



Research article

Preliminary study on the geographical origin of Chinese 'Cuiguan' pears using integrated stable isotope and multi-element analyses

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ABSTRACT

Distinguish the geographical origin of the pear is important due to the increasingly valued brand protection and reducing the potential food safety risks. In this study, the profiles of stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$) and the contents of 16 elements in pear pear from four production areas were analyzed. The $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$ and 12 elements were significantly different ($p < 0.05$) in the four production areas. Chemometrics analysis including principal component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA) and linear discriminant analysis (LDA) were exploited for geographical origin classification of samples. OPLS-DA analysis showed that crucial variables ($\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$, Ni, Cd, Ca, $\delta^{15}\text{N}$, Sr and Ga) are more relevant for the discrimination of the samples. OPLS-DA achieved pear origin accuracy rates of 87.76 % by combining stable isotope ratios and elemental contents. LDA had a higher accuracy rate than OPLS-DA, and the LDA analysis showed that the original discrimination rate reached to 100 %, while the cross-validated rate reached to 95.7 %. These studies indicated that this method could be used to assess the geographical discrimination of pear from different producing areas and could potentially control the fair trade of pear in fruit markets.

1. Introduction

The production and consumption of Chinese pears are the largest in the worldwide nowadays. According to the 2023 reports of United States Department of Agriculture (USDA), the production of pear in China was 17.9 million tons (97.7 % for national consumption) in 2022, making up 75 % of the world's production [1]. In order to improve the social and economic benefits of agricultural products, the Chinese government is implementing the "Protection of Geographical Indication (PGI) Products". Until now, *Pyrus* spp from 86 well-known production areas were protected by the General Administration of Quality Supervision, Inspection, and Quarantine of China (AQSIQ). In southern China, the early ripening cultivar 'Cuiguan' pear (*Pyrus pyrifolia*) is one of the most common and economically crucial fruit species [2]. Although cultivating the same Cuiguan variety, pears produced in some geographical regions (such as Ersheng pear, Zhuanghang pear, Taixing pear) have higher quality and price. Therefore, to keep their band value, it is

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necessary to establish internal tag and exploit creditable methods enabling the recognition of the geographic origin of pear.

Various approaches have been used to validate the geographical origin and authentication of food based on mass spectrometry (i.e., IRMS, ICP, and ICP-MS) [3,4], spectroscopy (i.e., NIR, NMR and Raman), separation analysis (i.e., GC and HPLC) [5], biochemistry analysis (DNA, protein and immunoassay) [6] and other methods (sensory analysis) in combination with statistics and chemometrics. In these methods, multi-elemental analysis combined with stable isotope analysis are the most common techniques in a wide range of foodstuff traceability studies due to their high accuracy. Successful studies included fruits (durian, peach, apple, jujube) [4,7–9], vegetables (potato, tomato, carrot, garlic, peanuts, Chinese yam) [10–16], Chinese herbs (*Panax ginseng*, *Angelica sinensis*, *Atractylodes macrocephala* Koidz) [17–19], livestock (yak meat, Tibetan chicken) [20,21], aquatic products (Chinese mitten crab, *Procambarus clarkii*, Bottarga) [22–24], wine [25,26], tea [27,28] and other kinds of food products [29,30]. Until now, there are minimal reports about pear origin traceability, one of which was based on multi-element and LDA analysis, and the authors successfully discriminate Rocha pear from two Portuguese regions [31].

Nevertheless, as far as we know, geochemical traceability techniques for Cuiguan pear have never been reported. In the present study, we investigated the effectiveness of discriminating the geographical origin of green appearance pear collected from different provinces in South China by analyzing multi-elements and stable isotopes combined with chemometrics. The results gives preliminary values that might help in solving the problem of mis-labelled origin of 'Cuiguan' pear in the Chinese fruit market.

2. Materials and methods

2.1. Pear collection and pre-treatment

70 pear samples with the typical green appearance of variety 'Cuiguan' were sampled from the following four production regions in Southern China in 2023 June to July (Fig. 1). CQ = Chong Qing city; SH = Shang Hai city; ZJ = Zhe Jiang Province; JS = Jiang Su Province. Sampling sites are shown in Fig. 1, and detailed information was collected at the same time. Sample numbers, coordination with locality (longitude and latitude), average altitude and mean annual temperature are presented in Table 1. All pears were harvested from local farms at the maturing time. After harvesting, approximately 8–10 pears were washed and separated from the seed, peel and pulp. All samples were dried in an oven at 80 °C to a constant weight, then finely ground to a homogeneous powder by hand

