

Application of Far-Infrared Irradiation in the Manufacturing Process of Green Tea

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Seven kinds of green tea leaves were manufactured with far-infrared (FIR) irradiation, and the physicochemical characteristics of the green tea were determined. Appropriate FIR irradiation during the manufacturing process significantly increased the polyphenolic content of green tea. FIR irradiation at 90 °C for 10 min, replacing the roasting step, and of the fully processed green tea leaves (GTP3) increased the total phenol content of green tea from 475.6 to 811.1 mg/g and the total flavanol content from 175.7 to 208.7 mg/g, as compared to the control. Epigallocatechin and epigallocatechin gallate increased from 57.68 and 9.60 mg/g in a nonirradiated control to 89.88 and 16.33 mg/g in GTP3, respectively. Ascorbic acid, caffeine, and nitrite scavenging activities were also increased in GTP3. However, the overall color change of GTP3 was negligible. These results indicate that the chemical properties of green tea are significantly affected by FIR irradiation at specific stages of the manufacturing process of green tea leaves and that this FIR irradiation results in high-quality green tea.

KEYWORDS: Green tea; far-infrared irradiation; catechin; nitrite scavenging ability

INTRODUCTION

Tea is a beverage made from the leaves of the *Camellia sinensis* L. species of the Theaceae family. Teas are generally classified into three major categories: nonfermented green tea, partially fermented oolong or pouchong tea, and fully fermented black tea. Approximately 20% of the world tea production is in the form of green tea. Green tea is made by steaming or roasting fresh tea leaves at elevated temperatures to inactivate the polyphenol oxidase present in the leaves.

Fresh shoots of green tea are very rich in polyphenols, especially the low molecular weight catechins (Cs) (flavan-3-ols) comprising up to 30% of the green tea (dry weight basis) (1). The Cs comprise a family of four major compounds, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), and four minor compounds, C, catechin gallate (CG), galocatechin (GC), and galocatechin gallate (GCG), which are epimers of the major Cs. The Cs have a role in protection against cancer, cardiovascular disease, and other degenerative diseases (2) and contribute to the characteristic bitter and astringent taste of tea. The brothy, sweet taste is due to amino acids such as theanine, glutamic acid, and arginine (3, 4). The caffeine in green tea has been shown to protect against colorectal cancer (5).

The variety, growing environment, manufacturing conditions, and particle size of tea leaves influence the composition of the tea leaf and the final infusion (6). Moreover, brewing temperature and time during the preparation of green tea influence the

polyphenol content and the anticlastogenicity of green tea (7, 8). Heat processing and storage also affect the flavanol composition and sensory quality of green tea. Many plant polyphenols, such as flavonoids, tannins, coumarins, curcuminoids, xanthenes, phenolics, and terpenoids, exist either as forms bound to high molecular weight compounds or as parts of repeating subunits of high molecular weight polymers (9). Several methods, including far-infrared (FIR) irradiation, are known to release and activate low molecular weight natural antioxidants (10–12). FIR rays are defined as electromagnetic waves of wavelengths longer than 4 μm but shorter than those of microwaves (>0.1 cm). FIR rays are biologically active (13) and transfer heat to the center of materials evenly without degrading the constituent molecules of the surface. FIR irradiation may be capable of cleaving covalent bonds and releasing antioxidants, such as flavonoids, carotene, tannin, ascorbate, flavoprotein, and polyphenols, from repeating polymers (10, 14).

The manufacturing process of roasted green tea can be divided into three steps. The first step is roasting the green tea leaves to inactivate enzymes, the second step is rolling the leaves, and the last step is drying the leaves. In this study, we applied FIR irradiation at the roasting and/or drying steps of the green tea manufacturing process and determined the effect of FIR irradiation on the physicochemical properties of green tea.

