

Article

# Metabolomic Technique in Green Tea Research: A Review

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## Abstract

Tea, consumed by two-thirds of the global population, had a production of 5.68 million tons in 2017. The cultivation and processing of tea are crucial variables that have a substantial impact on the final product. As a result, many types of tea, such as black, oolong, and green, are produced, each with its own unique flavors and health advantages. Metabolomic techniques, which employ advanced analytical methods, are employed to characterize chemical composition of tea, thereby enabling the assessment of its quality, origin, and bioactivity. This review focused on the application of these techniques to the green tea research. Analytical methods such as UPLC-MS and <sup>1</sup>H NMR, when employed in conjunction with multivariate analysis, have proven effective in correlating green tea quality with its chemical constituents, identifying key metabolites such as EGCG, ECG, and caffeine. These metabolomic techniques permit rapid and accurate assessments, enabling the differentiation between high- and low-quality green tea and enhancing the comprehension of its chemical composition and sensory attributes. In addition, a metabolomic approach was employed, utilizing HPLC-TOF-MS, to differentiate between various types of green tea based on their bioactivities. These methods have the capacity to reveal significant variations in metabolite profiles and bioactivities. The application of multivariate analysis enabled the identification of specific bioactive cultivars, thereby demonstrating the efficacy of the technique in evaluating the health-promoting effects of different tea samples. Thus, metabolomic methods represent an optimal approach for a comprehensive and expeditious investigation of the research related to green tea.

**Keywords:** green tea, metabolomic, *Camellia sinensis*, bioactivity, bioactive compounds

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## 1. Introduction

Tea is a beverage that is widely recognized as one of the most favored globally, with approximately two-thirds of the global population consuming it [1]. Global tea production in 2017 reached a total of 5.68 million tons, with China accounting for 2.55 million tons [2]. The quality of tea is influenced by a number of factors, including the cultivar, the criteria used for picking, the tea processing techniques

employed, the storage conditions to which it is subjected, and the duration for which it is stored. Consumers are increasingly concerned about the variability of tea quality. Thus, ensuring the quality of commercialized tea products is of significant importance [3]. Leaves of tea (*Camellia sinensis*) can be produced resulting in three different types of tea. Complete fermentation of the leaves produces in black tea, while semi-fermentation of the leaves results in oolong tea [4], [5]. Green tea is produced without any

fermentation process. Each type of tea has its own flavor characteristics due to metabolites resulting from fermentation. These characteristics are unique makes tea are favorable by consumers. However, tea is not only consumed for its flavor and ability to provide refreshment, but also for its health benefits. Green tea components have been demonstrated to exhibit a multitude of biological and pharmacological effects, including anti-metastatic, anti-carcinogenic, anti-hypertensive, anti-oxidative, and anti-hypercholesterolemic actions [6], [7].

The metabolomic technique is widely used to characterize the beneficial composition of tea. The method employs analytical techniques, including nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS), and chromatography. The analytical methods result in very diverse data sets describing chemical metabolites within food matrices [8]. These metabolite data can be modeled using multivariate analysis to provide a clear profile of the chemical composition of tea species [4]. The profile can be used to estimate important characteristics of green tea such as quality, origin, cultivation method, fermentation and even bioactivities. This paper aimed to review the application of metabolomics in determining the quality, origin and bioactivity of green tea. Furthermore, brief results from some studies on green tea were used as examples to demonstrate the robustness of the metabolite method in describing the green tea component profile.

## 2. Metabolomic technique to determine quality of green tea

The sensory evaluation of green tea has historically been conducted by expert evaluators who assess the quality of the product based on the visual appearance of the leaves, the aroma, and the taste of the brewed tea. Recently, however, a variety of analytical techniques and experimental methodology has been employed to examine the association between the quality and the chemical constituents of green tea. In green tea, the sensory attributes such as sweet and brothy taste are derived from amino acids, especially theanine [9]. Moreover, it is widely recognized that the astringency and bitterness of tea brews may be attributed to caffeine and

catechins. In addition, the characteristic taste of tea was composed of a complex interaction between astringency, bitterness, umami, light sourness and sweetness [10].

