{hx}	7.7^{cx}	10.9^{by}	7.2^{dx}	0.019
	10	-8.6^{hy}	-7.4^{fy}	-5.1^{ey}	21.8^{ax}	-8.2^{gy}	5.7^{cy}	16.0^{bx}	4.1^{dy}	0.012
	20	-9.9^{hz}	-8.9^{fz}	-8.2^{ez}	28.8^{aw}	-9.1^{gz}	2.5^{cz}	16.8^{bw}	-0.5^{dz}	0.009
	SEM[4)]	0.013	0.008	0.042	0.013	0.032	0.006	0.019	0.012	

Abbreviations: Control, no antioxidant added; VC, ascorbic acid; VE, vitamin E; Cys, csyteine; All, VC+VE+Cys.
[1)a-h]Different letters within the same row differ significantly ($p<0.05$).
[2)w-z]Different letters within the same column with the same storage temperature differ significantly ($p<0.05$).
[3)]Standard errors of the mean (n = 16). [4)](n = 8).

Table 6. Hunter color changes of green tea extract in 70% ethanol with various antioxidants and their combinations at 4°C for 3 weeks

Hunter	IR	Control	VC	VE	Cys	VC+VE	VC+Cys	VE+Cys	All	SEM[3)]
L*	0	56.3$^{fz1),2)}$	73.1^{az}	60.1^{cz}	59.4^{dx}	68.9^{bz}	57.1^{ez}	56.0^{gw}	55.8^{hz}	0.009
	5	61.7^{dw}	78.3^{ay}	64.1^{cw}	61.2^{dw}	72.4^{bx}	59.9^{ew}	55.6^{fx}	56.7^{gy}	0.010
	10	61.2^{dx}	79.4^{aw}	63.8^{cx}	56.8^{gy}	72.5^{bw}	59.6^{ex}	53.9^{hy}	57.7^{fx}	0.007
	20	59.4^{dy}	78.5^{ax}	61.5^{cy}	52.4^{hz}	71.0^{by}	58.7^{ey}	53.7^{gz}	57.8^{fw}	0.010
	SEM[4)]	0.009	0.009	0.011	0.006	0.010	0.007	0.010	0.009	
a*	0	34.9^{ax}	16.8^{hw}	33.5^{bx}	22.2^{gz}	24.3^{ex}	22.7^{fx}	31.3^{cy}	27.8^{dx}	0.023
	5	33.0^{az}	12.9^{hy}	31.6^{bz}	23.5^{ey}	21.9^{gz}	22.1^{fz}	30.9^{cz}	27.6^{dy}	0.012
	10	34.1^{ay}	12.6^{hz}	32.8^{cy}	28.5^{dx}	22.5^{gy}	22.5^{fy}	32.9^{bx}	27.4^{ez}	0.008
	20	36.0^{aw}	14.6^{hx}	35.4^{bw}	32.4^{dw}	25.3^{fw}	23.1^{gw}	34.5^{cw}	28.4^{ew}	0.008
	SEM[4)]	0.013	0.022	0.009	0.019	0.014	0.008	0.009	0.011	

Abbreviation: Control, no antioxidant added; VC, ascorbic acid; VE, vitamin E; Cys, csyteine; All, VC+VE+Cys.
[1)a-h]Different letters within the same row differ significantly ($p<0.05$).
[2)w-z]Different letters within the same column with the same storage temperature differ significantly ($p<0.05$).
[3)]Standard errors of the mean (n = 16). [4)](n = 8).

Table 7. Radical scavenging effect of DPPH and tyrosinase inhibition activity of green tea extract with added natural antioxidants or their combination at 4°C for 0 week[1)]

IR (kGy)	Control	VC	VE	Cys	VC+VE	VC+Cys	VE+Cys	All	SEM
• Scavenging effect of DPPH radical (%)									
0	39.0$^{y2)}$	40.9	42.0	43.1	39.1	42.7	41.8	41.4	2.37
5	41.5^{xy}	39.9	43.9	45.9	43.3	43.0	45.3	42.9	2.07
10	45.9^{x}	41.9	40.2	43.3	42.0	41.6	44.6	43.2	2.51
20	44.0^{xy}	42.6	41.4	43.4	42.1	42.9	43.7	41.8	2.05
SEM	1.60	2.13	1.70	4.17	1.77	2.05	1.66	1.80	
• Tyrosinase inhibition effect (%)									
0	24.2	37.6	25.2	43.4	29.3	36.7	36.1	36.3	6.59
5	34.2	42.9	29.4	42.8	31.1	33.3	41.7	26.6	5.42
10	33.2$^{ab3)}$	47.0^{a}	31.0^{b}	42.8^{ab}	33.8^{ab}	38.5^{ab}	36.5^{ab}	35.6^{ab}	4.67
20	39.9	39.7	38.0	49.2	36.9	41.4	32.3	34.9	7.20
SEM	5.04	6.71	6.51	6.60	6.67	4.59	6.22	5.70	

Abbreviation: Control, no antioxidant added; VC, ascorbic acid; VE, vitamin E; Cys, csyteine; All, VC+VE+Cys.
[1)]The data for 0, 1, 2, and 3 weeks were pooled for statistical analysis.
[2)x,y]Different letters within the same row differ significantly ($p<0.05$).
[3)a,b]Different letters within the same column with the same storage temperature differ significantly ($p<0.05$).

diation effect was not found in the sample with added antioxidants or their combination, and the use of a single natural antioxidant or their combinations made no difference in the radical scavenging effect. Previous results also indicated that there was no difference in the radical scavenging effect of various Korean medicinal herbs with irradiation (24).

Tyrosinase inhibition activity was also measured (Table 7) and the sample with added ascorbic acid showed a more effective inhibition than vitamin E. Although not statistically significant, the irradiated sample seemed to have a higher tyrosinase inhibition activity than the non-irradiated control, in general. Similarly, Byun et al. (21) reported that the tyrosinase inhibition activity of irradiated *Chungkukjang* and *Doenjang*, Korean traditional soybean based fermented food, was not changed by irradiation.

In conclusion, the effect of color changes in green tea leaf extract by irradiation was confirmed. However, the storage temperature after processing is important in preserving the enhanced color and ascorbic acid is the most effective way to maintain the color among the methods we tested. Irradiation technology has a great potential in the cosmetic and pharmaceutical industry as well as the food industry, for those wishing to produce value-added materials.

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