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Determination of the geographical origin of Chinese teas based on stable carbon and nitrogen isotope ratios*

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Abstract: The objective of this study was to investigate the geographical origin of Chinese teas using carbon and nitrogen stable isotope ratio technology. The results showed that inter-provincial dispersion of teas in Guangdong (GD), Guangxi (GX), Hainan (HA), Fujian (FJ), Shandong (SD), Sichuan (SC), Chongqing (CQ), and Henan (HN) provinces was high, while in Zhejiang (ZJ), Hubei (HB), Yunnan (YN), and Anhui (AH) provinces, it was low. Tea samples from GD, GX, HA, and FJ provinces were clustered in one group and separated from those from AH and HB provinces. Thus, carbon and nitrogen stable isotope ratio technology could discriminate teas from among some provinces of China, but not from among others. Better separation might be obtained with a combination of isotopic ratios and other indexes, such as elemental data and organic components.

Key words: Geographical origin, Stable carbon isotope ratios, Stable nitrogen isotope ratios, Tea
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1 Introduction

The leaves and buds of the evergreen tea shrub *Camellia sinensis* var. *sinensis* are used to produce different kinds of tea. The growth of tea in China started about 2000 years ago, and today China is the main producer of tea in the world. Tea in China grows in a geographical range from 18° N to 37° N and from 95° E to 122° E, and the climate and soil conditions vary considerably. Thus, the application of carbon and nitrogen isotopic ratios in tracing the geographical origin of tea in China may be feasible. Different regions create specific brands of tea, such as Longjing, Tieguanyin, Biluochun, Maofeng, and Puer, based on the special tea culture derived from the local processing technology and cultural background. The price of tea varies based on the brands and geographical origin, so it is important to identify the geographical origin of tea to protect consumers and the global food trade.

Stable isotope ratios are widely used to identify food fraud, for example, adulterated honey (Padovan *et al*., 2003), wine (Roßmann *et al*., 1996; Weber *et al*., 1997), juice (Antolovich *et al*., 2001), and olive oil (Angerosa *et al*., 1997). They have also been widely used to trace the geographical origin of food with animal derivations, such as beef in China (Guo *et al*., 2009; 2010), Japan (Nakashita *et al*., 2008), Argentina and other global regions (Heaton *et al*., 2008; Bong *et al*., 2010; Horacek and Min, 2010), lamb in Europe (Camin *et al*., 2007), pork in Spain (González-Martin *et al*., 1999), and honey in Europe (Kropf *et al*., 2010; Schellenberg *et al*., 2010). In addition, stable isotope ratios were used in the geographical assessment of plant materials, such as tea in

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global ranges (Pilgrim *et al*., 2010), cotton in China (Sun *et al*., 2010), potatoes in Italy (Longobardi *et al*., 2011), olive oil in Europe (Camin *et al*., 2010), and rice in Japan (Suzuki *et al*., 2008), America, Europe, and the Basmati of India growing regions (Kelly *et al*., 2002).

The objective of this research was to verify the feasibility of using carbon and nitrogen isotopes to identify the geographical origins of Chinese teas to help maintain the prestige and credibility of tea brands.

2 Materials and methods

2.1 Sampling

Forty-nine tea samples were collected from twelve provinces in China with significant geographical differences (Fig. 1). The abbreviations, provinces/city, and tea types were as follows: AH, Anhui Province, black tea; CQ, Chongqing City, green tea; FJ, Fujian Province, green and oolong tea; GD, Guangdong Province, green and oolong tea; GX, Guangxi Province, green tea; HA, Hainan Province, green tea; HB, Hubei Province, green tea; HN, Henan Province, green tea; SC, Sichuan Province, green tea; SD, Shandong Province, green tea; YN, Yunnan Province, black tea; and ZJ, Zhejiang Province, green tea. All samples were stored at room temperature until further preparation.