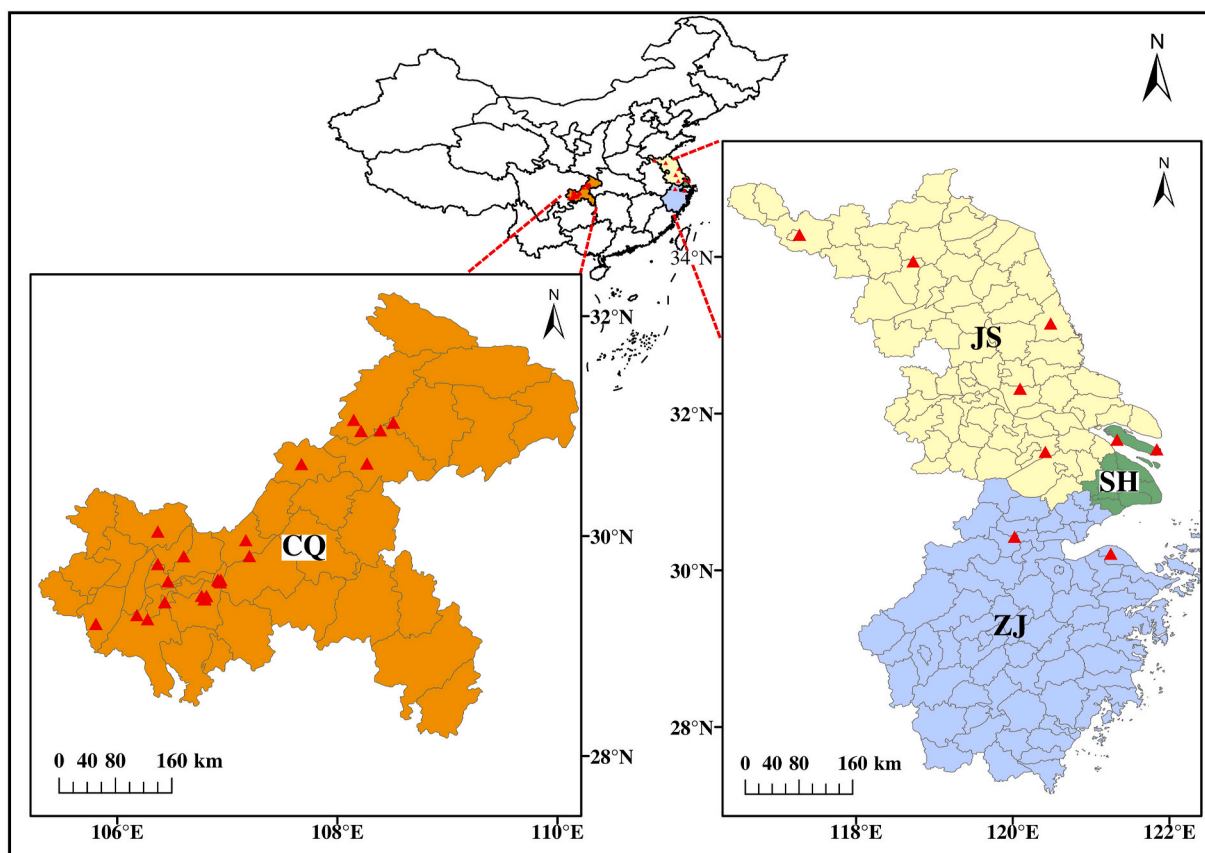


Fig. 1. Geographical location information of pear samples from different regions in China. CQ = Chong Qing city; SH = Shang Hai city; ZJ = Zhe Jiang Province; JS = Jiang Su Province.

Table 1
Region information of pear samples.

Origin	Number	Longitude/° E	Latitude/° N	Average Altitude/m	Mean annual temperature (°C)
CQ	52	105.81 to 108.27	29.20 to 31.66	491	16.1
SH	4	121.33 to 121.84	31.55 to 31.67	4	13.9
ZJ	4	120.02 to 121.25	30.21 to 30.43	26	14.4
JS	10	117.28 to 120.48	31.51 to 34.29	21	13.8

using an agate mortar and pestle and passed through a 100-mesh sieve for stable isotope ratio and elemental composition analysis.

