



Geographical origin of imported and domestic teas (*Camellia sinensis*) from Turkey as determined by stable isotope signatures

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ABSTRACT

In this study, stable isotope signatures ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and δD) of both tea leaves and tea infusions were investigated to identify the geographical origin of Turkish domestic and imported tea samples. Sixteen domestic tea samples collected from different locations in the Black Sea Region, which produces almost 100% of tea in Turkey, and 11 imported tea samples (Kenya, India, Sri Lanka, Indonesia, and China) purchased from importers were studied. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and δD in the samples were determined using isotope ratio mass spectrometry (IR-MS). $\delta^{13}\text{C}$ in the samples ranged from -29.18 ± 0.01 to -25.7 ± 0.2 , while $\delta^{15}\text{N}$ ranged between 1.1 ± 0.2 and 5.2 ± 0.8 . However, δD in the samples were found to be in the range from 56.5 ± 0.3 to 72 ± 1 . The classifications of the tea samples into domestic and imported tea samples were achieved with 100% accuracy using multivariate statistical analyses (principal component analysis, PCA, and hierarchical cluster analysis, HCA). In conclusion, the domestic tea samples had a distinctive isotopic fingerprint and the isotopic ratios used in the study can be significant predictors in determination of the geographical source of Turkish tea.

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Introduction

Tea is one of the world's most popular agro products and most widely consumed beverages. China is the largest tea producer in the world, with a production quantity of 1,700,000 tons/year, followed by India (1,000,000 tons/year), Kenya (369,400 tons/year), Sri Lanka (330,000 tons/year), and Turkey (225,000 tons/year).^[1] The price of tea varies depending on brand and geographical origin, so it is important to determine the geographical origins of tea to protect consumers and the global food trade.

Various analytical techniques have been employed to characterize the geographic origins of tea samples such as atomic absorption spectrometry (AAS), inductive coupled plasma atomic emission spectrometry (ICP-AES), and ICP mass spectrometry (ICP-MS) for determination of elemental contents;^[2–4] liquid chromatography mass spectrometry (LC-MS) for determination of metabolic markers;^[5] gas chromatography (GC) for detection of volatile components^[6,7]; capillary electrophoresis for testing major amino acid and theaflavin contents^[8]; high-performance liquid chromatography (HPLC) for detecting phenolic constituents and free amino acid contents;^[9,10] and colorimetric measurements for determination of free amino acid contents.^[11] However, recent reports show that the isotope ratio mass spectrometry (IR-MS) technique has gained increasing importance in discriminating the geographical origins of tea samples.^[2,12,13] This technique has also been widely used in the geographical assessment of a wide

variety of food commodities such as coffee beans,^[14,15] honeys,^[16] olive oils,^[17] cabbages,^[18] sugars,^[19,20] milks,^[21,22] potatoes,^[23] rice,^[24,25] sheep tissues,^[26] cattle,^[27–29] lamb,^[30–32] and swine.^[33]

IR-MS is a technique that can discriminate chemically identical compounds based on their isotopic compositions.^[34] In particular, determinations of isotopic ratios of carbon, nitrogen, hydrogen, and oxygen, so-called bio-elements, have been used successfully to provide information on the origin of food products. The bio-elements' isotopic ratios of agricultural products depend on various factors. The measurement of isotope ratios of oxygen and hydrogen in water is appropriate for the characterization of geographical differences because they are strongly dependent on latitude.^[35] The ratio of $^2\text{H}/^1\text{H}$ is also related to the meteorological cycle of evaporation, condensation, and precipitation of water for food provenance.^[36] The $^{13}\text{C}/^{12}\text{C}$ ratio is affected by the biochemical fixing of CO_2 (C3 or C4-plant) and only slightly modified by climatic effects. C3 plants use the Calvin photosynthetic pathway to assimilate CO_2 . During this cycle, the plants discriminate against ^{13}C and therefore possess relatively lower $^{13}\text{C}/^{12}\text{C}$ ratios than C4 plants that utilize the more energy efficient Hatch–Slack pathway. Because C4 plants are more common in warmer climates at lower latitudes (such as the tropics) and C3 plants predominate at higher latitudes, a gradient decrease of $^{13}\text{C}/^{12}\text{C}$ in plant material from the equator to the poles, which can also be used as a proxy for determination of geographical origin, is observed.^[36] There is a general agreement that C3 plants have $^{13}\text{C}/^{12}\text{C} = \delta^{13}\text{C}$ values from -23% to -28% , while C4 plants have values from -9% to -15% .^[37,38] In addition, $^{15}\text{N}/^{14}\text{N}$ signatures of plants can be affected by a wide variety of natural environmental factors such as water stress, climatic conditions, salinity, and soil processes, particularly de-nitrification and mineralization.^[39–41] Therefore, isotopic ratios of these elements can be used as a good descriptor for different food products, providing unique and representative fingerprints that make it possible to differentiate between food samples and their geographical origins.

IR-MS data can be used in combination with chemometric techniques for better determination of geographical originality. The chemometrical processing of the isotopic results can provide a more accurate classification of the samples according to their geographical origins. Multivariate statistical analysis (MSA) is a useful chemometrical technique for determination of common patterns in data distribution, leading to a reduction of the initial dimension of data sets and facilitating its interpretation.^[42] Several applications of MSA, mainly principal component analysis (PCA) and hierarchical cluster analysis (HCA), can be found in the literature with respect to establishing the origin of various food products.^[43–45] The objective of this research was to develop a novel method that uses carbon, nitrogen, and hydrogen isotopes in combination with MSA to discriminate Turkish tea from the foreign teas.