MATERIALS AND METHODS

Materials. Eight C standards, (–)-GC, (+)-C, (–)-EC, (–)-EGC, (–)-EGCG, (–)-GCG, (–)-ECG, (–)-CG, gallic acid (GA), and caffeine, were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol [high-performance liquid chromatography (HPLC) grade],

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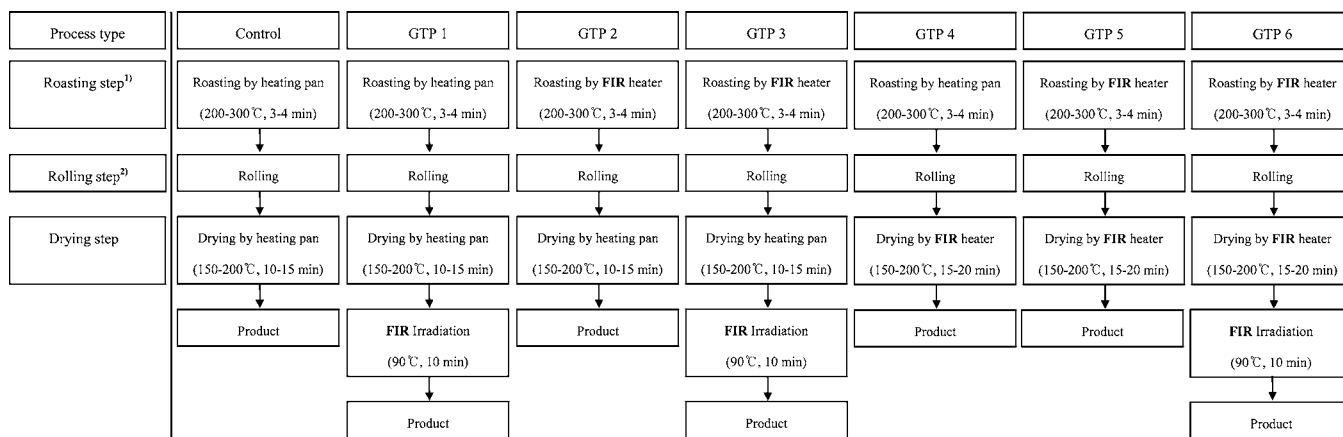


Figure 1. Diagram of processing of green tea leaves with FIR irradiation. The roasting step, 1), and rolling step, 2), were repeated two times.

85% orthophosphoric acid (analytical grade), vanillin, tannic acid, and 1,1-diphenyl-2-picrylhydrazyl were also purchased from Sigma Chemical Co. Folin-Ciocalteu reagent was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals were all of analytical grade and used as received. The water used in HPLC and sampling was prepared with a super purity water system (Purite Ltd., Oxon, United Kingdom) with a resistance of >17.5 M Ω cm.

Processing of Green Tea Leaves. Fresh tea leaves (*Camellia sinensis* var. *sinensis*) were harvested in August 2003 at Bosung, Korea. The leaves were processed by the traditional method—roasting, rolling, and drying. We added or substituted the heating step(s) and/or additional irradiation with FIR by a FIR heater. A schematic diagram of the seven kinds of green tea manufacturing processes used in this study is shown in Figure 1.

Control. The green tea leaves were processed without application of FIR. The leaves were roasted to inactivate endogenous enzymes by pan firing (200–300 °C for 3–4 min), and then, the leaves were rolled by hand for 5 min. Finally, the leaves were dried by heating (150–200 °C for 10–15 min) to a moisture content of 3–5%.

Green Tea Processing 1 (GTP1). The green tea leaves processed according to control were irradiated with FIR at 90 °C for 10 min.

Green Tea Processing 2 (GTP2). Tea leaves were FIR irradiated instead of the roasting step of processing at the same temperature and time as the control.

Green Tea Processing 3 (GTP3). Tea leaves were FIR irradiated instead of the roasting step of processing and additionally at the end of the processing at 90 °C for 10 min.

Green Tea Processing 4 (GTP4). Tea leaves were FIR irradiated instead of the drying step of processing at the same temperature and time as the control.