Fig. 1 Sampling sites of teas collected from different Chinese provinces

The sampling provinces are indicated in gray. AH, Anhui Province; CQ, Chongqing City; FJ, Fujian Province; GD, Guangdong Province; GX, Guangxi Province; HA, Hainan Province; HB, Hubei Province; HN, Henan Province; SC, Sichuan Province; SD, Shandong Province; YN, Yunnan Province; ZJ, Zhejiang Province

2.2 Sample preparation

After oven-drying at 60 °C for 2 h, 50 g of tea leaves were ground in a crusher and then passed through a 140-mesh sieve. The resulting powder, comprised of particles of less than 105 μm diameter, was collected for stable isotope analysis.

2.3 Isotopic analysis

For each sample, 1 mg of dry tea powder was weighed into a tin cup and folded following the standard procedure to extrude any air. The levels of carbon stable isotopes were measured using a Flash EA1112 elemental analyzer (Costech, Milan, Italy) connected to a Thermo-Finnigan Delta Plus XL gas isotope ratio mass spectrometer (Thermo-Finnigan GmbH, Bremen, Germany) via a Conflo III interface. The nitrogen and carbon in the samples were converted into N_2 and CO_2, respectively, and ionized. The ionized heavy and light isotopes were separated with a magnetic field and then collected and detected by the appropriate Faraday collector. The isotopic values (δ) were expressed using the formula:

$$\delta=[(R_{\text{sample}}-R_{\text{standard}})/R_{\text{standard}}]\times 1000‰,$$

where R_{sample} and R_{standard} are the isotopic ratios of the sample and the standard materials, respectively.

Carbon and nitrogen stable isotope ratios are reported in parts per thousand (‰) deviations with respect to Pee Dee Belemnite (PDB) and atmospheric nitrogen (AIR). The standards of carbon and nitrogen, casein and wheat flour, were analyzed at the beginning, middle, and end of each run to correct for instrumental drift and determine inter-batch variability of $\delta^{13}C$ and $\delta^{15}N$ analyses. The quality control (QC) standards for carbon and nitrogen were collagen and urea, respectively. Within carbon and nitrogen runs, isotopic precision for QC standards was 0.2‰. Carbon and nitrogen isotope ratio analyses were carried out in different runs and in triplicate for each element.

2.4 Statistical analysis

Data treatment, box-plot analysis, and one-way analysis of variance (ANOVA) were carried out with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Data are presented as mean±standard deviation (SD) and analyzed by ANOVA using Duncan's test at $P<0.05$ significance level.

3 Results

Means and SDs of the $\delta^{13}C$ and $\delta^{15}N$ contents of tea from different provinces in China and the results of ANOVA are shown in Table 1. Carbon isotope values ranged from −26.66‰ to −24.59‰, and nitrogen isotope values from 1.46‰ to 4.41‰.

The distribution of the $\delta^{13}C$ values is shown in Fig. 2. The order of geographical origin based on the $\delta^{13}C$ values in the tea is HB>CQ>SD>ZJ>YN>SC>HN>HA>AH>FJ>GD>GX. Along the coastal provinces of SD, ZJ, HA, FJ, GD, and GX, the values of $\delta^{13}C$ decreased gradually from north to south. The $\delta^{13}C$ values of HB samples were significantly different from those of central (HN and AH) and southern China (HA, FJ, GD, and GX); the $\delta^{13}C$ values of samples from southwestern China (CQ) were significantly different from those of central (HN and AH) and southern China (HA, FJ, GD, and GX); the $\delta^{13}C$ values of samples from northern China (SD) were significantly different from those of central (HN and AH) and southern China (HA, FJ, GD, and GX); $\delta^{13}C$ values of samples from ZJ were significantly different from those from HN, HA, AH, FJ, GD, and GX; and the $\delta^{13}C$ values of samples from YN were the significantly different from those from GD and GX. The values of $\delta^{13}C$ in samples from HA, FJ, GD, and GX were similar and could not be distinguished from each other. Box-plots (Fig. 2) revealed that the outlier sample from FJ was an oolong tea, which fell 1.5 times beyond the inter-quartile range (IQR) of the data (Moore and McCabe, 1993).