Materials and methods

Chemicals

The following carbon isotopic reference materials were used as reference materials in this study: sucrose (IAEA CH6) $\delta^{13}\text{C}_{\text{VPDB}} -10.45 \pm 0.04\%$, cellulose (IAEA CH3) $\delta^{13}\text{C}_{\text{VPDB}} -24.72 \pm 0.05\%$, and potassium nitrate (USGS 34) $\delta^{15}\text{N}_{\text{air}} -1.8 \pm 0.2\%$, $\delta^{18}\text{O}_{\text{VSMOW}} -27.9 \pm 0.6\%$. The isotopic standards were obtained from Cambridge Isotope Laboratories (Andover, MS, USA). The water, used for infusion, was produced by an ultrapure (18.2 M Ω cm at 25 °C) purification system from Millipore (Bedford, MA, USA).

Instrumentation

The $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and δD values of tea samples were determined on a Thermo-Electron Flash EA 2000 elemental analyser (EA) coupled via a Thermo Finnigan ConFlo IV Interface to a Delta V Thermo Finnigan Isotope Ratio Mass Spectrometer (Thermo, Waltham, MA, USA). Thermo Electron Isodat[®] version 2.38 was used as the data processing software.



Figure 1. Sampling locations of domestic teas collected from different districts of Turkey. 1, Kalkandere; 2, Salarha; 3, Ortopazar; 4, Arhavi; 5, Sürmene; 6, Iyidere; 7, Eskipazar; 8, Fındıklı; 9, Araklı; 10, Cayeli; 11, Tirebolu; 12, Cumhuriyet; 13, Hopa; 14, Pazar; 15, Derepaşarı; 16, Ardeşen.

Sampling

Sixteen black tea samples were collected from tea plantation areas located in the east Black Sea region of Turkey. It was reported that the eastern Black Sea region accounts for nearly 100% of all tea production in Turkey.^[46] All of the tea plantation areas in Turkey are located in this region and 66% of the total tea plantation areas of Rize, whilst the rest of them are of Trabzon (20%), Artvin (11%), Giresun and Ordu (3%), respectively.^[47] Collected samples from the eastern Black Sea region were marked as domestic tea samples. Sampling locations of the domestic tea samples are illustrated in Fig. 1 and these locations were Kalkandere (Rize), Salarha (Rize), Ortopazar (Rize), Arhavi (Artvin), Sürmene (Trabzon), Iyidere (Rize), Eskipazar (Trabzon), Fındıklı (Rize), Araklı (Trabzon), Cayeli (Rize), Tirebolu (Giresun), Cumhuriyet (Rize), Hopa (Artvin), Pazar (Rize), Derepaşarı (Rize), and Ardeşen (Rize). Latitude and longitude information of tea plantation locations are presented in Table 1. An additional 11 imported tea samples from India, Sri Lanka, Indonesia, Kenya, and China were purchased from commercial importers. The geographical origins of the imported tea samples were recorded using their brands. The samples were collected in November 2012 and stored at room temperature until further processing.

Sample preparation

Fifty grams of tea leaves were ground in a crusher after drying in an incubator at 70°C for 2 h and then passed through a 140-mesh sieve. The resulting powder, comprising particles of less than 100 µm diameter, was collected for stable isotope analysis. The sieved portions were directly analysed in the IR-MS instrument as a general agreement for standardized IR-MS analysis. Additionally, an extraction procedure was tried to achieve more reproducible results and the results obtained from the directly analysed samples and extracted samples were compared. For the extraction procedure, a 100 mL of boiled ultrapure water was added to 20 g of a black tea sample. The mixture was held for 20 min at 100°C in an ultrasonic bath and left to cool at room temperature. The prepared mixture was filtered to obtain a clear solution and 5 mL of the supernatant was evaporated to dryness overnight in an incubator at 70°C. The dried material was then introduced into the IR-MS. Approximately 150 ± 50 µg of materials (reference standard, ground tea sample, or extracted tea sample) was weighed in a small tin capsule by an MX-2 ultra microbalance and placed into the auto-sampler unit of an elemental analyser. Each material was measured three times. The results obtained

Table 1. Isotope ratios of C, N, and H in tea extracts.

