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Assessing Geographic Origins of Green Teas Using Instruments

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Abstract Parameters of soluble solids, amino acids, catechins, and color difference of 24 green tea samples from China and Korea were determined. The levels of soluble solids, amino acids, total catechins, and infusion lightness in tea samples from Korea were higher than those from China. Concentrations of epigallocatechin galate and epigallocatechin in teas from China were higher than tea samples from Korea. Geographical origin of teas from the 2 countries was discriminated using parameters of infusion lightness, gallocatechin, and total catechins and applying principal component analysis.

Keywords: *Camellia sinensis,* authenticity, catechin, tea infusion, principal component analysis

Introduction

Tea (*Camellia sinensis*) and its extract attract consumers because of their bioactive actions such as antimutagenic and antimicrobial, antioxidant, and anticancer activities (1-4) and tea is the second most widely consumed beverage in the world now (5). There are many factors influencing quality of tea, including cultivar, estate environments, fertilizing, and processing techniques. In some countries such as Korea and Japan, tea price is varied widely between domestic and imported teas owing to difference in quality. By counterfeiting brand names, fraudulently labeling and selling low quality products as premium products, this sector of industry has suffered from loss of income and the consumer has been deceived. The consumers and genuine tea processors are increasingly paying attention to the authentication of tea products.

The development of new and increasingly sophisticated techniques for the authentication of food products continues apace with increasingly consumer awareness of food safety and authenticity issues (6). Many techniques have been used to identify the authenticity and origins of food, including DNA-based methods (7), stable isotope analysis (8), chromatography (9), near infrared reflectance (NIR) spectroscopy (10), Fourier transform-infrared (FT-IR) and ultraviolet (UV) spectrometers (11), nuclear magnetic resonance (NMR) (12), and electronic nose (13). These methods can be classified as three groups, i.e., genetic, inorganic, and organic determinations. The authentication of cultivar can be accurately identified by DNA-based genetic analysis. However, if a same tea cultivar was planted in various regions and countries, it is difficult to distinguish the geographic origins of tea products by the DNA-based genetic analysis. Though stable isotope ratio of various elements is correlated to the estate soils and it provides useful information for origin assignment (14), it can not be carried out in general labs because it needs specific and sophisticated equipments. Metal content of teas is influenced by the soil composition and local environmental factors and so was used as marker of geographical classification (15). Little information on the identification of geographical origin of teas using organic parameters has been available. Tea quality was closely correlated to its color parameter and organic contents such as catechins and amino acids and they were applied as discriminating variables to assess quality of tea (16-18). Pattern recognition techniques including principal component analysis (PCA), cluster analysis (CA), and linear discriminant analysis (LDA) were used in classification of product brands and quality of origin (15). The combination of pattern recognition techniques and the quality control data will provide useful means to rapidly discriminate the quality and the geographical origin of teas.

The aim of this study was therefore to use high performance liquid chromatography (HPLC) and color difference analysis to determine the quality control parameters in green tea samples from China and Korea and then to evaluate the potential of geographical discrimination of teas using the quality control data and applying principal component analysis.

Materials and Methods

Materials Twenty-four green tea samples (500 g each) used in the present study were collected from China (sample No. 1-7) and Korea (sample No. 8-24). Details of the samples were listed in Table 1. The samples were ground and sifted through 0.45-mm sifter.

The reference compounds of 8 tea catechins for HPLC were products of Sigma-Aldrich Chemicals (St. Louis, MO, USA). The other chemical reagents used were of HPLC grade (Shild Biometric Technical Co., Ltd., Tianjing, China). Equipments for the chemical analysis were Shimadzu Model SCL-2010A HPLC; (Shimadzu Corporation, Kyoto, Japan) and that for tea infusion color difference analysis was an automatic color difference meter (Model TC-PIIG; Beijing Optical Instrument Factory, Beijing, China).

