

Accurate geographical origin identification of winter jujube PGIs by mineral elements and stable isotopes in flesh and pit

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ABSTRACT

Geographical mislabeling of PGI winter jujube undermines product integrity. Here, we analyzed 29 elements and six stable isotopes in flesh and pit of 206 samples from four PGIs. Significant compositional differences were found among four PGIs ($p < 0.05$). Key markers (Sb, Mo, Cd, Y, $\delta^{18}\text{O}_f$, $\delta^2\text{H}_f$, Se, La, $\delta^{13}\text{C}_p$, Cr, Th, U, and Br) were identified via OPLS-DA (VIP > 1) and LDA incorporating effect size (η^2) analysis. Integrating comprehensive stable isotope and element data from pits and flesh, the full-feature-fusion LDA model achieved 92.7% accuracy and demonstrated robustness in inter-annual validation (training set: 97.4%; test set: 95.6%). This confirms that tissue elemental and isotopic fingerprints combined with LDA provide reliable PGI winter jujube authentication.

1. Introduction

Jujube (*Ziziphus jujuba* Mill.) belongs to the *Ziziphus* Mill. of Rhamnaceae (Gao et al., 2013). The history of jujube cultivation in China exceeds 2000 years, and its cultivation area and production both account for about 98% of the world. Winter jujube (*Ziziphus jujuba* “Dongzao”) is of excellent quality and is the most important cultivar of fresh-eating jujube, accounting for more than 80% of the fresh-eating jujube production in China (Kong et al., 2024). It has unique flavor characteristics and nutritional values, and is rich in vitamins, phenolic compounds, flavonoids, triterpenoids, polysaccharides, and other bioactive components (Gao et al., 2013). In China, winter jujube is mainly cultivated in Shaanxi, Hebei, Shanxi, and Shandong provinces (Kong et al., 2024), and the Dali Winter Jujube (DWJ) of Shaanxi, the Huanghua Winter Jujube (HWJ) of Hebei, the Linyi Winter Jujube (LWJ) of Shanxi, and the Zhanhua Winter Jujube (ZWJ) of Shandong

have become the famous products of geographical indication (Guo et al., 2022; Nie, 2024; Su et al., 2024; National Intellectual Property Administration, PRC, 2023). With the increase in demand for the winter jujube of PGIs, non-PGI winter jujube may be used to misrepresent PGI, which will damage the reputation of authentic products and the economic interests of farmers (Kong et al., 2024; Li et al., 2023). Therefore, for winter jujube PGIs, establishing an accurate and reliable origin-tracing system is crucial to their protection.

Current metabolites, mineral elements, and stable isotopes serve as key markers for the geographical traceability of agricultural products (Gao et al., 2025; Ju et al., 2025; Pimenta et al., 2025), and these techniques have also been successfully applied to the origin identification of jujube (Kong et al., 2024; Wang et al., 2020; Wen et al., 2023). Numerous studies have demonstrated that mineral elements and stable isotopes possess unique advantages as traceability indicators. Plants absorb mineral elements from the soil through their root systems, and

Abbreviations: AIR, Atmospheric nitrogen; DWJ, Dali Winter Jujube; EA-IRMS, Elemental analyzer and isotope-ratio mass spectrometer; HWJ, Huanghua Winter Jujube; ICP-MS, Inductively coupled plasma-mass spectrometry; LDA, Linear discriminant analysis; LWJ, Linyi Winter Jujube; LOQ, the limit of quantification; One-way ANOVA, One-way Analysis of Variance; OPLS-DA, Orthogonal partial least squares discriminant analysis; PCA, Principal component analysis; REEs, Rare earth elements; TC/EA-IRMS, Thermal conversion/elemental analyzer-isotope ratio mass spectrometry; VPDB, Vienna-pee dee belemnite; VSMOW, Vienna standard mean ocean water; VIP, Variable Importance in the Projection; ZWJ, Zhanhua Winter Jujube.

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the composition and content of these elements are primarily inherited from the soil parent material. However, in agricultural ecosystems, the bioavailable element pool in the topsoil is also modulated by long-term, region-specific agronomic practices such as fertilization, pesticide application, and irrigation (Chen et al., 2022; Liu, Chen, et al., 2021; Liu, Zhao, et al., 2021). Consequently, the multi-element composition of crops reflects an integrated signature of both natural geological provenance and historical anthropogenic inputs, providing a robust “terroir fingerprint” for geographical origin authentication (Shuai, Peng, et al., 2022). Although agricultural practices can influence the mineral element content and composition of crops, geographical factors exert a significantly greater influence (Shuai, Peng, et al., 2023). ICP–MS can simultaneous detection of multiple mineral elements and has been widely employed in authenticity studies of agricultural products, including rice (Chen et al., 2022), meat (Qi et al., 2021), and tea (Ren et al., 2022). Specifically, a recent study successfully utilized ICP–MS combined with chemometric analysis to identify the geographical origin of winter jujube by characterizing the mineral element composition of its flesh (Kong et al., 2024).

The effectiveness of stable isotope ratios as indicators for plant geographical traceability is primarily due to the fractionation effects inherent in plant biochemical pathways and the long-term regulatory effects of environmental factors (Shuai, Yang, et al., 2022). On the one hand, the degree of isotope fractionation is determined by the plant's own genetic metabolic pathways (Dawson et al., 2002). On the other hand, plants are constantly affected by environmental factors as they grow, and the isotope ratios in their tissues are essentially influenced by the interaction of factors such as climate, irrigation, and soil over the years (Nie et al., 2023). The combination of genetic (metabolic pathways) and environmental (growth conditions) factors makes isotope ratios significantly location-specific (Shuai, Peng, et al., 2023). EA–IRMS has become a mainstream analytical tool for identifying origins due to its high accuracy (Li et al., 2022; Shuai, Peng, et al., 2023; Nie et al., 2023). Currently, most research combines stable isotopes with mineral elements to improve the accuracy of geographical identification for agricultural products, including tea (Liu, Chen, et al., 2021), fruit

(Liu, Zhao, et al., 2021; Strojnik et al., 2022; Wang et al., 2020; Wang et al., 2024), and nut (Ghisoni et al., 2020), etc. For example, Wang et al. (2020) combined OPLS–DA with the elements and stable isotopes to identify the geographical origin of jujube in Xinjiang province. The results showed that Na, Al, Ba, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ had significant geographical specificity, which could be used as key indicators for the geographical origin of jujube in Xinjiang. At present, no literature has used the combination of mineral elements and stable isotopes to identify the geographical origin of winter jujube.

In this study, we innovatively and systematically integrated ICP–MS and EA–IRMS data from the pit and flesh of 206 winter jujube samples of four winter jujube PGIs to comprehensively identify their geographical origin. The objectives of this study were: (1) To compare the discriminatory power of models based on elemental data, isotopic data, and their integrated profiles for authenticating winter jujube PGIs; (2) To validate the rationale for data fusion in subsequent modeling by assessing the relationships between flesh and pit isotopes and among pit elements; (3) Assess the impact of climatic variability on model performance through inter-annual validation.

2. Materials and methods

2.1. Sample collection and preparation

At the harvest season of 2020 and 2021, 206 winter jujube orchards were selected, and one sample was collected from each orchard, including 39 samples of DWJ, 38 samples of HWJ, 41 samples of LWJ, and 88 samples of ZWJ (Table S 1 and Fig. 1). In each selected orchard, five jujube trees were randomly chosen, 100 mature fruits (20 fruits per one chosen tree) were collected and composed the sample of this selected orchard. Sampling is based on the principle of maintaining a roughly similar sample density defined as the ratio of the total number of samples to the total cultivation area. For each sample, the collected winter jujubes were washed with deionized water before separating the flesh and pit for distinct processing.

The flesh was divided into two portions. One portion was dehydrated

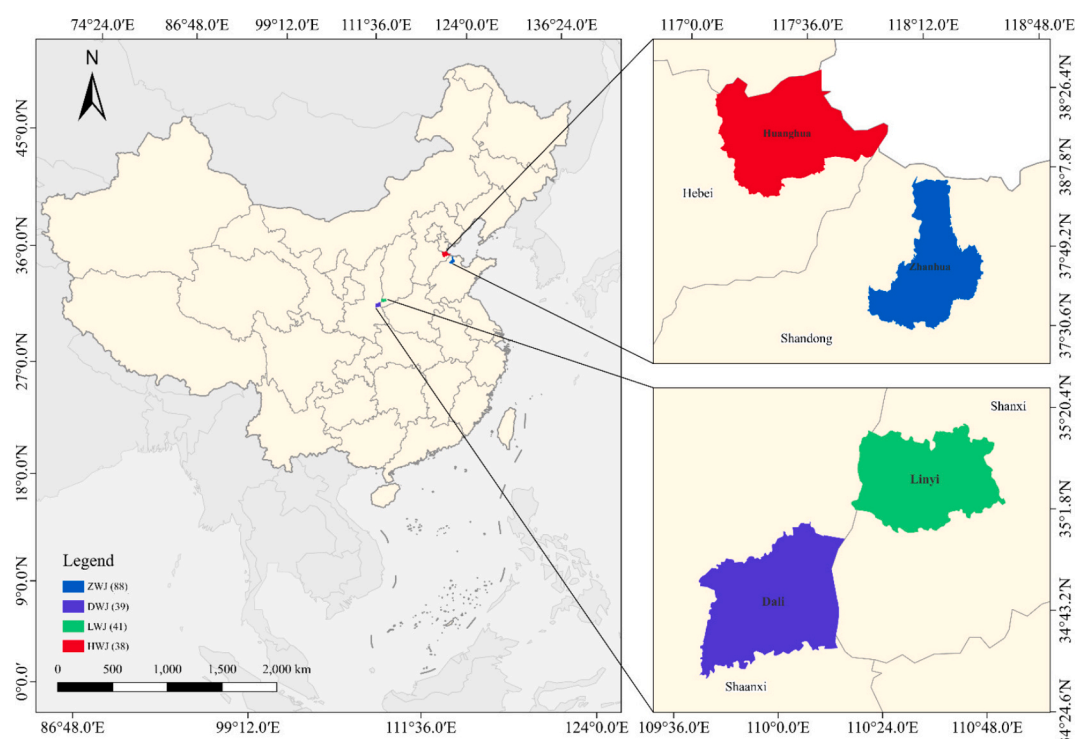


Fig. 1. Sampling locations of winter jujube PGIs.

in a forced-air drying oven (GFL-70, Tianjin Laboratory Instrument Equipment Co., Ltd., China) at 65 °C to constant weight, ground using a tungsten carbide vibratory disc mill (Retsch GmbH, Germany) to avoid metallic contamination, sieved through a 100-mesh (150 µm) nylon sieve, sealed in bags, and stored in silica-gel desiccators until $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ analysis. The other portion was flash-frozen in liquid nitrogen, homogenized into fine powder with a cryogenic ball mill (SPEX Sample Prep, USA), and stored at -80 °C until water extraction for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ analysis. The hard pits were dehydrated at 65 °C to constant weight, pulverized using a tungsten carbide vibratory disc mill (Retsch GmbH, Germany), sieved through a 100-mesh nylon sieve, and stored in desiccators for subsequent analysis of mineral elements, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$. The high hardness and inertness of tungsten carbide ensured effective grinding, and its potential elemental contaminants (e.g., W) were not among the analytical targets.

To ensure comparability of elemental concentrations across samples, all analyses were performed on a dry weight basis. The necessity of this rigorous drying step is underscored by the measured moisture content of the fresh samples. The average moisture content was 76.8% in flesh and 12.1% in pit. The high moisture content in flesh highlights that reporting data on a fresh weight basis would introduce substantial variability and obscure true compositional differences related to geography. Consequently, all elemental concentration data presented in the manuscript are reported on a dry weight basis. This normalization eliminates variability due to moisture differences among samples and ensures that the concentrations reflect the actual mineral composition of the tissue, a critical methodological step for authentic geographical origin discrimination as emphasized in standard practices (Kelly et al., 2005).

2.2. Chemicals and reagents

All analytical procedures were conducted using guaranteed reagent-grade chemicals. Deionized water (18.2 MΩ·cm) was obtained by the Milli-Q system (Merck Millipore, Germany). Nitric acid (HNO_3 , 65%–68% (w/w), GR grade, Sinopharm Chemical Reagents Co., Ltd., Beijing, China) and single-element standard solutions of 1000 mg/L (National Nonferrous Metals and Electronic Materials Analysis and Testing Center, Beijing, China) were used in this study. The elements analyzed include K, Ca, Mg, Ag, Cu, Zn, Mn, Ba, Pb, Mo, Cr, Se, Hg, Cd, Sb, Ni, As, V, Co, Ti, Th, U, Y, La, Ce, Pr, Tb, Er, and Lu.