No	Location	GPS Coordinates		Origin	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	δD
1	Kalkandere/Rize	40.928907 N	40.439113 E	Domestic	$-29.06 \pm 0.07^{\text{kl}}$	$1.71 \pm 0.08^{\text{ef}}$	$61.4 \pm 0.8^{\text{ghi}}$
2	Salarha/Rize	40.974542 N	40.530693 E	Domestic	$-28.30 \pm 0.03^{\text{ghij}}$	$1.4 \pm 0.4^{\text{ef}}$	$62.1 \pm 0.2^{\text{g}}$
3	Ortapazar/Rize	40.978302 N	40.472194 E	Domestic	$-28.4 \pm 0.3^{\text{ghijk}}$	$2.42 \pm 0.10^{\text{def}}$	$61.9 \pm 0.7^{\text{gh}}$
4	Arhavi/Artvin	41.351617 N	41.305218 E	Domestic	$-28.5 \pm 0.2^{\text{hijkl}}$	$2.60 \pm 0.06^{\text{cde}}$	$61.7 \pm 0.3^{\text{ghi}}$
5	Sürmene/Trabzon	40.911914 N	40.134416 E	Domestic	$-28.31 \pm 0.02^{\text{ghij}}$	$2.1 \pm 0.3^{\text{def}}$	$61.6 \pm 0.1^{\text{ghi}}$
6	Iyidere/Rize	40.991840 N	40.332580 E	Domestic	$-28.34 \pm 0.03^{\text{ghij}}$	$2.40 \pm 0.02^{\text{def}}$	$61.4 \pm 0.8^{\text{ghi}}$
7	Eskipazar/Trabzon	40.961717 N	40.294345 E	Domestic	$-28.13 \pm 0.03^{\text{fgh}}$	$1.1 \pm 0.2^{\text{f}}$	$61.2 \pm 0.1^{\text{hi}}$
8	Fındıklı/Rize	41.260651 N	41.130355 E	Domestic	$-28.944 \pm 0.003^{\text{ijkl}}$	$2.5 \pm 0.5^{\text{de}}$	$61.0 \pm 0.3^{\text{ijk}}$
9	Araklı/Trabzon	40.931289 N	40.063209 E	Domestic	$-28.43 \pm 0.09^{\text{ghijkl}}$	$2.7 \pm 0.67^{\text{cde}}$	$59.3 \pm 0.8^{\text{no}}$
10	Cayeli/Rize	41.081986 N	40.708144 E	Domestic	$-28.7 \pm 0.3^{\text{hijkl}}$	$1.67 \pm 0.03^{\text{ef}}$	$59.74 \pm 0.09^{\text{lmn}}$
11	Tirebolu/Giresun	41.005680 N	38.847362 E	Domestic	$-28.5 \pm 0.2^{\text{hijkl}}$	$3 \pm 1^{\text{cde}}$	$60.49 \pm 0.03^{\text{lmk}}$
12	Cumhuriyet/Rize	41.044447 N	40.587704 E	Domestic	$-28.7 \pm 0.3^{\text{hijkl}}$	$2.2 \pm 0.8^{\text{def}}$	$59.6 \pm 0.3^{\text{mno}}$
13	Hopa/Artvin	41.388817 N	41.414003 E	Domestic	$-29.1 \pm 0.1^{\text{kl}}$	$1.4 \pm 0.1^{\text{ef}}$	$58.4 \pm 0.4^{\text{qp}}$
14	Pazar/Rize	41.182981 N	40.900250 E	Domestic	$-29.177 \pm 0.009^{\text{l}}$	$3 \pm 1^{\text{bcd}}$	$58.8 \pm 0.3^{\text{op}}$
15	Derepazarı/Rize	41.024623 N	40.426618 E	Domestic	$-28.9 \pm 0.2^{\text{ijkl}}$	$3 \pm 1^{\text{cde}}$	$57.7 \pm 0.2^{\text{q}}$
16	Ardesen/Rize	41.189927 N	40.974819 E	Domestic	$-29.12 \pm 0.04^{\text{kl}}$	$2 \pm 2^{\text{def}}$	$56.5 \pm 0.3^{\text{r}}$
17	Indian			Imported	$-28.18 \pm 0.01^{\text{ghi}}$	$4.1 \pm 0.2^{\text{ab}}$	$64.7 \pm 0.3^{\text{f}}$
18	Sri Lanka			Imported	$-27.204 \pm 0.006^{\text{cd}}$	$4.7 \pm 0.6^{\text{a}}$	$65.7 \pm 0.9^{\text{e}}$
19	Indonesia			Imported	$-27.21 \pm 0.03^{\text{cd}}$	$3.4 \pm 0.2^{\text{bcd}}$	$60.2 \pm 0.4^{\text{klm}}$
20	Kenya			Imported	$-28 \pm 1^{\text{defg}}$	$3.9 \pm 0.4^{\text{abc}}$	$72 \pm 1^{\text{a}}$
21	Kenya			Imported	$-26.62 \pm 0.05^{\text{bc}}$	$5.2 \pm 0.8^{\text{a}}$	$70.7 \pm 0.5^{\text{b}}$
22	Sri Lanka			Imported	$-27.4 \pm 0.8^{\text{de}}$	$4.5 \pm 0.5^{\text{ab}}$	$67.9 \pm 0.3^{\text{c}}$
23	Sri Lanka			Imported	$-28.06 \pm 0.02^{\text{defg}}$	$4.2 \pm 0.3^{\text{ab}}$	$66.1 \pm 0.4^{\text{de}}$
24	Sri Lanka			Imported	$-28.44 \pm 0.02^{\text{hijkl}}$	$2.4 \pm 0.3^{\text{def}}$	$66.8 \pm 0.4^{\text{d}}$
25	China			Imported	$-27 \pm 1^{\text{def}}$	$3.35 \pm 0.02^{\text{bcd}}$	$65.3 \pm 0.1^{\text{ef}}$
26	China			Imported	$-26.4 \pm 0.7^{\text{b}}$	$4.1 \pm 0.3^{\text{ab}}$	$66.1 \pm 0.3^{\text{de}}$
27	China			Imported	$-25.6 \pm 0.2^{\text{a}}$	$2.3 \pm 0.9^{\text{def}}$	$59.3 \pm 0.1^{\text{no}}$

Values are presented as means \pm standard deviations ($n = 3$). For each parameter, different letters within a row indicate difference between cultivars with Duncan's multiple range test ($p < 0.05$).

from analysis before (directly analysed samples) and after extraction were compared to evaluate the efficacy of the method's performance.

Analytical conditions

The oxidation and reduction reactors of the IR-MS equipment were heated to 1020°C and 650°C, respectively. The oven temperature was set to 50°C. The carrier gas (He) flow was 110 mL min⁻¹. The flow rate of O₂ gas flash combustion was 175 mL min⁻¹. The carbon, nitrogen, and hydrogen in the samples were converted into CO₂, N₂, and H₂, respectively, and ionised. The ionised heavy and light isotopes were separated with a magnetic field and detected by the Faraday collector. CO₂ of known $\delta^{13}\text{C}$ content was introduced as a pulse of reference gas. The CO₂ reference gas pulse was introduced four times (20 s each) at the beginning of each run. The $\delta^{15}\text{N}$ composition was measured relative to a reference gas pulse of known $\delta^{15}\text{N}$ composition. Hydrogen gas of known δD was inserted as pulses of reference gas. The gases were passed into the IR-MS and the isotope compositions were calculated. The isotopic values were expressed using the following formula:

$$\delta = \left[\left(\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 1,000 \right] / \text{‰}$$

where R_{sample} and R_{standard} are the isotopic ratios of the sample and the standard materials, respectively. All analyses of the samples were replicated three times and the average and standard deviations were calculated. The calculated results were accepted if the standard deviation of three results of an introduced sample was $< 0.2\%$. The run time of the experiment was approximately 15 min for a single run. $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, and $^2\text{H}/^1\text{H}$ were measured as $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and δD , respectively.