Green Tea Processing 5 (GTP5). Tea leaves were FIR irradiated instead of the roasting and drying step of processing at the same temperature and time as the control.

Green Tea Processing 6 (GTP6). Tea leaves were FIR irradiated instead of the roasting and drying step of processing at the same temperature and time as control and additionally at the end of the processing at 90 °C for 10 min.

FIR Irradiation. The green tea leaves were irradiated by a FIR heater under controlled temperatures. Each batch of green tea leaves (2.0 g) was placed as a single layer in a Pyrex Petri dish (8.0 cm diameter) and irradiated by a FIR heater (350 mm \times 10 mm, output 300 W, Hakko Electric Machine Works Co., Ltd., Nagano, Japan), which emitted radiation at the wavelength from 2 to 14 μ m in a FIR dryer (A-Sung Machinery, Pusan, Korea). Samples were turned 360° continuously during the irradiation process to achieve uniform irradiation, and the distance between the FIR heater and the green tea leaves was 14 ± 1 cm.

Green Tea Samples. The processed green tea leaves (1.0 g) were extracted for 10 min with 100 mL of distilled water (boiling water) at room temperature. Then, the extracts (green tea) were filtered with a Whatman no. 1 filter paper. The green tea was immediately used for the following experiments.

Total Phenolic Contents (TPCs). The TPCs of the green tea were determined according to the method of Gutfinger (15). Each green tea (1 mL) was mixed with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na₂CO₃ and centrifuged at 13400g for 5 min after 30 min of incubation at room temperature. The absorbance was measured with a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 750 nm. TPCs were expressed as GA equivalents.

Total Flavanol Contents (TFCs). The TFC of the green tea was determined by vanillin method using C as a standard (16). Each green tea (1 mL) was mixed with 5.0 mL of 2.0% vanillin (8.0% methanolic HCl). The absorbance was measured with a spectrophotometer (Shimadzu UV-1601) at 500 nm after 20 min of incubation in the dark at room temperature. TFCs were expressed as (+)-C equivalents.

Ascorbic Acid Contents (AACs). AACs were determined according to the method of Sikić et al. (17). The green tea (1 mL) was centrifuged at 10000g for 10 min, and the supernatants (0.5 mL) were mixed with 2 mL of trichloroacetic acid (5%); then, the mixtures were centrifuged at 15000g for 10 min (4 °C). The supernatants (1 mL) were then mixed with 0.1 mL of 85% orthophosphoric acid, 0.1 mL of 8% α,α -dipyridyl, and 0.1 mL of 3% aqueous ferric chloride. The absorbance was measured with a spectrophotometer (Shimadzu UV-1601) at 525 nm after 1 h of incubation at room temperature. AACs were expressed as L-ascorbic acid equivalents.

Color Analyses. Color analyses on green tea were carried out using a colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-200 measuring head. The instrument was standardized against a white tile before each measurement. Color was expressed in *L*, *a*, and *b* Hunter scale parameters (18).

HPLC Analyses for Cs and Caffeine. The levels of Cs and caffeine in the green tea were measured by HPLC (19). The HPLC system consisted of Shimadzu LC-6AD pumps (Shimadzu Co. Ltd.) with a two-pump gradient system, a Shimadzu SPD-10AVP UV-vis detector, a Shimadzu SIL-10ADVP auto sample injector, and a Shimadzu CTO 10AVP column oven. The column was a Shim-pack VP ODS column (5 μ m, 250 mm \times 4.6 mm, Shimadzu Co. Ltd.) equipped with a Shim-pack CLC guard column (10 mm \times 4 mm, Shimadzu Co. Ltd). Mobile phases consisted of 0.1% orthophosphoric acid in water (v/v) (eluent A) and 0.1% orthophosphoric acid in methanol (v/v) (eluent B). The gradient was as follows: 0–5 min, 40% B; 5–12 min, linear gradient from 40 to 50% B; 12–27 min, 50% B; 27–30 min, linear gradient from 50 to 20% B; 30–35 min, linear gradient from 20 to 0% B. The post-run time was 5 min. Elution was performed at a solvent flow rate of 1 mL/min. Detection was accomplished with a UV-vis detector, and chromatograms were recorded at 210 nm. The column was maintained at 40 °C. The sample injection volume was 10 μ L. Peaks were identified by comparing their retention times with authentic standards. The concentration range of authentic Cs and caffeine for standard curve was 1.00–0.01 mg/mL.