Multiple point reference materials were employed to calibrate individual isotopic composition values. Stable isotope reference materials USGS 40 ($\delta^{13}\text{C}_{\text{VPDB}} = -26.39\text{‰}$; $\delta^{15}\text{N}_{\text{AIR}} = -4.52\text{‰}$), USGS 61 ($\delta^{13}\text{C}_{\text{VPDB}} = -35.05\text{‰}$; $\delta^{15}\text{N}_{\text{AIR}} = -2.87\text{‰}$), USGS 62 ($\delta^{13}\text{C}_{\text{VPDB}} = -14.79\text{‰}$; $\delta^{15}\text{N}_{\text{AIR}} = +20.17\text{‰}$), USGS 45 ($\delta^2\text{H}_{\text{VSMOW}} = -10.3\text{‰}$; $\delta^{18}\text{O}_{\text{VSMOW}} = -2.238\text{‰}$), USGS 46 ($\delta^2\text{H}_{\text{VSMOW}} = -235.8\text{‰}$; $\delta^{18}\text{O}_{\text{VSMOW}} = -29.80\text{‰}$), and USGS 47 ($\delta^{13}\text{C}_{\text{VPDB}} = -150.2\text{‰}$; $\delta^{18}\text{O}_{\text{VSMOW}} = -19.80\text{‰}$) were used to calibrate the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ values. The delta scales are based on VPDB ($\delta^{13}\text{C}_{\text{VPDB}} = 0\text{‰}$) for $\delta^{13}\text{C}$, AIR ($\delta^{15}\text{N}_{\text{AIR}} = 0\text{‰}$) for $\delta^{15}\text{N}$, and VSMOW ($\delta^2\text{H}_{\text{VSMOW}} = 0\text{‰}$, $\delta^{18}\text{O}_{\text{VSMOW}} = 0\text{‰}$) for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ based on quadruplicate analysis of the laboratory standard (Coplen, 2011).

2.3. Sample analysis

2.3.1. Mineral element analysis

A 0.500 g aliquot of winter jujube dry powder was precisely weighed into PTFE digestion vessels (BHW-09C Heating Block, Beijing Hengao Instrument Co., Ltd.), followed by addition of 8 mL nitric acid (65%–68%). Complete digestion was achieved using a Mars 6 microwave digestion system (CEM Corp, USA) with a programmed temperature gradient: initial ramp from 0 °C to 120 °C for 5 min, followed by heating to 150 °C in 1 min and final digestion at 180 °C for 20 min, with subsequent 30 min cooling. Digested solution was evaporated to less than 1 mL at 140 °C on a hot plate, then diluted to 25 mL with ultrapure water. The solution was filtered through a 0.22 µm water-based nylon

membrane and stored at 4 °C. The above 29 mineral elements were determined by ICP-MS (Agilent 7900, USA). The specific experimental procedures and instrument parameters followed those described in our previous study (Kong et al., 2024).

2.3.2. Stable isotope ratio analysis

A 2.500 mg aliquot of winter jujube flesh powder was weighed into a tin capsule for simultaneous $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis by EA (Flash 2000 HT, Thermo Fisher, Germany) and IRMS (MAT 253, Thermo Fisher, Germany). Winter jujube pit dry powder was weighed in aliquots of 1.500 mg for $\delta^{13}\text{C}$ and 10.000 mg for $\delta^{15}\text{N}$ analysis. The conditions for the EA-IRMS analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were as follows: the flow rate of the carrier and purge gas (helium, 99.999% purity) was 100 mL/min; the combustion temperature of carbon and nitrogen was 960 °C. Elemental C and N were changed into CO_2 and NO_x via burning with Cr_2O_3 at high temperatures, and nitrogen oxides were made into nitrogen gas via copper wires. The CO_2 and N_2 gases were then carried by helium and separated by a gas chromatography column. The gases were then analyzed using an IRMS (Ju et al., 2025).

A 2.000 g winter jujube fresh was weighted and packed into 8 mL brown glass bottle. The bottle was frozen at -18 °C for 24 h until completely frozen. The jujube juice was extracted using a fully automatic moisture vacuum condensation extraction system (BJJL-2200-D, Beijing Jianling Technology Co., Ltd). Extracted water was filtered through a 0.22 µm hydrophilic nylon membrane and then transferred into a glass vial for TC/EA-IRMS analysis. The conditions for TC/EA-IRMS analysis of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ were as follows: the flow rate of the carrier gas (helium, 99.999% purity) was 100 mL/min. The pyrolysis temperature was 1380 °C. Elemental H and O were converted into H_2 and CO via glassy carbon catalysis at high temperature. The H_2 and CO gases were carried by helium and separated by a gas chromatography column (Ju et al., 2025). The gases were then analyzed using an IRMS.

Stable isotope ratio (δ) was calculated using the standard delta notation formula: $\delta = \frac{R_{\text{sa}} - R_{\text{st}}}{R_{\text{st}}} \times 1000\text{‰}$, where R_{sa} and R_{st} represent the isotope ratios ($^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^2\text{H}/^1\text{H}$, or $^{18}\text{O}/^{16}\text{O}$) of the sample and the corresponding international standard, respectively. In this study, the subscripts “f” and “p” denote flesh and pit samples, respectively (e.g., $\delta^{13}\text{C}_f$, $\delta^{13}\text{C}_p$, $\delta^{15}\text{N}_f$, $\delta^{15}\text{N}_p$, $\delta^2\text{H}_f$, and $\delta^{18}\text{O}_p$).

2.4. Statistical analysis

2.4.1. Preliminary data analysis

Differences between groups were determined through one-way ANOVA, with post-hoc analysis conducted using either Kruskal-Wallis's test and Dunn's test using the GraphPad software package (Prism, San Diego, CA, USA). The Kruskal-Wallis's test identified significant overall differences in mineral elemental contents and stable isotopic values between multiple groups, followed by the Dunn's test to further clarify the region groups where specific differences existed. Differences between the four production areas were considered statistically significant at $p < 0.05$. Mineral element concentrations were $\log_{10}$ -transformed to mitigate skewness. Correlations were assessed via Pearson correlation analysis using Chiplot (<https://www.chiplot.online/>). The Pearson coefficient (r) was chosen because the $\log_{10}$ -transformed data reasonably met the assumptions of normality and linearity required for this parametric test, and it is the most widely used measure for assessing linear relationships in food traceability studies (Ahlgren et al., 2003; Kelly et al., 2005). For elemental concentrations below LOQ, values were substituted with LOQ/2 prior to $\log_{10}$ transformation. All variables were standardized using Z-score normalization prior to model construction to remove scale differences and ensure equal weighting across variables in the analysis (Henderi et al., 2021).

2.4.2. Multivariate modeling

PCA was performed using SPSS 26.0 based on the correlation matrix.

Components were retained based on eigenvalues >1, scree-plot inflection, and cumulative explained variance >70% (Wen et al., 2023). OPLS-DA model (SIMCA 16.0) was validated by 7-fold cross-validation and permutation testing ($n = 200$). Predictors with VIP scores >1.0 were considered influential. In addition to overall explained variance (R^2X , R^2Y) and predictive ability (Q^2), model performance was comprehensively assessed using a confusion matrix along with class-specific sensitivity and specificity (Jin et al., 2023).

LDA models (SPSS 26.0) employed a stepwise variable selection procedure based on minimizing Wilks' lambda. The discriminatory significance of each variable was assessed by examining the univariate F value (from one-way ANOVA) and the corresponding effect sizes (η^2). The multivariate effect size η^2 for the overall discriminant function was calculated as $\eta^2 = 1 - \text{Wilks' lambda}$. Classification was performed using the within-groups covariance matrix, with prior probabilities set proportional to group sizes. The analysis yielded three discriminant functions ($k - 1$ for $k = 4$ categories) (Maione & Barbosa, 2019). The relative contribution of each variable to these discriminant functions was presented as standardized canonical discriminant function coefficients (Table 5). The first two functions, which accounted for the majority of between-group variance, were primarily used for visualization (e.g., in scatter plots, Fig. 6). Model performance was rigorously evaluated using leave-one-out cross-validation (Shuai, Yang, et al., 2022; Kutner, Nachtsheim, Neter and Li, 2005).

Inter-annual validation was performed to assess model generalization. Due to the larger sample size in 2021, the full-feature fusion LDA model was trained on 2021 samples ($n = 115$) and applied to predict the independent 2020 samples ($n = 91$). Predicted group memberships were compared with actual PGI denominations using cross-tabulation, and the classification accuracy for each PGI was calculated as row percentages. This temporal hold-out validation provides a more rigorous assessment of model robustness against inter-annual climatic variability than internal cross-validation alone.

3. Results and discussion

3.1. Variations in mineral elements of winter jujube pit among PGIs

The accumulation of mineral elements in winter jujube is influenced by multiple pathways, including soil absorption, atmospheric deposition, and agricultural management. Four PGI winter jujube (LWJ, DWJ, ZWJ, and HWJ), revealed significant regional differentiation in their elemental profiles ($p < 0.001$) (Table 1). Distinct patterns in element contents were observed among the regions, demonstrating that mineral element content in winter jujube is highly region-dependent, providing a reliable chemical basis for authenticating GI products.

The soil type, physicochemical properties (e.g., pH), and parent material are key determinants of elemental background values (Tyler, 2004). Developed from homogeneous loess, the DWJ producing area of DaLi exhibits a stable elemental composition, with elements like Se and Ca demonstrating both high mean contents and low variability (Kong et al., 2024). Mg content varied little among the four PGIs (0.146–0.182 g/kg), being physiologically regulated as an essential plant nutrient that maintains stable concentrations (Gao et al., 2013). The ZWJ producing area of Zhanhua, located within the Yellow River alluvial zone, exhibits moderate and controlled variability in elements such as Ag and Ba, consistent with the area's complex microelement sources and transitional soil characteristics (Kelly et al., 2005). Notably, Ni in ZWJ showed a relatively high mean content (7.67 ± 8.1 mg/kg) with considerable variability (Table 1). This high variability likely reflects the heterogeneous background of Ni in alluvial deposits or historical metal enrichment during sedimentation (Tyler, 2004). The HWJ producing area of Huanghua is characterized by coastal saline-alkali soils with high pH and elevated salinity, which create geochemical conditions that notably influence element solubility and mobility (Li et al., 2023). This is evidenced by markedly higher and more variable levels of Cr (1.78 ± 4.7

Table 1

Mineral elemental content in winter jujube pit of four PGIs.