2.2. Stable isotope ratio analysis

The determination of four stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$) in pear peel samples was performed using an elemental analyzer (Vario Pyro Cube, Elementar, Germany) coupled with an isotope ratio mass spectrometer (EA-IRMS) (IsoPrime100, England) referenced previous published methods [14,15]. Approximately, 8.0 mg of samples were weighed and folded into tin capsules for the determination of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. For $\delta^2\text{H}$ and $\delta^{18}\text{O}$ analysis, about 0.5 mg of samples were weighed and folded into silver capsules, which were freeze-dried for five days and then put in laboratory for three days to reach hydrogen equilibrium. The isotope compositions were expressed as delta value, which displayed as the following Eq. (1):

$$\delta X = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000\text{‰} \quad (1)$$

Where X represents heavier mass numbers of element, like ^{13}C , ^{15}N , ^2H or ^{18}O , whereas, R_{sample} and $R_{\text{reference}}$ denotes the abundance ratio of heavy isotope against light isotope, e.g. $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ ratio in samples and references, respectively. These isotopic delta values are usually presented as per mil (‰). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are relative to Vienna Pee Dee Belemnite (VPDB) and atmospheric N_2 (AIR), respectively. The $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values are relative to Vienna Standard Mean Ocean Water (VSMOW). In this study, reference materials included B2155 ($\delta^{13}\text{C} = -26.98\text{‰}$, $\delta^{15}\text{N} = +5.94\text{‰}$), BCR-657 ($\delta^{13}\text{C} = -10.76\text{‰}$), USGS 64 ($\delta^{13}\text{C} = -40.81\text{‰}$), IAEA-N-2 ($\delta^{15}\text{N} = +20.3\text{‰}$), USGS 40 ($\delta^{15}\text{N} = -4.52\text{‰}$) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ calibration, and USGS 54 ($\delta^2\text{H} = -150.4\text{‰}$, $\delta^{18}\text{O} = +17.79\text{‰}$), USGS 55 ($\delta^2\text{H} = -28.2\text{‰}$, $\delta^{18}\text{O} = +19.12\text{‰}$), and USGS 56 ($\delta^2\text{H} = -44.0\text{‰}$, $\delta^{18}\text{O} = +27.23\text{‰}$) were used for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ calibration. A laboratory-made pear peel QC sample was employed for precision check, which was lower than 0.1 ‰ for $\delta^{13}\text{C}$, 0.2 ‰ for $\delta^{15}\text{N}$, 3.0 ‰ for $\delta^2\text{H}$ and 0.5 ‰ for $\delta^{18}\text{O}$, respectively.

2.3. Elemental composition analysis

Elemental composition analysis was conducted based on Chinese National Standard GB 5009.268–2016 by using ICP-OES (Optima

Table 2
Overview of the stable isotopes and mineral elements of all pear peel samples.

Isotopic Ratio [‰] or Elements (mg/kg)	Min	Max	Median	Mean	SE	Std.dev	Coef.var	LOD (mg/kg)
$\delta^{13}\text{C}_{\text{VPDB}}$	-29.1	-26.5	-27.6	-27.6	0.1	0.6	0.02	-
$\delta^{15}\text{N}_{\text{Air}}$	-0.328	8.70	3.83	3.77	0.26	2.17	0.58	-
$\delta^2\text{H}_{\text{VSMOW}}$	-65.2	-34.9	-51.2	-51.4	0.7	6.2	0.12	-
$\delta^{18}\text{O}_{\text{VSMOW}}$	20.1	22.7	21.2	21.2	0.1	0.5	0.25	-
K	2504	7657	4456	4561	139	1167	3.06	0.04
P	777	2456	1288	1349	43	358	3.17	0.0633
Mg	792	1403	1050	1072	15	129	1.44	0.0064
Ca	547	2800	1237	1322	47	390	3.52	0.0125
Fe	13.7	193.2	27.1	34.1	3.4	28.1	9.86	0.0011
Na	15.3	191.4	34.4	41.8	3.0	25.3	7.23	0.0262
Mn	8.0	48.4	17.7	19.4	0.9	7.8	4.84	0.0006
B	6.5	24.5	12.7	12.8	0.4	3.7	3.46	0.9613
Cu	3.5	10.5	6.7	6.7	0.2	1.7	2.94	0.0006
Zn	5.5	37.5	9.9	11.3	0.7	5.7	6.05	0.0084
Sr	1.01	9.11	3.11	3.64	0.21	1.78	5.86	0.05
Ba	0.45	13.36	4.10	4.69	0.37	3.12	7.96	0.0496
Ni	0.04	3.32	0.92	1.04	0.10	0.80	9.19	0.0177
Ga	0.0546	2.3295	0.8608	0.9443	0.0756	0.6323	8.00	0.0125
Co	0.0194	0.6664	0.1082	0.1629	0.0189	0.1577	11.57	0.0071
Cd	0.0069	0.1732	0.0547	0.0604	0.0044	0.0372	7.35	0.0043

Notes.

Median, median values from the four regions; mean, average values from the four regions; SE, Standard error; std.dev, standard deviation; coef. Var, coefficient of variation; LOD, limit-of-detection values.