Method validation

The validation of the method was carried out by the following parameters: selectivity, stability, linearity, accuracy, and repeatability.^[48] Selectivity was controlled by introducing a reference gas pulse and reference materials (IAEA CH6, IAEA CH3, and USGS 34). Stability of an analytical method means that the sample provides equivalent quantitation both during and after storage. Stability testing of the method was performed by determining long-term stock stability by using a reference material. Linearity of an analytical method is its ability to elicit test results that are directly, or by means of well-defined mathematical transformations, proportional to the concentration of analytes in samples within a given range. The linearity study was carried out using standard samples of IAEA CH6 and IAEA CH3. Accuracy can be described as the closeness of agreement between the value that is adopted, as a conventional, true, or accepted reference value, and the value found. Accuracy was calculated as the absolute error between the reference material and the found value. The repeatability of the isotope ratio measurement was found by measuring a set of prepared and mounted samples on different occasions, preferably separated by several days under normal operating conditions. The repeatability of the method was calculated using 12 consequent measurements and expressed as the per cent relative standard deviation (RSD%).

Statistical analyses

The results were statistically analysed using analysis of variance (ANOVA, $p < 0.01$). Significant means were subjected to analysis using Duncan's multiple range test ($p < 0.05$). The statistical analyses were performed using the Statistical Analysis System software.^[49] In this study, pattern recognition methods were applied involving display methods such as PCA and HCA. The statistical package Minitab® Release 14.1 was used for these analyses.^[50]

Results and discussion

The method used demonstrated acceptable performance for the analysis of isotopic ratios of tea samples. Isotopic peaks of $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, and $^2\text{H}/^1\text{H}$ were well separated with no interfering peaks and noises. According to the result of stability, there were no significant differences among the isotopic values of the reference materials during the time interval (2 weeks). The linearity of the method has high correlation ($r^2 = 0.992$). The accuracy of the method was satisfactory (0.53‰). The RSD value for the determination of repeatability was found to be 0.08. The results from the method validation study showed that the method adequately met the validation criteria and was successfully used to analyse the isotopic ratios in tea samples. However, the other parameters such as sample preparation step should be optimized.

The sample introduction step of IR-MS analysis is a critical stage for obtaining reproducible, accurate, and precise results. The amount of sample used for the EA-based IR-MS system is extremely small. It has been reported in the literature that the sample weight introduced to the IR-MS system should be approximately 150–200 μg .^[51–53] However, a weighted sample may be represented by different parts of a plant body such as stem or leaf and/or other non-interested materials such as dust and debris. This can result in an increased standard deviation in the repetitive analysis for each sample. In this study, ground tea samples were introduced into the IR-MS in two ways in order to evaluate the effects of sample uniformity on the analytical results: a) direct introduction (without extraction), and b) after an extraction process. The results showed that there were significant differences between the standard deviations of the directly introduced samples and the extracted samples. The obtained results from the extracted samples were found to be more reproducible than those of the directly introduced samples. Therefore, the results of the extracted samples were used for the determination of geographical origins. The results of isotopic ratios of C, N, and H for the extracted tea samples are shown in Table 1 and a statistical evaluation of the data is presented in Table 2. $\delta^{13}\text{C}$ in the samples ranged from -29.18 ± 0.01 to -25.7 ± 0.2 while $\delta^{15}\text{N}$ ranged between 1.1 ± 0.2 and 5.2 ± 0.8 . δD values in the samples were found to be in the range from 56.5 ± 0.3 to 72

Table 2. The range, mean, and median isotope ratios obtained in domestic and imported tea samples.

Isotopes	Domestic			Imported		
	Range	Mean	Median	Range	Mean	Median
$\delta^{13}\text{C}$	-29.18 ↔ -28.13	-28.7 ± 0.3	-28.62	-28.44 ↔ -25.66	-27.3 ± 0.8	-27.41
$\delta^{15}\text{N}$	1.08 ↔ 3.19	2.2 ± 0.6	2.39	2.35 ↔ 5.15	3.8 ± 0.9	4.11
δD	56.54 ↔ 62.14	60 ± 2	60.74	59.33 ↔ 72.24	66 ± 4	66.12

± 1. The separation among geographical origins of domestic tea samples was checked by a multiple range test. According to the result of the test, the orders of geographical origin based on the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and δD values in the domestic tea samples were not found to be significant. This result can be explained by the fact that the locations of domestic samples were close to each other. The ranges of isotopic ratios in the samples were in good agreement with those obtained in some previous studies.^[2,13] $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ distributions of three different Chinese teas analysed in the study were found to be in the range from -27.46 to -25.66 and from 2.35 to 4.14, respectively. These results are consistent with those of Zhang et al (2012)^[13] who reported the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ levels of Chinese black teas were from -26.42 to -25.49 and from 1.46 to 2.47, respectively.

There were significant differences between the domestic and imported tea samples. The $\delta^{13}\text{C}$ results of domestic tea samples were slightly lower than those of imported tea samples. These low values could be explained by the high humidity in the locations of sample sites. Black Sea region, where the samples were collected, has an oceanic climate with high and evenly distributed rainfall the year round. The coast of the region receives the greatest amount of precipitation and is the only region of Turkey that receives high precipitation throughout the year. Similarly, the $\delta^{15}\text{N}$ values of the domestic tea samples were found to be lower than those of imported tea samples. The isotopic compositions of nitrogen mainly depend on temperature variation, soil nutrition, and de-nitrification and are influenced by the type of fertilizer used. In addition, significant differences were observed between the δD results of domestic and imported tea samples. Although the mean level of δD was found to be 60 ± 2 in domestic tea samples, the level was 65 ± 4 for imported tea samples. Variation among the δD compositions of the samples can be attributed to the differences of latitudes and natural environmental factors such as meteorological conditions, water stress, and mineralization in the locations tea plants are grown.