Element	LWJ (Mean ± SD)	DWJ (Mean ± SD)	ZWJ (Mean ± SD)	HWJ (Mean ± SD)	<i>p</i>
K (g/kg)	1.98 ± 0.3 a	1.99 ± 0.5 a	1.60 ± 0.3 b	1.74 ± 0.3 ab	***
Ca (g/kg)	1.47 ± 0.6 b	1.86 ± 0.5 a	1.10 ± 0.5 c	0.856 ± 0.3 c	***
Mg (g/kg)	0.158 ± 0.02 a	0.182 ± 0.05 a	0.146 ± 0.03 b	0.155 ± 0.02 a	***
Cu (mg/ kg)	3.26 ± 1.1 b	3.68 ± 3.6 b	5.79 ± 4.8 ab	7.42 ± 5.5 a	**
Mn (mg/ kg)	3.26 ± 0.3 a	3.33 ± 0.5 a	2.94 ± 0.5 b	3.22 ± 0.7 ab	***
Zn (mg/ kg)	3.97 ± 2.2 ab	5.61 ± 3.4 a	2.51 ± 1.6 c	3.17 ± 1.9 bc	***
Mo (μg/ kg)	44.1 ± 35.6	ND	ND	ND	***
Cr (mg/ kg)	1.37 ± 4.0 a	0.294 ± 0.1 b	0.461 ± 0.3 a	1.78 ± 4.7 a	**
Ni (mg/ kg)	6.03 ± 6.8 ab	5.09 ± 9.7 b	7.67 ± 8.1 a	7.41 ± 6.5 a	**
Ag (mg/ kg)	0.224 ± 0.6 d	2.99 ± 1.4 c	11.2 ± 3.1 b	18.5 ± 2.5 a	***
Ba (mg/ kg)	1.03 ± 0.3 d	3.57 ± 1.0 c	8.99 ± 3.0 b	13.8 ± 3.8 a	***
V (μg/kg)	5.12 ± 5.85 a	2.45 ± 2.4 b	4.00 ± 2.7 a	6.73 ± 7.9 a	*
Hg (μg/ kg)	0.249 ± 0.5 a	0.014 ± 0.1 b	0.043 ± 0.2 b	ND	***
Pb (μg/ kg)	20.7 ± 68.9 a	0.888 ± 5.5 b	2.25 ± 7.6 b	ND	***
Co (μg/ kg)	6.10 ± 11.6 a	3.17 ± 2.5 ab	2.42 ± 2.4 b	4.89 ± 5.9 a	***
As (μg/ kg)	9.54 ± 6.7 ab	11.5 ± 8.1 a	8.34 ± 4.0 b	13.2 ± 11.7 a	*
Se (μg/ kg)	0.092 ± 0.1 a	0.008 ± 0.0 b	0.008 ± 0.0 b	0.008 ± 0.0 b	***
Cd (μg/ kg)	1.51 ± 1.5 a	0.141 ± 0.2 b	ND	ND	***
Sb (μg/ kg)	10.2 ± 5.5 a	0.703 ± 1.4 b	ND	ND	***
Ti (μg/kg)	0.342 ± 0.6 a	0.016 ± 0.1 b	0.053 ± 0.2 b	ND	***
Th (μg/ kg)	0.579 ± 1.0 a	0.059 ± 0.4 b	0.195 ± 0.6 b	ND	***
U (μg/kg)	0.159 ± 0.3 a	0.012 ± 0.1 b	0.092 ± 0.3 ab	ND	***
Y (μg/kg)	0.512 ± 0.6	ND	ND	ND	***
La (μg/ kg)	0.576 ± 1.5	ND	ND	ND	***
Ce (μg/ kg)	16.2 ± 9.5 a	12.70 ± 0.7 b	8.22 ± 3.5 c	6.15 ± 2.7 c	***
Pr (μg/kg)	0.119 ± 0.3 d	1.06 ± 0.4 c	3.47 ± 1.0 b	5.58 ± 0.7 a	***
Tb (μg/ kg)	ND	0.203 ± 0.5 a	0.163 ± 0.3 ab	0.013 ± 0.0 b	**
Er (μg/ kg)	2.97 ± 1.4 a	2.13 ± 1.2 b	2.20 ± 1.4 b	1.20 ± 1.3 c	***
Lu (μg/ kg)	ND	0.391 ± 0.9 a	0.436 ± 0.7 a	0.415 ± 0.4 a	**

Note: All elemental data are expressed on a dry weight. The different letters (a, b, c, etc) on the graph indicate the results of Dunn's multiple comparison test: a shared letter indicates no significant difference between groups ($p > 0.05$), whereas the absence of a shared letter indicates a significant difference ($p < 0.05$). *, **, and *** indicate differences in regional elemental content significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Abbreviations: SD, standard deviation. ND: not detected.

mg/kg) and As (13.2 ± 11.7 μg/kg), along with elevated Ni (7.41 ± 6.5 mg/kg), which may reflect the combined effects of the region's natural geochemical background and local pollution sources (Shan et al., 2022; Shao et al., 2019). LWJ producing area of Linyi, located in the long-cultivated Fenwei Plain under strong anthropogenic disturbance, exhibits unique geochemical features. The Mo was detected only in LWJ

($44.1 \pm 35.6 \mu\text{g/kg}$) with extremely high variability, and multiple heavy metals such as Pb ($20.7 \pm 68.9 \mu\text{g/kg}$) and Cd ($1.51 \pm 1.5 \mu\text{g/kg}$) showed high means and high variability (Table 1). These patterns strongly suggest the presence of historical composite pollution sources in the area, such as industrial emissions or long-term wastewater irrigation (Shao et al., 2019; Xu et al., 2022).

Agricultural management practices are important anthropogenic drivers of regional differences in element contents (Kong et al., 2024). Pesticide usage, driven by variations in disease pressure across the four PGIs, serves as a key pathway for exogenous heavy metal input. This is clearly demonstrated by Cu: HWJ exhibited significantly higher and more variable concentrations, followed by ZWJ with elevated levels. The marked variability in both regions points to uneven field applications of copper-based fungicides like Bordeaux mixture (Nie, 2024). Differences in Zn content of DWJ, showing a significantly higher concentration ($5.61 \pm 3.4 \text{ mg/kg}$) than that of ZWJ ($2.51 \pm 1.6 \text{ mg/kg}$) ($p < 0.05$), may be attributed to differing use of zinc fertilizers or zinc-containing pesticides (Kong et al., 2024). Fertilizer application directly influences element inputs and accumulation (Kelly et al., 2005). The mean K content was highest in DWJ ($1.99 \pm 0.5 \text{ g/kg}$) and significantly higher than in ZWJ ($1.60 \pm 0.3 \text{ g/kg}$) ($p < 0.05$), attributable to the widespread use of standardized water-soluble fertilizers in facility cultivation in DWJ (Liu, 2015). These fertilizers ensure stable K supply and, due to their controlled composition, generally result in lower heavy metal contents (Nie, 2024). In the production of ZWJ and HWJ, local organic or compound fertilizers are commonly applied, which may introduce or amplify certain elemental signals. For example, the elevated and variable levels of Ag (HWJ: $18.5 \pm 2.5 \text{ mg/kg}$, ZWJ: $11.2 \pm 3.1 \text{ mg/kg}$) and Ba (HWJ: $13.8 \pm 3.8 \text{ mg/kg}$, ZWJ: $8.99 \pm 3.0 \text{ mg/kg}$) in these regions might reflect the complex and heterogeneous composition of such locally sourced fertilizers (Kong et al., 2024; Xu et al., 2022). Similarly, higher Zn content in DWJ ($5.61 \pm 3.4 \text{ mg/kg}$) and LWJ ($3.97 \pm 2.2 \text{ mg/kg}$) compared to ZWJ ($2.51 \pm 1.6 \text{ mg/kg}$) suggests regional differences in the use of zinc-enriched fertilizers or soil amendments ($p < 0.05$) (Table 1). These differences collectively highlight that how fertilizer type and sourcing shape the elemental profile of winter jujube (Kong et al., 2024). In addition, irrigation with industrially polluted water serves as a source of heavy metal accumulation in soils of winter jujube PGIs (Shao et al., 2019; Xu et al., 2022), as potentially reflected in the elevated levels of Cd ($1.51 \pm 1.5 \mu\text{g/kg}$) and Pb ($20.7 \pm 68.9 \mu\text{g/kg}$) in LWJ, as well as Cr ($1.78 \pm 4.7 \text{ mg/kg}$) and As ($13.2 \pm 11.7 \mu\text{g/kg}$) in HWJ (Table 1). The higher Cd and Pb contents in LWJ, as well as the Cr and As levels in HWJ, may be related to historical irregular irrigation practices, leading to the accumulation of heavy metals in the soil-plant system (Shao et al., 2019; Xu et al., 2022).

It should be noted that heavy metals such as Pb, Cd, and As are strongly bio-accumulative and toxic; long-term consumption of foods with heavy metals above safety limits can cause irreversible damage to the nervous, renal, and cardiovascular systems, with potential carcinogenic risks (Chung et al., 2017). The highest contents detected in all samples were well below the corresponding regulatory limits (Table 1): Pb ($20.7 \mu\text{g/kg}$) below $100 \mu\text{g/kg}$, Cd ($1.51 \mu\text{g/kg}$) below $50 \mu\text{g/kg}$, Cr (1.78 mg/kg) below 2.0 mg/kg (GB 2762-2025), and inorganic As ($13.2 \mu\text{g/kg}$) below $100 \mu\text{g/kg}$ (European Commission, 2006; National Health Commission of the People's Republic of China, 2025). Elements such as Ni, Cu, and Zn were within typical background ranges and are not subject to specific safety limits. The observed variations in elemental content, all of which fall within safe ranges, primarily reflect region-specific environmental and agronomic conditions. These variations further support the use of elemental profiles as effective and distinctive fingerprints for geographical origin traceability (National Health Commission of the People's Republic of China, 2025).

Beyond macro, trace, and heavy metal elements, REEs provide a valuable geochemical fingerprint for tracing the geographical origin of winter jujube, although their interpretation requires consideration of both natural and anthropogenic sources (Tyler, 2004). In plants,

although REEs in agricultural systems are primarily derived from soil parent materials, potential anthropogenic inputs, particularly from phosphate fertilizers and atmospheric deposition, still need to be considered (Li et al., 2019; Shuai, Peng, et al., 2022). Significant differences in REE contents were observed among four PGIs, with notable enrichment of La and Ce. The LWJ region exhibited the highest diversity of REEs, with elevated levels of Sb ($10.2 \pm 5.5 \mu\text{g/kg}$), Ti ($0.342 \pm 0.6 \mu\text{g/kg}$), Th ($0.579 \pm 1.0 \mu\text{g/kg}$), U ($0.159 \pm 0.3 \mu\text{g/kg}$), Y ($0.512 \pm 0.6 \mu\text{g/kg}$), La ($0.576 \pm 1.5 \mu\text{g/kg}$), Ce ($16.2 \pm 9.5 \mu\text{g/kg}$), and Er ($2.97 \pm 1.4 \mu\text{g/kg}$) (Table 1). Notably, Y and La were exclusively detected in this region. The high Ce content and extremely high La/Er ratio may suggest that the parent material is rich in yttrium minerals (e.g., xenotime), and weathering conditions favor element mobilization and accumulation (Tyler, 2004). Notably, elements like Sb, Th, U, Cd, and Pb can originate from both natural weathering and human activities (e.g., industrial dust, fertilizer) (Shan et al., 2022; Xu et al., 2022). In geographical traceability, their combined signal serves as a regional fingerprint if it shows spatial consistency (low inter-sample variability) and temporal stability (reproducibility across vintages), regardless of origin (Kelly et al., 2005; Zhao et al., 2020). HWJ had no detectable levels of Sb, Ti, Th, U, Y, or La. The absence of these elements may reflect both natural geological background and limited anthropogenic inputs (Shan et al., 2022; Tyler, 2004). However, its mean Pr content ($5.58 \pm 0.7 \mu\text{g/kg}$) was significantly higher than other PGIs ($p < 0.05$), and it exhibited an extremely high Pr/Er ratio. This characteristic may be related to the alkaline saline soils in this coastal area, which promote strong geochemical fractionation of Pr from other REEs (Tyler, 2004). Ce in ZWJ ($8.22 \pm 3.5 \mu\text{g/kg}$) was significantly lower than that in DWJ ($12.70 \pm 0.7 \mu\text{g/kg}$). But the Pr content in ZWJ ($3.47 \pm 1.0 \mu\text{g/kg}$) was higher than in the other three PGIs ($p < 0.05$). Notably, La was quantifiable in the other three PGIs, whereas in ZWJ samples it was detected only in some individuals, with a mean concentration below the detection limit (reported as ND) (Table 1), exhibiting considerable within-region variability. This observation further highlights the pronounced regional heterogeneity in REE profiles (Shuai, Yang, et al., 2022).

The differences in REEs among the PGIs are influenced by a combination of geological background, soil environment, and potentially long-term anthropogenic inputs (Kelly et al., 2005; Tyler, 2004). The LWJ producing area (Fenwei Basin, Linyi) is underlain developed on ancient REE-rich strata, but the co-occurrence of Sb, Th, U, and Y with REEs suggests that the observed fingerprint integrates both natural and anthropogenic signatures. The coastal region of HWJ producing area promotes strong REE fractionation, with minimal interference from anthropogenic REE sources as evidenced by the absence of typical pollution indicators (Shan et al., 2022; Xu et al., 2022). In contrast, DWJ producing area, derived from loess, presents a generally low REE background (despite aeolian signals in Ce and Lu), and ZWJ producing area, as a Yellow River alluvial zone, exhibits a transitional, mixed-source composition (Tyler, 2004). Soil properties, such as alkalinity in HWJ producing area, further modulate REE bioavailability. It is worth noting that elements such as Sb and Pb may originate from both natural weathering processes and human activities (e.g., industrial pollution, agricultural inputs) (Shan et al., 2022; Xu et al., 2022). Ultimately, each PGI has developed a distinct REE fingerprint that reflects its unique combination of natural background and anthropogenic influences. The specific characteristics are as follows: the LWJ region exhibits multi-element enrichment (particularly Y and La), which likely results from the combined effects of REE-rich parent material and anthropogenic inputs. The HWJ region is characterized by Pr enrichment and Ce depletion, primarily attributable to natural geochemical fractionation processes in alkaline coastal soils (Tyler, 2004; Xu et al., 2022). In contrast, the DWJ and ZWJ regions can be effectively distinguished by differences in their Ce/Pr/Lu ratios (Tyler, 2004).

