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Quantitative analysis of metabolites in Korean green tea using NMR

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Abstract The plucking season of green tea leaves is one of the important parameters that decide their metabolic quality. Here, we performed the identification and quantity analysis of the metabolites of the green tea using NMR spectroscopy. We assigned the ^{1}H resonances for sixteen metabolites. This analysis found that four metabolites, gallic acid, quinic acid, theobromine and ECG, exhibited clear discrimination of green teas by the three different grades, Ujeon, Sejak and Jungjak. Our results suggest that these four metabolites could be used for diagnostics for quality control of green tea.

Keywords NMR, metabolomics, quality control, green tea, metabolite analysis

Introduction

Green tea is the most widely consumed in Asia and Northern Africa and contains various natural medicines.[1,2] The quality of a green tea depends on the content of metabolites such as catechins, caffeine and amino acids.[3,4] In East Asia, the plucking time of green tea leaves is the important factor that decides the quality of green teas.[5] The best plucking time of green teas is the first flush in April or May than July or August.[5] In Korea, green tea is classified as four grades which depend on the plucking timing of leaves: "Ujeon" (plucked before 20th, April), "Sejak" (plucked in late April or early May), "Jungjak" (plucked in the middle of May) and "Daejak" (plucked in late May or early June). Among them, the Ujeon grade is the green tea with highest quality and is the most expensive grade of green teas.

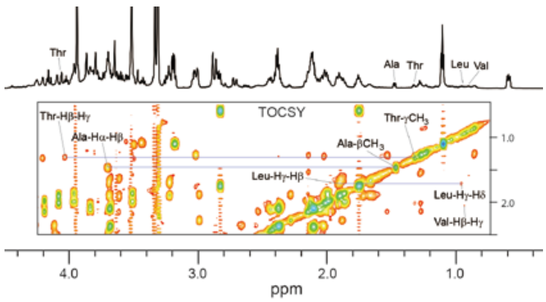

Figure 1. Resonance assignments of amino acids. (upper) 1D ^{1}H and (lower) 2D TOCSY spectra.

Previous NMR study using the orthogonal projection on latent structure-discriminant analysis (OPLS-DA) showed clear discrimination of green teas by three different grades depending on plucking seasons: Ujeon, Sejak and Jungjak.[5] This study found that the nine peak groups could be used for diagnostics for identification of high quality Ujeon grade of green tea.[5] However, this study could not explain which metabolites played the important role in the quality control of green tea classified by the plucking season. Thus, we performed the identification and quantitative analysis of the metabolites of the green tea, which are strongly correlated with the plucking timing of leaves and quality (including flavor and aroma) of green teas.

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Table 1. ^{1}H chemical shifts of compounds of green tea (Ujeon grade).

Compound	Chemical shifts (ppm)						
DSS	2.84 (H1)	1.76 (H2)	0.60 (H3)	0.00 (5-CH_3)			
Alanine	3.72 (Hα)	1.48 (β-CH_3)					
Leucine	n.d.* (Hα)	1.87 (Hβ)	1.60 (Hγ)	0.95 (δ-CH_3)			
Threonine	n.d. (Hα)	4.04 (Hβ)	1.32 (γ-CH_3)				
Valine	n.d. (Hα)	2.06 (Hβ)	0.92 (γ-CH_3)				
Theanine	3.69 (H2)	2.11 (H3)	2.39 (H4)	3.19 (H7)	1.11 (8-CH_3)		
GABA	2.29 (H2)	1.90 (H3)	3.00 (H4)				
Fumaric acid	6.47 (H2)						
Caffeine	3.53 (1-CH_3)	3.35 (3-CH_3)	3.96 (7-CH_3)	7.89 (H8)			
Theobromine	n.d. (3-CH_3)	n.d. (7-CH_3)	7.92 (H8)				
Gallic acid	7.14 (H2)						
Quinic acid	1.88 (H2)	3.96 (H3)	3.52 (H4)	3.97 (H5)	2.00 (H6)		
Theogallin	2.07 (H2)	4.21 (H3)	3.84 (H3)	5.38 (H5)	2.13 (H6)	7.05 (H2′)	
EC	4.89 (H2)	4.25 (H3)	2.71/2.86 (H4)	6.02 (H6,H8)	6.88 (H2′,H5′)	6.78 (H6′)	
ECG	5.11 (H2)	5.50 (H3)	2.88/3.03 (H4)	6.08 (H6,H8)	6.87 (H2′,H5′)	6.79 (H6′)	6.96 (H2″)
EGC	4.89 (H2)	4.25 (H3)	2.73/2.88 (H4)	6.02 (H6,H8)	6.58 (H2′)		
EGCG	5.04 (H2)	5.49 (H3)	2.87/3.02 (H4)	6.06 (H6,H8)	6.58 (H2′)	6.96 (H2″)	