A three-dimensional scatter plot (Fig. 2) of the three variables ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and δD) was generated in order to see possible clustering of the 27 tea samples. As seen from the figure, imported tea samples have larger $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and δD values. The figure also shows that some of the domestic tea samples overlapped with imported samples. As a result, it is difficult to deduce an overall conclusion on the differences in isotopic contents between domestic and imported tea samples due to the limitations of single-variable statistical analysis. Therefore, pattern recognition procedures such as PCA and HCA were used to achieve more reliable results about the geographical locations of teas.

Principal component analysis

PCA describes the relationships of multidimensional data arrays between variables and objects in a rigorous and comprehensive way. PCA greatly reduces data and presents different manners of interpretation.^[45] In mathematical terms, PCA relies upon an eigenvector decomposition of the covariance or correlation matrix.^[54] In the study, 89.6% of the total variability among the three variables in the original data was explained by the first two principal components (PC1–2), where PC1 and PC2 contributed 72.12% and 17.63% of the total variance, respectively. The results suggest that the first two components are significant factors in determining the geographical origin of tea samples. The scatter plot of the first two discriminate functions from the analysis of the samples is presented in Fig. 3. While examining the scatter plot graphic, a clear separation between domestic and imported tea samples from different geographical origins was revealed.

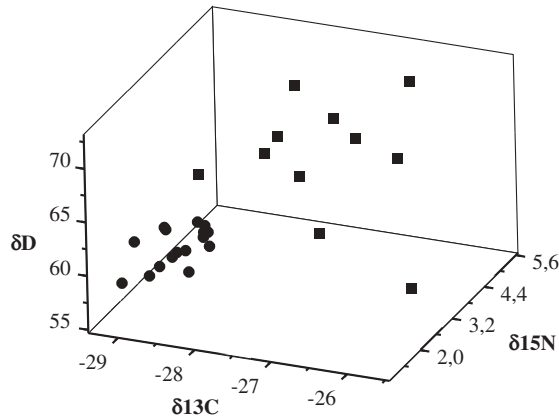


Figure 2. Scatter plot of the isotope ratios of C, N, and H, showing separation between domestic (●) and imported tea samples (■).

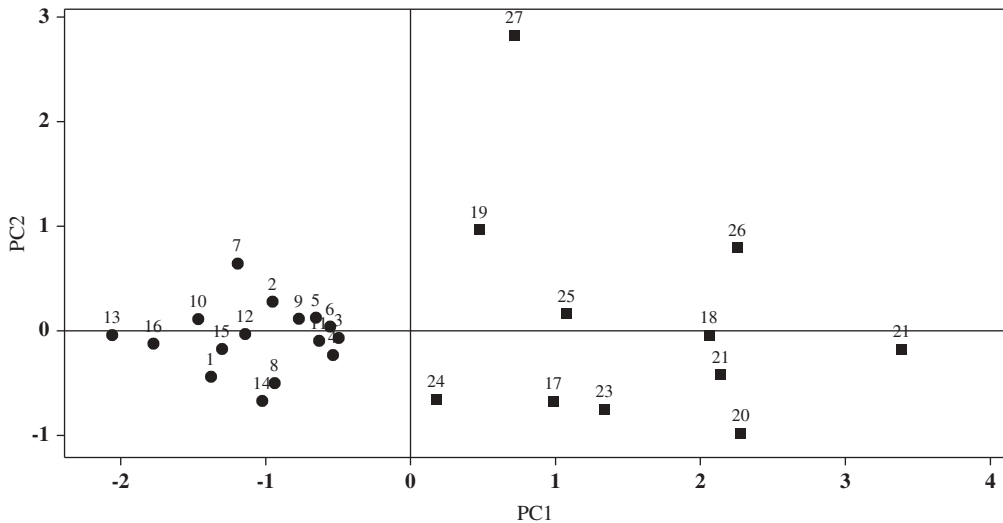


Figure 3. Scatter plot of the first two principal components (PC1-2) showing separation between domestic (●) and imported tea samples (■).

HCA analysis

HCA can be considered to be an alternative to PCA and is the most commonly applied cluster analysis (CA) method for environmental analysis. HCA examines distances between data set and samples. The result obtained could be presented in a two-dimensional plot called a dendrogram.^[45] HCA of samples was carried out using the selected isotopic contents as variables, the squared Euclidean distance as a similarity measurement, and Ward's method as an amalgamation rule. The resulting cluster dendrogram is shown in Fig. 4. As can be seen from the figure, there is a clear discrimination between the domestic and imported tea samples. The first cluster was composed of domestic tea samples (from 1 to 16), followed by a second cluster that included imported tea samples (from 17 to 27). Therefore, it can be concluded that two different origins of the samples can be clearly clustered together, using HCA based on the isotopic content in tea extracts. Similar results were obtained in both PCA and HCA.