3.2. Variations in stable isotopes of winter jujube among PGIs

$\delta^{13}\text{C}_f$ and $\delta^{13}\text{C}_p$. The $\delta^{13}\text{C}$ values in plant tissues are primarily regulated by photosynthetic pathway and water-use efficiency. They originate from atmospheric CO_2 and reflect the long-term integration of photosynthetic carbon assimilation (Coplen, 2011; Farquhar et al., 1989; Liu et al., 2020). In C_3 plants such as winter jujube, $\delta^{13}\text{C}$ is influenced by environmental conditions that affect photosynthetic physiology. Specifically, under drought or high-temperature stress, stomatal conductance decreases to improve water-use efficiency, which concurrently reduces discrimination against $^{13}\text{CO}_2$ during carboxylation, leading to less negative $\delta^{13}\text{C}$ values. Temperature further modulates photosynthetic activity and stomatal behavior, thereby influencing $\delta^{13}\text{C}$ fractionation (Farquhar et al., 1989; Shuai, Peng, et al., 2022). Moreover, water-limited conditions suppress carbon isotope fractionation, resulting in elevated $\delta^{13}\text{C}$ in plant tissues (Zhao et al., 2020).

The $\delta^{13}\text{C}$ values of the winter jujube across the PGIs ranged from -28.7‰ to -26.6‰ , which falls within the typical C_3 range (-35.0‰ to -25.0‰) (Chung et al., 2017). Mean $\delta^{13}\text{C}$ values of the four PGIs also aligned with this range (Chung et al., 2017; Farquhar et al., 1989). A clear spatial pattern emerged, with $\delta^{13}\text{C}_f$ in flesh ranked as ZWJ (-27.8‰) $\approx$ HWJ (-27.7‰) $>$ LWJ (-28.5‰) $>$ DWJ (-28.7‰); a similar trend was observed for $\delta^{13}\text{C}_p$ in pits. DWJ exhibited the lowest mean $\delta^{13}\text{C}_p$ value (-28.0‰), compared to -27.6‰ in LWJ and -26.6‰ in both HWJ and ZWJ. Extreme values further highlighted regional contrasts: the highest $\delta^{13}\text{C}_f$ and $\delta^{13}\text{C}_p$ were recorded in HWJ (-25.8‰ and -24.4‰ , respectively), while the lowest occurred in DWJ (-30.1‰ and -29.4‰) (Table 2; Figure S 1). These spatial and extremal variations can be attributed to differences in integrated water-use efficiency driven by regional climatic conditions. HWJ likely experiences drier and/or warmer environments that promote higher water-use efficiency and thus less negative $\delta^{13}\text{C}$ values, whereas in cooler and/or more humid conditions, DWJ favors greater stomatal conductance and stronger isotopic discrimination during photosynthesis. This climatic interpretation is further reinforced by the consistently lower $\delta^{13}\text{C}$ across tissues in DWJ (Liu et al., 2020). Collectively, the results support a region-specific climatic explanation for the observed $\delta^{13}\text{C}$ variation and align with the established mechanism whereby water stress suppresses carbon isotope fractionation, a process rooted in photosynthetic regulation, which lead to elevated $\delta^{13}\text{C}$ (Zhao et al., 2020). Thus, $\delta^{13}\text{C}$ in winter jujube serves not only as an indicator of water-use efficiency but also reflects the integrated outcome of photosynthetic activity under distinct environmental controls. Within the same PGIs, $\delta^{13}\text{C}_p$ values in pits (-28.0‰ to -26.6‰) were generally slightly higher than $\delta^{13}\text{C}_f$ values in flesh

(Table 2). This inter-tissue difference may be related to carbon allocation during transport between fruit tissues, as well as to further carbon isotope fractionation associated with metabolic processes such as lipid synthesis during later developmental stages of the pit (Badeck et al., 2005; Sha et al., 2020).

$\delta^{15}\text{N}_f$ and $\delta^{15}\text{N}_p$. The $\delta^{15}\text{N}$ values in plant tissues are primarily regulated by the $\delta^{15}\text{N}$ composition of the soil, which in turn is influenced by nitrogen sources and soil nitrogen cycle processes (e.g., nitrification, denitrification) (Zhao et al., 2020). Among these, fertilization practice is a core factor and organic fertilizers (e.g., animal manure) typically have higher $\delta^{15}\text{N}$ values ($+10\text{‰}$ to $+20\text{‰}$), whereas synthetic nitrogen fertilizers have $\delta^{15}\text{N}$ values (-4‰ to $+4\text{‰}$) encompassing the value of atmospheric nitrogen (0‰) (Chung et al., 2017).

This study showed the mean $\delta^{15}\text{N}_f$ values ranked as HWJ (0.467‰) $>$ ZWJ (0.027‰) $>$ LWJ (-0.924‰) $>$ DWJ (-1.39‰), and a similar trend was observed for $\delta^{15}\text{N}_p$: HWJ (-0.584‰) $>$ ZWJ (-2.04‰) $>$ DWJ (-3.05‰) $>$ LWJ (-3.43‰) ($p < 0.05$) (Table 2). All measured $\delta^{15}\text{N}$ values fell within or close to the range characteristic of synthetic nitrogen fertilizers (-4‰ to $+4\text{‰}$) and were significantly lower than those typical of organic fertilizers (Chung et al., 2017; Shuai, Li, et al., 2023). This pattern strongly suggests that synthetic nitrogen fertilizers were the predominant nitrogen source in four winter jujube PGIs, with minimal contribution from organic amendments. Nevertheless, systematic variations in $\delta^{15}\text{N}$ were observed in four PGIs. Particularly, HWJ exhibited the highest mean $\delta^{15}\text{N}$ values (-0.584‰ in pits and -0.467‰ in flesh), followed by ZWJ (-2.04‰ and -0.027‰ , respectively), while LWJ and DWJ displayed the lowest values (e.g., -3.43‰ and -3.05‰ in $\delta^{15}\text{N}_p$, respectively) (Table 2). These differences likely reflect region-specific agricultural practices and soil biogeochemical conditions. The relatively elevated $\delta^{15}\text{N}$ in HWJ may be attributed to a greater historical or current use of organic amendments, higher soil organic matter content, and more active nitrification processes. In contrast, the lower $\delta^{15}\text{N}$ in DWJ and LWJ suggests a stronger reliance on synthetic fertilizers, possibly combined with soil conditions (e.g., pH, texture, or moisture) that promote nitrogen loss through pathways such as denitrification, which preferentially removes ^{14}N and further lowers the $\delta^{15}\text{N}$ of the available nitrogen pool (Bateman & Kelly, 2007; Craine et al., 2015). Notably, despite geographical proximity, ZWJ and HWJ still differed significantly in $\delta^{15}\text{N}$ ($p < 0.05$), highlighting subtle distinctions in local soil isotopic baselines, organic matter cycling efficiency, or fertilizer input regimes (Chung et al., 2017; Zhao et al., 2020). Overall, $\delta^{15}\text{N}$ serves as a sensitive indicator of both soil fertility characteristics and agricultural management practices, demonstrating clear potential for discriminating four winter jujube PGIs (Kelly et al., 2005; Nie et al., 2023).

Notably, in all PGIs except HWJ, the $\delta^{15}\text{N}_p$ values in pits were significantly lower than the $\delta^{15}\text{N}_f$ values in flesh ($p < 0.05$). This systematic isotopic fractionation between flesh and pit may originate from isotope effects during nitrogen transport, assimilation, and remobilization within the plant (Kolb & Evans, 2002). During the later stages of fruit development, nitrogen compounds are transported and metabolically converted in the pit, particularly for the synthesis of proteins and nucleic acids. These biochemical processes may preferentially incorporate the lighter isotope ^{14}N , resulting in its relative enrichment and consequently more negative $\delta^{15}\text{N}$ values in pit tissues (Craine et al., 2015; Kolb & Evans, 2002). Furthermore, differences in fertilization practices, soil nitrogen forms, and the efficiency of nitrogen metabolism among producing regions may modulate the intensity of this physiological fractionation process, leading to the distinct tissue-distribution pattern observed in HWJ (Bateman & Kelly, 2007; Craine et al., 2015).

$\delta^{18}\text{O}_f$ and $\delta^2\text{H}_f$. Water-stable isotopes ($\delta^{18}\text{O}$, $\delta^2\text{H}$) in plants are governed by the isotopic composition of local water sources and transpiration-driven fractionation, varying systematically with geographic-climatic factors (Houghton et al., 1990; Kelly et al., 2005; Shuai, Li, et al., 2023). In this study, significant differences in the mean $\delta^{18}\text{O}_f$ and $\delta^2\text{H}_f$ values were observed among four winter jujube PGIs ($p <$

Table 2
Stable isotope ratios in winter jujube flesh and pit of four PGIs.

Stable isotope	LWJ (Mean $\pm$ SD)	DWJ (Mean $\pm$ SD)	ZWJ (Mean $\pm$ SD)	HWJ (Mean $\pm$ SD)	<i>p</i>
$\delta^{13}\text{C}_f$ (‰)	-28.5 ± 0.5 b	-28.7 ± 0.6 b	-27.8 ± 0.6 a	-27.7 ± 0.8 a	***
$\delta^{15}\text{N}_f$ (‰)	-0.924 ± 1.3 b	-1.39 ± 1.7 b	0.027 ± 1.5 a	0.467 ± 1.4 a	***
$\delta^2\text{H}_f$ (‰)	-65.3 ± 12.9 c	-65.9 ± 17.7 c	-43.8 ± 6.4 a	-51.8 ± 7.3 b	***
$\delta^{18}\text{O}_f$ (‰)	-8.15 ± 2.8 b	-9.08 ± 3.6 b	-4.83 ± 2.4 a	-5.80 ± 2.5 a	***
$\delta^{13}\text{C}_p$ (‰)	-27.6 ± 0.5 b	-28.0 ± 0.7 b	-26.6 ± 0.8 a	-26.6 ± 1.0 a	***
$\delta^{15}\text{N}_p$ (‰)	-3.43 ± 1.7 c	-3.05 ± 1.8 c	-2.04 ± 1.7 b	-0.584 ± 2.2 a	***

Note: The different letters (a, b, c, etc.) on the graph indicate the results of Dunn's multiple comparison test: a shared letter indicates no significant difference between groups ($p > 0.05$), whereas the absence of a shared letter indicates a significant difference ($p < 0.05$). *, **, and *** indicate differences in regional elemental content significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Abbreviations: SD, standard deviation.

0.001). The mean $\delta^{18}\text{O}_f$ values ranked as ZWJ (-4.83‰) > HWJ (-5.80‰) > LWJ (-8.15‰) > DWJ (-9.08‰), while $\delta^2\text{H}_f$ values were significantly higher in the coastal group (ZWJ: -43.8‰ ; HWJ: -51.8‰) than in the inland group (LWJ: -65.3‰ ; DWJ: -65.9‰) (Table 2). Variability within each PGI (e.g., $\delta^2\text{H}_f$ SD up to 17.7‰) also indicated influences from inter-annual climate fluctuations and micro-environmental differences (Table S 1) (Houghton et al., 1990; Sier & Monteith, 2016). These spatial patterns reflect systematic differences in regional hydroclimatic conditions. The highest $\delta^{18}\text{O}_f$ and $\delta^2\text{H}_f$ values in ZWJ correspond to its distinctly higher annual precipitation (YR: $814.0\text{--}988.2\text{ mm}$) and mean annual temperature (YT: $14.2\text{--}14.5\text{ °C}$), conditions that favor isotopically heavier precipitation (Bowen & Revenaugh, 2005; Gat et al., 2011). Conversely, the lower and statistically indistinguishable values for LWJ and DWJ align with the lighter isotopic signature characteristic of precipitation in their more continental temperate climate (Table S 1). Notably, despite geographic proximity, ZWJ and HWJ showed significant differences in $\delta^2\text{H}_f$ ($p < 0.01$). This divergence likely arises from their distinct climate subtypes (temperate vs. warm-temperate humid monsoon) and the more pronounced hydrothermal regime in ZWJ, which may enhance evaporation and transpiration-driven fractionation (Dawson et al., 2002; Gao et al., 2025).

