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# Metabolomic Analysis of the Effect of Shade Treatment on the Nutritional and Sensory Qualities of Green Tea

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**ABSTRACT:** We analyzed metabolites from a 50% aqueous methanol extract of green teas treated with different shade periods (0, 15, 18, and 20 days) to investigate the effect of low light on their nutritional and sensory qualities. The shaded groups could be clearly distinguished from the control (0 day), and the 20 day group was separated from the 15 and 18 day groups. The shade treatment increased quercetin-galactosylrutinoside, kaempferol-glucosylrutinoside, epicatechin gallate, epigallocatechin gallate, tryptophan, phenylalanine, theanine, glutamine, glutamate, and caffeine levels but decreased quercetin-glucosylrutinoside, kaempferol-glucoside, gallocatechin, and epigallocatechin levels. Further studies on the nutritional benefits of these metabolites are needed. However, this result, along with the sensory evaluation and color measurement data, suggests that shade treatment improves the nutritional and sensory quality of green tea. Thus, we proposed a metabolomic pathway related to the effect of low light, which could elucidate the relationship between low light and tea quality.

**KEYWORDS:** *Green tea, low light, metabolomics, shade, sensory, UPLC−Q-TOF*

## INTRODUCTION

Tea obtained from the plant *Camellia sinensis* by the process of steaming, rolling, and/or fermentation is the most widely consumed beverage in the world after water and is known for its various health benefits.[1,2] However, there exists some disagreement about its benefits, owing to the harmful effects of overconsumption, such as high caffeine content, aluminum content, and inhibition of non-heme iron absorption.[2] Despite these arguments, numerous animal, clinical, and epidemiological studies suggest that tea may be pertinent to the promotion of health and prevention or treatment of chronic diseases, including cardiovascular diseases and cancer.[1,2] These possible health benefits are mainly associated with secondary metabolites in tea. However, the accumulation/buildup of these metabolites is inversely related to the taste quality of tea.[3,4] Therefore, various methods involving a change in environmental conditions during growth have been developed to regulate the production of secondary metabolites in tea plants.[5,6] Of these methods, the control of light transmission by shade treatment is the most effective method for tea plants.[7]

Light is one of the key environmental factors for the regulation of growth and development of plants. Moreover, many studies have clearly reported that the stimulation of secondary metabolites, including anthocyanin, catechins, and flavanols, in various plants is proportional to light radiation.[8−10] Although control/manipulation of light is important for regulating the nutritional and sensory quality of tea as well as plant growth and development, only a few studies have been performed on the correlation between shade variation and tea secondary metabolites.[11−14] In these studies, conventional methods, such as liquid chromatography−mass spectrometry (LC−MS) and gas chromatography−mass spectrometry (GC−MS), were used to analyze the target compounds for investigating the effect of shade treatment on the quality of tea, but the target analysis was irrelevant for a comprehensive study.

Although no single instrument can analyze all metabolites, the recent development of the metabolomic technology for a systematic and comprehensive study of small-molecule metabolites from biological samples of humans and animal and plant models[15] has enabled evaluation of the nutritional quality of tea during its cultivation and processing.[16,17] These metabolomic studies have provided a new approach for monitoring the change in nutritional quality during the processing and cultivation of tea plants.[13] The changes in the composition of secondary metabolites obtained from tea plants with different shade periods have not been studied using a LC−MS-based metabolomic technology, except for the metabolomic study of green tea with 80% shade treatment for 10 days using LC−MS and GC−MS.[13] However, a time-dependent study with different shade levels has not been performed to date.

Therefore, in this study, metabolomic profiling of green teas cultivated with different shade periods (0, 15, 18, and 20 days) was analyzed by ultraperformance liquid chromatography−quadrupole-time-of-flight mass spectrometry (UPLC−Q-TOF MS), and the resultant data were applied to multivariate statistical analysis to find metabolites for monitoring the nutritional status and the taste quality of tea according to the shade period. Additionally, sensory evaluation of the teas was

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**Table 1. Characteristics of the Tea with Different Shade Periods**

| | | shade period (days) | | | |
|---|---|---|---|---|---|
| | | 0 | 15 | 18 | 20 |
| TPC (GAE mg/g)[a] | | 140.85 ± 7.76 a | 135.03 ± 5.51 a | 127.98 ± 2.50 b | 123.71 ± 4.30 b |
| lutein (mg/g)[a] | | 0.50 ± 0.01 b | 0.58 ± 0.60 a | 0.58 ± 0.06 a | 0.56 ± 0.07 a |
| chlorophyll *a* (mg/g)[a] | | 0.77 ± 0.07 c | 2.48 ± 0.40 a | 2.41 ± 0.25 ab | 2.15 ± 0.34 b |
| chlorophyll *b* (mg/g)[a] | | 0.88 ± 0.02 b | 1.56 ± 0.15 a | 1.56 ± 0.11 a | 1.45 ± 0.18 a |
| color value[a] | *L** | 56.78 ± 1.39 a | 55.82 ± 1.60 ab | 53.72 ± 2.62 ac | 54.88 ± 1.43 bc |
| | *a** | −12.41 ± 0.29 a | −16.72 ± 0.65 c | −16.24 ± 0.41 bc | −15.84 ± 0.62 b |
| | *b** | 33.72 ± 0.56 ns | 33.28 ± 1.44 | 32.62 ± 0.85 | 33.17 ± 1.21 |
| | chroma* | 35.94 ± 0.59 b | 37.24 ± 1.58 a | 36.44 ± 0.94 ab | 36.76 ± 1.35 ab |
| | hue° | 110.21 ± 0.36 c | 116.68 ± 0.18 a | 116.46 ± 0.16 a | 115.53 ± 0.24 b |