The coefficient of variation (CV) measures the degree of variation within an element (less than 0.2, has a low degree of variation; between 0.2 and 0.3, a medium variation; greater than 0.35, highly variable).

8300, PerkinElmer, Waltham, MA, USA) and ICP-MS (ELANDRC II, PerkinElmer, Waltham, MA, USA) with some modifications. Before analysis, approximately 0.3 g dried powdered samples were weighed and digested in 5.0 mL nitric acid (HNO₃) at 120 °C for 3 h, then 1.0 mL H₂O₂ was added and kept at 120 °C for 1 h. Then evaporate the acid until the digestion liquid volume was decreased to about 1.0 mL. After cooling to room temperature, the digested liquids were diluted to the same volume of 25 mL with deionized water. For the certified reference material (GBW10020, GSB-11; GBW10021, GSB-12), 0.2 g was processed accordingly in each digestion batch for quality control, and the results of analyses of the certified reference materials were provided in Table S2.

2.4. Multivariate statistical analysis

One way analysis of variance (One-way ANOVA), Kaiser-Meyer-Olkin (KMO) test, Shapiro-Wilk test, nonparametric test and stepwise linear regression analysis (LDA) was run on SPSS 27.0 (SPSS Inc. Chicago, IL, USA). The Shapiro-Wilk test was first applied to check the normal distribution of data. One-way ANOVA with the least significant difference (LSD) method or its alternative nonparametric test (i.e. Kruskal-Wallis test) was used to evaluate whether there were statistically significant differences in the average stable isotope values and elemental contents of the geographical origins at the 95 % confidence level. Principle-component analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA) and permutation test were performed using SIMCA software (ver. 14.0, Umetrics, Sweden).

3. Results and discussion

3.1. Analysis of stable isotope ratios

Analytical results of four stable isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$) contained in pear peel samples obtained in 2023 are presented in Table 2. The mean \pm standard error of CQ, SH, ZJ, and JS were calculated to compare the differences between the stable isotope values from four regions (Table 3). Based on the one-way ANOVA analysis, significant differences ($p < 0.05$) was identified in the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ values across four different geographical origins. Among them, the $\delta^{13}\text{C}$ values for all the samples ranged between -29.1 and -26.5 ‰, which is consistent with previous report that $\delta^{13}\text{C}$ values in C3 plant were ranging between -33 and -23 ‰ [13, 32]. The average pear peel of $\delta^{13}\text{C}$ values from four major production areas were as follows: CQ > ZJ > SH > JS. Among them, the CQ area showed significant differences from SH and JS, while there was no significant difference between ZJ, SH, and JS. As for the specific plant species, $\delta^{13}\text{C}$ values mainly related to the fractionation of ^{13}C during plant growth, rather than the distinction of CO₂ fixation pathways during photosynthesis. The former would be affected by the plant growth environment factors such as temperature, sunlight intensity, CO₂ deviation pressure in air and management practices [33–35].

The $\delta^{15}\text{N}$ values of pear peel samples ranged from 3.72 to 6.94 ‰, and significant higher values were observed for SH pears. It needs to be noted that the $\delta^{15}\text{N}$ signature is highly associated with agricultural practices such as fertilization type. As well known that organic N is generally enriched in ^{15}N compared with atmospheric N₂, which would lead to the higher $\delta^{15}\text{N}$ values in organic fertilizer [16].

The $\delta^2\text{H}$ values for all the samples ranged between -65.2 and -34.9 ‰ (Table 2). The mean value of $\delta^2\text{H}$ from CQ pear peel samples was significantly higher (-49.0 ‰) than the values of the other areas ($p < 0.05$) (Table 3). This may be owing to the higher mean annual temperature (about 16.1 °C) of CQ compared to those in ZJ (about 14.4 °C), JS (about 13.8 °C), and SH (about 13.9 °C) (Table 1). Similar results are reported in tobacco that higher mean $\delta^2\text{H}$ values in several origins are likely due to exposure to a more

Table 3

Mean stable isotopes values, multi-element contents and standard deviations of pear peel samples from different provinces.