3.3. Correlation analysis of elemental and isotopic signatures

3.3.1. Correlation analysis of mineral elements

The spatial distribution and inter-element relationships of mineral elements in winter jujube were investigated through correlation analysis with geographical coordinates and among elemental concentrations. Ag and Pr exhibited positive correlations with latitude ($r = 0.5392$ and 0.4985 , respectively) and longitude ($r = 0.5349$, and 0.4889 , respectively), indicating their contents increase systematically with geographical coordinates (Fig. 2) (Sier & Monteith, 2016). This spatial pattern is closely linked to regional hydrothermal conditions, where lower temperatures and reduced evaporation at higher coordinates influence soil weathering, leaching, and element bioavailability (Sier & Monteith, 2016). Furthermore, geographical coordinates integrate the effects of soil parent material, pedogenesis, and long-term agricultural practices, collectively amplifying these elemental signals at a regional scale (Kelly et al., 2005). Correlation analysis among elements revealed systematic patterns in their sources and behaviors. Combinations with extremely high correlations ($r > 0.9$), such as Cd—Sb ($r = 0.9718$), Pb—Th—U ($r = 0.9228\text{--}0.9774$), and Hg—Ti ($r = 0.9716$), predominantly indicate specific industrial pollution sources or mineral assemblages, demonstrating strong anthropogenic or geological specificity (Fig. 2) (Shan et al., 2022; Xu et al., 2022). Groups with high correlations ($r = 0.7\text{--}0.9$), such as Hg—Pb/Th/U ($r = 0.7310\text{--}0.8384$) and Ti—U/Th/Pb (r

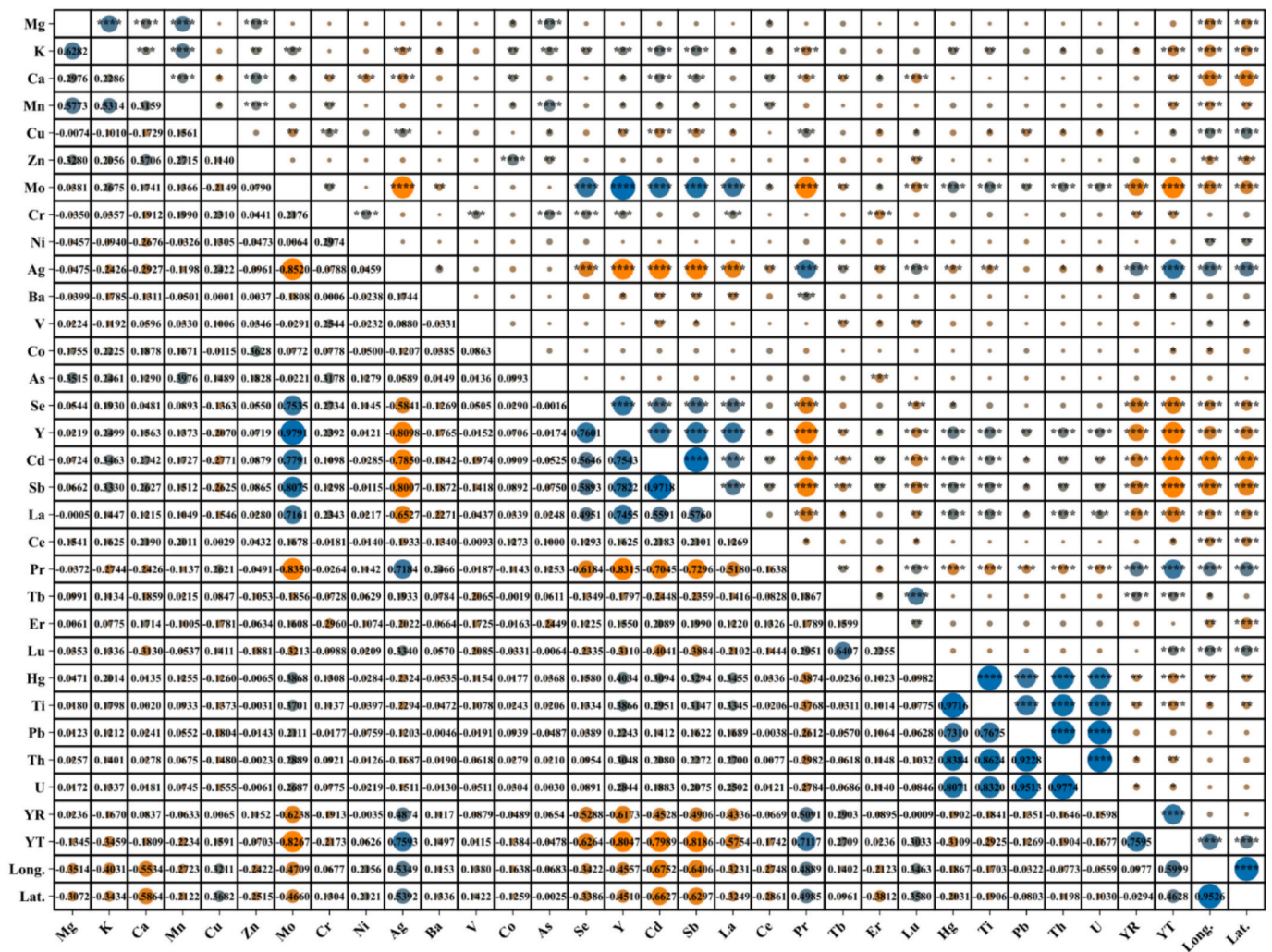


Fig. 2. Correlation among the environmental variables and the element content of the winter jujube samples. (Note: The deepening of the blue color represents a gradual increase in the positive correlation, while the deepening of the orange color represents a gradual increase in the negative correlation. YR, YT, Long., and Lat. stand for yearly rainfall, yearly temperature, longitude, and latitude).

= 0.7675–0.8624), often reflect multi-source pollution superposition or complex geochemical processes. Correlations within the moderate range ($r = 0.5$ – 0.7), such as those between Mg and Mn/K ($r = 0.5773$ – 0.6282) and between Se and Y ($r = 0.7601$), contribute to the multi-dimensional fingerprint by reflecting natural geochemical backgrounds, coupled nutrient cycling, or specific sedimentary influences (Kelly et al., 2005; Tyler, 2004).

3.3.2. Correlation analysis among stable isotopes

$\delta^2\text{H}_f$ and $\delta^{18}\text{O}_f$ were strongly correlated ($r = 0.8900$, $p < 0.001$) (Fig. 3), reflecting their common moisture origin and coupled fractionation driven by transpiration (Bowen & Revenaugh, 2005; Gat et al., 2011). Although $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ all show positive correlations with longitude and latitude ($r = 0.3646$ – 0.6529), the strength of these correlations is moderate to weak (Fig. 3). While geographical gradients are a factor influencing their spatial variation, they may not be the sole or primary driver. These results indicate that the spatial variation of these isotopes is shaped by a combination of geographical and climatic factors, and may be significantly modulated by localized processes such as soil nitrogen cycling, fertilization practices, water sources, and transpiration-driven fractionation (Craine et al., 2015).

$\delta^{13}\text{C}_f$ and $\delta^{13}\text{C}_p$ exhibited strong inter-tissue consistency ($r = 0.7372$, $p < 0.001$), indicating a shared photosynthetic carbon source and coherent stomatal regulation across the fruit. This high correlation primarily stems from the direct translocation of recently fixed carbohydrates via the phloem, with the major isotope fractionation occurring uniformly during initial photosynthesis mediated by Rubisco. The robust, yet not absolute, correlation can be attributed to minor isotopic fractionation during post-photosynthetic carbon allocation and potential differences in the developmental timing of carbon assimilation between flesh and pit tissues (Badeck et al., 2005; Farquhar et al., 1989).

In contrast, $\delta^{15}\text{N}$ showed weaker tissue-level correspondence ($r = 0.5180$, $p < 0.001$), suggesting more distinct pathways of nitrogen assimilation and metabolism between flesh and pit. The disparity arises from the complex biogeochemical cycling of nitrogen, which involves multiple potential sources (e.g., newly absorbed vs. remobilized storage N) and tissue-specific metabolic demands. Furthermore, substantial and variable isotope fractionation can occur during numerous steps of nitrogen uptake, assimilation, transport, and re-allocation (Kolb & Evans, 2002; Schmidt et al., 2004). The tissue-specific difference in $\delta^{15}\text{N}$ ($\Delta^{15}\text{N} = \delta^{15}\text{N}_f - \delta^{15}\text{N}_p$) further revealed distinctive profiles among origins. For

example, DWJ displayed a mean $\Delta^{15}\text{N}$ of $+0.8\text{‰}$ (indicating ^{15}N enrichment in flesh), whereas LWJ averaged -0.3‰ , with these differences being statistically significant across PGIs. This pattern likely stems from divergent temporal nitrogen integration. The pit mainly incorporates nitrogen during early fruit development, capturing post-anthesis nitrogen pools, while the flesh continues to assimilate nitrogen throughout fruit expansion, rendering it sensitive to seasonal agronomic practices such as fertilization timing (Bateman & Kelly, 2007; Craine et al., 2015; Kolb & Evans, 2002). Thus, $\Delta^{15}\text{N}$ serves as a temporally integrated signature that encodes region-specific management and environmental information, thereby enhancing the discriminatory power for geographical origin identification of winter jujube (Craine et al., 2015; Kelly et al., 2005; Kolb & Evans, 2002).

Correlation analysis demonstrates that elemental and isotopic signatures not only possess intrinsic geographical indicative potential but, more importantly, their complex internal correlations collectively form a robust “chemical fingerprint” network (Kelly et al., 2005; Liu et al., 2020). This multi-layered feature system is crucial, as it ensures that the subsequent discrimination model can rely on stable origin-specific signals from the entire network (Nie et al., 2023; Wang et al., 2020). Accordingly, chemometrics will be employed to identify key discriminatory markers and evaluate the discriminatory power of multi-elements and stable isotopes for geographical origin discrimination of winter jujube PGIs.

3.4. Chemometrics for geographical origin discrimination of winter jujube PGIs

3.4.1. Principal component analysis (PCA)

PCA was employed as an initial step for dimensionality reduction, aiming to reduce the number of correlated variables while preserving as much original variance as possible (Shuai, Peng, et al., 2022). The results showed distinct differences in the cumulative variance explained by the first two principal components across the models.

The cumulative variance of the first two PCs exceeded 75% in PCA models using only stable isotopes (Fig. 4 a–c), but fell below 40% when elemental indicators were included (Fig. 4 d–f). This demonstrates that stable isotope data are internally consistent, with most of their variation captured by the first two PCs, whereas mineral elements add substantial dimensionality and diversity to the dataset. These results underscore the importance of integrating multiple indicators to build a robust and highly discriminative origin fingerprint (Kelly et al., 2005). The PCA score plots revealed substantial overlap among samples from the four PGIs when only isotopic data were used (Fig. 4 a–c). Although the inclusion of pit elemental data (Fig. 4 d–f) somewhat reduced the overlap among the four PGI jujube samples, clear clustering by origin was still not achieved even with all features integrated. These results demonstrate that while PCA effectively summarized the overall data structure, it was insufficient for capturing the fine-scale geochemical differences necessary to discriminate four PGIs (Jin et al., 2023; Wen et al., 2023). Consequently, supervised discriminant analysis methods were subsequently applied to achieve more accurate classification.

3.4.2. Orthogonal partial least squares discriminant analysis (OPLS-DA)

To classify four winter jujube PGIs, this study constructed six OPLS-DA models (a–f). The permutation test results (Fig. 5 C) confirmed that none of the models were overfitted (R^2 : 0.0–0.2, Q^2 : –0.2–0.05). **Model a** (pit isotopes only) showed substantial sample overlap ($R^2Y = 0.25$, $Q^2 = 0.23$), with only ZWJ partially separated, corresponding to an overall accuracy of 53.9% (Fig. 5 A; D). Single model exhibited weak discriminatory performance for four PGIs, resulting in abnormally low accuracy rate. In **Model a**, the accuracies for LWJ and HWJ were only 29.3% and 10.5%, respectively, while in **Model d**, the accuracy for LWJ was as high as 90.2%. Furthermore, significant classification confusion was observed between ZWJ and HWJ in single-source models. **Model a** showed severe classification confusion between LWJ and DWJ, and this

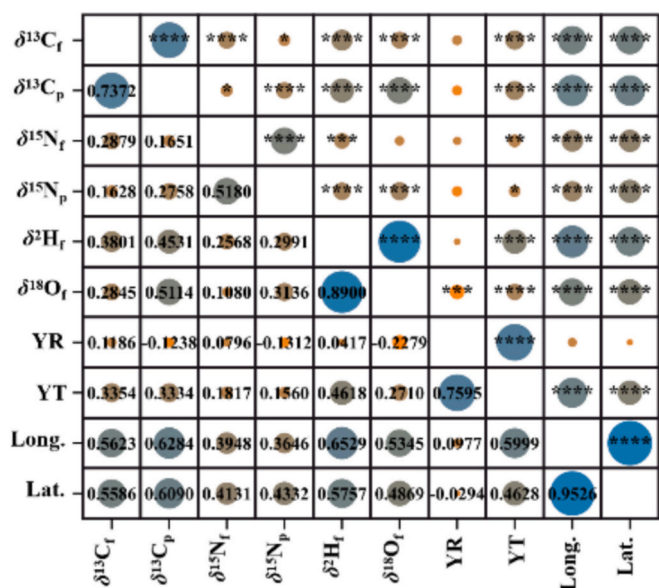


Fig. 3. Correlation among the environmental variables and the stable isotope ratios of the winter jujube samples.

