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Effect of Far-Infrared Irradiation on Catechins and Nitrite Scavenging Activity of Green Tea

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The processed green tea leaves were irradiated by far-infrared (FIR) at eight temperatures (80, 90, 100, 110, 120, 130, 140, and 150 °C) for 10 min. After FIR irradiation, green teas were prepared by soaking the leaves in boiling water, and the physicochemical characteristics of the green tea were determined. FIR irradiation at 90 °C increased total phenol contents of green tea from 244.7 to 368.5 mg/g and total flavanol contents from 122.0 to 178.7 mg/g, compared with non-irradiated control. FIR irradiation also significantly affected the amounts of epigallocatechin and epigallocatechin gallate. Nitrite scavenging activity also increased with increasing FIR irradiation until the temperature reached 110 °C. However, the overall color changes of green tea irradiated with FIR at 90 and 100 °C were negligible. These results indicate that the chemical quality of green tea is significantly affected by FIR irradiation temperature of the green tea leaves.

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

INTRODUCTION

Green tea, a water extract of the nonfermented leaf of *Camellia sinensis* L., is a popular drink in East Asian countries and is becoming progressively more popular worldwide. Green tea contains catechins, which are low molecular weight polyphenols belonging to the flavan-3-ol class of flavonoids. The catechins comprise a family of four major substances—epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG)—and four minor catechins—catechin (C), catechin gallate (CG), gallocatechin (GC), and gallocatechin gallate (GCG)—as epimers of the major catechins. The catechins have a role in protection against cancer, cardiovascular disease, and other degenerative diseases (*1*) and contribute to the characteristic bitter and astringent taste of tea, along with the brothy and sweet taste, which is due to amino acids such as theanine, glutamic acid, and arginine (*2*, *3*).

The variety, growing environment, manufacturing conditions, and particle size of tea leaves influence the composition of the tea leaf and the final infusion (*4*). Moreover, soaking temperature and soaking time during the preparation of green tea influence the content of polyphenols and anticlastogenicity of green tea (*5*, *6*). Heat processing and storage also affect the flavanol composition and sensory quality of green tea. Many plant polyphenols, such as flavonoids, tannins, coumadins, curcuminoids, xanthons, phenolics, and terpenoids, exist either as forms bound to high molecular weight compounds or parts of repeating subunits of high molecular weight polymers (*7*). Several methods, including far-infrared (FIR) irradiation, are known to release and activate low molecular weight natural antioxidants (*8*−*10*). FIR rays are defined as electromagnetic waves having wavelengths longer than 4 μm but shorter than those of microwaves (>0.1 cm). FIR rays are biologically active (*11*) and transfer heat to the center of materials evenly without degrading the constituent molecules of the surface. FIR may be capable of cleaving covalent bonds and releasing antioxidants, such as flavonoids, carotene, tannin, ascorbate, flavoprotein, and polyphenols, from repeating polymers (*8*, *12*). The objective of this work is to determine the effect of FIR irradiation temperature of green tea leaf product on the physicochemical properties of green tea.

MATERIALS AND METHODS

Materials. Eight catechin standards, (−)-gallocatechin (GC), (+)-catechin (C), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epicatechin gallate (ECG), (−)-catechin gallate (CG), and caffeine were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol (HPLC grade), 85% orthophosphoric acid (analytical grade), vanillin, and gallic acid 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, U.K.) with a resistivity of >17.5 MΩ cm.

Processing of Green Tea Leaves and FIR Irradiation. Fresh tea leaves (*Camellia sinensis*) were harvested in August 2003 at Bosung, Korea. In this study, the leaves were roasted to inactivate enzymes by pan firing (230−280 °C for 5−10 min), and then the leaves were rolled by hand. Finally, the leaves were dried to a moisture content of 3−5%. The processed green tea leaves were irradiated by FIR under controlled temperatures. Each batch of processed 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 (35 × 10 cm, output 300 W, Hakko Electric Machine Works Co., Ltd., Nagano, Japan), which emitted radiation at

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Table 1. Effect of Far-Infrared Irradiation Temperature on Total Phenol Contents, Total Flavanol Contents, and Ascorbic Acid Contents of Green Tea[a]