A previous study developed methods to evaluate the quality of green tea originating from Japan utilizing ultra-performance liquid chromatography (UPLC) coupled with electrospray ionization mass spectrometry (ESI-MS). There were 56 samples of green tea (registered in the commercial tea competition among the specific area in Japan, which called Kansai) were dried in advance using a freeze dryer. The quality of the tea was evaluated by a panel of tea experts, who assessed the appearance, aroma, color, and taste of the brewed tea. Prior to UPLC/MS analysis, preparations were conducted by combining a solvent mixture consisting of methanol, water, and chloroform in a volumetric ratio of 2.5:1:1. The mixture was then incubated at a temperature of 37°C for 30 minutes, followed by centrifugation at a speed of 16,000 g at 4°C for 10 minutes. The liquid portion was transferred to an Eppendorf tube and dehydrated via vacuum centrifugation and freeze drying to obtain a dry, unrefined extract. The crude extract was then dissolved in methanol and water (0.1% formic acid) and filtered through a 0.2 µm PTFE filter. The crude extracts were analyzed using UPLC-MS with C18 columns measuring 1.7 µm in diameter and 2.1 × 150 mm in size. The analysis employed two mobile phases: mobile phase A, comprising water containing 0.1% formic acid, and mobile phase B, comprising acetonitrile containing 0.1% formic acid. The technique of mass spectrometry is employed, utilizing an ESI source operating in the negative mode. This results in the acquisition of mass-to-charge ratio (m/z) values ranging from 100 to 1000. Spectral data from UPLC/MS are processed using Marker-Lynx and MZmine software. The data from the study underwent multivariate analysis using Principal Component Analysis (PCA) with SIMCA-P version 11.0. This analysis was conducted to assess the relationship between green tea samples and various qualities, as well as to project latent structures using Partial Least-Square (PLS) and establish quality prediction models [9].

Figure 1A presents the complete ion chromatogram of the green tea samples. This

research indicates that both high- and low-quality green teas exhibit identical chromatographic trends. The distinction is evident in the maximum strength of several key components that influence the quality of green tea, including epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). The multivariate analysis revealed that teas with low and high quality were distinctly clustered along the principal component axis. Samples with higher rankings were grouped towards the negative side of PC1, whereas samples with lower rankings were predominantly found in the positive region (Figure 1B). These findings indicated variations in the composition of the green metabolite across different characteristics. Nevertheless, the specific metabolites that are responsible for distinguishing the chromatograms of low- and high-quality green teas are well-documented [9].

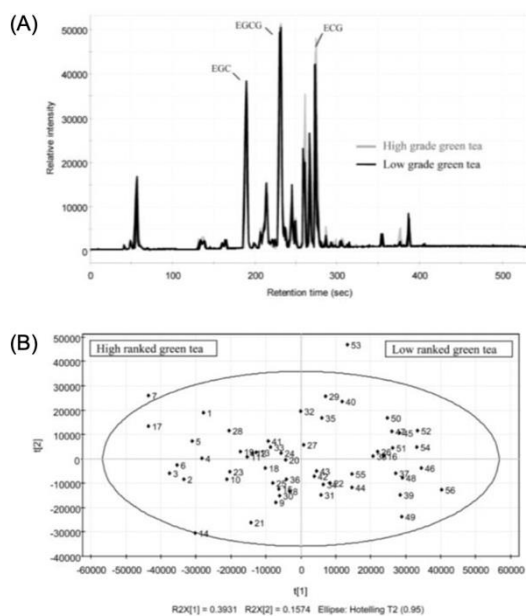


Fig 1. (A) Chromatogram of high- and low-quality green tea leaves, (B) PCA analysis shows the difference between high- and low-quality green tea leaves

The relationship between quality ( $y$ ) and metabolite profile ( $x$ ) of green tea was investigated using PLS regression (Figure 2). The quality parameter ( $y$ ) is determined by expert tea panelists, whereas the metabolite profile parameter ( $x$ ) is derived from the intensity of each metabolite's peak. The data set comprised

56 samples, of which 50 were allocated to the training set and 6 to the test set. The analysis showed that two significant components of the extraction results were determined based on cross-validation. The two components accounted for 83.3% of the variation in  $Y$ , as indicated by an  $R^2Y$  value of 0.833. Additionally, they were able to predict 71.4% of the variation in  $Y$ , with a  $Q^2$  value of 0.714. Furthermore, the test set predicted by PLS demonstrated superior accuracy for the test sample ( $RMSEP = 7.22$ ) in comparison to the projected model based on the training set sample ( $RMSEE = 6.75$ ). The considerable values of the goodness of fit ( $R^2Y$ ) and predictability ( $Q^2$ ) models indicate that this method is a dependable approach for non-specialists to assess the green tea quality and need specific quality indicators [9].