Measurement of Nitrite Scavenging Ability (NSA). The NSA of green tea was determined according to a method using Griess reagent (20). First, 1 mL of each green tea was mixed with 1 mL of 1 mM nitrite sodium. Then, the mixture was added to 8 mL of 0.2 M citrate

Table 1. Effect of FIR Irradiation in the Manufacturing Process on TPC, TFC, and AAC of Green Tea^a

	control	GTP1	GTP2	GTP3	GTP4	GTP5	GTP6	SEM b
TPC	460.8 e	506.8 c	538.7 b	811.1 a	416.5 f	475.6 d	485.8 d	0.04
TFC	175.7 c	184.4 b	165.5 d	208.7 a	130.7 f	150.9 e	165.8 d	1.43
AAC	13.4 b	13.7 b	13.2 bc	14.7 a	12.4 c	11.9 d	11.8 d	0.25

^a Green tea leaves were irradiated by a FIR heater during processing as shown in Figure 1, and green tea was made by soaking the leaves in boiling water (1.0 g/100 mL). SEM, standard error of the means. All values are on a dry green leaf basis (mg/g). Different letters (a–f) within a row are significantly different ($P < 0.05$); $n = 3$.

buffer (pH 3.0, 4.2, or 6.0). After the mixtures were incubated for 1 h at 37 °C, 1 mL was withdrawn and added to 2 mL of 2% acetic acid and 0.4 mL of Griess reagent (1% sulfanilic acid and 1% naphthylamine in a methanol solution containing 30% acetic acid). After vigorous mixing with a vortex, the mixture was placed at room temperature for 15 min, and absorbance was measured at 520 nm. The NSA (%) was calculated by the following equation.

$$\text{NSA (\%)} = [1 - (S/B)] \times 100$$

where S is the absorbance of treated sample solution and B is the absorbance of water instead of sample.

Statistical Analysis. The experiment was performed in triplicate, and all measurements were analyzed in three repetitions. Analyses of variance were conducted by the General Linear Model procedure using SAS software (21). Student–Newman–Keul's multiple range tests were used to test for significant differences between the mean values for the treatments ($P < 0.05$).

RESULTS AND DISCUSSION

TPC, TFC, and AAC. Green tea contains polyphenols, which include flavanols, flavandiols, flavonoids, and phenolic acids. The polyphenols are the most biologically active group of the tea components and are connected with tea's antioxidative and anticarcinogenic properties (22–25). Phenolic compounds are key elements that determine both the color and the taste of green tea (26). As shown in Table 1, FIR irradiation during the processing of green tea significantly affects the TPC of green tea. For example, the TPC of GTP3, where the roasting step of processing was substituted with FIR irradiation at 90 °C for 10 min and the processed green tea leaves were additionally irradiated under the same conditions, increased by 76.0% (811.1 mg/g) as compared to nonirradiated control (460.8 mg/g). Almost all types of FIR irradiation during the processing of green tea leaves increased the TPC as compared to control; however, the TPC of GTP4 decreased. The treatments containing FIR irradiation during the drying step of green tea process, GTP4, GTP5, and GTP6, had relatively lower contents of TPC as compared with the treatments not irradiated during the drying step, control, GTP2, and GTP3.