* n.d.: not determined

Results and Discussion

Alanine. The signals of several amino acids, such as alanine, leucine, threonine and valine, were assigned by TOCSY cross-peaks from their methyl groups (Fig. 1). Among amino acids found in green tea, the relative amounts of alanine could be quantified easily by the peak intensities of the methyl resonance at 1.48 ppm (Fig. 1) and its content was compared within three grades. It is known that green tea leaves cultivated in spring had higher levels of amino acids than that of later seasons.[6] Recently, it was reported that the alanine content in green tea leaves slightly depends on the plucked position of leaves.[3] However, Ujeon grade showed no clear differences in alanine content from other two grades (Fig. 2a), indicating that the green tea leaves exhibit no differentiation in the level of alanine within spring (from April to May).

Theanine. The resonances of theanine, which is the most abundant amino acid, were assigned by the analysis of its TOCSY spectra at 25 ºC (Fig. 3). The relative amounts of theanine were quantified by the peak intensities of the well-resolved 8-CH_3 resonance at 1.11 ppm (Fig. 3). Like alanine, there is no clear differentiation in the relative contents of theanine among three grades of green tea (Fig. 2I). This finding can be explained by the previous report that theanine synthesis in green tea mainly increased during spring season and then the theanine contents decreased during late spring and early summer due to degradation by light to polyphenolic compounds.[7]

γ-Aminobutyric acid (GABA). Resonance assignment of GABA is made by the analysis of its TOCSY spectra (Fig. 4). The relative amounts of GABA were quantified by the peak intensities of the well-resolved 4-CH_2 resonance at 3.00 ppm (Fig. 4). GABA also did not exhibit any differentiation in its content as varying the plucking season (Fig. 2F). It