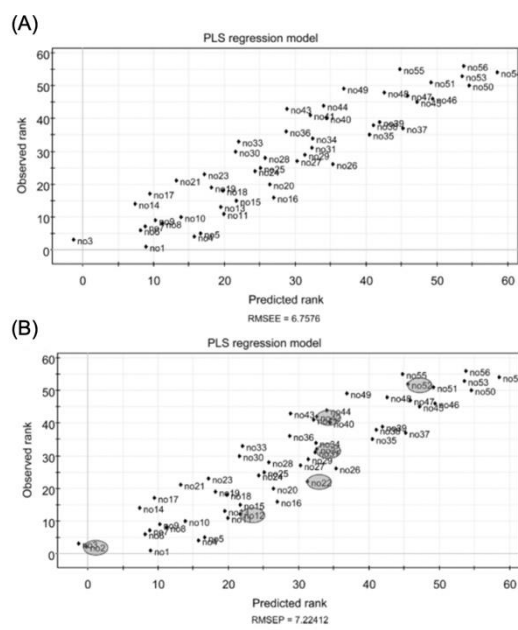


Fig 2. A calibration model incorporating two criteria is used to forecast the green tea quality based on the data obtained from the chromatogram. (A) The training set comprises 50 samples of green tea and (B) the test set includes all rankings marked with a circle, in addition to the training set.

Furthermore, the model design revealed that the epicatechin groups, specifically EGCG, ECG, and EGC, as well as unidentified compounds, had the most impact based on VIP values. The study's findings indicated that the metabolomic fingerprinting technique, employing UPLC/TOF

MS, offers rapid analysis and efficient sample processing. This technology enables the identification of a diverse array of substances, including those present in the extraction process, and can be detected within a span of 10 minutes. This method provides the most efficient analysis time and detects the maximum number of metabolites compared to procedures utilizing GC/MS and GC/MS, as well as PY-GC/MS paired pyrolyzers. Moreover, the quality-prediction model exhibited a high level of accuracy with regard to the R2Y and Q2 values, exhibiting minimal prediction errors. Additionally, the epicatechin group was identified as a noteworthy biomarker for assessing product quality [9].

In contrast to the previous study [8], research by another research group developed Japanese green tea quality evaluation methods using 1H NMR with a combination of pattern recognition techniques [8]. The study included a total of 53 samples of green tea. The green tea sample underwent evaluation by expert tea connoisseurs at the Kansai Green Tea Contest, encompassing the examination of numerous aspects, including appearance, flavor, and odor. Additionally, the researchers purchased the same type of green tea from the Experiment Station. The samples were divided into two groups, designated as Sample No. 1-25 which categorized as high quality, while Sample No. 26-53 were categorized as low quality. All samples were prepared for 1H NMR analysis by adding D<sub>2</sub>O, incubating 60°C at 1400 rpm for 30 minutes, and centrifuging at 25°C for 10 minutes. The supernatant was then taken and filtered, after which it was dissolved in a buffer containing DSS. This solution was then subjected to NMR analysis. The temperature at which this analysis was conducted was 25°C. This work included a number of typical compounds, including quinic acid, p-coumaric acid, myoinositol, 2-O-β-L-arabinopyranosylmyo-inositol, chlorogenic acid, L-arginine, EGCG, ECG, EGC, and epicatechin. The objective was to compare the chemical shift resonance obtained from 1H NMR. The peak determination was assisted by the Chenomx library. Additionally, various software was used for data processing purposes such as Chenomx NMR Suite 4.0 (adjusting first phase and baseline data), Pirouette 3.11 (data conversion to ASCII and PCA analysis), and SIMCA-P (SIMCA-P (creating PLS, OSC, and OSCW models) [8].

The aforementioned studies yielded three distinct segments of the 1H NMR analysis results. The first region of interest was the low-frequency region (0.5-3.0 ppm), the second was the medium-frequency region (3.0-4.5 ppm), and the third was the high-frequency region (5.0-8.0 ppm). In the low-frequency region, the most prevalent compounds were quinic acids and theanine. In addition, caffeine, arginine, quinic acid, and chlorogenic acid were frequently observed at modest frequencies. The chemicals that were most frequently observed at high frequencies include 2-O-β-L-arabinopyranosylmyo-inositol, p-coumaric quinic acid, cinnamic acid, EGCG, and ECG. These results indicate that 1H NMR has the capacity to detect a significant number of metabolites in green samples. The high-quality green tea exhibited a higher concentration of caffeine than the low-quality green tea. Caffeine characteristics are also employed by taste panelists to distinguish between low- and high-quality teas. The differentiation of the spectral curve disparity between the high- and low-quality samples proved challenging in both the low and high frequency spectra. However, the middle frequency spectra exhibited a slight divergence in the curve. PCA can overcome this difficulty, as the separation of the 1H NMR signal is clearly demonstrated by the two groups observed on the PCA results in medium and high frequencies (Figures 3A and B). Caffeine and theanine play a significant role in the grouping of samples. Moreover, phenolic compounds, including EGCG, ECG, EGC, and EC, contribute to the separation of clusters. The study concluded that the metabolomic approach, in conjunction with a multivariate analysis, was the optimal methodology for determining the profile of compounds associated with a specific sensory quality factor. The simplicity of sample preparation and the satisfactory sensory profile results obtained through multivariate analysis make this method an appropriate choice for subsequent analyses [8].