Isotopic Ratio [‰] or Elements (mg/kg)	CQ (n = 52)	SH (n = 4)	ZJ (n = 4)	JS (n = 10)	p
$\delta^{13}\text{C}_{\text{VPDB}}$	$-27.4 \pm 0.5\text{a}$	$-28.0 \pm 0.1\text{b}$	$-27.8 \pm 0.2\text{ ab}$	$-28.3 \pm 0.5\text{b}$	***
$\delta^{15}\text{N}_{\text{Air}}$	$3.72 \pm 1.81\text{b}$	$6.94 \pm 1.82\text{a}$	$3.33 \pm 2.34\text{b}$	$2.92 \pm 3.03\text{b}$	*
$\delta^2\text{H}_{\text{VSMOW}}$	$-49.0 \pm 4.7\text{a}$	$-61.9 \pm 2.23\text{c}$	$-56.5 \pm 6.2\text{b}$	$-57.5 \pm 4.0\text{bc}$	***
$\delta^{18}\text{O}_{\text{VSMOW}}$	$21.4 \pm 0.4\text{a}$	$20.1 \pm 0.1\text{b}$	$20.8 \pm 0.4\text{b}$	$20.8 \pm 0.6\text{b}$	***
K	$4754 \pm 1072\text{a}$	$4264 \pm 531\text{ ab}$	$5366 \pm 1978\text{a}$	$3353 \pm 467\text{b}$	***
P	$1393 \pm 366\text{a}$	$1309 \pm 239\text{ ab}$	$1393 \pm 419\text{ ab}$	$1116 \pm 238\text{b}$	*
Ca	$1263 \pm 325\text{b}$	$1077 \pm 48\text{b}$	$1466 \pm 233\text{ ab}$	$1669 \pm 599\text{a}$	***
Fe	$36.7 \pm 31.9\text{a}$	$19.4 \pm 1.5\text{b}$	$25.8 \pm 9.1\text{ ab}$	$29.6 \pm 10.9\text{a}$	**
Na	$38.6 \pm 17.4\text{a}$	$79.7 \pm 70\text{a}$	$30.3 \pm 4.7\text{a}$	$47.8 \pm 25.7\text{a}$	ns
Mn	$20.8 \pm 8.2\text{a}$	$9.73 \pm 0.7\text{b}$	$16.7 \pm 1.2\text{ ab}$	$17.1 \pm 5\text{a}$	***
B	$12.4 \pm 3.6\text{a}$	$16.7 \pm 5\text{a}$	$12.1 \pm 1.8\text{a}$	$13.8 \pm 4.3\text{a}$	ns
Cu	$6.86 \pm 1.83\text{a}$	$7.11 \pm 0.37\text{a}$	$5.45 \pm 1.23\text{a}$	$6.4 \pm 1.11\text{a}$	ns
Zn	$10.1 \pm 3\text{b}$	$19.7 \pm 2.8\text{a}$	$13.1 \pm 4.3\text{ ab}$	$13.1 \pm 11.7\text{b}$	***
Sr	$3.53 \pm 1.69\text{b}$	$1.79 \pm 0.36\text{c}$	$6.08 \pm 1.38\text{a}$	$3.93 \pm 1.75\text{ ab}$	***
Ba	$5.43 \pm 2.43\text{a}$	$0.597 \pm 0.16\text{b}$	$6.83 \pm 6.71\text{a}$	$1.61 \pm 0.61\text{b}$	***
Ni	$1.29 \pm 0.79\text{a}$	$0.133 \pm 0.1\text{b}$	$0.64 \pm 0.6\text{ ab}$	$0.244 \pm 0.13\text{b}$	***
Ga	$1.13 \pm 0.51\text{a}$	$0.0798 \pm 0.03\text{b}$	$1.14 \pm 1.15\text{a}$	$0.248 \pm 0.11\text{b}$	***
Co	$0.1896 \pm 0.1738\text{a}$	$0.0809 \pm 0.0267\text{ ab}$	$0.1024 \pm 0.0126\text{ ab}$	$0.081 \pm 0.0372\text{b}$	*
Cd	$0.072 \pm 0.0366\text{a}$	$0.0291 \pm 0.0123\text{b}$	$0.0381 \pm 0.0164\text{b}$	$0.0219 \pm 0.0094\text{b}$	***

Values are expressed as the mean \pm SD. Different superscript letters (a-d) in each row designate significant differences ($p < 0.05$). ns: p -value > 0.05 , *: $0.05 > p$ -value > 0.01 , **: $0.01 > p$ -value > 0.001 , ***: p -value ≤ 0.001 .

tropical climate [36]. Except for temperature, the distance to the sea, irrigation water type, altitude, longitude and humidity also impart significant variations in $\delta^2\text{H}$ value [7,8].