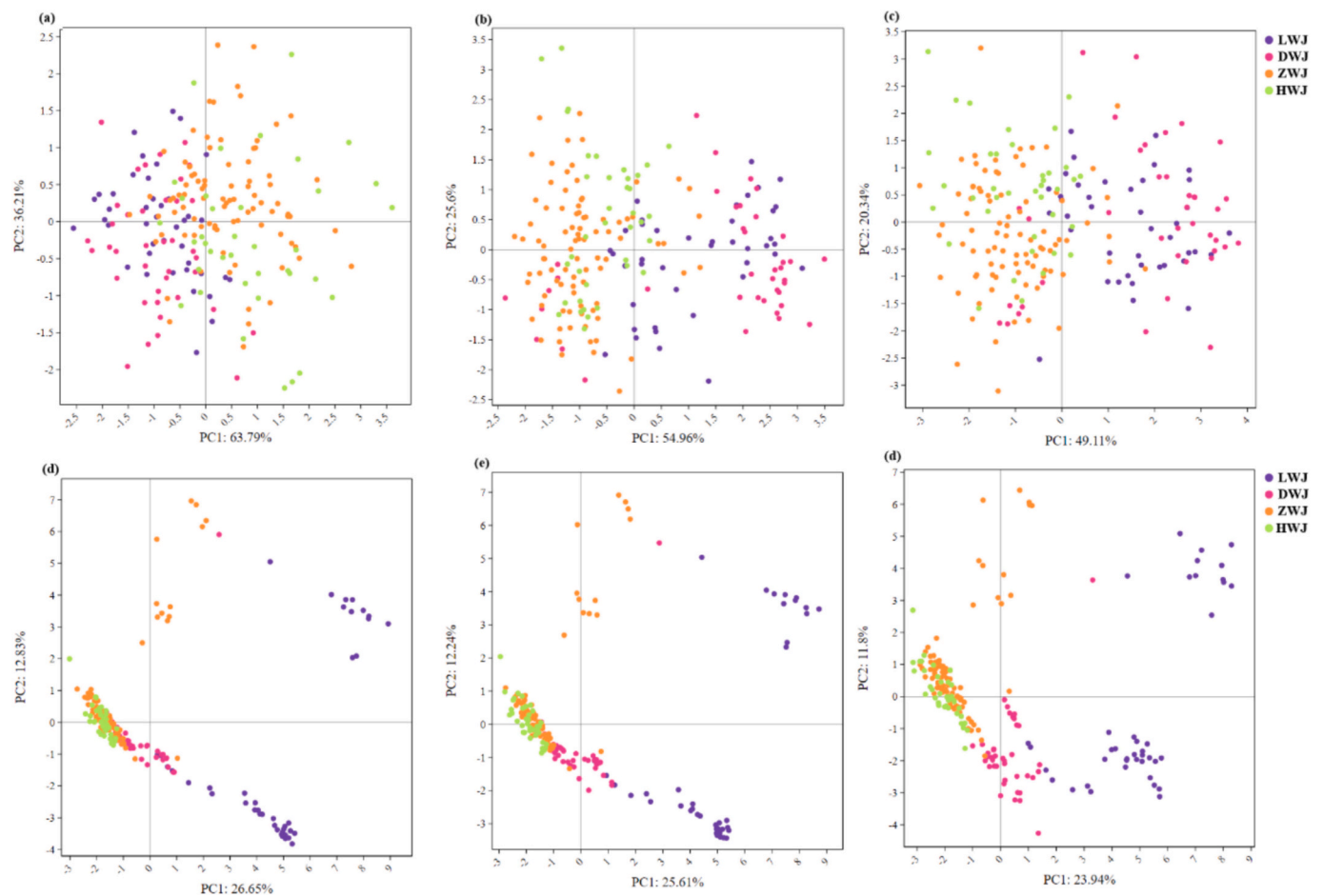


Fig. 4. Classification of the winter jujube samples of four PGIs by PCA. (Note: PCA based on the stable isotopes of pit (a), the stable isotopes of flesh (b), the stable isotopes of pit and flesh (c), the elements of pit (d), the stable isotopes and elements of pit (e), and the stable isotopes of pit and flesh combined with the elements of pit (f), respectively, for geographical origin winter jujubes PGIs. 95% confidence ellipse).

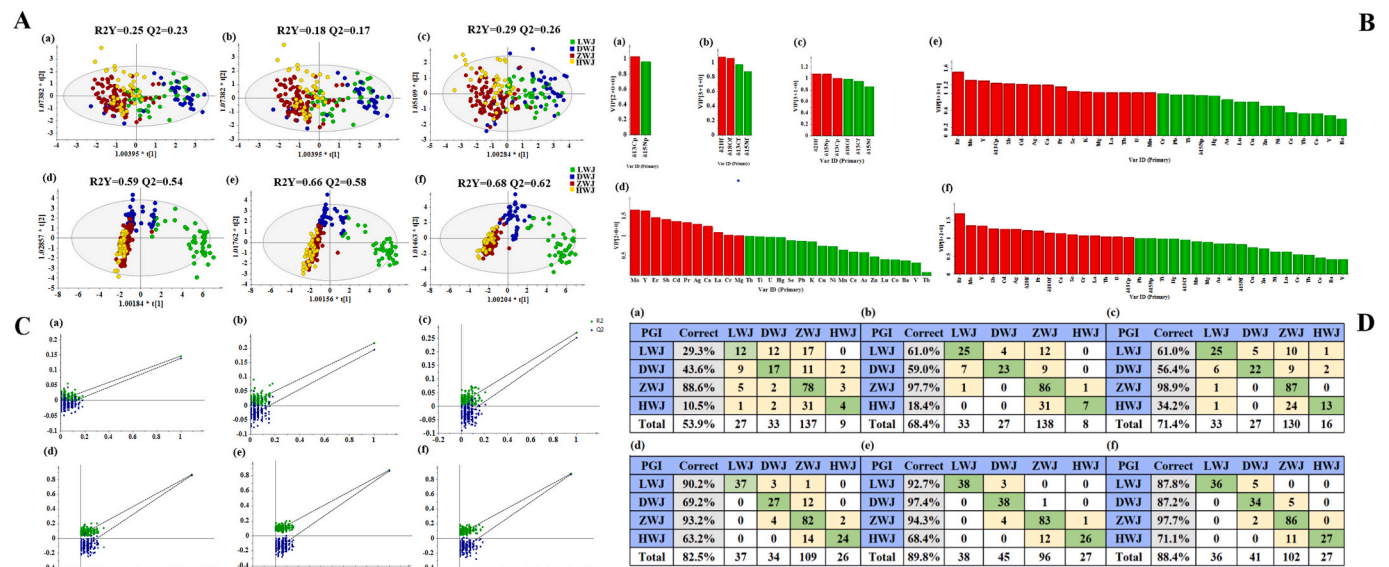


Fig. 5. Score plots and classification rate of OPLS-DA model (A, D) using elements and stable isotopes of the winter jujube samples of four PGIs based on cross-validation with 200 calculations using a permutation test (B), and (C) and variable importance in projection (VIP) analysis (D). (Note: OPLS-DA based on the stable isotopes of pit (a), the stable isotopes of flesh (b), the stable isotopes of pit and flesh (c), the elements of pit (d), the stable isotopes and elements of pit (e), and the stable isotopes of pit and flesh combined with the elements of pit (f), respectively, for geographical origin of winter jujube PGIs. 95% confidence ellipse).

misclassification phenomenon was significantly improved in the optimal multi-feature fusion **Model f**. The correct classification rates for LWJ

and DWJ in **Model f** were close to those of ZWJ and HWJ, with only a slight gap remaining for LWJ. This “low separability” indicates a substantial similarity in the chemical profiles (isotopic and elemental composition) between LWJ and DWJ in single-feature models. Even after multi-feature fusion, the intrinsic differences between these two origins were still slightly insufficient for completely consistent discrimination with ZWJ and HWJ by the models. The main reason for the low accuracy in single-source models is that a single type of feature (e.g., pit isotopes alone) fails to comprehensively capture the origin-specific signals of PGIs, leading to inadequate recognition of four winter jujube PGIs (Kelly et al., 2005; Shuai, Peng, et al., 2022). With the inclusion of flesh isotopes (**Model b**) and pit elements (**Model d**), sample separation gradually improved (R^2Y/Q^2 of **Model b** was 0.18/0.17, and **Model d** increased to 0.59/0.54, respectively), and the corresponding accuracy rates rose to 68.4% and 82.5% (Fig. 5 A; D). Feature-fusion models (e–f) significantly enhanced classification balance: **Model e** increased the accuracy for HWJ from 10.5% in **Model a** to 97.4%; **Model f** further achieved perfect discrimination for HWJ (100%) and reached optimal spatial separation ($R^2Y = 0.68$, $Q^2 = 0.62$), with an overall accuracy of 88.4%. Nevertheless, in **Model f**, 20% of DWJ samples were still misclassified as ZWJ, indicating similarities in chemical composition between these PGIs (Fig. 5 A; D). The VIP values identified the key variables driving PGIs discrimination. In the full feature-fusion **Model f**, thirteen key markers ($VIP > 1$) were identified, including Br, Mo, Y, Sb, Cd, Ag, δ^2H_f , Pr, $\delta^{18}O_f$, Ca, Se, Cr, La, Th, U, and $\delta^{13}C_p$. Among them, δ^2H_f and Ag contributed most significantly (Fig. 5 B). Notably, the full feature-fusion model achieved a discrimination accuracy of 88.4% (Fig. 5 D, Figure S 4 D). However, the OPLS-DA models still did not fully distinguish four winter jujube PGI. Therefore, LDA will be employed in the following section to further investigate classification performance (Kong et al., 2024; Wen et al., 2023).

3.4.3. Linear discriminant analysis (LDA)

In all LDA models, the first two discriminant functions cumulatively accounted for more than 90% of the variance, indicating highly efficient discrimination and that most classification-relevant information was condensed into just two dimensions (Fig. 6), providing a reliable basis for subsequent visualization and interpretation. However, models relying on a single data source exhibited clear limitations: models using only pit stable isotopes (**Models a and b**) yielded accuracies of 56.8% and 68.0%, respectively, and their combination (**Model c**) improved only to 70.9% (Fig. 6), but failed to discriminate the HWJ (42.1% accuracy; Table 3), suggesting a lack of specific stable isotopes markers for this origin. In contrast, the model based solely on pit elements (**Model d**) achieved a cross-validated overall accuracy of 83.5%. Pit stable isotope models showed greater consistency across origins but had lower overall discriminatory power than pit element. This comparison confirms the complementary value of while elements underscoring the higher specificity of mineral–element fingerprints (Kelly et al., 2005; Kong et al., 2024).