### 3. Metabolomic technique to determine geographical difference

A multitude of factors, including growth conditions, harvesting time, climate, cultivar type, soil composition, and geographical location, as

well as processing, influence the chemical composition of tea. A separate study has demonstrated that the chemical composition of green tea leaves is influenced by environmental conditions and geographical locations in which the plants are cultivated. These environmental factors are analogous to the concept of terroir, which is used in wine science [8]. Furthermore, another set of studies examined the metabolic characteristics of green tea obtained from the highly esteemed areas which produce tea in Japan, China, and South Korea. In addition, these investigations documented the correlation between the chemical makeup of the metabolites generated and the environmental or "terroir" characteristics specific to each planting site [11].

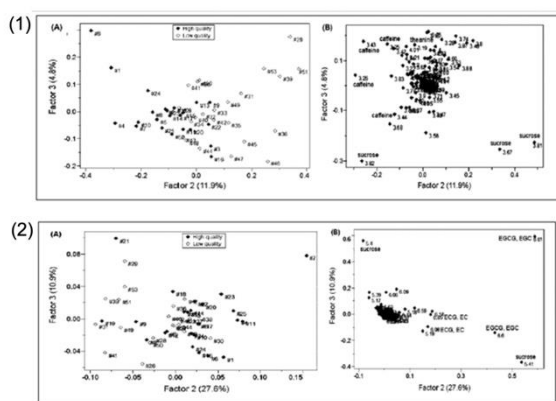


Fig 3. (1) clustering of  $^1\text{H}$  NMR Spectra prolife center frequency spectra of high quality ( $\blacklozenge$ ) and low quality ( $\diamond$ ) groups; (2) high-frequency ( $\blacklozenge$ ) and low-quality ( $\diamond$ )  $^1\text{H}$  NMR spectra clustering. (A): Loading PCA plot; (B): PCA classification.

A total of 284 tea samples, encompassing green, oolong, and white tea varieties, were gathered from Japan, China, and South Korea. A meticulous selection process was conducted to gather a comprehensive data set for each type of tea. This involved choosing tea with the same type of products but from different company. By doing so, any potential variations in tea metabolites caused by different production methods used by the same tea factory were eliminated. In parallel, fresh tea leaves were procured from three tea-producing regions on Jeju Island, South Korea, for the purpose of obtaining green tea samples. The tea samples were collected, blended with a blender, sieved, and distilled water was added. Subsequently, the mixture was extracted in a water bath shaker at

$60^\circ\text{C}$  for 30 minutes and centrifuged. The supernatant was then obtained, separated, and lyophilized. The lyophilized tea extracts were reconstituted in deuterium oxide and propionic acid sodium salt in distilled water for one-hour NMR analysis using the Avance 500 Bruker spectrometer operating at a frequency of 500.13 MHz at a temperature of 300 K. This was achieved through the use of a resonant cryogenic probe and an automated injector. The entire  $^1\text{H}$  NMR spectrum was subjected to normalization, harmonization, and optimization for subsequent analysis using multivariate statistical models, including PCA, PLS-DA, and orthogonal partial least squares-discriminant analysis (OPLS-DA). The climate data utilized in this study were procured from publicly accessible data library supplied by South Korea, China, and Japan Meteorological Agency. The data employed is restricted to a monthly database, encompassing information on average temperature, total sun exposure duration, minimum grass temperature, and rainfall. The data obtained is considered to represent climate characteristics that are a key factor for tea cultivation in this study [11].

The aforementioned studies demonstrated the identification of various types of green metabolites using  $^1\text{H}$  NMR spectroscopy, including catechin, EC, ECG, and EGCG compounds. The most prevalent molecules identified were theanine and caffeine, while other minor components included gallic acid, glucose, sucrose, valine, isoleucine, leucine, alanine, threonine, and quinic acid (Figure 1). The concurrent detection of these metabolites provides a comprehensive understanding of the metabolites generated by green tea in diverse geographical locations. The application of PCA and OPLS-DA to  $^1\text{H}$  NMR spectra enables the distinguishment of green tea originating from Japan, South Korea, and China. The approaches were employed to assess the significance of varying metabolite concentrations in the tea samples from each nation. Nevertheless, it is noteworthy that certain green tea from South Korea exhibit characteristics similar to those from China, and in certain instances, they are identical to samples from Japan. Nevertheless, the pairwise comparison in the OPLS-DA model revealed that the tea samples from Korea were statistically distinct from those from China and Japan. This finding was corroborated by the

results of the permutation tests, which were conducted with 200 repetitions. This indicates that the tea-planting area is subject to diverse environmental conditions. In addition, all samples of green tea gathered from disparate locations within each country were also distinguished, including those obtained from neighboring countries where tea is cultivated. The extensive dispersion of green tea from South Korea in the OPLS-DA model is presumed to be a consequence of the heterogeneous environmental variables present in the local tea-growing region [11].