The most abundant polyphenols in green tea are the flavanols, which are commonly known as Cs. Green tea also contains ascorbic acid. The TFC and AAC of the processed green tea showed the same pattern as was seen for TPC (Table 1). The highest TFC and AAC values were 208.7 and 14.7 mg/g as compared to 175.7 and 13.4 mg/g, respectively, for the control. However, the TFC and AAC of GTP4 also showed lower values than those of the control.

FIR irradiation of rice hulls, peanut hulls, and defatted sesame meals increased the phenolic content and antioxidant activity of their extracts (10–12). Lee et al. also found that FIR irradiation of the processed green tea leaves at 90 °C for 10 min significantly increased the TPC and TFC of green tea (27).

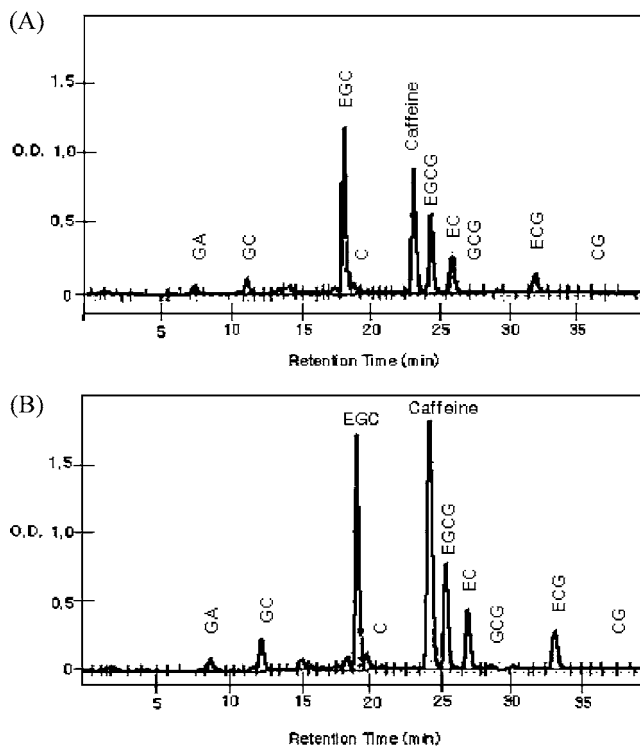


Figure 2. Typical HPLC chromatography of (A) nonirradiated control green tea and (B) GTP3 green tea.

The results of this study showed that a combination of FIR irradiation at the roasting step and of the processed green tea leaves resulted in the highest levels of TPC, TFC, and AAC. FIR irradiation of the drying step, however, was detrimental, in terms of the contents of TPC, TFC, and AAC. These results can be attributed to the following possibilities. The FIR irradiation in the step of roasting might denature enzymes like polyphenol oxidases more effectively than general heating, resulting in the prevention of oxidation or polymerization of polyphenol compounds. Second, the specific conditions of FIR irradiation during the drying step might be detrimental to the stability of polyphenol compounds. It is difficult to make a conclusion on these questions, and more detailed studies will be needed on these mechanisms.

Cs and Caffeine Content. Green tea is an excellent source of polyphenol antioxidants, particularly C. Green tea Cs possess potent antioxidant activities, and preventive effects against various oxidative diseases have been reported (28, 29). Eight types of Cs, caffeine, and GA of the FIR-irradiated green tea were analyzed by HPLC as shown in Figure 2. As shown in Table 2, EGC and EGCG are the main flavanols of green tea Cs in the control. Because gallyl and galloyl moieties of GCs possess three hydroxyl groups and easily form radicals during oxidation, they show the high hydrogen-donating ability of antioxidants and, for this reason, EGC and EGCG have been reported to be the most important flavanols in green tea (2). FIR irradiation during processing of green tea significantly affects the amount of EGC and EGCG of green tea. In particular, EGC and EGCG in GTP3 showed the highest values among the processed green teas. EGC and EGCG increased from 57.68 and 9.60 mg/g in nonirradiated control to 89.88 and 16.33 mg/g in GTP3, respectively. The other epicatechins (EC and ECG) also showed the highest values in GTP3.