	FIR temperature (°C)								
	control	80	90	100	110	120	130	140	150	SEM
TPC	244.7c	274.2b	368.5a	270.9b	236.3c	185.0e	217.7d	174.7f	122.4g	0.1
TFC	121.4d	140.4b	178.7a	133.1c	113.2e	82.5g	91.1f	45.7h	29.0i	1.1

[a] Green tea leaves were irradiated by FIR at given temperatures for 10 min, and green tea was made by soaking the leaves in boiling water (1.0 g/100 mL). TPC, total phenol contents; TFC, total flavanol contents. SEM, standard error of the means. All values are on a dry green leaf basis (mg/g). Different letters (a–i) within a row indicate significant difference ($P < 0.05$), $n = 3$.

the wavelength range from 2 to 14 μm in a FIR dryer (A-Sung Machinery, Kyungki-Do, Korea). FIR irradiation was carried out for 10 min at 80, 90, 100, 110, 120, 130, 140, or 150 °C. Samples were turned 360° continuously during the irradiation process to achieve uniform irradiation, and the distance between the FIR heater and green tea leaves was 14 ± 1 cm. After irradiation, the green tea leaves were allowed to cool to ambient temperature before they were used in the preparation of green tea.

Green Tea Samples. The green tea leaves (1.0 g), irradiated by FIR or not irradiated (controls), were extracted for 10 min with 100 mL of distilled water (boiling water) at room temperature. Then, the extracts (green tea) were filtered through a Whatman no. 1 filter paper. The green tea was immediately used for the following experiments.

Total Phenolic Contents (TPC). TPC of the green tea were determined according to the method of Gutfinger (*13*). Each green tea (1 mL) was mixed with 1 mL of 50% Folin−Ciocalteu reagent and 1 mL of 2% Na_2CO_3 and centrifuged at 13400*g* 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. TPC were expressed as gallic acid equivalents.

Total Flavanol Contents (TFC). TFC of the green tea were estimated according to the vanillin method using catechin as a standard (*14*). 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, Tokyo, Japan) at 500 nm after 20 min of incubation in the dark at room temperature. TFC were expressed as (+)-catechin equivalents.

Ascorbic Acid Contents. The ascorbic acid contents were determined using the method of Sikic et al. (*15*). The green tea (1 mL) was centrifuged at 10000*g* for 10 min, and the supernatants (0.5 mL) were mixed with 2 mL of trichloroacetic acid (5%); then the mixtures were centrifuged at 15000*g* 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. Ascorbic acid contents are 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 (*16*).

HPLC Analyses for Catechins and Caffeine. The levels of catechins and caffeine in the green tea were measured by HPLC (*17*). The HPLC system consisted of Shimadzu LC-6AD pumps (Shimadzu Co. Ltd., Kyoto, Japan) with a two-pump gradient system, a Shimadzu SPD-10AVP UV−vis detector, a Shimadzu SIL-10ADVP autosample injector, and a Shimadzu CTO 10AVP column oven. The column was a Shim-pack VP ODS column (5 μm, 250 × 4.6 mm, Shimadzu Co. Ltd.) equipped with a Shim-pack CLC guard column (10 × 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 solvent composition started at 80% solvent A and 20% solvent 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. Postrun 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 μL. Peaks were identified by comparing their retention times with authentic standards.

Measurement of Nitrite Scavenging Ability (NSA). The NSA of green tea was determined according to a method using Griess reagent (*18*). 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 buffer (pH 3.0, 4.2, or 6.0). After the mixtures had been 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 nitrite scavenging activity (percent) was calculated with the equation

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

where A is the absorbance of treated sample, C is the absorbance of green tea, and B is the absorbance of 1 mM $NaNO_2$.

Statistical Analyses. All measurements were performed in triplicate, and analyses of variance were conducted by the General Linear Model procedure using SAS software (*19*). 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 and TFC. Green tea contains several kinds of polyphenols, including flavanols, flavandiols, flavonoids, and phenolic acids. The polyphenols are the most biologically active group of the tea component. Flavanols are the main polyphenol compounds in green tea (*20*). Recently, flavanols have received much attention because of their pharmaceutical functions, such as antioxidative, antitumor, and anticarcinogenic activities (*21*−23). As shown in **Table 1**, FIR irradiation at 90 °C for 10 min increased TPC by >50% (final content = 368.5 mg/g) compared with non-irradiated control (244.7 mg/g), but there was a decreasing trend over 90 °C. FIR irradiation of green tea also exhibited the same pattern in TFC. The highest TFC was detected as 178.7 mg/g at 90 °C compared with that of 122 mg/g for the control, whereas there was a decreasing trend above 90 °C.