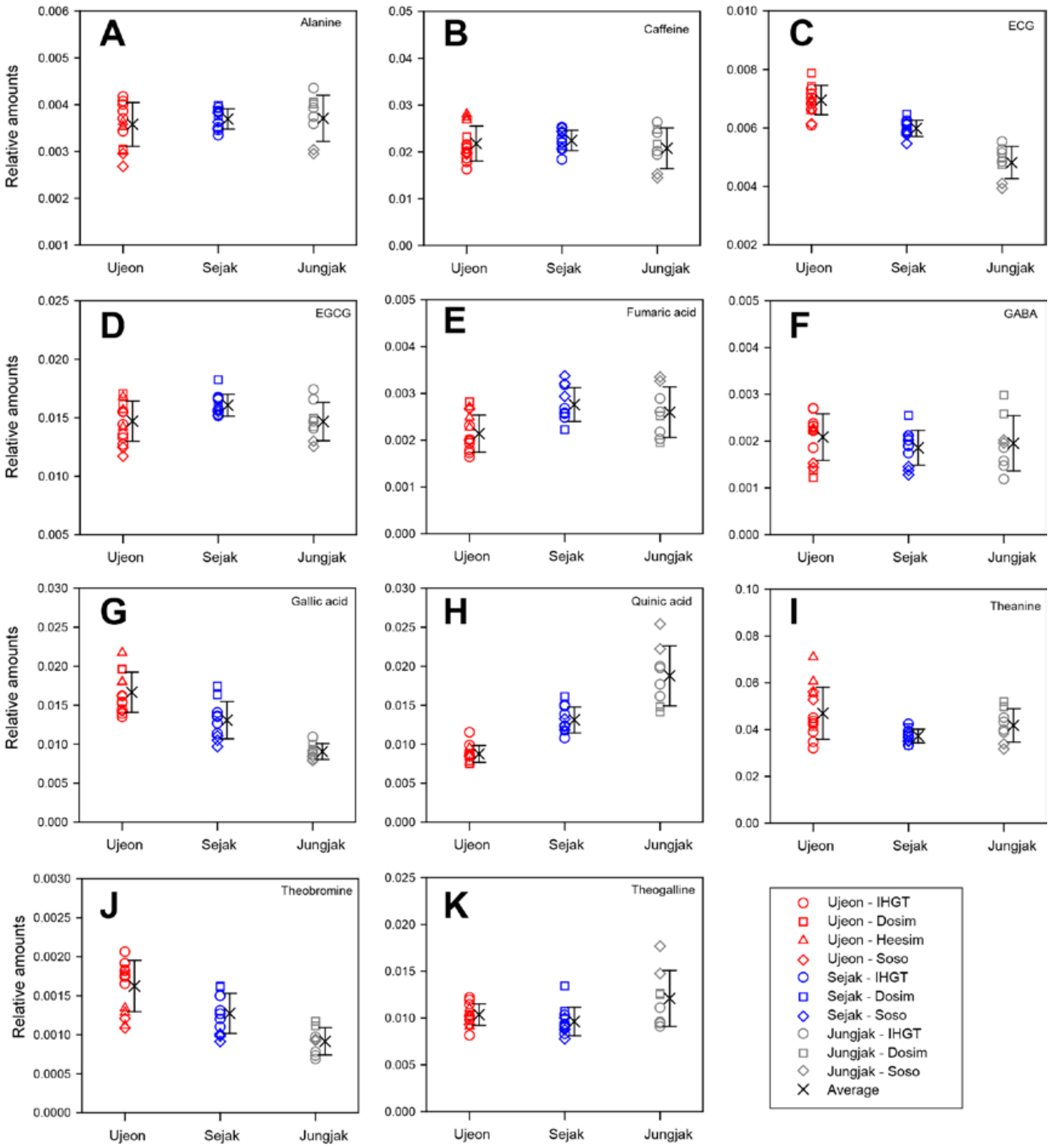

Figure 2. Quantification of (A) Alanine, (B) Caffeine, (C) ECG, (D) EGCG, (E) Fumaric acid, (F) GABA, (G) gallic acid, (H) quinic acid, (I) theanine, (J) theobromine and (K) theogallin identified from extracts of Hadong green tea. Symbol X indicates average value and two horizontal bars indicate the standard deviation from the average value.

was concluded that the three amino acids quantitatively analyzed could not be used for diagnostics for quality identification of green tea.

Fumaric Acid, gallic acid and theobromine. Resonances for fumaric acid, gallic acid, caffeine (1,3,7-trimethylxanthine) and theobromine (3,7-dimethylxanthine) were assigned by comparison with previous report (Fig. 5).[2] The relative amounts of fumaric acid were quantified by the peak intensities

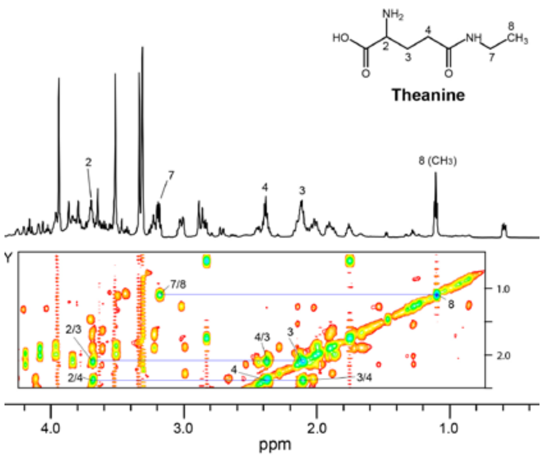

Figure 3. Resonance assignment of theanine. (upper) 1D ^{1}H and (lower) 2D TOCSY spectra. The chemical structure of theanine is shown in upper right.

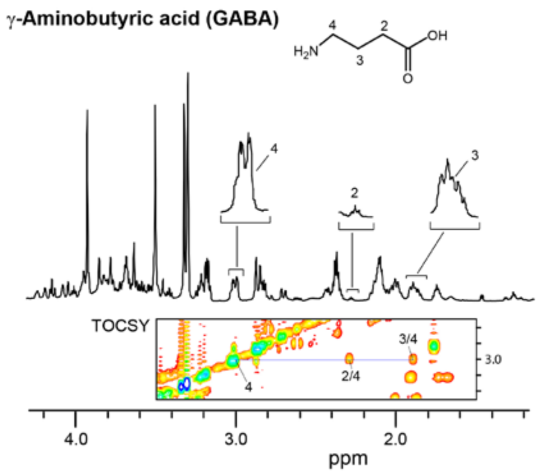

Figure 4. Resonance assignment of GABA. (upper) 1D ^{1}H and (lower) 2D TOCSY spectra. The chemical structure of GABA is shown in upper right.

binned at 6.46-6.48 ppm for H2 resonance at 6.47 ppm (Fig. 5). The fumaric acid content shows no clear differences among three grades (Fig. 2E).