[a]The contents of total phenolic compounds, lutein, and chlorophyll, and color parameters were statistically analyzed by ANOVA with Duncan's multiple range test.

conducted, and the color and the content of color agents were measured.

## MATERIALS AND METHODS

**Chemicals.** Catechin, epicatechin (EC), epicatechin gallate (ECG), catechin gallate (CG), epigallocatechin (EGC), gallocatechin (GC), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), gallic acid, theanine, caffeine, theobromine, chlorophyll *a*, chlorophyll *b*, leucine-enkephalin, and Folin−Ciocalteu's phenol reagent were purchased from Sigma-Aldrich Co. (St. Louis, MO). All of the reagents used in the high-performance liquid chromatography (HPLC) and UPLC−Q-TOF MS analyses were of HPLC and LC−MS grade.

**Tea Plant Cultivation and Shade Treatment.** Tea plants (*C. sinensis* O. Kuntz, variety of Yabukita) were grown at Damian's tea garden located in Haenam (34°33′ N, 126°34′ E), Republic of Korea, under natural conditions (mean temperature, 13.0 °C; total sunshine, 1971.9 h/year; rainfall, 1498.9 mm/year; and mean annual relative humidity, 74%). Three areas in the plant cultivation field were randomly selected for the shade variation. The plants were covered by black polyethylene (5% light transmission, 70 × 70 m) for different times (15, 18, and 20 days, June 2−7, 2011). Plants without shade were used as the control group and were harvested on June 7, 2011. The leaves (buds, first and second) collected from the plants were steamed, dried, and pulverized.

**Sensory Evaluation.** The 20 panelists were trained to evaluate the taste and green color of the shaded tea samples using reference standards[18] for bitterness and astringency and using a commercial green tea (Matcha, Aiya Co., Ltd., Japan) for green color. The sensory characteristic of tea powder and its infusion was carried out by a group of trained panelists, after obtaining the preparation from a 5 min hot water extraction (100 mL, 80 °C) of 0.2 g of powdered green tea. Samples were served in a random order, which was controlled to ensure that panelists did not see all samples. Sensory evaluation was achieved by assigning scores between 1 and 9, with 1 being "weak or extremely disliked" and 9 being "strong or extremely liked", for color intensity and color acceptability of the tea powder and bitterness and astringent intensity of the tea. On the basis of the sensory evaluation of taste and color of the tea, the overall acceptability of the tea was evaluated by the same scoring scale.

**Determination of the Total Polyphenol and Chlorophyll Contents.** The total phenolic content in the tea powder (500 mg) was determined by the modified Folin−Ciocalteu assay carried out according to the method described by Singleton and Rossi[19] after an initial extraction by aqueous methanol (50%, 25 mL). The extract (0.1 mL) was diluted 10-fold with 50% aqueous methanol and mixed with 0.2 N Folin−Ciocalteu reagent (1 mL). The mixture was allowed to stand at room temperature for 3 min, after which 1 mL of saturated sodium carbonate was added. Upon incubation at room temperature for 60 min, the absorbance was recorded at 735 nm. The total polyphenol content was calculated by comparing to an external standard calibration curve of gallic acid and was expressed as gallic acid equivalents (GAE, mg of gallic acid) per gram of sample.

The chlorophyll content was determined by the method described by Caldwell et al.,[20] with a minor modification. The tea powder (10 mg) was mixed with 1 mL of cold 80% aqueous acetone. After vortexing and centrifugation, the supernatant was collected and the pellet was re-extracted for 2 additional times. The combined supernatant was applied to an XTerra RP $C_{18}$ column (3.5 $\mu$m, 4.6 × 150 mm, Waters, Milford, MA) connected to a HPLC system (Jasco Co., Tokyo, Japan) for chlorophyll analysis. The extract was injected into the column and eluted in a linear gradient of solvent A (75% aqueous methanol) and B (ethyl acetate) at a flow rate of 0.8 mL/min. The gradient initial condition was 40% solvent B with a linear gradient to 50% for 10 min, followed by a linear gradient to 90% at 25 min, and then maintained at 90% for 10 min. The eluted sample was detected at 430 nm. Authentic standards (chlorophyll *a* and chlorophyll *b*) were used for the quantitative and qualitative analysis of chlorophyll.