FIR irradiation of rice hulls at 100 °C and of peanut hulls at 150 °C also increased the content of phenolic compounds and antioxidant activity in their extracts (*8*, *9*). Niwa and Miyachi (*24*) found that FIR irradiation could increase the antioxidant activities of natural medicinal products. At high temperatures, however, our results coincided with the observations for heated coffee brews in that the phenolic compounds in crude coffee were progressively lost during roasting because of their destruction and/or transformation (*25*). FIR irradiation at 90 °C for 10 min could increase phenolic contents and flavanols in green tea.

Catechins and Caffeine Content. Eight types of catechins, caffeine, and gallic acid of the FIR-irradiated green tea were analyzed by HPLC (**Table 2**).

Table 2. Effect of Far-Infrared Irradiation Temperature on Catechins and Caffeine in Green Tea[a]

		FIR temperature (°C)								
	control	80	90	100	110	120	130	140	150	SEM
EC	6.05bc	6.67b	7.95a	6.51b	5.89bc	5.57bc	1.86d	0.36de	0.14e	0.50
ECG	6.92bcd	10.34b	9.71bc	9.71bc	15.98a	5.67cd	3.31de	0.52e	0.15e	1.08
EGC	77.55b	86.32a	89.49a	77.5b	72.09bc	70.28c	19.60d	3.28e	0.95f	1.37
EGCG	13.54c	16.19b	14.91c	14.84c	21.06a	17.90b	14.58c	14.3c	6.79d	1.20
C	1.22c	1.36c	1.68bc	1.59bc	2.34a	1.81 b	1.18d	0.23e	0.09f	0.96
CG	0.05f	0.64c	0.34d	0.34d	1.50a	0.28e	1.10b	0.21e	0.05 f	0.07
GC	4.88de	8.05cd	9.88c	10.42c	22.43a	16.72b	10.22c	1.70ef	0.31f	1.26
GCG	0.35e	3.04cde	5.31c	5.50c	20.34a	4.16cd	10.46b	1.04de	0.29e	0.88
caffeine	98.24b	116.54ab	119.11ab	120.92ab	149.52a	83.54b	39.76c	4.85d	1.49d	9.45

[a] Green tea leaves were irradiated by FIR at given temperatures for 10 min, and green tea was made by soaking the leaves in boiling water (1.0 g/100 mL). EC, epicatechin; ECG, epicatechingallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; C, catechin; CG, catechingallate; GC, gallocatechin; GCG, gallocatechingallate; SEM, standard error of the means. All values are on a dry green leaf basis (mg/g). Different letters (a–f) within a row indicate significant difference ($P < 0.05$), $n = 3$.