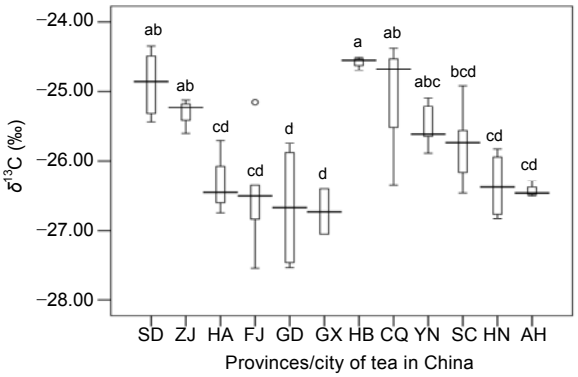

Fig. 2 $\delta^{13}C$ (vs. V-PDB) values of tea from different provinces/city of China

All the abbreviations are the same as shown in Fig. 1. Data are analyzed by ANOVA using Duncan's test and different letters above the columns showed significant differences at $P<0.05$

Table 1 Means and SDs of $\delta^{13}C$ and $\delta^{15}N$ of tea from different provinces/city of China

Province/city	Type	No.	$\delta^{13}C$ (‰)*		$\delta^{15}N$ (‰)#	
			Mean	SD	Mean	SD
AH	Black	3	−26.42cd	0.11	1.46d	0.14
CQ	Green	3	−25.14ab	1.06	1.50d	0.31
FJ	Green and oolong	6	−26.48cd	0.78	3.45abc	1.08
GD	Green and oolong	6	−26.66d	0.78	3.50abc	0.48
GX	Green	2	−26.73d	0.46	4.41a	0.55
HA	Green	3	−26.30cd	0.54	3.83ab	0.48
HB	Green	3	−24.59a	0.10	1.78cd	0.55
HN	Green	4	−26.35cd	0.48	3.89ab	1.92
SC	Green	5	−25.77bcd	0.59	3.02abcd	1.60
SD	Green	6	−24.89ab	0.45	2.76abcd	1.54
YN	Black	5	−25.49abc	0.33	2.47bcd	0.56
ZJ	Green	3	−25.32ab	0.25	2.71abcd	0.08
Total		49	−25.85	0.87	2.94	1.25

AH, Anhui Province; CQ, Chongqing City; FJ, Fujian Province; GD, Guangdong Province; GX, Guangxi Province; HA, Hainan Province; HB, Hubei Province; HN, Henan Province; SC, Sichuan Province; SD, Shandong Province; YN, Yunnan Province; ZJ, Zhejiang Province. * $\delta^{13}C$ (‰) vs. V-PDB; # $\delta^{15}N$ (‰) vs. V-AIR. Data are analyzed by ANOVA using Duncan's test and different letters after each value in the same column showed significant differences at $P<0.05$

The distribution of the δ^{15}N values is shown in Fig. 3. The order of the geographical origin based on the δ^{15}N values of the tea is GX>HN>HA>GD>FJ>SC>SD>ZJ>YN>HB>CQ>AH. Along the coastal provinces of ZJ, FJ, GD, HA, and GX, the values of δ^{15}N increased from north to south, but the differences among them were not significant. The δ^{15}N values of samples from HN, SD, and SC were variable and overlapped each other. The δ^{15}N values of samples from GX were significantly different from those from AH, CQ, HB, and YN; the δ^{15}N values of samples from HA were significantly different from those from AH, CQ, and HB; the δ^{15}N values of samples from GD were significantly different from those from AH and CQ; and the δ^{15}N values of samples from FJ were significantly different from those from AH and CQ (Table 1). Box plots revealed that the outlier samples in FJ and GD belonged to oolong and green tea, respectively (Fig. 3).