The OPLS-DA model was employed to identify metabolites in samples of green tea from South Korea and China. A notable contrast was observed between the samples, with a high level of prediction (Q<sup>2</sup><sub>Y</sub>) of 0.90. The plots coefficient of OPLS-DA model facilitates the differentiation of various metabolites in Chinese and Korean green tea samples. The OPLS-DA coefficient plot indicates that Korean green tea has higher levels of tea metabolites compared to Chinese green tea in the upper section. In contrast, the bottom part of the plot shows lower levels of tea metabolites in Korean green tea. Korean green tea has markedly elevated concentrations of, leucine, isoleucine, valine, EGC, EC, caffeine, glucose, and sucrose. In comparison, it demonstrates reduced amounts of theanine, alanine, and threonine. A comparative analysis of metabolites from green tea in samples from South Korea, China, and Japan revealed that South Korean green tea exhibited the greatest expression of sucrose, glucose, EC, EGCG, EGC, and caffeine. In contrast, Chinese green tea exhibited the greatest expression of threonine, alanine, and theanine. Korean green tea exhibited considerably elevated quantities of EGCG, EG, and quinic acid in comparison to Chinese green tea, while Chinese green tea demonstrated higher levels of theanine [11].

The difference in the content of green metabolites between countries is due to geographical dependence such as climate and green tea cultivars. Yabukita, which can be classified into various varieties, constitutes up to 75.6% of the total cultivated region in Japan. A significant proportion of these varieties are imported and cultivated in Korea, resulting in the production of Korean green tea. There are differences in metabolites between Japanese and

Korean green tea due to climatic differences during tea planting. Based on previous research, several factors such as harvest time, season, and temperature affect the metabolite content of green tea. It has been demonstrated that elevated temperatures and prolonged exposure to sunlight can result in the transformation of theanine into catechin molecules. The lower theanine levels in green tea from South Korea compared to green tea from Japan are a consequence of the colder temperatures in South Korea, which are more severe than in Japan. Conversely, the levels of EGCG, EGC, EC, and glucose in Korean green tea are higher than in Japanese green tea. Moreover, the quantity of catechins in green tea is also contingent upon the picking season, in addition to factors such as temperature and sun exposure [11].

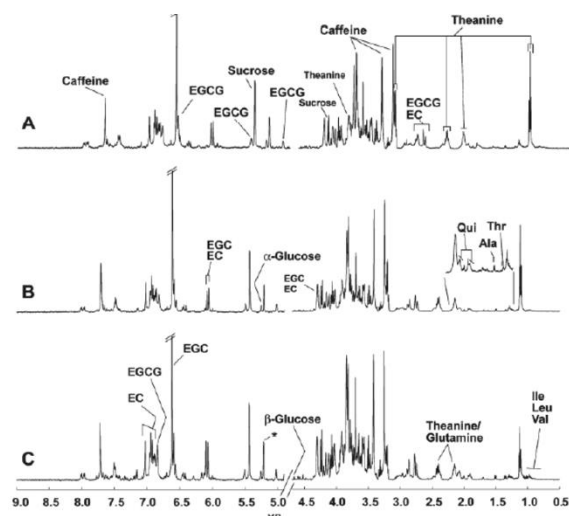


Fig 4. The results of <sup>1</sup>H NMR analysis of green tea metabolites from 3 different planting regions namely (A) Hannam, (B) Dosun, and (C) Seogwang.

A separate study was conducted to investigate the impact of climate on the characteristics of green tea produced in three distinct regions on Jeju Island, South Korea, using a metabolomic approach with the <sup>1</sup>H NMR instrument. They obtained samples from 3 regions in Jeju Island, namely Hannam, Dosun and Seogwang. Meteorological data for each location was obtained from the local meteorological office. The obtained samples were then prepared including extraction and centrifugation. The supernatant obtained was then subsequently lyophilized for storage and

analysis by  $^1\text{H}$  NMR. The obtained  $^1\text{H}$  NMR spectra were analyzed using VnmrJ 2.1B software (basic data correction and data conversion to ASCII format), MATLAB (normative  $^1\text{H}$  NMR spectra normalization), and SIMCA-P (multivariate analysis). In this study, a multivariate analysis using PCA to examine intrinsic data factors, and OPLS-DA to determine the principles in compounds in distinguishing the parameters produced. In addition, they visualized data quality and predictability using R2 and Q2, which represent the proportion of variance in the data model that shows goodness of fit and the variance in the data model that shows predictability, respectively [12].