The monomeric flavanols undergo oxidative polymerization, which leads to the formation of bisflavanols, theaflavins, thearubigins, and other oligomers. Gulati et al. (30) found that

Table 2. Effect of FIR Irradiation in the Manufacturing Process on Cs and Caffeine in Green Tea^a

	control	GTP1	GTP2	GTP3	GTP4	GTP5	GTP6	SEM
ECs								
EC	4.68 c	4.86 c	5.65 b	6.52 a	3.78 d	5.01 c	5.07 c	0.11
ECG	4.22 c	4.83 c	6.92 a	7.15 a	4.49 c	4.46 c	6.30 b	0.16
EGC	57.68 d	72.01 b	75.14 b	89.88 a	54.14 d	66.21 c	64.58 c	1.18
EGCG	9.60 d	11.82 c	14.64 ab	16.33 a	10.47 cd	13.43 b	15.03 ab	0.53
total	76.18	93.52	102.35	119.88	72.88	89.11	90.98	1.98
EC epimers								
C	0.96 d	1.00 c	0.77 e	1.27 a	0.69 f	0.89 d	1.18 b	0.02
CG	0.05 d	0.04 d	0.03 d	0.24 b	0.04 d	0.15 c	0.28 a	0.01
GC	4.87 c	5.88 b	4.81 c	7.93 a	3.34 d	4.96 c	7.57 a	0.21
GCG	0.44 e	0.87 d	0.85 d	1.70 b	0.48 e	1.04 c	1.86 a	0.03
total	6.32	7.79	6.46	11.14	4.55	7.04	10.89	0.27
caffeine	70.72 c	75.19 c	95.87 a	99.21 a	63.62 d	65.13 d	88.87 b	1.57
GA	1.39 ac	1.51 c	2.01 a	1.67 b	1.18 d	1.42 c	2.11 a	0.05

^a Green tea leaves were irradiated by a FIR heater during processing as shown in Figure 1, and green tea was made by soaking the leaves in boiling water (1.0 g/100 mL). SEM, standard error of the means. All values are on a dry green leaf basis (mg/g). Different letters within a row are significantly different ($P < 0.05$); $n = 3$.

total phenols and Cs of green tea were increased by microwave treatment during manufacture and suggested that the application of microwave energy prevented the binding of polyphenol and C to the leaf matrix, which could increase Cs in green tea. Although it is not certain that FIR acts in a similar way to microwave irradiation, FIR could be a method to increase the C content of green tea.

EC epimers (C, CG, GC, and GCG) are not originally present in green tea leaves but are produced by a thermal-induced epimerization reaction of ECs (EC, ECG, EGC, and EGCG) (31). When canned and bottled tea drinks are heat-treated for pasteurization at 120 °C for several minutes, considerable amounts (around 50%) of Cs are epimerized at the 2-position, and C, GC, CG, and GCG are formed (32, 33). On the other hand, both C and EC contents were increased significantly by FIR irradiation during the processing of green tea in this study. For example, the total amount of ECs in green tea increased from 76.18 to 119.98 mg/g in GTP3 as compared to the nonirradiated control, and the total amount of EC epimers also increased from 6.32 to 11.14 mg/g under the same processing. These results indicated that FIR of green tea leaves induces epimerization of Cs as well as increasing the C content of green tea. However, it is difficult to conclude why GTP3 (FIR irradiation at the roasting step of processing and additional irradiation of the processed green tea leaves) showed the highest green tea C levels among the processing methods studied.

The content of caffeine, a plant alkaloid having stimulatory effects, was also significantly affected by FIR irradiation during the manufacturing process. In green tea of GTP3, the caffeine content increased to 99.21 from 70.72 mg/g in control.