**Color Evaluation.** The color values of the tea powder were determined by measuring the parameters $L^*$, $a^*$, and $b^*$ with a chroma meter (CR-400, Konica Minolta, Osaka, Japan) calibrated with a white plate. The value of chroma ($C^*$), indicating color saturation, was calculated as $(a^{*2} + b^{*2})^{1/2}$, and the hue angle ($h°$) was calculated with the formula $\tan^{-1}(b^*/a^*)$.

**UPLC−Q-TOF MS Analysis of Tea Metabolites.** The tea metabolites were extracted by 50% aqueous methanol containing 4-acetoaminophenol as an external standard and were analyzed by an UPLC system (Waters, Milford, MA). The extracts were injected into an Acquity UPLC BEH $C_{18}$ column (2.1 × 50 mm, 1.7 $\mu$m, Waters) equilibrated with water containing 0.1% formic acid. The sample was eluted in a gradient with acetonitrile (ACN) containing 0.1% formic acid at a flow rate of 0.35 mL/min for 14 min. The eluted metabolites were analyzed by Q-TOF MS (Waters) with positive electrospray ionization (ESI). The voltages of the capillary and sampling cone were set at 3 kV and 30 V, respectively, and the temperatures of the source and desolvation and the desolvation flow were set to 110 °C, 300 °C, and 700 L/h, respectively. The TOF MS data were collected in the $m/z$ 100−1000 range with a scan time of 0.2 s and an interscan delay time of 0.02 s. Lock spray with leucine-enkephalin (556.2771 Da in positive ESI mode) was used to ensure accuracy and reproducibility for all analyses with a flow rate of 5 $\mu$L/min and frequency of 10 s. For quality control, the mixture of all samples was injected after every 10 samples. The tandem mass spectrometry (MS/MS) spectra of the metabolites were obtained by a collision energy ramp from 10 to 30 eV. All MS data, including retention time, $m/z$, and ion intensity, obtained by MassLynx software (Waters) were extracted with MarkerLynx software (Waters).

**Data Process of Metabolites Analyzed by UPLC−Q-TOF MS.** Data processing, including data collection, alignment, and normalization of tea metabolites analyzed by UPLC−Q-TOF MS, was performed by MarkerLynx (Waters). Metabolite peaks were obtained using a peak width at 5% height of 1 s, noise elimination of 6, and an intensity threshold of 40. The data were aligned with a 0.05 Da mass tolerance and a retention time window of 0.2 s. All MS spectra were normalized to an


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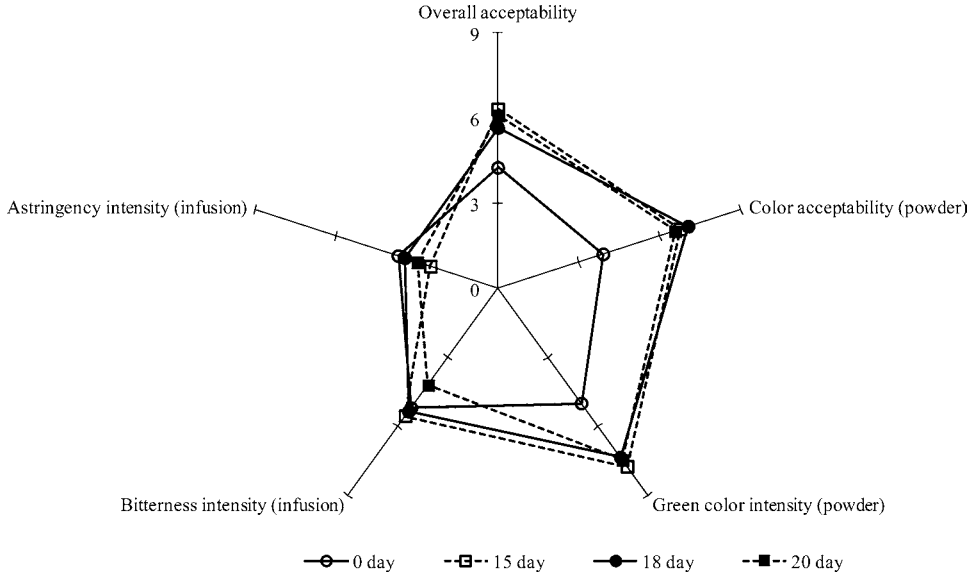


**Figure 1.** Sensory evaluation of green tea cultivated with different shade periods (0, 15, 18, and 20 days). The sensory evaluation of tea powder and its infusion was carried out by a team of 20 trained panelists and was assigned scores between 1 and 9, with 1 being weak or "extremely disliked" and 9 being strong or "extremely liked".