Various metabolites of green tea origin can be observed based on  $^1\text{H}$  NMR spectra (Figure 4). This study identified a total of 14 metabolites from green tea using  $^1\text{H}$  NMR spectra. These metabolites include quinic acid, theanine, glutamine, leucine, isoleucine, threonine, valine, alanine, caffeine, R- and  $\beta$ -glucose, sucrose, EC, EGC, and EGCG. Furthermore, this study also mentions the visual differences observed from theanine signals, EC, EGC catechins, and EGCG, amino acids caffeine and (Figure 4). Further analysis was performed using PCA and OPLS-DA to provide a comparative comparison between observed metabolites. The analysis showed that there were significant differences between PCA and OPLS-DA, among  $^1\text{H}$  NMR spectra of metabolites from three growing areas (Figure 5). Although there were slight overlaps between metabolite spectra samples from Hannam and Dosun in PCA, OPLS-DA results showed a clear differentiation between these spectra [12].

Lee *et al.* [12] presented the correlation coefficient, R2, more than 0.5%, which meant that the compound had a significant contribution in differentiating the metabolite profile between areas. The significant contribution was marked as yellow to red colors. For example, the higher level of alanine, isoleucine, sucrose, EC, and EGCG, as well as the lower level of quinic acid, glutamine, and an unknown compound contributed significantly to the metabolite profile differences between green tea from Dosun and Hannam (Figure 4). These studies were confident to state that there were significant correlations between metabolite profiles and geographical,

due to climatic variables. For example, theanine content was higher in green tea from Dosun than that from Seogwang. The study predicted that Dosun had higher rainfall and longer exposure to sunlight. They also explained that theanine degrades to catechin at a faster rate when theanine is exposed to sunlight. Another example was the caffeine content, which was probably dependent on rainfall. This study stated that green tea from Seogwang possessed caffeine synthesis ability higher than that of Hannam area [12]. Thus, metabolomic study is of paramount importance in the study of plant metabolites and factors determining their biosynthesis. In relation to the previous study, the geographical area determines the growing conditions of green tea, resulting in a different metabolomic profile.

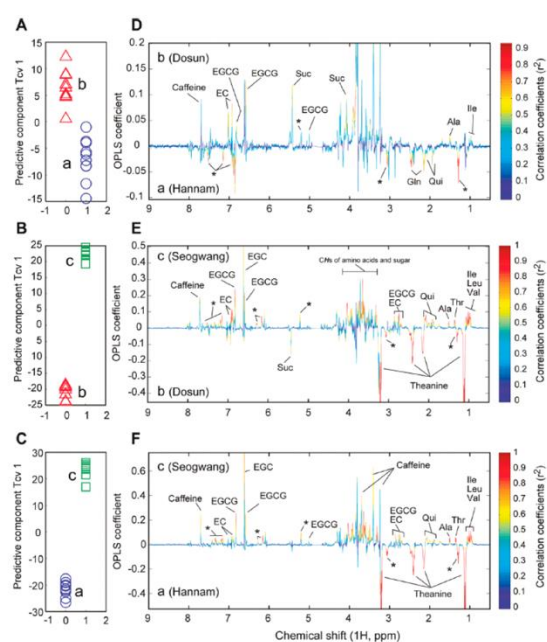


Fig 5. Comparison of  $^1\text{H}$  NMR Pair of green tea spectrum from different planting regions: a. Hannam, b. Dosun, and c. Seogwang, which have been subjected to OPLSDA analysis (A-C) and Loading Plots (D-F).

#### 4. Metabolomic technique to determine differences green tea cultivars for their bioactivities

Tea (*Camellia sinensis* L.) is a widely consumed beverage globally and has garnered significant interest as a medicinal plant due to its possible therapeutic advantages. The fermentation process

is the primary determinant of the fundamental categories of tea, including green tea (unfermented), oolong tea (partially fermented), and black tea (completely fermented). Green tea contains numerous biological and pharmacological components, including antioxidant, anti-carcinogenic, anti-hypertensive, anti-metastatic, and anti-hypercholesterolemic actions. The positive impacts of tea may be attributed to the presence of metabolites, such as theanine [13]. However, it is probable that there would be differences in metabolites among different varieties of green tea. A previous study sought to assess the correlation between the metabolome and bioactivity (the ability to promote health) of several tea cultivars. Furthermore, this study aimed to examine the impact of various green tea varieties on the activity of thrombin-induced myosin regulatory light chain (MRLC) in human umbilical vein endothelial cells (HUVECs) [3].