Preparation of tea infusions Three g of the ground tea

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Table 1. Details of green tea samples

Sample nunber	Origin	Grade	Sample number	Origin	Grade
1	Zhejiang, China	1	13	Nanjieju, Korea	3
2	Zhejiang, China	2	14	Boseong, Korea	1
3	Zhejiang, China	3	15	Boseong, Korea	2
4	Anhui, China	1	16	Boseong, Korea	3
5	Anhui, China	2	17	Gangjin, Korea	1
6	Hunan, China	1	18	Gangjin, Korea	2
7	Hunan, China	3	19	Gangjin, Korea	3
8	Seogwipo, Korea	1	20	Hadong, Korea	1
9	Seogwipo, Korea	2	21	Hadong, Korea	2
10	Seogwipo, Korea	3	22	Hadong, Korea	3
11	Nanjieju, Korea	1	23	Gimhae, Korea	1
12	Nanjieju, Korea	2	24	Gimhae, Korea	2

sample was extracted in 150 mL freshly boiled distilled water for 5 min and then filtered though a 'Double-ring' No. 102 filter paper (Xinhua Paper Industry Co., Ltd., Hangzhou, China) and finally centrifuged at 5,500×g for 15 min.

Color difference analysis The ground tea leaf or tea infusion was placed in the sample cell of the automatic color difference meter and color difference parameters of *L, a,* and *b* were printed directly by the equipment. The data of *L, a,* and *b* are parameters on the 3 dimensional Hunter Lab color scale. The *L* axis runs from top to bottom. The maximum for *L* is 100, which could be a reflecting diffuser. The minimum for *L* would be 0, which would be black. Positive *a* is red and negative *a* is green. Positive *b* is yellow and negative *b* is blue.

HPLC analysis of tea infusion The above prepared tea infusion was filtered through a 0.22-μm Millipore filter before injected into HPLC. Concentrations of catechins and caffeine were determined by HPLC (19) and the HPLC conditions were as follows:

Injection volume:	10 μL
Column:	5 μm-Diamonsil™ C18 4.6×250 mm
Temperature:	40ºC
Mobile phase:	Solvent A: acetonitrile/acetic acid/ water (6:1:193,v/v/v) Solvent B: acetonitrile/acetic acid/ water (60:1:193, v/v/v)
Gradient:	100%(v/v) solvent A to 100%(v/v) Solvent B by linear gradient in 45 min
Flow rate:	1 mL/min
Detector:	Shimadzu SPD UV detector, 280 nm

Determination of amino acids Amino acids concentration was determined by ninhydrin assay method. Two mL of the tea infusion was transferred to a 50-mL volumetric flask with 1 mL of reagent (20 g/L of ninhydrin and 0.8 g/L of $SnCl_2 \cdot 2H_2O$) and 1 mL of buffer (6.7×10^{-2} M Na_2HPO_4 and 6.7×10^{-2} M KH_2PO_4, pH 8.0) and then reacted in boiling water bath for 15 min. The control flask contained 2 mL of distilled water, 1 mL of reagent and 1 mL of buffer. The reacted sample was then transferred to quartz cell with black aperture (1 cm light-path) and absorbance at 570 nm was determined by an HP8453E UV-VIS spectrophotometer (Hewlett Packard, Palo Alto, CA, USA). Glutamic acid (Sigma-Aldrich) was used as amino acids standard to make calibration graph and amino acids concentration of the tea sample (glutamic acid equivalent) was determined from the calibration graph according to its absorbance at 570 nm (17).

Determination of soluble solids Twenty mL of the tea infusion was transferred to a weighted glass dish (A) and dried at 80ºC for 24 hr and then dried at 105ºC for 1 hr. The dish with dried tea extract was weighted (B) when it was cooled to room temperature. The solids concentration was calculated according to the difference in weight between A and B and the result was presented as mg/g on dry base.

Statistics The tests in the present paper were carried out in duplicates for each sample and the mean values of the duplicates were presented. Statistic analysis was carried out on software SPSS 11.0 for Windows (SPSS Inc. Chicago, IL, USA).

Results and Discussion

Comparison of color parameters Figure 1 showed that the values *L* of both tea leaf and infusion of samples from Korea were higher than those from China, suggesting green teas from Korea were more diffuse in light than those from China. Although the mean values of *a*, the red-green parameter for tea infusions and leaf were negative, there was difference in parameter *a* between leaf and infusion. The infusions had less negative *a* but higher positive *b* than leaf, suggesting that the tea infusions was lighter green but deeper yellow than leaf. The parameters *a* and *b* also showed that the infusion and leaf in samples from China was more deep in green and yellow color than those from Korea (Fig. 1). During the green tea processing, the fresh leaf fixation was carried out in a heated drum in China whereas the fresh leaf was usually steamed in a steamer in Korea. The difference in color parameters between