NSA. Nitrite ions in the acidic environment of the stomach induce mutagenic and cell-damaging reactions (34). Exposure to excess nitrite from the diet is implicated as a potential etiological factor in the development of stomach and colorectal cancers. EGCG in green tea has been known to act as a most efficient inhibitor of N-nitrosation. We determined the effect of the manufacturing process with FIR on the NSA of green tea. As shown in Table 3, NSA increased during the manufacturing process of green tea with FIR, at various pH levels, but with greater NSA at lower pH levels. At pH 3.0, the highest levels of the NSA were 98.8 and 98.6% in GTP1 and GTP3, as compared with 92.8% in control. At pH 4.2, the highest levels of the NSA were 20.7 and 21.0% in GTP1 and GTP3, as compared with 15.2% in the control. At pH 6.0, the highest levels of the NSA were similar in GTP1 and GTP3 (3.6 and 4.2%, respectively) as compared with 1.6% in control. Naka-

Table 3. Effect of FIR Irradiation in the Manufacturing Process on Nitrite Scavenging Activity (%) of Green Tea under Different pH Conditions^a

	control	GTP1	GTP2	GTP3	GTP4	GTP5	GTP6	SEM
pH 3.0	92.8 c	98.8 a	97.4 b	98.6 a	85.7 f	92.1 d	89.8 e	0.1
pH 4.2	15.2 c	20.7 a	19.3 a	21.0 a	13.9 c	14.1 c	17.4 b	0.5
pH 6.0	1.6 c	3.6 b	3.6 b	4.2 a	0.7 d	3.2 b	3.6 b	0.1

^a Green tea leaves were irradiated by a FIR heater during processing as shown in Figure 1, and green tea was made by soaking the leaves in boiling water (1.0 g/100 mL). Different letters (a–f) within a row are significantly different ($P < 0.05$); $n = 3$. SEM, standard error of the means.

Table 4. Effect of FIR Irradiation in the Manufacturing Process on Color L Value, a Value, and b Value of Green Tea^a

	L value	a value	b value	DE
control	97.74 b	-1.08 b	2.60 e	0.00 g
GTP1	97.74 b	-1.01 a	2.60 e	0.07 f
GTP2	97.67 c	-1.27 c	3.12 c	0.56 c
GTP3	97.57 d	-1.46 d	3.82 a	1.29 a
GTP4	97.80 a	-1.03 a	2.46 f	0.16 e
GTP5	97.75 b	-1.27 c	3.04 d	0.48 d
GTP6	97.59 d	-1.28 c	3.53 b	0.96 b
SEM	0.01	0.01	0.01	0.01

^a Green tea leaves were irradiated by FIR at given temperatures for 10 min, and green tea was made by soaking in boiling water (1.0 g/100 mL). Different letters (a–i) within a column are significantly different ($P < 0.05$); $n = 3$. L, degree of lightness; a, degree of redness; b, degree of yellowness; ΔE , overall color difference: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$; and SEM, standard error of the means.

gawa and Yokozawa (35) observed that the galloyl group enhanced the nitric oxide scavenging activity of tannin, whereas caffeine did not affect nitric oxide production. The amount of Cs containing galloyl groups, EGCG, GCG, ECG, and CG, in this study was highest in GTP3 (Table 2); these results closely coincide with the results of Table 3.

Color Analysis. The Hunter color values of green tea extracts after the various manufacturing processes with FIR are shown in Table 4. The Hunter color L value was changed slightly by the manufacturing process. The L values of green tea were in the range of 97.57–97.80 and showed the highest value in GTP4. The redness (a value) and yellowness (b value) were also slightly changed in the range of -1.46 to -1.01 and 2.60 to 3.82, respectively. The maximum overall color change (ΔE)

of green tea was shown in GTP3 with the value of 1.29. Such a change is difficult to distinguish with the naked eye.

In conclusion, FIR irradiation at 90 °C for 10 min, replacing the roasting step of the manufacturing process of green tea leaves, and on the fully processed green tea leaves could significantly increase C contents and nitrite scavenging activity of green tea, with negligible effects on color. These results suggest that FIR irradiation could be a useful method for increasing the health-promoting properties of green tea.

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