The relative amounts of gallic acid were quantified by the peak intensities of the H2 resonance at 7.14 ppm (Fig. 5). Interestingly, the Ujeon grade exhibits the higher level of gallic acid than other two grades (Fig. 2G). Gallic acid is a natural polyphenol antioxidant compound and is regarded as an important constituent

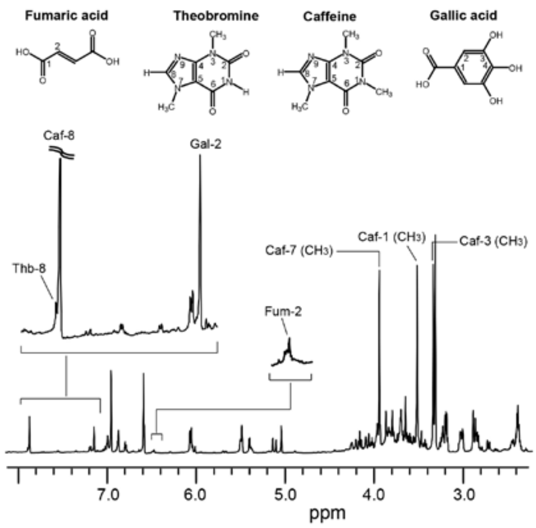

Figure 5. Resonance assignment of fumaric acid, theobromine, caffeine and gallic acid. (upper) chemical structures and (lower) 1D ^{1}H NMR spectra of fumaric acid, theobromine, caffeine and gallic acid.

for the health benefits in food.[7] Gallic acid was higher as green tea leaves became younger, indicating that gallic acid is metabolized in growing phase of tea plant. Therefore, gallic acid is thought to contribute to high quality of green tea classified by plucking season.

The relative amounts of theobromine were quantified by the peak intensities binned at 7.92 ppm for H8 resonance (Fig. 5). Similar to gallic acid, the levels of theobromine in young green tea leaves were higher than in old leaves (Fig. 2J). This means that theobromine also could be used as a metabolite for discriminating the grade of green tea.

Caffeine. The relative amounts of caffeine were quantified by the peak intensities of the 1-CH_3 resonance at 3.53 ppm (Fig. 5). It was reported that caffeine produced in young leaves and continues to accumulate gradually during the maturation of tea plant.[7] However, in this study, we could not find the differentiation in the caffeine contents among three grades of tea products (Fig. 2B).

Quinic Acid. Resonance assignment of quinic acid is made by the analysis of its TOCSY spectra (Fig. 6).

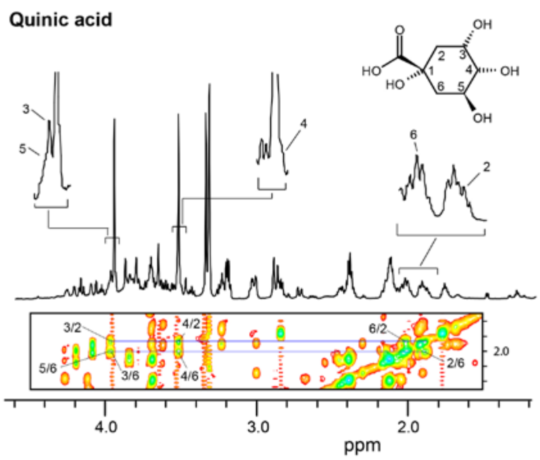

Figure 6. Resonance assignment of quinic acid. (upper) 1D ^{1}H and (lower) 2D TOCSY spectra. The chemical structure of quinic acid is shown in upper right.

The relative amounts of quinic acid were quantified by the peak intensities binned at 3.98 ppm for of H5 resonance at 3.97 ppm (Fig. 6). Quinic acid is wildly biosynthesized in the plants and is a metabolite involved in the shikimate pathway.[8] In contrast to gallic acid, the Jungjak grade contains relatively larger amounts of quinic acid compared to other grades and the amount of quinic acids increases as the plucking time is delayed (Fig. 2H).

Theogallin. Resonance assignment of theogallin is made by the analysis of its TOCSY spectra (Fig. 8). The relative amounts of theogallin were quantified by the peak intensities binned at 4.00 ppm for of H3 resonance at 4.01 ppm (Fig. 7). The theogallin had no clear differentiation in its content among three grades (Fig. 2K).