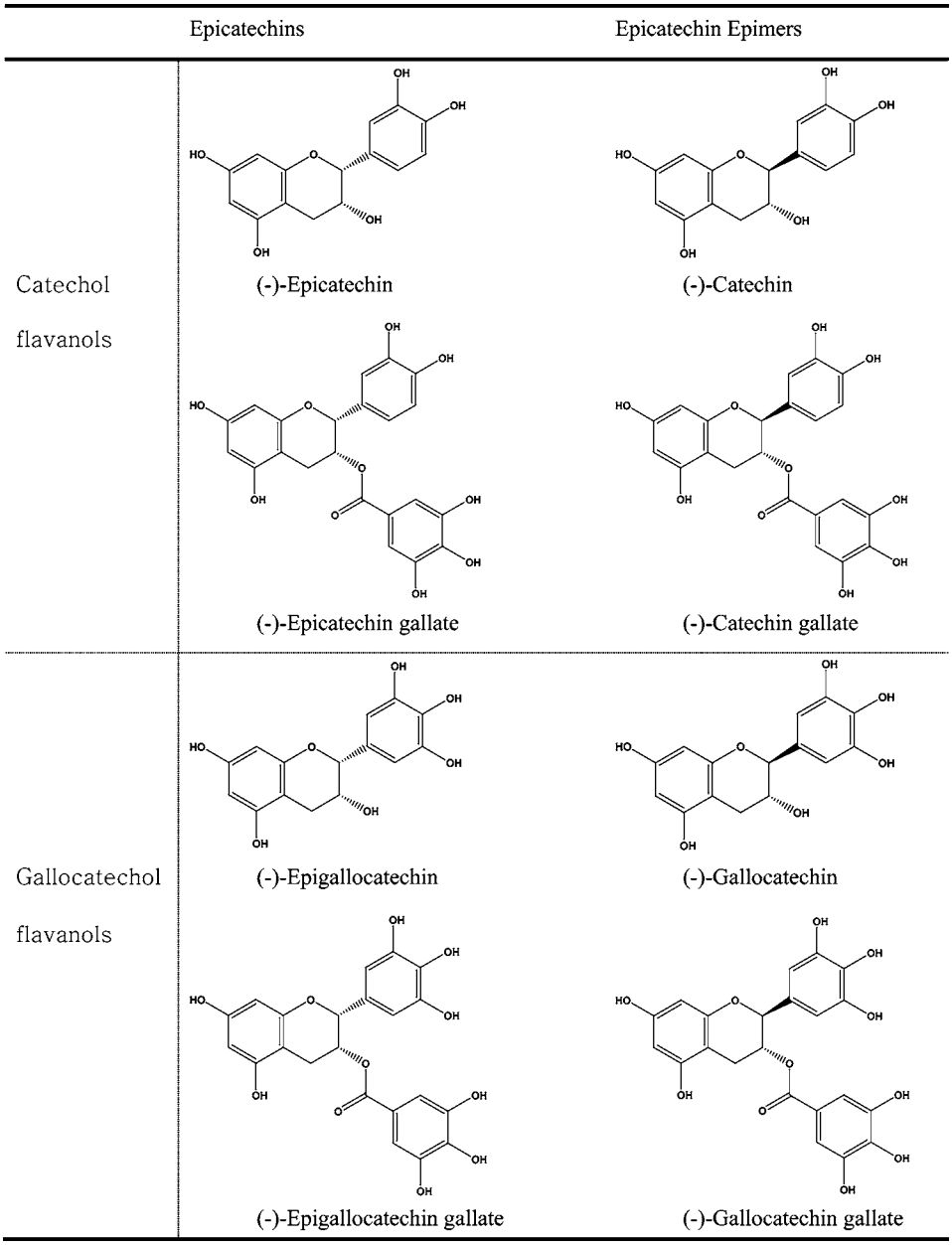

Figure 1. Structures of catechins of green tea.

Green tea flavanols can be also divided into catechol-flavanols and gallo-flavanols according to the number of hydroxyl groups attached to the B rings (**Figure 1**). Because gallyl and galloyl moieties of gallocatechins 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 (*1*). FIR irradiation significantly affected

Table 3. Color *L*, *a*, and *b* Values of Green Tea Extracts on Far-Infrared-Treated Temperature[a]

temperature (°C)	*L* value	*a* value	*b* value	Δ*E*	temperature (°C)	*L* value	*a* value	*b* value	Δ*E*
control	99.28d	–0.72d	2.34h	0.00i	120	98.49f	–0.69c	5.49d	3.25d
80	99.60a	–0.50c	1.59i	0.84f	130	98.00g	–1.00e	6.78b	4.63b
90	99.39c	–0.73d	2.41g	0.13h	140	97.35i	–0.18b	7.18a	5.24a
100	99.16e	–0.76d	2.64f	0.32g	150	97.53h	–0.03a	6.35c	4.43c
110	99.44b	–1.13f	3.51e	1.25e	SEM	0.00	0.02	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 the leaves in boiling water (1.0 g/100 mL). Different letters (a–i) within a column indicate significant difference ($P < 0.05$), $n = 3$. *L*, degree of lightness; *a*, degree of redness; *b*, degree of yellowness; Δ*E*, overall color difference, $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$; SEM, standard error of the means.