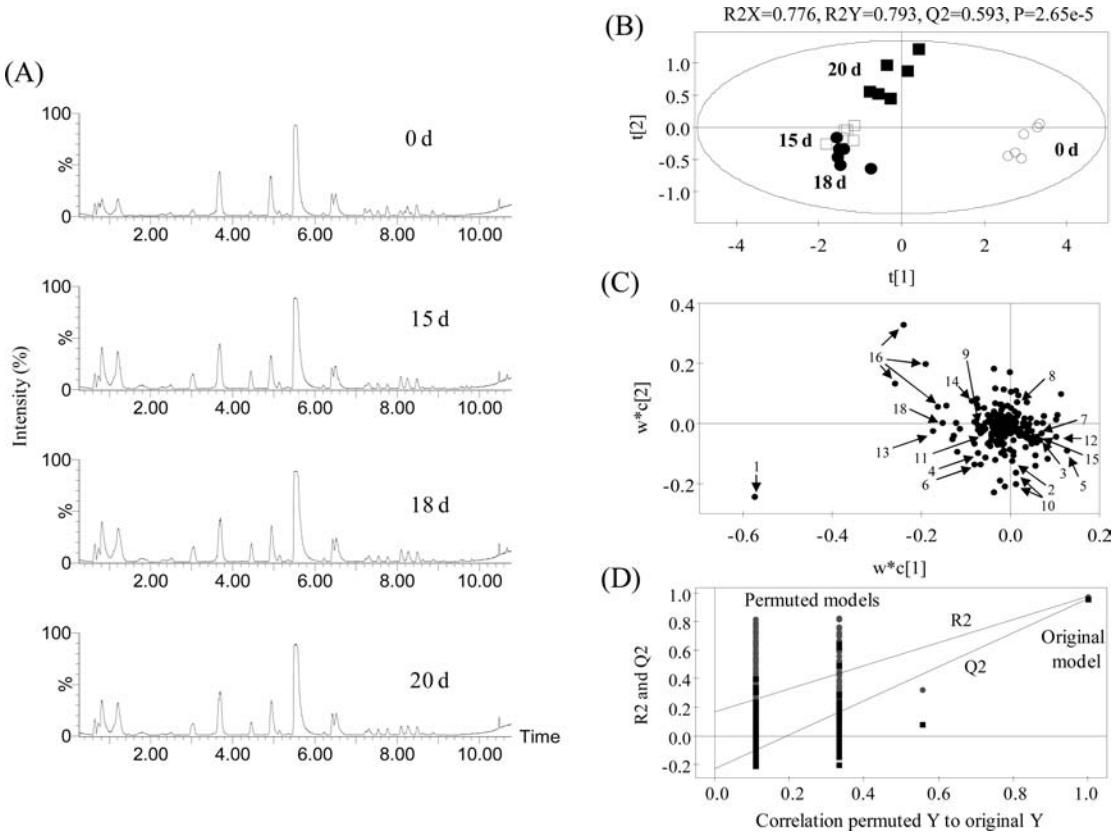


**Figure 2.** (A) UPLC−Q-TOF MS profiles of green tea cultivated with different shade periods (0, 15, 18, and 20 days), (B) partial least-squares discriminant analysis scores, (C) loading plots, and (D) permutation test. The tea metabolites were analyzed by a UPLC−BEH $C_{18}$ column (2.1 × 50 mm, 1.7 $\mu$m). The eluted metabolites were analyzed by Q-TOF MS with an ESI mode. Lock spray and quality control were used to ensure accuracy and reproducibility. Identified metabolites were marked with numbers on the loadings plot, and the metabolite list was the same as given in Table 1.

external standard. The metabolites were identified using ChemSpider (www.chemspider.com), Human metabolome databases (www.hmdb.ca), Metlin database (metlin.scripps.edu), literature references,[11,21] and authentic standards.

**Statistical Analysis.** The processed data sets were analyzed by carrying out a multivariate statistical analysis using SIMCA-P$^+$, version 12.0.1 (Umetrics, Umeå, Sweden). Partial least-squares discriminant analysis (PLS-DA) was used to visualize the dissimilarity/distinction among samples. The quality of PLS-DA models was evaluated by employing three parameters: $R^2X$, $R^2Y$, and $Q^2Y$. The goodness-of-fit measure was quantified by $R^2X$ and $R^2Y$, and the predictive ability was indicated by $Q^2Y$. PLS-DA models were validated by a 7-fold cross-