The $\delta^{18}\text{O}$ values of our pear peer samples varied from 20.1 to 22.6 ‰ (Table 2). The mean value of $\delta^{18}\text{O}$ from CQ pear peer samples was significantly higher (21.4 ‰) than the values of the other areas ($p < 0.05$). CQ is characterized by the higher annual mean temperature among all sites (Table 1) indicating a possible positive relationship between temperature and $\delta^{18}\text{O}$ values of pear peer. Previous reports showed that many factors, such as altitude, latitude, and the distance to the sea, influence plant oxygen isotopic ratios [37]. Besides, a statistics relationship has been observed between $\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ ($p < 0.001$) in our study. This is consistent with previous reports that both $\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in plants rely on factors of influence in the similar pattern [38].

3.2. Analysis of elemental composition

In this study, 16 elements in pear peel samples from four different geographical origins were analyzed, including four macroelements (K, P, Ca, and Mg), six microelements (Na, Fe, Mn, B, Zn, Cu), and six trace elements (Ba, Sr, Ni, Ga, Co and Cd) (Table 2). Among macroelements, the K content (4561 ± 1172 mg/kg) in 70 pear peel samples was the highest, followed by P (1349 ± 358 mg/kg), Ca (1322 ± 393 mg/kg), Mg (1072 ± 130 mg/kg). The content of microelements that were in the pear peer samples in the four regions are as follows: Na (41.8 ± 25.3 mg/kg) > Fe (34.1 ± 28.1 mg/kg) > Mn (19.4 ± 7.8 mg/kg) > B (12.8 ± 3.7 mg/kg) > Zn (11.3 ± 5.7 mg/kg) > Cu (6.7 ± 1.7 mg/kg). As for trace elements, the content concentration order was Ba > Sr > Ni > Ga > Co > Cd. Only Mg was lowly variable, K, P, Ca, B, and Cu were moderately variable, while the other elements including Na, Fe, Mn, Zn, Ba, Sr, Ni, Ga, Co and Cd were highly variable.

In order to identify significant markers among four production origins pear, one-way ANOVA coupled with a nonparametric test (Kruskal-Wallis test) was analyzed (Table 3). Results showed that most of the elements were statistically different among the four origins, except for Mg, Cu, Na and B ($p > 0.05$). CQ samples were more prosperous in Fe, Mn, Ni, Ga, Co and Cd than other pear peer samples. At the same time, the Zn content from CQ area was the lowest in the four production areas. These difference might be associated with pear growth environments, such as soil conditions (pH and mineral compositions), fertilizer applications, regional climates and irrigation water [39,40]. Thus, based on these element differences in pear peer, we will try to use multivariate statistical analysis to help established the discrimination model for the different origins of pear.