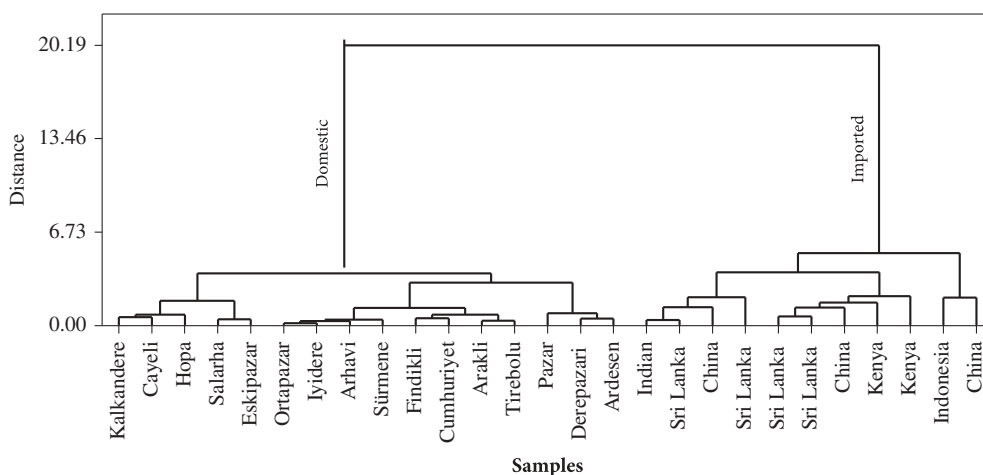


Figure 4. Dendrogram of cluster analysis.

Conclusion

This study has shown that the geographical discrimination of tea origins can be easily achieved through the extraction technique employed here. The ranges of isotopic ratios of $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, and $^2\text{H}/^1\text{H}$ in extracts of tea samples with different geographical origins were in good agreement with the levels obtained in previous studies. However, it was found that there are considerable isotopic differences between domestic and imported tea samples. The classification of these samples was accomplished with 100% accuracy using PCA and HCA. In conclusion, domestic tea samples had a distinctive isotopic fingerprint, and the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and δD values can be significant predictors in discriminating the geographical source of tea. This technique may be adapted to determine the geographical origins of other infused products such as medicinal herbs (linden, thyme, ginger, cinnamon, etc.) and coffee.

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References

1. FAOSTAT. Statistics Division 2016. Available via FAO (Food and Agriculture Organization). <http://faostat.fao.org/site/567/default.aspx#ancor>. (accessed 10 June 2016). **2012**.
2. Pilgrim, T.S.; Watling, R.J.; Grice, K. Application of Trace Element and Stable Isotope Signatures to Determine the Provenance of Tea (*Camellia sinensis*) Samples. *Food Chemistry* **2010**, *118*, 921–926.
3. Marcos, A.; Fisher, A.; Rea, G.; Hill, S.J. Preliminary Study Using Trace Element Concentrations and a Chemometrics Approach to Determine the Geographical Origin of Tea. *Journal of Analytical Atomic Spectrometry* **1998**, *13*, 521–525.
4. Moreda-Piñeiro, A.; Fisher, A.; Hill, S.J. The Classification of Tea According to Region of Origin using Pattern Recognition Techniques and Trace Metal Data. *Journal of Food Composition and Analysis* **2003**, *16*, 195–211.