**Table 2. Identification of Tea Metabolites Analyzed Using UPLC−Q-TOF MS**

| number[a] | identity | R/T | exact mass (M + H) | actual mass (M + H) | mass error (mDa) | MS fragments [ESI+] | VIP[b] | *p* value[c] | reference |
|---|---|---|---|---|---|---|---|---|---|
| 1 | caffeine | 5.555 | 195.0882 | 195.0973 | 9.1 | 138, 110 | 9.71 | $3.72 \times 10^{-10}$ | standard |
| 2 | catechin | 5.371 | 291.0882 | 291.0984 | 10.2 | 207, 165, 139 | 0.43 | 0.6140 | standard |
| 3 | EC | 6.452 | 291.0869 | 291.0941 | 7.2 | 207, 165, 139 | 0.72 | 0.0922 | standard |
| 4 | ECG | 8.284 | 443.0978 | 443.1016 | 3.8 | 291, 273, 139, 123 | 1.18 | $7.57 \times 10^{-7}$ | standard |
| 5 | EGC | 4.964 | 307.0818 | 307.0860 | 4.2 | 139 | 2.33 | 0.0325 | standard |
| 6 | EGCG | 6.544 | 459.0927 | 459.0921 | −0.6 | 289, 139 | 2.27 | 0.0054 | standard |
| 7 | GC | 3.590 | 307.0818 | 307.0926 | 10.8 | 139 | 1.15 | $6.29 \times 10^{-8}$ | standard |
| 8 | glutamate | 0.766 | 148.0610 | 148.0686 | 7.6 | 130, 84 | 1.40 | $4.47 \times 10^{-12}$ | standard |
| 9 | glutamine | 0.749 | 147.0770 | 147.0846 | 7.6 | 130, 84 | 0.91 | $3.36 \times 10^{-11}$ | standard |
| 10 | kaempferol 3-*O*-glucoside | 8.507 | 449.1084 | 449.1134 | 5.0 | 287, 213, 153 | 2.26 | $9.95 \times 10^{-6}$ | 11 and 20 |
| 11 | kaempferol 3-*O*-glucosylrutinoside | 8.115 | 757.2186 | 757.2213 | 2.7 | 595, 449, 287 | 1.82 | $5.93 \times 10^{-5}$ | 20 |
| 12 | myricetin 3-*O*-glucoside/-galactoside | 7.265 | 481.0982 | 481.1034 | 5.2 | 319 | 1.70 | 0.0002 | 11 and 20 |
| 13 | phenylalanine | 3.042 | 166.0868 | 166.0956 | 8.8 | 120 | 2.81 | $3.79 \times 10^{-16}$ | standard |
| 14 | quercetin3-*O*-galactosylrutmoside | 7.564 | 773.2135 | 773.2165 | 3.0 | 611, 465, 303 | 0.95 | $1.40 \times 10^{-5}$ | 11 and 20 |
| 15 | quercetin 3-*O*-glucosylrutinoside | 7.784 | 773.2135 | 773.2160 | 2.5 | 611, 465, 303 | 1.29 | $9.35 \times 10^{-9}$ | 11 and 20 |
| 16 | theanine | 0.810/1.209 | 175.1083 | 175.1155 | 7.2 | 158, 130 | 4.11/2.70 | $4.26 \times 10^{-14}$ | standard |
| 17 | theobromine | 3.653 | 181.0726 | 181.0808 | 8.2 | 149, 118 | 2.66 | $2.08 \times 10^{-6}$ | standard |
| 18 | tryptophan | 4.455 | 205.0977 | 205.1083 | 10.6 | 188, 146, 118 | 2.49 | $1.97 \times 10^{-18}$ | standard |

[a]The number of metabolites marked in Figure 2. [b]VIP is variable importance in the project, and its value of above 1.00 shows high relevance for explaining the differences of sample groups. [c]*p* values were analyzed by ANOVA.

validation, and the reliabilities of the models were rigorously confirmed by a permutation test ($n$ = 200). For identification of metabolites contributing to the discrimination, the intensity differences of the metabolites with a variable importance in the projection (VIP) value of >0.5, showing a high relevance for explaining the differences among ample groups, were analyzed by analysis of variation (ANOVA) using SPSS 17.0 (SPSS, Inc., Chicago, IL). The loading plots displaying a combination of w*c(1) and w*c(2) from the PLS-DA model were used to better visualize the metabolites contributing to the discrimination, and identified metabolites with significant differences ($p < 0.05$) were visualized in a heat map drawn by R with ggplot2. All metabolomic analyses were duplicated.

In addition to metabolomic analyses, color parameters and the contents of total phenolic compounds and chlorophyll were analyzed by ANOVA with Duncan's multiple range test.