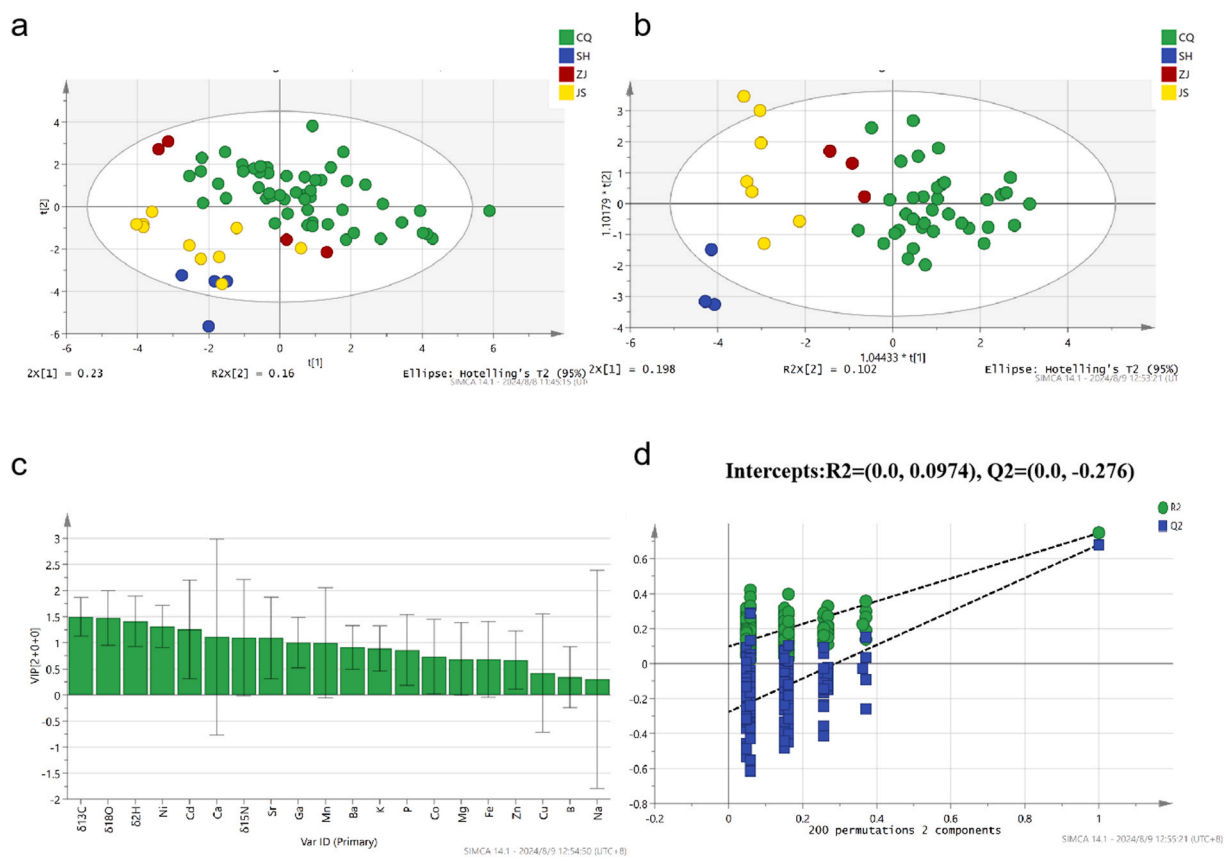


Fig. 2. (a) PCA score plots, (b) OPLS-DA score plots of pears from four origins with the data of stable isotope and multi-element. (c) Variable importance in the projection (VIP) was obtained from the OPLS-DA model. (d) Permutation test of the OPLS-DA model.

3.3. Multivariate statistical analysis

Although the significant variance was identified in stable isotope and some elemental compositions of pear peer from different origins, it was very hard to clearly discriminate all pear origins using any single or paired variable. To identify reliable markers for discrimination between the various geographical origins of pear, PCA was first performed to evaluate the potential of discriminating pear peer samples from four origins depending on the combination of multi-elements and stable isotopes. PCA is an unbiased and unsupervised method and typically used to analyze data without taking into account class membership [41]. The KMO test result was $KMO = 0.487$ ($KMO < 0.5$) indicating that our data is not suitable for PCA. Table S1 shows that there are six PCs and can explain 71.04 % variance. Fig. 2a shows the score plot of PCA with parameters of the model, including $R2X = 0.710$ and $Q2(\text{cum}) = -0.0158$, which can explain and predict 23 % and 16 % of the total variation, respectively. As shown in Fig. 2a, the pear from different origins were overlapped. Therefore, discriminating pear origin by PCA is not feasible, and it is necessary to further explore other supervised methods such as OPLS-DA [42] or LDA [43].

An OPLS-DA model was further developed to explore the pear origin. The training dataset matrices with a size of 49×20 (four stable isotopes and 16 elemental contents of samples harvested from four origins) were first built using OPLS-DA modelling, and external validation with a size of 21×20 was used to demonstrate the performance of the model (Fig. 2b). Analysis results showed that the predictive ability ($Q2Y(\text{cum})$) and cumulative variation ($R2Y(\text{cum})$) were 0.238 and 0.451, respectively, which indicated that the model has poor predictive and good explanatory effect. In the classification model, one SH sample was misclassified into JS sample, one sample in ZJ were misclassified into JS, while two JS samples were misclassified into CQ and SH, respectively. The accuracy of the classification can account for 87.76 %. In addition, Variable Importance of Projection (VIP) analysis was performed for the identified isotopic and elemental identification markers and results showed that variables $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, $\delta^2\text{H}$, Ni, Cd, Ca, $\delta^{15}\text{N}$, Sr and Ga were the most critical discriminant variables (with $VIP > 1$) (Fig. 2c). At last, a permutation test was used to check whether the model is over-fitting. After permuting the specified number 200 times, the y-intercept values for R^2 was 0.0974 and for Q^2 was -0.276 (Fig. 2d), which less than 0.40 and 0.05, indicating that the model is effective and there is no over-fitting [44]. Some previous isotope and multi-element reports investigating the geographical origin of peach and milk have acquired similar effective results by establishing OPLS-DA models [8,45].