In a previous study, 43 samples of various Japanese green tea varieties were acquired from the National Institutes of Vegetables and Tea Science in Japan. Green tea samples were introduced into water that was brought to a boiling point, then separated by passing through a filter and subjected to centrifugal force. Polyvinyl polypyrrolidone was introduced into the resultant supernatant to hinder the thrombin-induced phosphorylation of MRLC in HUVECs. The inhibitory effect of green tea extract on MRLC phosphorylation was quantified by Western blot analysis. This research also used high performance liquid chromatography tandem with time-of-flight mass spectrometry to identify components of green tea samples. The obtained chromatogram was processed using multivariate analysis. Several software were used for the analysis such as XCMS (peak extraction from LC-MS), SIMCA-P (multivariate analysis including PCA and OPLS-DA) and Multi-Experiment Viewer (MeV v4.6.1) to summarize the z-value of the peak obtained in LC-MS and visualize it in the form of heat maps [4].

The result showed that 43 samples had different effects on the inhibition of MRLC phosphorylation. Some samples can show very strong inhibition of phosphorylation (No. 2 (Chuukanbohon) and 43 (Sunrouge)), but the other samples promote phosphorylation (No. 20 (Meiryoku) and 38 (Okumidori)). In this study, it

was also shown that there was a total of 541 peaks identified by LC-MS from all 43 samples. Heatmap of 541 peaks showed that there were differences in metabolite profiles, which believed that these differences affect the vary bioactivity among samples (Figure 7A). Samples with higher ability to inhibit phosphorylation (Sample No. 42 and No. 43) were clearly characterized in PCA score plot (Figure 7B). The research findings indicate that the identified metabolites, including EC, ECG, EGC, EGCG, caffeine, theanine, theogallin, myricetin, and other unidentified m/z peaks, played a significant role in differentiating the clusters [4].

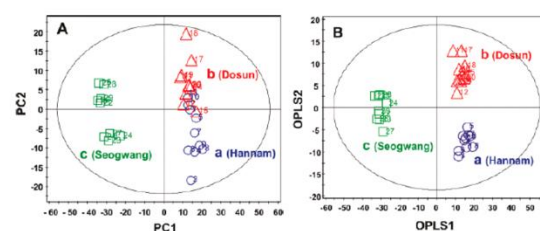


Fig 6. Results of <sup>1</sup>H NMR spectral analysis of samples from three green tea planting locations: Hannam (a), Dosun (b) and Seogwang (c). The analyses were performed using PCA (A) and OPLSDA (B).

Three samples were analyzed to confirm the result described in Figure 6B and C. The samples included the non-bioactive cultivar designated as Yabukita (YB), the bioactive cultivar designated as SR, and the less bioactive cultivar designated as Benifuuki (BF). Based on these results, a similarity was found in the cluster formation and component distribution of the three samples against all samples. The OPLS-DA score plots between SR vs. YB (Figure 7F and 7H) and SR vs. BF (Figure 7G and 7I) indicated that there were many differences among the metabolites among these samples. In addition, sample No. 42 and No. 43 possess unique bioactive characteristics. Therefore, it can be concluded that the metabolomic technique proved to be useful in identifying the bioactive functionality of samples No. 42 and No. 43 in inhibiting MRLC phosphorylation in HUVECs [4].

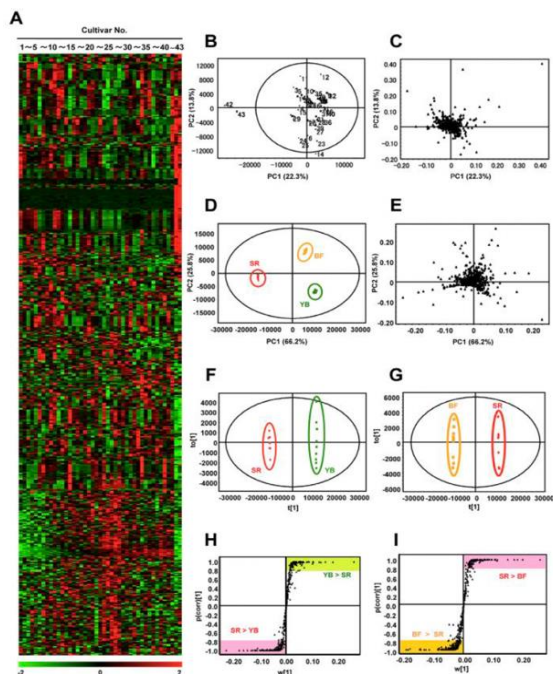


Fig 7. (A) Heat map of different green tea extracts in Japan; B) The PCA score plot indicates the presence of distinct clusters of MS profiles corresponding to Nou-6 and SR, and other cultivars; C) Corresponding loading plots of all samples show MS peaks that differ among samples. D) PCA score plot derived from different tea cultivars; Yabukita (YB), Benifuuki (BF) and SR. E) Corresponding loading plots of three tea cultivars (YB, BF, and SR) show MS peaks that differ among samples. (F and G) OPLS-DA score plots and plots (H and I) loading S- were derived from each LC-MS data set (F and H: YB vs SR; G and I: BF vs SR).