5. Fraser, K.; Lane, G.A.; Otter, D.E.; Hemar, Y.; Quek, S.-Y.; Harrison, S.J.; Rasmussen, S. Analysis of Metabolic Markers of Tea Origin by UHPLC and High Resolution Mass Spectrometry. *Food Research International* **2013**, *53*, 827–835.
6. Tontul, I.; Torun, M.; Dincer, C.; Sahin-Nadeem, H.; Topuz, A.; Turna, T.; Ozdemir, F. Comparative Study on Volatile Compounds in Turkish Green Tea Powder: Impact of Tea Clone, Shading Level and Shooting Period. *Food Research International* **2013**, *53*, 744–750.
7. Yang, Z.; Baldermann, S.; Watanabe, N. Recent Studies of the Volatile Compounds in Tea. *Food Research International* **2013**, *53*, 585–599.
8. Hsieh, M.-M.; Chen, S.-M. Determination of Amino Acids in Tea Leaves and Beverages Using Capillary Electrophoresis with Light-emitting Diode-induced Fluorescence Detection. *Talanta* **2007**, *73*, 326–331.
9. Beelders, T.; Sigge, G.O.; Joubert, E.; de Beer, D.; de Villiers, A. Kinetic Optimisation of the Reversed Phase Liquid Chromatographic Separation of Rooibos Tea (*Aspalathus linearis*) Phenolics on Conventional High Performance Liquid Chromatographic Instrumentation. *Journal of Chromatography A* **2012**, *1219*, 128–139.
10. Wang, L.; Xu, R.; Hu, B.; Li, W.; Sun, Y.; Tu, Y.; Zeng, X. Analysis of Free Amino Acids in Chinese Teas and Flower of Tea Plant by High Performance Liquid Chromatography Combined with Solid-phase Extraction. *Food Chemistry* **2010**, *123*, 1259–1266.
11. Chen, L.; Chen, Q.; Zhang, Z.; Wan, X. A Novel Colorimetric Determination of Free Amino Acids Content in Tea Infusions with 2,4-dinitrofluorobenzene. *Journal of Food Composition and Analysis* **2009**, *22*, 137–141.
12. Lagad, R.A.; Alamelu, D.; Laskar, A.H.; Rai, V.K.; Singh, S.K.; Aggarwal, S.K. Isotope Signature Study of the Tea Samples Produced at Four Different Regions in India. *Analytical Methods* **2013**, *5*, 1604–1611.
13. Zhang, L.; Pan, J.R.; Zhu, C. Determination of the Geographical Origin of Chinese Teas Based on Stable Carbon and Nitrogen Isotope Ratios. *Journal of Zhejiang University Science B* **2012**, *13*, 824–830.
14. Liu, H.-C.; You, C.-F.; Chen, C.-Y.; Liu, Y.-C.; Chung, M.-T. Geographic Determination of Coffee Beans Using Multi-element Analysis and Isotope Ratios of Boron and Strontium. *Food Chemistry* **2014**, *142*, 439–445.
15. Rodrigues, C.L.; Maia, R.; Miranda, M.; Ribeirinho, M.; Nogueira, J.M.F.; Máguas, C. Stable Isotope Analysis for Green Coffee Bean: A Possible Method for Geographic Origin Discrimination. *Journal of Food Composition and Analysis* **2009**, *22*, 463–471.
16. Dinca, O.-R.; Ionete, R.E.; Popescu, R.; Costinel, D.; Radu, G.-L. Geographical and Botanical Origin Discrimination of Romanian Honey Using Complex Stable Isotope Data and Chemometrics. *Food Analytical Methods* **2015**, *8*, 401–412.
17. Camin, F.; Larcher, R.; Nicolini, G.; Bontempo, L.; Bertoldi, D.; Perini, M.; Schlicht, C.; Schellenberg, A.; Thomas, F.; Heinrich, K.; Voerkelius, S.; Horacek, M.; Ueckermann, H.; Froeschl, H.; Wimmer, B.; Heiss, G.; Baxter, M.; Rossmann, A.; Hoogewerff, J. Isotopic and Elemental Data for Tracing the Origin of European Olive Oils. *Journal of Agricultural and Food Chemistry* **2010**, *58*, 570–577.
18. Bong, Y.-S.; Shin, W.-J.; Gautam, M.K.; Jeong, Y.-J.; Lee, A.R.; Jang, C.-S.; Lim, Y.-P.; Chung, G.-S.; Lee, K.-S. Determining the Geographical Origin of Chinese Cabbages Using Multielement Composition and Strontium Isotope Ratio Analyses. *Food Chemistry* **2012**, *135*, 2666–2674.
19. Rodushkin, I.; Baxter, D.C.; Engström, E.; Hoogewerff, J.; Horn, P.; Papesch, W.; Watling, J.; Latkoczy, C.; van der Peijl, G.; Berends-Montero, S.; Ehleringer, J.; Zdanowicz, V. Elemental and Isotopic Characterization of Cane and Beet Sugars. *Journal of Food Composition and Analysis* **2011**, *24*, 70–78.
20. Martin, G.J.; Danho, D.; Vallet, C. Natural Isotope Fractionation in the Discrimination of Sugar Origins. *Journal of the Science of Food and Agriculture* **1991**, *56*, 419–434.
21. Lin, G.P.; Rau, Y.H.; Chen, Y.F.; Chou, C.C.; Fu, W.G. Measurements of δD and $\delta^{18}O$ Stable Isotope Ratios in Milk. *Journal of Food Science* **2003**, *68*, 2192–2195.
22. Luo, D.; Dong, H.; Luo, H.; Xian, Y.; Guo, X.; Wu, Y. Multi-Element (C, N, H, O) Stable Isotope Ratio Analysis for Determining the Geographical Origin of Pure Milk from Different Regions. *Food Analytical Methods* **2016**, *9*, 437–442.
23. Longobardi, F.; Casiello, G.; Sacco, D.; Tedone, L.; Sacco, A. Characterisation of the Geographical Origin of Italian Potatoes, based on Stable Isotope and Volatile Compound Analyses. *Food Chemistry* **2011**, *124*, 1708–1713.
24. Suzuki, Y.; Chikaraishi, Y.; Ogawa, N.O.; Ohkouchi, N.; Korenaga, T. Geographical Origin of Polished Rice Based on Multiple Element and Stable Isotope Analyses. *Food Chemistry* **2008**, *109*, 470–475.
25. Chung, I.-M.; Kim, J.-K.; Prabakaran, M.; Yang, J.-H.; Kim, S.-H. Authenticity of Rice (*Oryza sativa* L.) Geographical Origin Based on Analysis of C, N, O and S Stable Isotope Ratios: A Preliminary Case Report in Korea, China and Philippine. *Journal of the Science of Food and Agriculture* **2016**, *96*(7), 2433–2439.
26. Shumin, S.; Boli, G.; Yimin, W.; Ming-tao, F. Analysis of Stable Carbon and Nitrogen Isotope Compositions and Geographical Origins of Sheep Tissues. *China Agriculture Science* **2010**, *43*, 1670–1676.
27. Bo-Li, G.; Yi-Min, W.; Simon, K.D.; Jia-Rong, P.; Shuai, W. Application of Stable Hydrogen Isotope Analysis in Beef Geographical Origin Traceability. *Chinese Journal of Analytical Chemistry* **2009**, *37*, 1333–1336.
28. Nakashita, R.; Suzuki, Y.; Akamatsu, F.; Iizumi, Y.; Korenaga, T.; Chikaraishi, Y. Stable Carbon, Nitrogen, and Oxygen Isotope Analysis as a Potential Tool for Verifying Geographical Origin of Beef. *Analytica Chimica Acta* **2008**, *617*, 148–152.