## RESULTS

**Characteristics of the Shaded Teas.** Characteristics of the powdered teas with different shade periods are shown in Table 1. The color analysis showed that the shade treatment decreased the $a^*$ value, accompanied by a slight decrease or no change in the $L^*$ and $b^*$ values and a slight increase in the chroma ($C^*$) and hue angle ($h°$) values. The content of chlorophyll *a* and chlorophyll *b* was elevated by the shade treatment, and their values for the 20 day group (2.15 ± 0.34 and 1.45 ± 0.18 mg/g, respectively) were 2.8 and 1.7 times higher those of the control group (0 day; 0.775 ± 0.07 and 0.88 ± 0.02 mg/g, respectively). However, no significant difference was observed among the other shaded groups, and the amount of lutein with an orange−red color was not changed by the shade treatment. The total polyphenol contents decreased with an increase in the duration of the shade period. The polyphenol contents in the 20 day group (123.7 ± 4.3 GAE mg/g) was 12% lower than that in the control group (140.9 ± 7.8 GAE mg/g), whereas the lutein level was not changed by the shade treatment. The sensory characteristic of shaded tea powder and its infusion (tea) was evaluated in terms of color and taste (Figure 1). The scores of color acceptability and green color intensity of the shaded tea powders were 1.8 and 1.5 times higher than those of the control, whereas the shade period did not affect the color characteristics. Intensities of astringency and bitterness of tea slightly declined or remained unchanged by the shade treatment. The lowest astringency and bitterness intensities were observed for the 15 and 20 day groups and the 20 day group, respectively. Overall, acceptability of the shaded tea was higher than that of the control, whereas no significant differences were noted among the shaded groups.

**Metabolomic Analysis of Green Tea Extracts.** The metabolite profiles of tea cultivated with different shade periods (0, 15, 18, and 20 days) analyzed by UPLC−Q-TOF were statistically analyzed by a PLS-DA (Figure 2). Shade-treated groups were clearly distinguished from the control (0 day) on the first two-component PLS-DA scores plot generated using Par scaling by the principal component $t(1)$ (68.87%). Among the shade-treated groups, the 20 day group was clearly separated from the 15 and 18 day groups by the principal component $t(2)$ (5.11%), whereas no discrimination was observed between the 15 and 18 day groups. We observed good quality parameters for the PLS-DA model validated by a 7-fold cross-validation ($p$ value = 0.001): fitness ($R^2X$ = 0.732 and $R^2Y$ = 0.814), predictability ($Q^2$ = 0.556), and permutation values ($R^2$ and $Q^2$ intercepts = 0.171 and −0.233, respectively), indicating that the models were not overfitted and regarded as a predictable model (panels B and D of Figure 2). The metabolites contributing to the discrimination were determined by statistically analyzing the intensities of all of the metabolites from all groups by ANOVA and calculating their VIP values using the Simca-P+ software. Moreover, PLS-DA loadings plot was generated for all groups, indicating that the metabolites far from the cross-intersection of w*c(1) and w*c(2) were the more relevant ions for explaining the discrimination (Figure 2C). The major metabolites were


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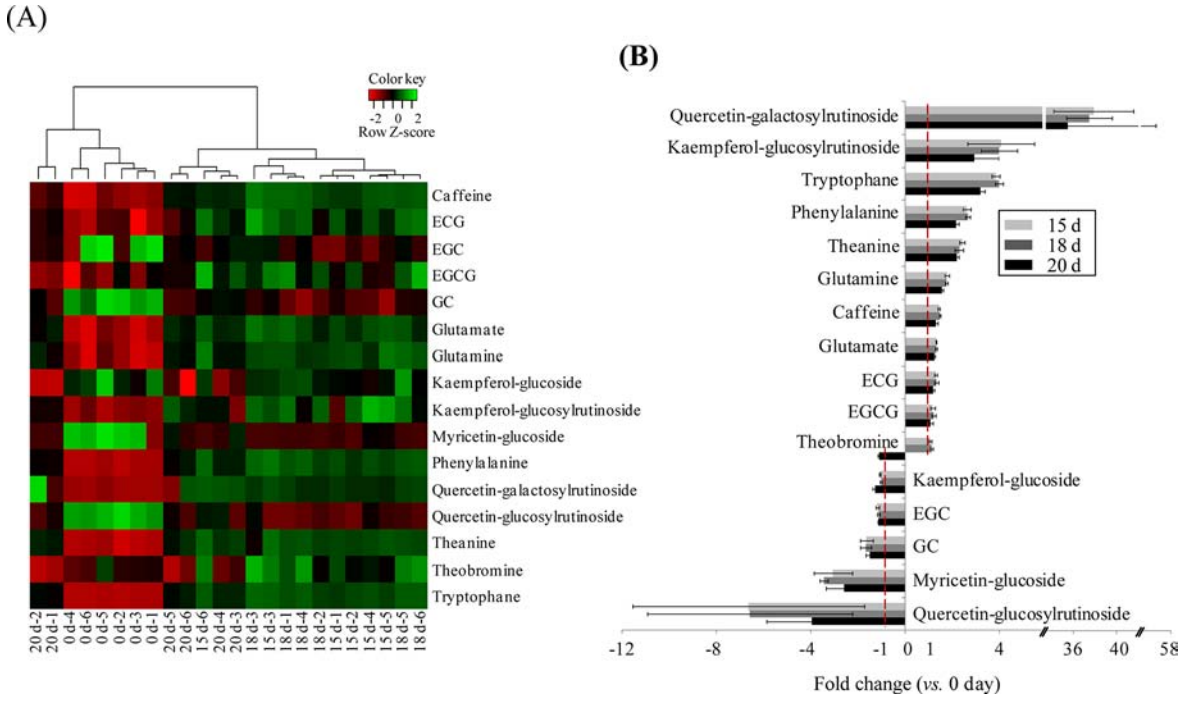


**Figure 3.** (A) Heat map of the identified metabolites and (B) their fold changes. The heat map was drawn by R with ggplot2. The quantitative fold changes of tea metabolites obtained from 15, 18, and 20 day groups were calculated against those obtained from 0 day.

identified on the basis of the *p* values and VIP values (Table 2) and marked with numbers on the plots (Figure 2C).