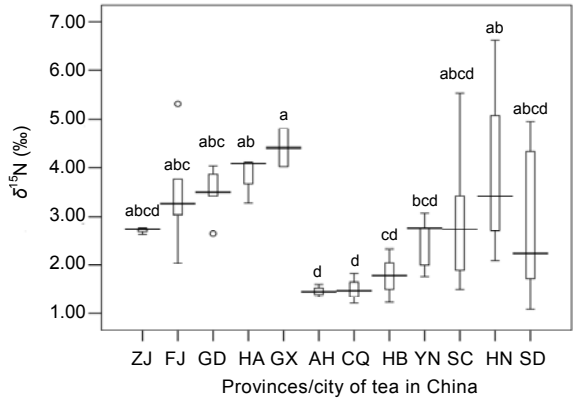

Fig. 3 δ^{15}N (vs. V-AIR) values of tea from different provinces/city of China

All the abbreviations are the same as shown in Fig. 1. Data are analyzed by ANOVA using Duncan's test and different letters above the columns showed significant differences at $P<0.05$

The separation among geographical origins of the tea was checked by plotting the values of δ^{13}C and δ^{15}N. Fig. 4 shows that the inter-provincial dispersion of teas was low in samples from ZJ, HB, YN, and AH, but high in samples from GD, GX, HA, FJ, SD, SC, CQ, and HN. The inter-provincial dispersion in one sample collected from AH overlapped with that in one sample each from CQ and SC. The samples from HB could not be separated from the samples from SD and CQ. It was difficult to separate the samples from ZJ, FJ, GD, GX, HA, YN, and HN. Because the teas from South China, GD, GX, HA, and FJ were similar to each other, combining them into one group may be beneficial. The combined samples could be separated from samples from AH and HB provinces, but would have some overlap with samples from YN and ZJ.

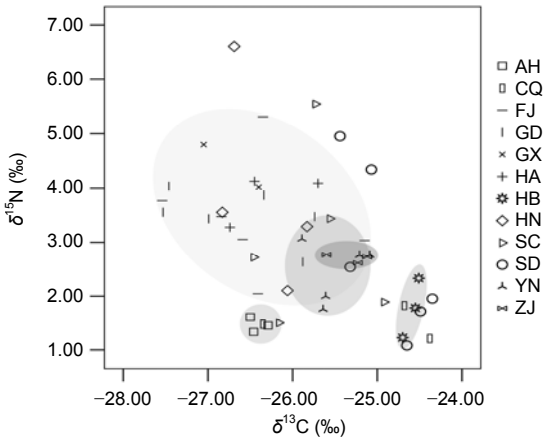

Fig. 4 Cross plot of δ^{13}C (vs. V-PDB) values vs. δ^{15}N (vs. V-AIR) values

All the abbreviations are the same as shown in Fig. 1

We conclude that teas cannot be separated based on provinces because of overlap, but could be grouped to provide regional comparisons. This study indicated that carbon and nitrogen stable isotope ratio technology could not discriminate the teas from many provinces in China, and better separation may be gained through a comparison of chemical components, such as elemental data, or organic components.

4 Discussion

Carbon isotopic ratios are influenced by climate. Carbon isotope compositions are calculated using the equation of $\delta^{13}C_p=\delta^{13}C_{env}-a-(b-a)\times C_i/C_a$ (Farquhar *et al*., 1982), where $\delta^{13}C_p$ is the carbon isotopic composition (‰) of the plant, $\delta^{13}C_{env}$ is the composition (‰) of the CO_2 in the environment, a is the fractionation (‰) caused by diffusion (in the air, a is 4.4), b is the fractionation (‰) caused by ribulose bisphosphate (RuBP) carboxylation of the Calvin-Benson photosynthetic pathway (in C_3 plants, b is 27) (Farquhar *et al*., 1982; Farquhar, 1983), and C_i and C_a

are the intercellular and atmospheric partial pressures of CO_2, respectively. The atmospheric partial pressure of CO_2 is relatively stable. Thus, it is obvious that carbon isotopic ratios are influenced by C_i. Humidity, temperature, soil water conditions, and air water conditions are all related to C_i. The climate of China differs significantly between the north and the south and between the coastal and inland regions.