29. Bong, Y.S.; Shin, W.J.; Lee, A.R.; Kim, Y.S.; Kim, K.; Lee, K.S. Tracing the Geographical Origin of Beefs Being Circulated in Korean Markets Based on Stable Isotopes. *Rapid Communications in Mass Spectrometry: RCM* **2010**, *24*, 155–159.
30. Camin, F.; Bontempo, L.; Heinrich, K.; Horacek, M.; Kelly, S.D.; Schlicht, C.; Thomas, F.; Monahan, F.J.; Hoogewerff, J.; Rossmann, A. Multi-element (H, C, N, S) Stable Isotope Characteristics of Lamb Meat from Different European Regions. *Analytical and Bioanalytical Chemistry* **2007**, *389*, 309–320.
31. Piasentier, E.; Valusso, R.; Camin, F.; Versini, G. Stable Isotope Ratio Analysis for Authentication of Lamb Meat. *Meat Science* **2003**, *64*, 239–247.
32. Perini, M.; Camin, F.; Bontempo, L.; Rossmann, A.; Piasentier, E. Multielement (H, C, N, O, S) Stable Isotope Characteristics of Lamb Meat from different Italian Regions. *Rapid Communications in Mass Spectrometry* **2009**, *23*, 2573–2585.
33. Gonzalez-Martin, I.; Gonzalez-Perez, C.; Hernandez Mendez, J.; Marques-Macias, E.; Sanz Poveda, F. Use of Isotope Analysis to Characterize Meat from Iberian-breed Swine. *Meat Science* **1999**, *52*, 437–441.
34. Brenna, J.T.; Corso, T.N.; Tobias, H.J.; Caimi, R.J. High-Precision Continuous-flow Isotope Ratio Mass Spectrometry. *Mass Spectrometry Reviews* **1997**, *16*, 227–258.
35. Kelly, S.; Brereton, P.; Guillou, C.; Broil, H.; Laube, I.; Downey, G.; Rossmann, A.; Hözl, S.; Veer, G.v.d. New Approaches to Determining the Origin of Food. In: *Woodhead Publishing Series in Food Science, Technology and Nutrition*; Number 212, Woodhead Publishing Ltd: Cambridge, **2011**; 238–258.
36. Kelly, S.; Heaton, K.; Hoogewerff, J. Tracing the Geographical Origin of Food: The Application of Multi-element and Multi-isotope Analysis. *Trends in Food Science & Technology* **2005**, *16*, 555–567.
37. Ruiz-Matute, A.I.; Weiss, M.; Sammartaro, D.; Finely, J.; Sanz, M.L. Carbohydrate Composition of High-Fructose Corn Syrups (HFCS) Used for Bee Feeding: Effect on Honey Composition. *Journal of Agricultural and Food Chemistry* **2010**, *58*, 7317–7322.
38. Cabañero, A.I.; Recio, J.L.; Rupérez, M. Liquid Chromatography Coupled to Isotope Ratio Mass Spectrometry: A New Perspective on Honey Adulteration Detection. *Journal of Agricultural and Food Chemistry* **2006**, *54*, 9719–9727.
39. Yousfi, S.; Serret, M.D.; Voltas, J.; Araus, J.L. Effect of Salinity and Water Stress During the Reproductive Stage on Growth, Ion Concentrations, $\Delta^{13}C$, and $\delta^{15}N$ of Durum Wheat and Related Amphiploids. *Journal of Experimental Botany* **2010**, *61*, 3529–3542.
40. Inglett, P.W.; Reddy, K.R.; Newman, S.; Lorenzen, B. Increased Soil Stable Nitrogen Isotopic Ratio Following Phosphorus Enrichment: Historical Patterns and Tests of Two Hypotheses in a Phosphorus-Limited Wetland. *Oecologia* **2007**, *153*, 99–109.
41. Bol, R.; Ostle, N.J.; Petzke, K.J.; Chenu, C.; Balesdent, J. Amino Acid ^{15}N in Long-term Bare Fallow Soils: Influence of Annual N fertilizer and Manure Applications. *European Journal of Soil Science* **2008**, *59*, 617–629.
42. Franco-Uría, A.; López-Mateo, C.; Roca, E.; Fernández-Marcos, M.L. Source Identification of Heavy Metals in Pastureland by Multivariate Analysis in NW Spain. *Journal of Hazardous Materials* **2009**, *165*, 1008–1015.
43. Tokaloğlu, Ş. Determination of Trace Elements in Commonly Consumed Medicinal Herbs by ICP-MS and Multivariate Analysis. *Food Chemistry* **2012**, *134*, 2504–2508.
44. Alali, W.Q.; Scott, H.M.; Norby, B. Assessing the Similarity of Antimicrobial Resistance Phenotypes Among Fecal *Escherichia coli* Isolates from two Aggregated Occupational Cohorts of Humans Versus Swine Using Cluster Analysis and Multivariate Statistics. *Preventive Veterinary Medicine* **2010**, *94*, 77–83.
45. Idris, A.M. Combining Multivariate Analysis and Geochemical Approaches for Assessing Heavy Metal Level in Sediments from Sudanese Harbors along the Red Sea Coast. *Microchemical Journal* **2008**, *90*, 159–163.
46. Korkmaz Görür, F.; Keser, R.; Akçay, N.; Dizman, S.; Okumuşoğlu, N.T. Radionuclides and Heavy Metals Concentrations in Turkish Market Tea. *Food Control* **2011**, *22*, 2065–2070.
47. FAO. http://www.fao.org/fileadmin/templates/est/meetings/IGGtea21/Presentation-MarketReportTurkey_02.pptx. **2014**.
48. Cengiz, M.F.; Durak, M.Z.; Ozturk, M. In-house Validation for the Determination of Honey Adulteration with Plant Sugars (C4) by Isotope Ratio Mass Spectrometry (IR-MS). *LWT - Food Science and Technology* **2014**, *57*, 9–15.
49. SAS. The SAS system for windows. Release 7.00 TS Level 00P1. Cary, NC, USA: SAS Institute, Inc. **1998**.
50. Minitab. Minitab® Release 14.1 Statistical Software, USA. **2003**.
51. Tosun, M. Detection of Adulteration in Honey Samples Added Various Sugar Syrups with $^{13}C/^{12}C$ Isotope Ratio Analysis Method. *Food Chemistry* **2013**, *138*, 1629–1632.
52. Simsek, A.; Bilsel, M.; Goren, A.C. $^{13}C/^{12}C$ Pattern of Honey from Turkey and Determination of Adulteration in Commercially Available Honey Samples Using EA-IRMS. *Food Chemistry* **2012**, *130*, 1115–1121.
53. Kracht, O.; Hilker, A. Detection of Honey Adulteration with FlashEA Elemental Analyzer and DELTA V Isotope Ratio Mass Spectrometer. Thermo Fisher Scientific Application **2009**, Application Note: 30177, pp 4.
54. Tokaloğlu, Ş.; Kartal, Ş. Multivariate Analysis of the Data and Speciation of Heavy Metals in Street Dust Samples from the Organized Industrial District in Kayseri (Turkey). *Atmospheric Environment* **2006**, *40*, 2797–2805.