**Identification of Metabolites and Analysis of Their Fold Change.** Q-TOF MS detected 301 metabolites (including ion fragments), of which only 18 metabolites (caffeine, catechin, EC, ECG, EGC, EGCG, GC, glutamate, glutamine, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-glucosylrutinoside, myricetin 3-*O*-glucoside/galactoside, phenylalanine, quercetin 3-*O*-galactosylrutinoside, quercetin 3-*O*-glucosylrutinoside, theanine, theobromine, and tryptophan) were identified (Table 2). We found that all of these metabolites, except catechin and EC, were significantly affected by the shade treatment and their VIP values were more than 0.9, indicating that they were highly relevant to the differences among groups. A heat map prepared with released identified metabolites indicated that the control group (0 day) was clearly separated from the shaded groups, and among the shaded groups, the 20 day group was also separated from the 15 and 18 day groups (Figure 3A). The contents of 10 metabolites (quercetin 3-*O*-galactosylrutinoside, kaempferol 3-*O*-glucosylrutinoside, tryptophan, phenylalanine, theanine, glutamine, caffeine, glutamate, ECG, and EGCG) were significantly increased by shade treatment, but the levels of the other metabolites, except for theobromine, were decreased (Figure 3B). In particular, the levels of quercetin-galactosylrutinoside and kaempferol-glucosylrutinoside were elevated by the shade treatment, and their levels were about 37 and 4 times higher than those of the control, respectively. However, we observed no remarkable change among the shaded groups.

## DISCUSSION

In this study, we investigated the effect of shade treatment on the quality of tea in terms of tea characteristics, sensory evaluation, and metabolite analysis. Differences in color, colored compounds, and taste of the teas cultivated with different periods of shade treatment (0, 15, 18, and 20 days) were evaluated, and their metabolite profiles were analyzed by UPLC−Q-TOF MS.

Color, one of the most important factors for determining tea quality, is directly affected by the growth and environmental conditions of tea plants, such as rainfall, fertilizer, and light. Of various environmental conditions, light, which is very important as an energy source for the regulation of plant growth and development, is the most important factor for plant color. Light is absorbed by the chloroplasts to produce energy through photosynthesis. This system is physiologically regulated by the intensity of light radiation. Upon exposure to bright light, chloroplasts move to the side walls of the cell to regulate the amount of light absorbed, whereas in low light, they are positioned near the upper surface of the cells to absorb the maximum amount of light.[22] As a result, plants growing in low light have a higher concentration of chlorophyll compared to those growing in bright light.[23] We also found that the shade treatment increased the amount of chlorophyll, which plays a role in light absorption in the chloroplast to maximize light absorption. The elevation in chlorophyll levels consequently causes an increase in the green color value (negative $a^*$ value) of the tea. For the sensory evaluation, the accumulation of green color affected the visual quality of tea, resulting in a higher green color intensity and color acceptability in the shaded teas than the control tea.

In addition to determination of color characteristics, metabolites from 50% aqueous methanol extract of the tea were analyzed. The shaded tea plants were found to be clearly distinguishable from the control plants grown under full sunlight, and metabolites contributing to the separation were assigned as phenolic compounds, caffeine metabolites, and amino acids and associated with the nutritional and sensory quality of green tea.

Of the phenolic compounds, the levels of quercetin-galactosylrutinoside, kaempferol-glucosylrutinoside, ECG, and EGCG were elevated by the shade treatment, but the levels of kaempferol-glucoside, EGC, GC, myricetin-glucoside, and