To overcome these limitations, we constructed a full-feature fusion model (**Model f**) that integrated isotopic and elemental profiles from both flesh and pit tissues. This model achieved a high discrimination accuracy of 92.7%, and its superior performance stems from the synergistic action of multi-source data: isotopes provide a robust geographical framework that is resistant to short-term environmental noise, whereas elements supply granular discriminatory signals. The combination of both achieves an optimal balance between accuracy and robustness. The effectiveness of **Model f** is rooted in its internal structure and rigorous variable screening. The standardized coefficients revealed that δ^2H_f (−1.530) and Sb (1.173) were the dominant variables defining Function 1 (Shuai, Yang, et al., 2022), while Function 2 captured distinct geographical signals, prominently including U (−0.934), Er (−0.871), Th (0.848), Ti (−0.804), and δ^2H_f (−0.711), Function 3 was primarily characterized by δ^2H_f (1.110) and $\delta^{18}O_f$ (−0.816) (Kelly et al., 2005;

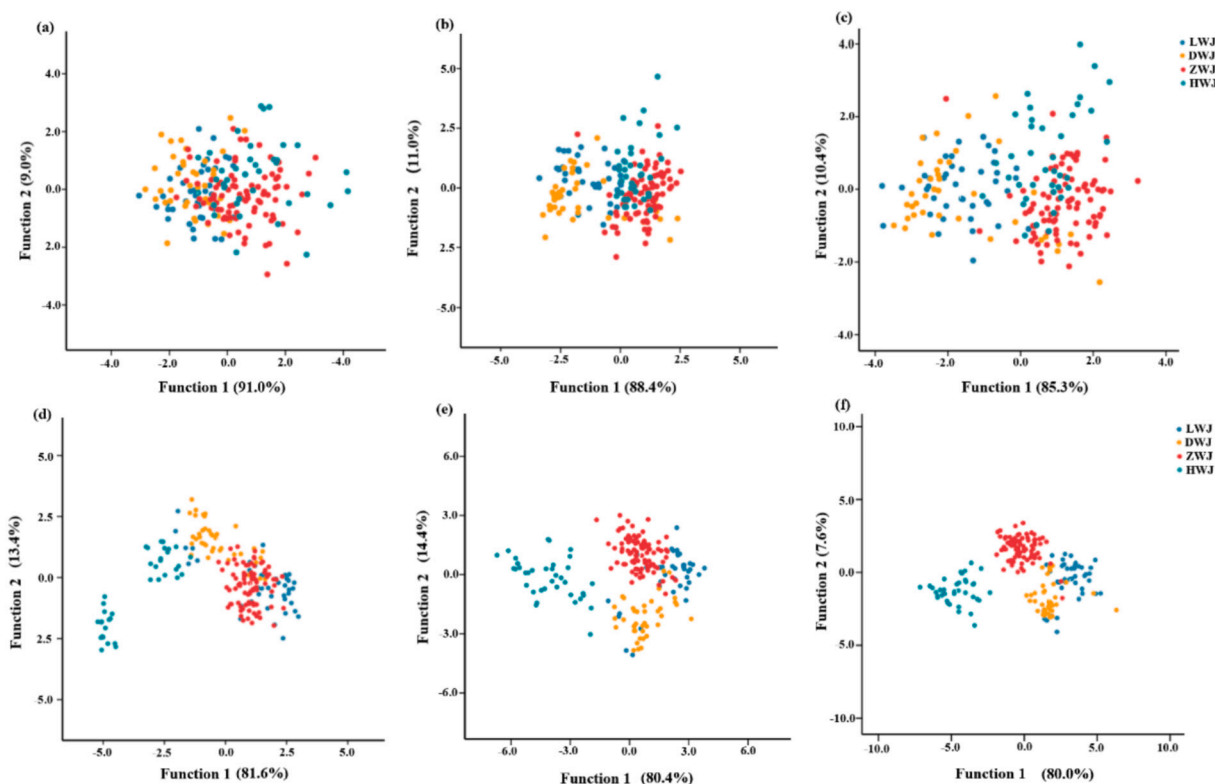


Fig. 6. Classification of the winter jujube samples of four PGIs by LDA. (Note: LDA based on the stable isotopes of pit (a), the stable isotopes of flesh (b), the stable isotopes of pit and flesh (c), the elements of pit (d), the stable isotopes and the elements of pit (e), and the stable isotopes of pit and flesh combined with the elements of pit (f), respectively, for geographical origin of winter jujube PGIs, 95% confidence ellipse).

Table 3

Classification of the samples of four winter jujube PGIs by LDA models.

Model	Accuracy (%)	LWJ	DWJ	ZWJ	HWJ	Total
^a LDA	Original cases	46.3 (19/41)	43.6 (17/39)	81.8 (72/ 88)	31.6 (12/ 38)	58.3 (120/ 206)
	Cross-validated	39.0 (16/41)	43.6 (17/39)	81.8 (72/ 88)	31.6 (12/ 38)	56.8 (117/ 206)
^b LDA	Original cases	61.0 (25/41)	59.0 (23/39)	95.5 (84/ 88)	28.9 (11/ 38)	69.4 (143/ 206)
	Cross-validated	58.5 (24/41)	56.4 (22/39)	95.5 (84/ 88)	26.3 (10/ 38)	68.0 (140/ 206)
^c LDA	Original cases	73.2 (30/41)	64.1 (25/39)	96.6 (85/ 88)	44.7 (17/ 38)	76.2 (157/ 206)
	Cross-validated	68.3 (28/41)	51.3 (20/39)	93.2 (82/ 88)	42.1 (16/ 38)	70.9 (146/ 206)
^d LDA	Original cases	95.1 (39/41)	87.2 (34/39)	93.2 (82/ 88)	89.5 (34/ 38)	91.7 (189/ 206)
	Cross-validated	85.4 (35/41)	82.1 (32/39)	85.2 (75/ 88)	78.9 (30/ 38)	83.5 (166/ 206)
^e LDA	Original cases	95.1 (39/41)	100.0 (39/39)	94.3 (83/ 88)	86.8 (33/ 38)	94.2 (194/ 206)
	Cross-validated	87.8 (36/41)	97.4 (38/39)	87.5 (77/ 88)	73.7 (28/ 38)	86.9 (179/ 206)
^f LDA	Original cases	100.0 (41/41)	97.4 (38/39)	96.6 (85/ 88)	92.1 (35/ 38)	96.6 (199/ 206)
	Cross-validated	97.6 (40/41)	97.4 (38/39)	93.2 (82/ 88)	81.6 (31/ 38)	92.7 (192/ 206)

Note: a, b, c, d, e, and f stand for the stable isotopes of pit, the stable isotopes of flesh, the stable isotopes of pit and flesh, the elements of pit, the stable isotopes and elements of pit, and the stable isotopes of pit and flesh combined with the elements of pit.

Kong et al., 2024). The variables used to build the model were strictly screened. In line with standard practice for selecting discriminative features in food origin studies, variables were first evaluated for their univariate between-PGI differences (Kelly et al., 2005). Except for V and Co, all others showed highly significant between-PGI differences ($p < 0.05$) (Table 5). To quantify their practical discriminatory power, effect-size (η^2) analysis was applied, as recommended for robust variable evaluation (Shuai, Yang, et al., 2022). This further identified a set of key discriminators with exceptionally large effect sizes, including Sb ($\eta^2 = 0.833$), Mo ($\eta^2 = 0.818$), Cd ($\eta^2 = 0.809$), Y ($\eta^2 = 0.766$), Ag ($\eta^2 = 0.745$), Pr ($\eta^2 = 0.631$), $\delta^2\text{H}_f$ ($\eta^2 = 0.455$), Se ($\eta^2 = 0.432$), La ($\eta^2 = 0.413$), $\delta^{13}\text{C}_p$ ($\eta^2 = 0.396$), Er ($\eta^2 = 0.393$), Ca ($\eta^2 = 0.359$) and $\delta^{13}\text{C}_f$ ($\eta^2 = 0.322$). Conversely, variables with minimal effect sizes, such as Zn ($\eta^2 = 0.068$), contributed relatively little to discrimination (Table 6). Notably, these high-contribution factors correspond closely to their underlying driving mechanisms, confirming the effective differentiation of signal sources. Specifically, a set of factors with $\eta^2 > 0.6$ dominated by rare earth elements (Y, Pr, La, Er) and selected trace elements (Sb, Mo, Cd, Ag) are primarily controlled by parent rock weathering and soil parent material, directly reflecting geological background characteristics. Meanwhile, $\delta^2\text{H}_f$ ($\eta^2 = 0.455$) captures climatic and hydrological conditions (e.g., precipitation sources, evaporation effects), while $\delta^{13}\text{C}_p$ and $\delta^{13}\text{C}_f$ ($\eta^2 \approx 0.3$ – 0.4) respond to agronomic growth conditions (e.g., water use efficiency, photosynthetic pathways). This inherent consistency between statistical results and mechanistic interpretation demonstrates that the climatic, agronomic, and geological signals carried by different isotope and element systems have been effectively integrated

and distinguished in the discrimination process. Thus, the high performance of **Model f** is not incidental; it is built upon a foundation of multi-source features statistically screened for strong discriminatory potential, providing a solid basis for a reliable, high-accuracy traceability system (Kong et al., 2024).

To comprehensively evaluate the discriminative performance and generalization capability of the full-feature fusion LDA model, multiple validation strategies were employed in this study. When modeling with all samples collected in 2020 and 2021 ($n = 206$), the model achieved an accuracy of 92.7% (192/206) via leave-one-out cross-validation. After optimization using the full dataset, the cross-validated accuracy further increased to 96.6% (199/206) (Table 4; Fig. 7). To further examine the model's robustness against inter-annual climatic variability and assess its generalization ability, an inter-annual validation was conducted. Samples collected in 2021 ($n = 115$) were used exclusively as the training set, and the derived discriminant functions were applied to predict the geographical origins of independent samples collected in 2020 ($n = 91$). The results showed that the model achieved an overall classification accuracy of 95.6% (87/91) on the 2020 independent test set, with per-origin accuracies of 100.0% for LWJ (25/25), 95.7% for ZWJ (44/46), and 90.0% for HWJ (18/20). Due to the COVID-19 pandemic, no DWJ samples were collected in 2020, so inter-annual validation could not be performed for this PGI. The similar distribution patterns between the two panels demonstrate the strong inter-annual stability of the model (Fig. 7). The classification accuracies for the 2021 training set and the 2020 test set were 97.4% and 95.6%, respectively, with a minimal decrease of only 1.8%, indicating strong inter-annual stability of the model, which confirms the **Model f**'s strong discriminative power and without overfitting. Notably, the high prediction accuracy achieved on independent samples from a different year demonstrates that the discriminative information captured by the model reflects stable geographical characteristics rather than transient climatic conditions. In conclusion, this study demonstrates that LDA based multi-element and isotopic fingerprinting provided a reliable tool for winter jujube geographical authentication. Future work could explore machine learning approaches to further enhance model performance.

4. Methodological discussion

4.1. Tissue-specific signatures

Seeds and pits have emerged as promising biological matrices for geographical origin authentication, as demonstrated in studies on almonds, pistachios, hazelnuts, and lotus seeds (Amorello et al., 2016; Boukid et al., 2019; Ghisoni et al., 2020; Su et al., 2025). Building on this foundation, this study presents the first systematic evaluation of winter jujube pits as a novel material for tracing four PGIs. Our results confirm that pits retain substantial origin-discriminatory information. The OPLS-DA model based on fused data from pits and flesh achieved an accuracy of 88.4% (Fig. 5), which is slightly higher than that reported for jujube flesh alone (85.0%) in the literature (Wang et al., 2020). More

Table 4

Inter-annual validation results of the full-feature fusion LDA model.

Dataset	LWJ	DWJ	ZWJ	HWJ	Overall
2021 Training set ($n = 115$)	100.0% (16/16)	100.0% (25/25)	97.6% (41/42)	94.4% (17/18)	97.4% (112/115)
2020 Test set ($n = 91$)	100.0% (25/25)	–	95.7% (44/46)	90.0% (18/20)	95.6% (87/91)
All samples ($n = 206$)	100.0% (41/41)	97.4% (38/39)	96.6% (85/88)	92.1% (35/38)	96.6% (199/206)

Note: No DWJ samples were collected in 2020 due to the COVID-19 pandemic. The model was trained on samples collected in 2021 ($n = 115$) and applied to predict the origin of independent samples collected in 2020 ($n = 91$).