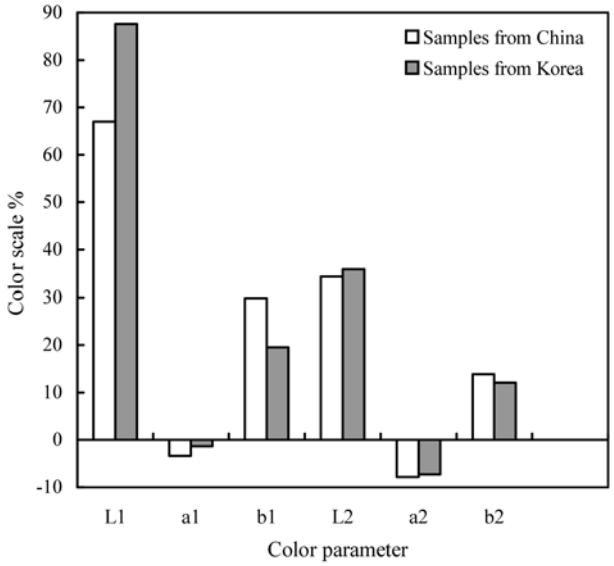

Fig. 1. Mean values of color parameters. L, Lightness-darkness; a, greenness-redness; b, blueness-yellowness. 1, Tea infusion; 2, tea leaf.

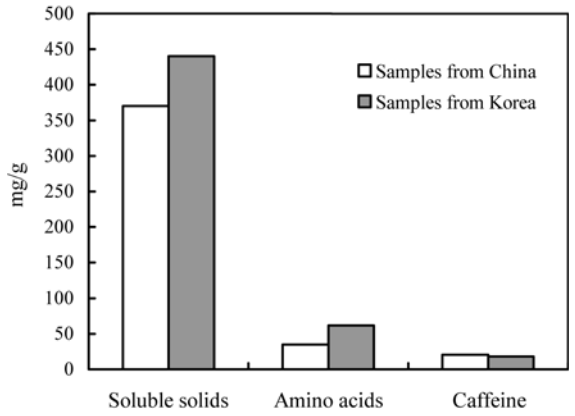

Fig. 2. Mean levels of soluble solids, amino acids, and caffeine.

teas from the two geographical origins might be related to the processing methods.

Comparison of chemical compositions Concentrations of soluble solids and amino acids in samples from Korea were significantly higher than those from China. There was no significant difference in caffeine concentration between the samples from the two countries (Fig. 2). Soluble solids and amino acids decreased with development of tea shoots (20). During sensory assessment, it was found that the infused tea leaves of samples from Korea were tender and softer than samples from China. The difference in tenderness of plucked shoots might be the reason that resulted in higher levels of soluble solids and amino acids in samples from Korea than those from China.

Figure 3 showed that (-)-epigallocatechin gallate (EGCg) was the most abundant compound of tea catechins and (-)-epigallocatechin (EGC) was the next in tea samples from the two origins. However, composition of tea catechins was different between samples from China and Korea. Samples from China had higher concentrations of EGCg

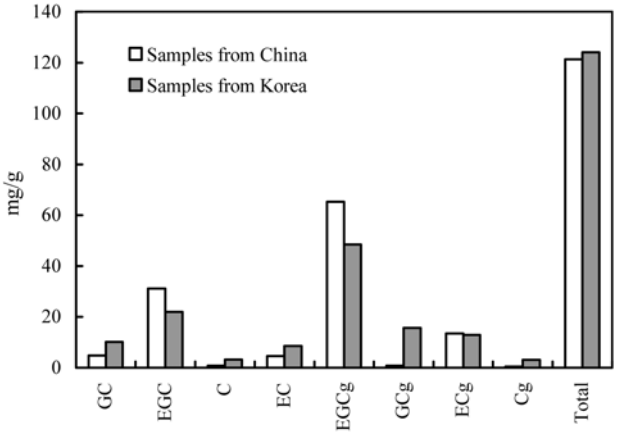

Fig. 3. Concentrations of tea catechins. GC, (+)-gallocatechin; EGC, (-)-epigallocatechin; C, (+)-catechin; EC, (-)-epicatechin; EGCg, (-)-epigallocatechin gallate; GCg, (+)-gallocatechin gallate; ECg, (-)-epicatechin gallate; Cg, (+)-catechin gallate; Total, total concentration of catechins.