## 5. Conclusion

Metabolomic techniques such as UPLC-MS, 1H NMR and HPLC-TOF-MS have proven effective in assessing the quality, origin and bioactivity of green tea by identifying key metabolites and correlating them with tea quality and health benefits. These methods allow rapid and accurate differentiation between high- and low-quality teas and between different cultivars based on their bioactive properties. The application of multivariate analysis in these studies demonstrates the robust ability of metabolomics to improve the understanding of the chemical composition and sensory attributes of green tea, as well as its health-promoting effects. Thus, these advanced analytical

approaches can significantly contribute to the development of green tea research and its application in the food and health industries.

## References

- [1] I. Raskin *et al.*, "Plants and human health in the twenty-first century," *Trends Biotechnol.*, vol. 20, no. 12, pp. 522–531, Dec. 2002.
- [2] Z. J. Ng, M. A. Zarin, C. K. Lee, and J. S. Tan, "Application of bacteriocins in food preservation and infectious disease treatment for humans and livestock: a review," *RSC Adv.*, vol. 10, no. 64, pp. 38937–38964, 2020.
- [3] C. S. Yang, X. Wang, G. Lu, and S. C. Picinich, "Cancer prevention by tea: animal studies, molecular mechanisms and human relevance," *Nat. Rev. Cancer*, vol. 9, no. 6, pp. 429–439, Jun. 2009.
- [4] Y. Fujimura *et al.*, "Metabolomics-Driven Nutraceutical Evaluation of Diverse Green Tea Cultivars," *PLoS One*, vol. 6, no. 8, p. e23426, Aug. 2011.
- [5] K. Hayat, H. Iqbal, U. Malik, U. Bilal, and S. Mushtaq, "Tea and Its Consumption: Benefits and Risks," *Crit. Rev. Food Sci. Nutr.*, vol. 55, no. 7, pp. 939–954, Jun. 2015.
- [6] T. Chisaka, H. Matsuda, Y. Kubomura, M. Mochizuki, J. Yamahara, and H. Fujimura, "The effect of crude drugs on experimental hypercholesteremia: Mode of action of (-)-epigallocatechin gallate in tea leaves.," *Chem. Pharm. Bull.*, vol. 36, no. 1, pp. 227–233, 1988.
- [7] T. Zhao, C. Li, S. Wang, and X. Song, "Green Tea (*Camellia sinensis*): A Review of Its Phytochemistry, Pharmacology, and Toxicology," *Molecules*, vol. 27, no. 12, p. 3909, Jun. 2022.
- [8] L. Tarachiwin, K. Ute, A. Kobayashi, and E. Fukusaki, "1 H NMR Based Metabolic Profiling in the Evaluation of Japanese Green Tea Quality," *J. Agric. Food Chem.*, vol. 55, no. 23, pp. 9330–9336, Nov. 2007.
- [9] W. Pongsuwan, T. Bamba, K. Harada, T. Yonetani, A. Kobayashi, and E. Fukusaki, "High-Throughput Technique for Comprehensive Analysis of Japanese Green Tea Quality Assessment Using Ultra-performance Liquid Chromatography with Time-of-Flight Mass Spectrometry (UPLC/TOF MS)," *J. Agric. Food Chem.*, vol. 56, no. 22, pp. 10705–10708, Nov. 2008.
- [10] L. Zhang, Q.-Q. Cao, D. Granato, Y.-Q. Xu, and C.-T. Ho, "Association between

- chemistry and taste of tea: A review,” *Trends Food Sci. Technol.*, vol. 101, pp. 139–149, Jul. 2020.
- [11] J.-E. Lee *et al.*, “Metabolomic unveiling of a diverse range of green tea (*Camellia sinensis*) metabolites dependent on geography,” *Food Chem.*, vol. 174, pp. 452–459, May 2015.
- [12] J.-E. Lee *et al.*, “Geographical and Climatic Dependencies of Green Tea (*Camellia sinensis*) Metabolites: A  $^1\text{H}$  NMR-Based Metabolomics Study,” *J. Agric. Food Chem.*, vol. 58, no. 19, pp. 10582–10589, Oct. 2010.
- [13] D. Türközü and N. Şanlıer, “L-theanine, unique amino acid of tea, and its metabolism, health effects, and safety,” *Crit. Rev. Food Sci. Nutr.*, vol. 57, no. 8, pp. 1681–1687, May 2017.