Catechin. Major catechin derivatives in green tea leaves are EC, ECG, EGC and EGCG as shown in Figure 8. We quantified the relative amounts of ECG and EGCG by the peak intensities at 5.10 and 5.04

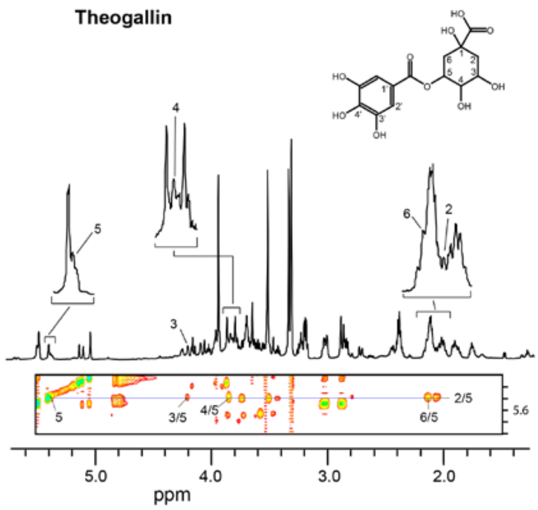

Figure 7. Resonance assignment of theogallin. (upper) 1D ^{1}H and (lower) 2D TOCSY spectra. The chemical structure of theogallin is shown in upper right.

ppm, respectively, whereas the contents of EC and EGC could not be determined because of resonance overlapping. This study revealed that the Ujeon grade exhibits the higher level of ECG than other two grades (Fig. 2C), whereas the EGCG content shows no clear differences among three grades (Fig. 2D). Lin et al. reported that in some green tea samples, the ECG level in the young leaves was higher than that in the old leaves,[9] consistent with our results.

In summary, we performed the identification and quantity analysis of the metabolites of the green tea using NMR spectroscopy. We assigned the ^{1}H resonances for sixteen metabolites. This analysis found that four metabolites, gallic acid, quinic acid, theobromine and ECG, exhibited clear discrimination of green teas by the three different grades, Ujeon, Sejak and Jungjak. Our results suggest that these four metabolites could be used for diagnostics for quality control of green tea.

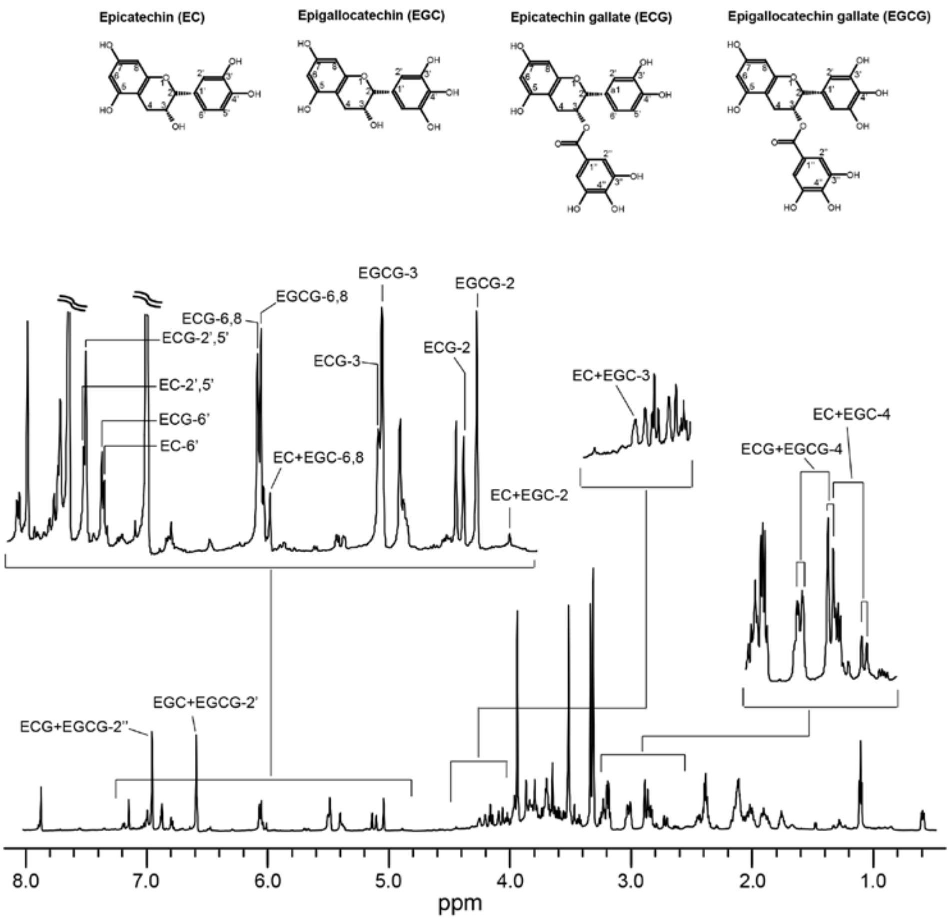

Figure 8. Resonance assignment of catechin. (upper) chemical structures and (lower) 1D ^{1}H NMR spectra of EC, EGC, ECG and EGCG.

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