and EGC, while samples from Korea had higher levels of (+)-gallocatechin (GC), (+)-gallocatechin gallate (GCg), and (-)-epicatechin (EC). Fresh tea leaf did not contain GCg and GC. Epimerisation of tea catechins from epistructure to non-epistructure took place when they were placed under elevated temperature conditions, during which EGCg and (-)-epicatechin gallate (ECg) were partially changed into GCg and Cg, respectively (19,21). The higher levels of GCg and GC in samples from Korea suggest that the heating treatment was more severe during their processing. During green tea processing in China, fresh leaf was fixed in a heated drum, during which the leaf was partially dried. In Korea, the fresh leaf was steamed and the leaf moisture increased after the steaming. This led a longer drying process for the teas from Korea. Furthermore, temperature during drying process of the teas from Korea might be higher than those from China. This was confirmed by sensory evaluation that the samples from Korea had a strong burnt sugar smell. This might explain why samples from Korea had higher levels of GCg and Cg.

Principal component analysis (PCA) PCA is a project method to reduce dimension for observing a primary evaluation of the between-class similarity, which allows an easy visualization of all the information contained in a data set and helps to find out in what aspect one sample is different from another and which variables contribute most to this difference (15). In the present study, PCA was used to reduce the dimension from 18 variables to 2 or 3 principal components which kept most of the original information intact in the data set. The PCA analysis showed that lightness parameter of infusion (L1) had the largest loading (0.966), and GCg (0.928) the next with component 1. The two parameters were the dominating features in the first principal component (Table 2). Accordingly, the dominating features with the second principal component were GC (0.788) and EGC (0.716), and those with the third principal component were ECg (0.790) and total catechins (0.723) (Table 2). L1, GC, and ECg showed the highest weights in the principal

Table 2. Component matrix

Principal component[1)]	1	2	3
GC	0.540	0.788	0.156
EGC	-0.545	0.716	0.118
C	0.919	0.351	0.100
EC	0.808	0.337	0.006
EGCg	-0.684	-0.103	0.686
GCg	0.928	0.273	0.151
ECg	-0.119	-0.542	0.790
Cg	0.901	0.052	0.220
Total catechins	0.107	0.590	0.723
Soluble solids	0.860	-0.149	0.370
Amino acids	0.695	-0.457	-0.188
Caffeine	-0.442	-0.178	0.635
L1	0.966	0.003	0.100
A1	0.743	-0.343	0.346
B1	-0.867	0.296	0.176
L2	0.322	-0.671	-0.011
A2	0.202	-0.658	0.156
B2	-0.655	-0.148	-0.139

[1)]3-Components extracted by PCA. GC, (+)-gallocatechin; EGC, (-)-epigallocatechin; C, (+)-catechin; EC, (-)-epicatechin; EGCg, (-)-epigallocatechin gallate; GCg, (+)-gallocatechin gallate; ECg, (-)-epicatechin gallate; Cg, (+)-catechin gallate; Total, total concentration of catechins.

Table 3. Extraction sums of squared loadings[1)]

Principal component	Total	% Variance	Cumulative %
1	8.48	47.11	47.11
2	3.47	19.30	66.41
3	2.52	13.97	80.38
4	1.76	9.75	90.13

[1)]Early 4 principal components were extracted by PCA.

components 1-3, respectively. The cumulative loading of components 1-3 was 80.38% of total loadings in the data set with the 18 variables (Table 3).

If a 2-dimensional plot in the space defined by the dominating variables of first and the second principal components was examined, a separation between samples from China (sample No.1-7) and Korea (sample No. 8-24) was found (Fig. 4). On a 3-dimensional plot of L1, GC, and ECg, samples from China and from Korea were differentiated distinctly (Fig. 5). Concentration of total catechins was confirmed to be an important indicator of tea quality (16) and it was the next most correlated to the third principal component (Table 2). When ECg was replaced by total catechins on the 3-dimensional plot, the tea samples of the two origins were clearly differentiated and within-group samples were distributed more closely (Fig. 6) because within-group variation of total catechins level was less than that of ECg.