Table 4. Effects of Far-Infrared-Treated Temperature on Nitrite Scavenging Activity (Percent) of Green Tea Extracts, under Different pH Conditions[a]

		FIR temperature (°C)								
pH	control	80	90	100	110	120	130	140	150	SEM
3.0	79.2e	99.8d	102.4b	101.4c	106.2a	95.1f	90.0g	61.6h	37.3i	0.1
4.2	15.5f	21.3d	25.9b	24.1c	32.6a	17.4e	20.2d	19.8d	13.0g	0.6
6.0	1.9e	6.0d	7.1d	8.9d	14.8a	5.8d	13.1b	15.4a	11.1c	0.4

[a] Different letters (a–i) within a row indicate significant difference ($P < 0.05$), $n = 3$. SEM, standard error of the means.

the amount of EGC and EGCG. For example, FIR irradiation at 90 °C for 10 min increased the EGC content of green tea from 77.55 to 89.49 mg/g, and the EGCG content of green tea irradiated at 110 °C increased from 13.54 to 21.06 mg/g, compared with the non-irradiated control.

The monomeric flavanols undergo oxidative polymerization, which leads to the formation of bisflavanols, theaflavins, thearubigins, and other oligomers. Gulati et al. (*26*) found that total phenols and catechins of green tea were increased by microwave treatment during manufacture and suggested that the application of microwave energy prevented the binding of polyphenol and catechin to the leaf matrix, which could increase catechins 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 catechin content of green tea.

Epicatechin epimers (C, CG, GC, and GCG) were not originally present in green tea leaf but were produced by the thermally induced epimerization reaction of epicatechins (EC, ECG, EGC, and EGCG) (*27*). When canned and bottled tea drinks are heat-treated for pasteurization at 120 °C for several minutes, considerable amounts (~50%) of catechins are epimerized at the 2-position and C, GC, CG, and GCG are formed (*28*, *29*). On the other hand, both catechin and epicatechin contents were increased significantly by FIR treatment at some temperatures in this study. For example, the total amount of epicatechins of green tea increased from 104.06 to 122.06 mg/g after irradiation by FIR at 90 °C for 10 min, and the epicatechin epimer also increased from 6.50 to 17.21 mg/g under the same conditions. These results indicate that FIR irradiation of green tea leaves induces epimerization of catechins as well as increasing the catechin content of green tea.

Caffeine is a plant alkaloid present in some popular beverages such as tea, coffee, and cocoa and is known for its stimulatory effect. As shown in **Table 2**, temperature significantly affected the caffeine content of green tea following FIR irradiation. At 90−110 °C, FIR irradiation increased the caffeine content of green tea by >20%; however, the caffeine content rapidly decreased at temperatures above 120 °C. At high temperatures >120 °C, it is difficult to conclude whether caffeine was not liberated by FIR irradiation or the liberated caffeine was degraded for high temperature.

Color Analysis. The Hunter color value changes of FIR-irradiated green tea are shown in **Table 3**. The Hunter color *L* value was changed slightly by FIR irradiation and temperature. The *L* value showed the highest value in FIR treatment at 80 °C and decreased as the irradiation temperature increased. The redness (*a* value) was also slightly changed by FIR irradiation, and the lowest value was at 110 °C. Yellowness (*b* value) at 140 °C increased significantly from 2.34 to 7.18, compared with nontreated control. The overall color changes (Δ*E*) of green tea irradiated with FIR at 90 and 100 °C were below 0.5, which is difficult to detect with the naked eye. On the contrary, γ irradiation (5−20 kGy) onto the 70% ethanol extracts of green tea leaves significantly increased *L* value and decreased *a* and *b* values (*30*) without noticeable change of physiological activities (*31*, *32*). These indicated that the characteristic changes of green tea induced by irradiation were variable depending on irradiation rays.

NSA. Nitrite ions in the acidic environment of the stomach induce mutagenic and cell-damaging reactions (*33*). 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 (*34*). We determined the effect of FIR irradiation on the NSA of green tea. As shown in **Table 4**, NSA increased with increasing FIR irradiation at temperatures up to 110 °C and then decreased at higher temperatures at all pH levels studied but with greater NSA at lower pH levels. Nakagawa 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 contents of catechins containing galloyl groups, EGCG, GCG, ECG, and CG, in this study were highest in FIR-irradiated green tea at 110 °C (**Table 2**); these results closely coincide with the results presented in **Table 4**.

In conclusion, FIR irradiation of green tea leaves at some temperatures could increase catechin contents and nitrite scavenging activity of green tea, with negligible effects on color. These results support the idea that FIR irradiation could be a useful method for increasing the health-promoting properties of green tea.

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