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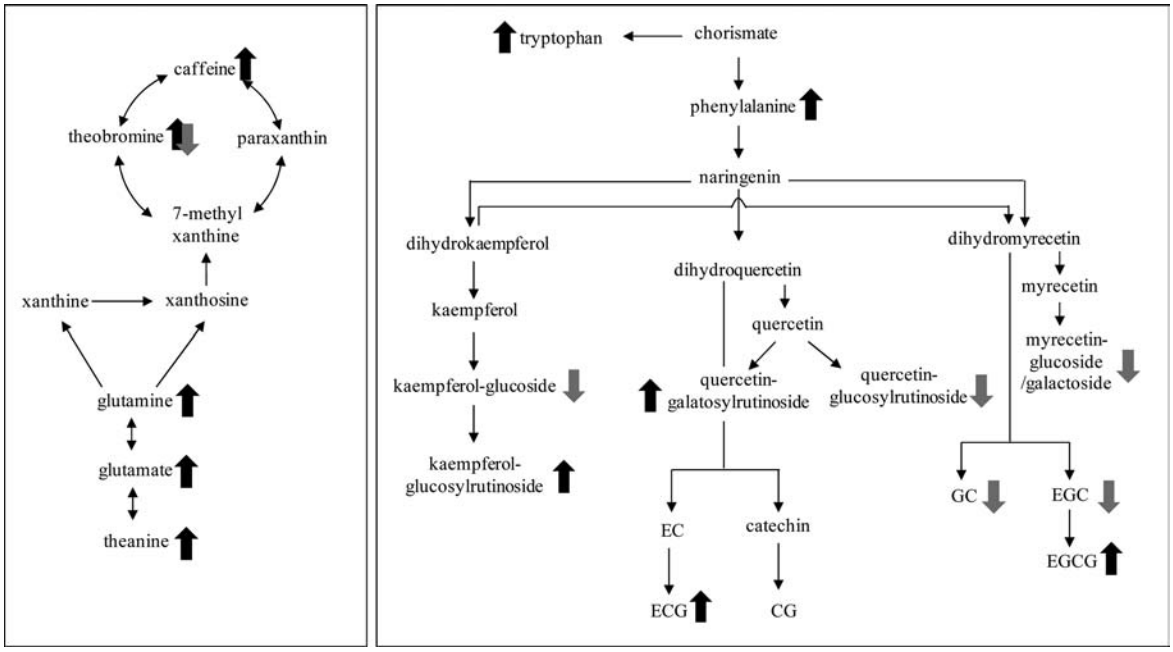


**Figure 4.** Metabolomic pathway of the shaded green tea based on the metabolites analyzed by UPLC−Q-TOF MS. Colored arrows represent the quantitative changes of metabolites with the shade treatment: black, increase; gray, decrease.

quercetin-glucosylrutinoside were decreased. Particularly, the raised levels of quercetin-galactosylrutinoside and kaempferol-glucosylrutinoside were much higher than the reduced levels of quercetin-glucosylrutinoside and kaempferol-glucoside, whereas the levels of other compounds were not remarkably affected. The elevated level of these individual phenolic compounds resulted in increased levels of total phenolic compounds in the shaded tea plants, which was in partial disagreement with previous reports. This finding suggests that the levels of phenolic compounds, including *O*-glycosylated flavonols, proanthocyanins, and catechins, and the activities of enzymes associated with their biosynthesis declined on shade treatment with 20% light transmission for 21 and 10 days.[11,13] This disagreement could be due to a difference in the shade level, which was in accordance to a previous study that indicated that the effect on the release of the tea catechins differed with the shade levels.[12] Although the relationship between the metabolites and their health benefits was not investigated in this study, our findings suggest that the stimulation of phenolic compounds may be proportional to the improvement of health promoted by green tea. Numerous preclinical, clinical, and epidemiological studies, in which tea phenolic compounds were relevant to the promotion of health, including a reduced risk of developing several cancers and cardiovascular diseases,[1,2] strongly support our findings.

The levels of amino acids (tryptophan, phenylalanine, theanine, glutamine, and glutamate) and caffeine associated with the taste quality of green tea were elevated by the shade treatment in coherence with previous studies.[24] The accumulation of some amino acids, such as tryptophan and phenylalanine, along with caffeine and other tea phenolic compounds, is known to increase the intensity of bitter and astringent taste of the tea.[4,25,26] Our sensory evaluation data showed that their intensities were not increased but rather slightly decreased, which may have been caused by the buildup of umami and sweet taste compounds, such as theanine, glutamine, and glutamate,[4,25] because of the shade treatment. Moreover, the accumulation of theanine, which is reported to be associated with the health benefits of tea, including relaxation and improvement in learning ability,[24] might be related to the improvement of health benefits of teas subjected to shade treatment.

On the basis of the analysis of the tea metabolites by UPLC−Q-TOF MS, we propose metabolomic pathways, including those for the metabolism of caffeine and catechin, associated with shade treatment (Figure 4). Although further studies on the relationship between low light and metabolites will be needed to evaluate the pathway, this pathway suggests that the regulation of light intensity and light treatment period can control the level of specific metabolites in the pathway. Moreover, we believe that this metabolomic-based pathway will improve the understanding of the relationship between low light and tea quality.

In summary, a LC−MS-based metabolomic study of green teas provided important information on the nutritional and taste quality of the green tea growing under low light. The levels of phenolic compounds, caffeine metabolites, and amino acids associated with nutritional and taste qualities of tea were elevated with different shade periods. Although further studies on the relationship between metabolites and the health benefits are needed, this metabolomic study, along with a sensory evaluation and color measurement data, supported the hypothesis that the nutritional and taste quality of tea could be improved by the shade treatment. Moreover, the metabolomic pathway proposed in this study could help us better understand the relationship between low light and the nutritional and taste quality of tea.

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### Notes

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