To investigate the potential effect of the data type on the classification and identification of the pear origins, the LDA was conducted on the single stable isotope ratios, or the single mineral elements or the combination of these two data, and the classification results were summarized in Table 4. For the stable isotope ratios panel, the classification model achieved 90.0 % and 84.3 % accuracy by original validation and cross-validation (Table 4A), respectively, while achieving 97.1 % and 91.4 % correct assignment when only using the mineral element contents (Table 4B). Notably, when the values of the stable isotope ratios were coupled with the values of the element compositions, the original discrimination rate reached to 100 %, while the cross-validated rate reached 95.7 % (Table 4C). Fig. 3 displays the scatter plot of pear peer samples from four origins. Based on the Wilks' lambda values, we established three discriminant functions, which can explain 100 % of the variation (Table S3). Table S3 presents the extent of the association between chemical composition and the discriminant functions. Function 1 can interpret 75.6 % of the total variation, while function 2 can interpret 18.6 %, and function 3 can interpret 5.8 %. The Wilks' lambda values of the functions 1, 2 and 3 were 0.012, 0.146 and 0.542, respectively, with p -values $3.23\text{E}-25$, $7.29\text{E}-9$ and 0.01. All these results showed that the LDA based on the combined chemical data sets achieved good results for the discrimination of the geographical origin of pear from the four origins.

Table 4
Classification of pear peer samples via LDA.

Geographical origin	Predicted Group (Original/Cross-Validated)				Correctly Classified% (Original/Cross-Validated)
	CQ	SH	ZJ	JS	
A. Stable isotopes					
CQ (52)	52/49	0/0	0/0	1/3	98.1/94.2
SH (4)	0/0	4/4	0/0	0/0	100.0/100.0
ZJ (4)	1/2	1/1	1/0	1/1	25.0/0
JS (10)	3/4	0/0	0/0	7/6	70/60
Total					90.0/84.3
B. Element profiles					
CQ (52)	52/51	0/0	0/1	0/0	100/98.1
SH (4)	0/0	4/1	0/0	0/3	100/25
ZJ (4)	0/0	0/0	4/4	0/0	100/100
JS (10)	0/0	2/2	0/0	8/8	80/80
Total					97.1/91.4
C. Stable isotopes and elements combined					
CQ (52)	52/52	0/0	0/0	0/0	100.0/100.0
SH (4)	0/0	4/3	0/0	0/1	100.0/75
ZJ (4)	0/0	0/0	4/4	0/0	100.0/100.0
JS (10)	0/0	0/2	0/0	10/8	100.0/80
Total					100.0%/95.7%

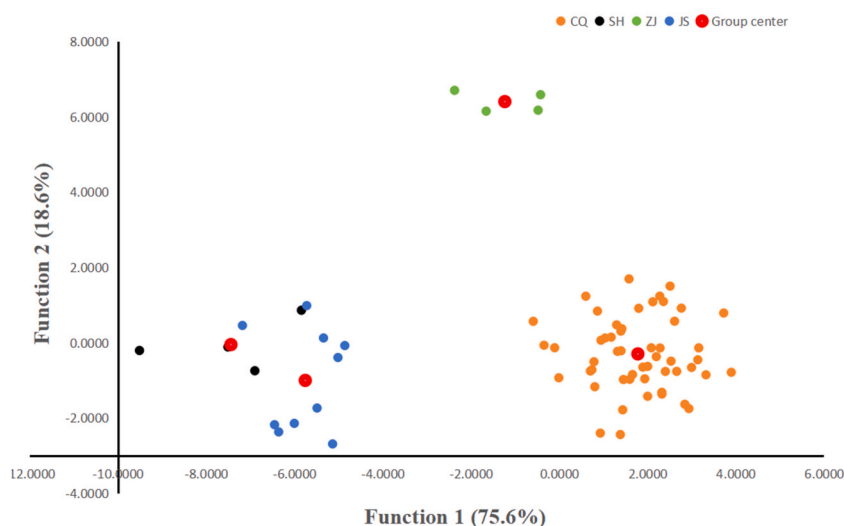


Fig. 3. Scattering points of the first two typical discriminant functions of pear peers from different regions by LDA.

4. Conclusions

In the present study, a total set of 70 pear samples was collected from four origins, and isotopic ratio ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, $\delta^{18}\text{O}$) and 16 element content signatures were determined. The multivariate statistical analysis LDA revealed combining the stable isotopes with mineral elements could be used to trace pear samples' geographical origin. The LDA models presented high discrimination ability and were able to discriminate accurately 100 % of samples in the initial classification. Further improvement can be made by expanding the sampling sites, enlarging the number of samples from more regions or years to verify our method.

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Data availability statement

All data generated or analyzed during this study are included in this published article.

CRediT authorship contribution statement

Tingting Zeng: Writing – original draft, Methodology, Formal analysis. **Tingting Fu:** Writing – original draft, Software, Conceptualization. **Yongchuan Huang:** Methodology, Data curation. **Wei Zhang:** Supervision, Resources. **Jiuping Gong:** Software, Data curation. **Bingjing Ji:** Writing – review & editing, Supervision. **Xiaoxia Yang:** Writing – review & editing, Software. **Mingfeng Tang:** Writing – review & editing, Supervision, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e37450>.

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