It is known that characteristics of tea from various origins are related to tea cultivar, environment conditions, and techniques of cultivating and processing (16). The above results showed that L1, GC, ECg, and total catechins

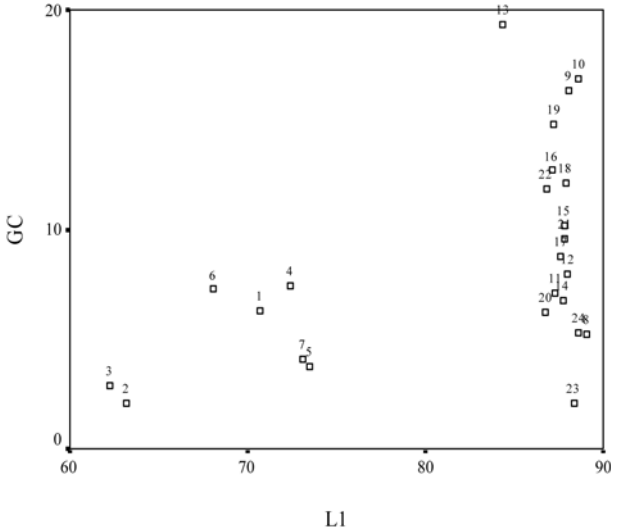

Fig. 4. Separation of tea samples from different origins on 2-dimensional plot. L1, Infusion lightness-darkness; GC, (+)-gallocatechin. Samples No.1-7 were from China and No. 8-24 were from Korea.

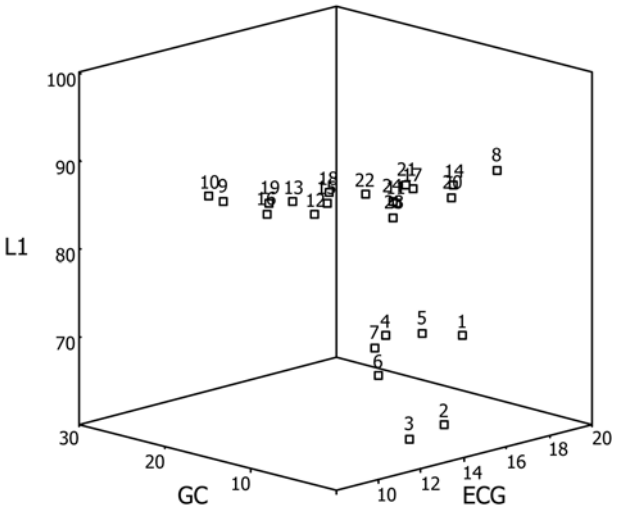

Fig. 5. Separation of tea samples from different origins on 3-dimensional plot. L1, Infusion lightness-darkness; GC, (+)-gallocatechins; ECG, (-)-epicatechin gallate. Samples No.1-7 were from China and No. 8-24 were from Korea.

were potential indicators of origins of tea samples from China and from Korea. L1 is a parameter of lightness-darkness of tea infusion and it is related to chemical composition of the infusion including catechins which were dependent on tea cultivar, cultivation, and processing (16,17,22,23). L1 was one of the integrated indicators of the tea samples (17,23). GC is absent in fresh leaf and it is transformed from EGC under conditions of elevated temperature during processing (19). The level of GC will reflect the processing procedures of various tea estates. This explains why L1, GC, ECg, and total catechins were dominating features to discriminate tea samples from China and those from Korea.

The present study shows that it is possible to differentiate

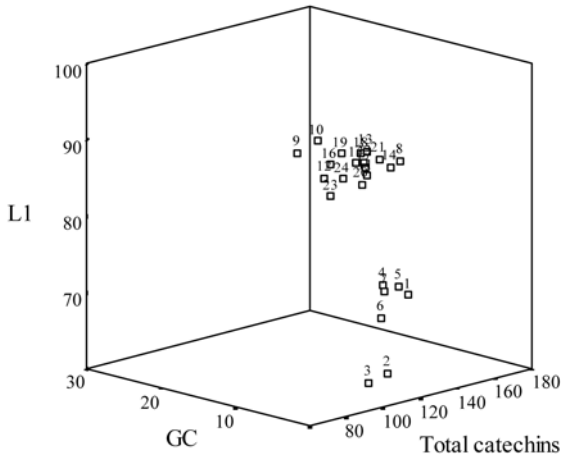

Fig. 6. Separation of tea samples from different origins on 3-dimensional plot. L1, Infusion lightness-darkness; GC, (+)-gallocatechins. Total catechins: total concentration of catechins. Samples No.1-7 were from China and No. 8-24 were from Korea.

and classify green tea samples from China and Korea using quality control parameters and applying PCA. The significance of this study was that the assessment of quality and the discrimination of geographical origin could be carried out using a same set of quality control data.

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