On the coast, in association with increasing temperature, the $\delta^{13}C$ values of tea from SD, ZJ, FJ, GD, GX, and HA decreased progressively. In SD, the low mean temperature and low average humidity reduced the $\delta^{13}C$ values compared to those from central (HN and AH) and southern China (HA, FJ, GD, and GX) (Fig. 2). The $\delta^{13}C$ values of tea from HN and AH provinces were as low as those from southern China (HA, FJ, GD, and GX). These low values could be explained by the high humidity in the sample sites, which were adjacent to inland lakes (Henan and Nanwan) or rivers (Anhui and Xin'an). HB, CQ, HN, and AH are all inland provinces. The $\delta^{13}C$ values of HB and CQ were higher than those of HN and AH, which could be explained by differences in altitude between the growing sites (Diaz *et al*., 1996): an increase in altitude induces a decrease in mean temperature and average humidity. The box-plots show differences between populations of the data and identify outliers, and were constructed using five values: the maximum value, the 75th percentile, the 50th percentile, the 25th percentile, and the minimum value. The vertical line protruding from the CQ box extends from the 25th percentile to the minimum values of $\delta^{13}C$ and overlaps with the boxes of HN, GD, and AH. However, after ANOVA analysis, the $\delta^{13}C$ values in CQ were significantly different from those from HN, GD, and AH. This could be explained by the fact that the box-plot is a descriptive statistic, while the significant difference in $\delta^{13}C$ values between CQ and HN, GD, and AH was a quantitative statistic, and therefore the two analyses are not in conflict.

The nitrogen isotopic compositions depend mainly on soil nutrition (Kohl *et al*., 1973; Shearer and Legg, 1975) and are influenced by the type of fertilizer used. In the present study, the $\delta^{15}N$ values of tea from ZJ, FJ, GD, HA, and GX increased progressively, which might be explained by increasing temperatures along the coast, but the differences among them were not significant. The variability within HN, SD, and SC covered a wide range and their values overlapped each other. The climate of HN, SD, and SC is relatively dry, and previous studies indicated that the range in $\delta^{15}N$ values for dry regions (−4‰ to 11‰) showed higher dispersion than that of humid regions (2‰ to 10‰) (Shibuya *et al*., 2007) in accordance with the higher dispersion of $\delta^{15}N$ in HN, SD, and SC provinces. The fertility of the tea gardens might be another factor. The values of $\delta^{15}N$ in inland provinces/city (AH, CQ, and HB) were different from those of coastal provinces (GD, GX, and HA). Heaton (1987) and Ehleringer *et al.* (2001) showed the same results, which can be explained by a previous study that linked an increase in $\delta^{15}N$ values to an increase in humidity (Gremaud and Hilkert, 2009). The problem is that nitrogen is indiscriminant, and nitrogen-containing reserves (amino acids, e.g., arginine, glutamine, and aspartic acid) in xylem could be contributing (Cyr and Bewley, 1989). Because of the perennial growth properties of tea, the early development and growth of buds and leaves is sustained using nitrogen reserves stored in the xylem (Ta *et al*., 1990; Moing *et al*., 1994) in different years under different fertilization conditions.

In the present study, the distribution of tea, both oolong and green, in FJ and GD covered a wide range (Fig. 4), which might explain the outliers in the box-plots. There was not enough evidence to show that the type of tea influenced the stable isotope ratios of carbon and nitrogen, and in a previous study the effect of the type of tea was not considered (Camin *et al*., 2010). To our knowledge, there have been few studies on the variation in carbon and nitrogen stable isotopes in different kinds of tea. Thus, new studies about the effect of the kind of tea on the ratio of stable carbon and nitrogen isotopes should be carried out.

In the present study, stable carbon and nitrogen isotopes could not discriminate among tea samples from different provinces of China exactly; thus, in order to trace the geographical origin of the tea, the stable isotope ratio must be combined with other chemical properties. For example, satisfactory results have been obtained using a combination of stable isotopic ratios and mineral element contents for the geographic origin of teas (Pilgrim *et al*., 2010), potatoes (Longobardi *et al*., 2011), and olive oils (Camin *et al*., 2010).

5 Conclusions

The teas from one province of China could not easily be separated from those from other provinces, but grouping several provinces might yield useable results. Another possible solution is to also use chemical components, such as elemental data, or organic components.

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