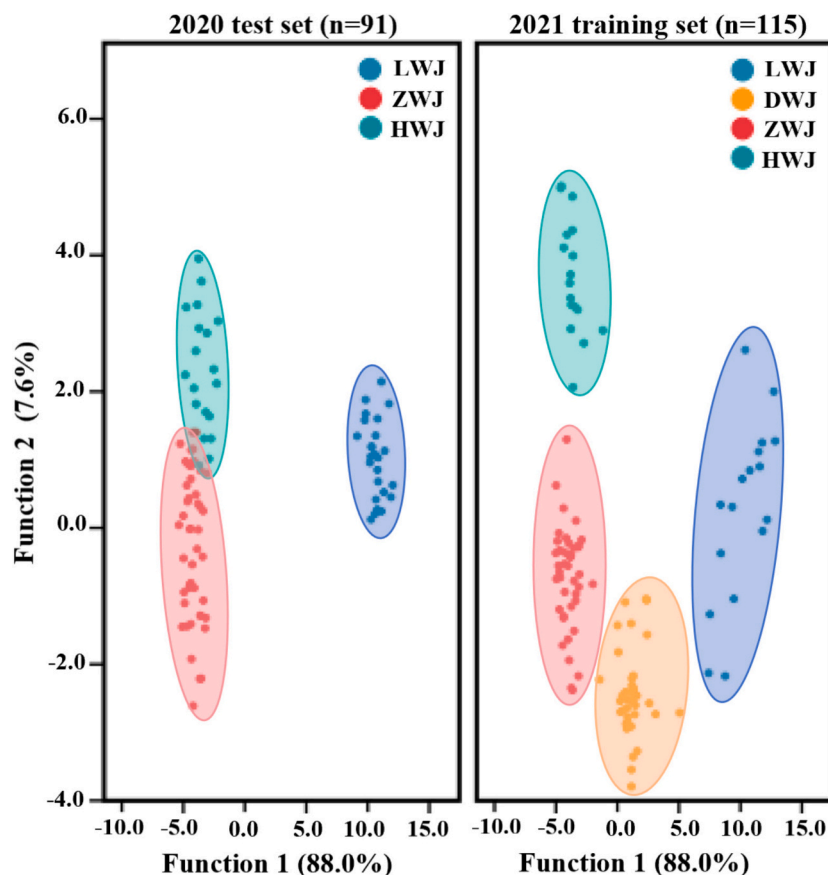


Fig. 7. Discriminant score plots of the full-feature fusion LDA model for inter-annual validation. Note: No DWJ samples were collected in 2020 due to the COVID-19 pandemic. (95% confidence ellipse).

importantly, the optimal full-feature fusion LDA model (**Model f**) attained a cross-validated accuracy of 92.7% (Table 3). While this is slightly lower than the highest accuracy achieved using advanced models on flesh data alone in prior work (e.g., 96.4% for a multilayer perceptron) (Kong et al., 2024), it unequivocally demonstrates that pits encode geographical signatures of comparable utility to those in the edible flesh. This finding underscores a key tissue-specific insight: the pit is not merely a by-product but an effective, information-rich alternative for origin tracing when flesh samples are unavailable or destructive sampling is undesirable. Furthermore, it highlights the complementary potential of a multi-tissue approach. Integrating signatures from both pits and flesh could create a more resilient and informative traceability system, a strategy likely applicable to a wide range of fruits and nuts.

4.2. Data fusion

Data fusion strategies have been widely employed for the geographical origin identification of various agricultural products, such as ginseng, tea, vegetables, and fruits (Bin et al., 2022; Liu, Chen, et al., 2021; Shuai, Li, et al., 2023; Shuai, Yang, et al., 2022; Wang et al., 2024). These studies confirm that integrating multi-dimensional data constructs a more comprehensive chemical fingerprint, thereby effectively enhancing discrimination accuracy. This capability is especially critical when discriminating geographically proximate, small-scale production regions (Jin et al., 2023). This study adopted a multi-technique fusion strategy to achieve precise traceability of four winter jujube PGIs. It is noteworthy that the accuracy of origin discrimination is closely related to geographical scale. Single techniques (e.g., isotopes) are suitable for discrimination at large scales (e.g., inter-provincial), whereas for small-scale, geographically proximate regions with similar

climatic and soil conditions (such as ZWJ/HWJ and LWJ/DWJ in this study, Fig. 1), reliance on multi-indicator fusion is essential to overcome the issue of overlapping features (Ren et al., 2022). Our results validate this observation: the model based solely on pit elements (**Model d**) achieved an accuracy of 83.5%; with the introduction of pit stable isotope data (**Model e**), accuracy increased to 86.9%; and the full-feature fusion model (**Model f**), which integrated isotopes from both flesh and pits along with pit elements, ultimately reached 92.7% (Table 3). This significant performance improvement is highly consistent with the complementary roles of isotopes in large-scale discrimination (**Model c**, 70.9%) and elements in fine-scale differentiation (Ren et al., 2022), providing strong evidence that data fusion is indispensable for the traceability of PGIs at small geographical scales. Admittedly, the model accuracy in this study (92.7%) is lower than that achieved by some advanced methods based on GC-MS/XGBoost (98%), NIR/SVM (97.7%), or even NIR/Adaboost-CLDA (100%) (Wen et al., 2023; Wu et al., 2025; Xia et al., 2025). However, more importantly, there are fundamental differences in the discrimination mechanisms among different techniques. For instance, NIR spectroscopy primarily relies on differences in organic molecular vibrations, and its spectral features offer limited interpretability regarding specific geographical causes (Wu et al., 2025; Xia et al., 2025). In contrast, the multi-element and isotope model established in this study not only achieves high discrimination accuracy but also demonstrates strong temporal robustness. Notably, inter-annual validation demonstrated strong temporal robustness: the model, trained on 2021 samples and tested on independent 2020 samples, achieved accuracies of 97.4% and 95.6%, respectively (Table 4). This confirms that the identified “chemical fingerprints” possess clear environmental mechanistic interpretations and are stable against natural climatic fluctuations, making them inherently resistant to interference and difficult to falsify. Building on these findings, future research

Table 5

Standardized canonical discriminant function coefficients for the variables used in the full-feature fusion LDA model (*Model f*).

Variables	Function 1	Function 2	Function 3
$\delta^{13}\text{C}_f$	-0.093	0.031	-0.114
$\delta^{13}\text{C}_p$	-0.304	0.411	0.501
$\delta^{15}\text{N}_f$	-0.145	0.232	0.324
$\delta^{15}\text{N}_p$	0.050	-0.019	-0.294
$\delta^2\text{H}_f$	-1.530	-0.711	1.110
$\delta^{18}\text{O}_f$	0.371	0.601	-0.816
Mg	0.352	-0.216	-0.307
K	-0.318	-0.094	-0.101
Ca	0.014	-0.549	-0.222
Mn	-0.049	0.353	0.107
Cu	-0.043	0.288	0.015
Zn	0.095	-0.347	0.153
Mo	0.461	0.536	0.540
Cr	0.160	-0.099	-0.113
Ni	-0.219	-0.036	0.085
Ag	-0.024	-0.033	-0.285
Ba	-0.143	0.040	-0.054
V	0.162	0.209	0.270
Co	-0.017	0.225	-0.185
As	-0.041	0.142	0.085
Se	0.220	-0.094	-0.050
Y	0.147	-0.002	-0.381
Cd	0.263	-0.258	-0.282
Sb	1.173	0.390	0.030
La	-0.140	-0.132	0.121
Ce	0.072	-0.196	-0.062
Pr	0.049	0.224	-0.116
Tb	0.033	-0.518	0.277
Er	0.161	-0.871	0.649
Lu	0.023	0.691	-0.433
Hg	0.201	0.665	-0.104
Ti	0.007	-0.804	0.018
Pb	0.358	0.196	0.277
Th	0.041	0.848	-0.459
U	-0.393	-0.934	0.545

could explore the deep integration of rapid, non-destructive NIR spectral data with elemental and isotopic profiles to develop multimodal fusion models. Such an integrated approach holds promise for maintaining high discrimination accuracy while significantly enhancing the interpretability, practical applicability, and overall robustness of the traceability system.

4.3. Sample balance

In this study, the sample sizes of the four PGIs varied (ZWJ: 88; HWJ: 38; LWJ: 41; DWJ: 38), reflecting a sampling strategy intentionally aligned with the relative cultivation area of each PGI to enhance the ecological representativeness of the dataset (Chu & Zhao, 2022; Guo, 2013; Li et al., 2025; Wang, 2024). However, from a statistical modeling perspective, such class imbalance can inherently bias models toward the majority class and inflate performance estimates for minority classes (Qi et al., 2021). To address potential biases arising from class imbalance, a multi-layered strategy was implemented to ensure robust and unbiased evaluation of the models. (1) Algorithm-level adjustment: Class weighting inversely proportional to sample size was applied in OPLS-DA, and prior probabilities were set based on group sizes in LDA (Maione & Barbosa, 2019). (2) Rigorous validation: All models were evaluated using stratified 7-fold cross-validation (OPLS-DA) and leave-one-out cross-validation (LDA), supplemented by 200 permutation tests to exclude random effects in OPLS-DA. (3) Comprehensive metrics: In addition to overall accuracy, model performance was assessed using confusion matrices, class-specific sensitivity, and specificity, providing a complete view of classification efficacy (Maione & Barbosa, 2019). (4) Inter-annual validation: To further assess model generalizability and temporal robustness, an independent inter-annual validation was conducted. The full-feature fusion LDA model was trained on samples

Table 6

Tests of equality of group means for the variables used in the full-feature-fusion LDA model (*Model f*).

Variables	Wilks' Lambda	η^2	F value	p
$\delta^{13}\text{C}_f$	0.678	0.322	31.978	***
$\delta^{13}\text{C}_p$	0.604	0.396	44.165	***
$\delta^{15}\text{N}_f$	0.827	0.173	14.042	***
$\delta^{15}\text{N}_p$	0.778	0.222	19.224	***
$\delta^2\text{H}_f$	0.545	0.455	56.215	***
$\delta^{18}\text{O}_f$	0.709	0.291	27.692	***
Mg	0.843	0.157	12.582	***
K	0.814	0.186	15.394	***
Ca	0.641	0.359	37.700	***
Mn	0.902	0.098	7.348	***
Cu	0.852	0.148	11.665	***
Zn	0.932	0.068	4.882	**
Mo	0.182	0.818	301.919	***
Cr	0.885	0.115	8.721	***
Ni	0.945	0.055	3.947	**
Ag	0.255	0.745	196.799	***
Ba	0.960	0.040	2.819	**
V	0.977	0.023	1.569	ns
Co	0.963	0.037	2.574	ns
As	0.947	0.053	3.766	*
Se	0.568	0.432	51.177	***
Y	0.234	0.766	220.153	***
Cd	0.191	0.809	284.732	***
Sb	0.167	0.833	335.549	***
La	0.587	0.413	47.278	***
Ce	0.916	0.084	6.149	***
Pr	0.369	0.631	114.956	***
Tb	0.938	0.062	4.442	**
Er	0.607	0.393	43.606	***
Lu	0.832	0.168	13.594	***
Hg	0.851	0.149	11.790	***
Ti	0.860	0.140	10.984	***
Pb	0.924	0.076	5.529	***
Th	0.898	0.102	7.651	***
U	0.903	0.097	7.234	***

Note: *, **, and *** indicate differences in regional elemental content significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

collected in 2021 ($n = 115$) and applied to predict the origin of independent samples collected in 2020 ($n = 91$). The model achieved a high classification accuracy of 95.6% on the 2020 test set, with per-origin accuracies of 100.0% for LWJ (25/25), 95.7% for ZWJ (44/46), and 90.0% for HWJ (18/20). This performance closely matched the internal cross-validation result on the 2021 training set (97.4%), with a minimal decrease of only 1.8% (Table 4). The near-equivalent performance between training and test sets demonstrates strong year-to-year generalizability and confirms that the identified geographical signatures are stable against natural climatic variability. Nevertheless, we acknowledge that no statistical remedy can fully substitute for a balanced sample design. While the area-proportional sampling strategy enhanced real-world representativeness, future work should aim to increase sampling density in smaller regions. Standardized grid sampling could help construct more balanced datasets, enabling further independent external validation and strengthening the robustness of conclusions (Kelly et al., 2005; Strojnik et al., 2022).

5. Conclusion

This study demonstrates the feasibility of using multi-element and stable isotope fingerprinting for geographical origin traceability of four winter jujube PGIs. While stable isotopes alone provided limited discrimination accuracy (56.8–68.0%), the integration of elemental data from pit tissues significantly enhanced classification performance. The optimal full-feature fusion model (*Model f*), which combined pit elements with flesh and pit stable isotopes, achieved a high cross-validated accuracy of 92.7%. Inter-annual validation was performed to assess model robustness against temporal climatic variability. *Model f*, trained

on 2021 samples, successfully predicted the origin of independent 2020 samples, demonstrating strong year-to-year generalizability. Importantly, the near-equivalent performance between the training (97.4%) and test sets (95.6%) confirms that the identified geographical signatures are stable against natural climatic fluctuations, rather than being artifacts of a specific sampling year. Additionally, the absence of corresponding soil samples represents a limitation of this study, as it precludes definitive source apportionment between geogenic and anthropogenic element contributions. Future work should incorporate the collection of paired rhizosphere soil in the sampling design to enable more precise tracing of element sources and to further strengthen the interpretability of the traceability model.

CRedit authorship contribution statement

Mengying Shuai: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Zhichao Li:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Xiabing Kong:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **An Li:** Writing – review & editing, Writing – original draft, Visualization, Validation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Qiusheng Chen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Duoyong Zhao:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Min Xu:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mengyao Yang:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation, Conceptualization. **Kangni Yang:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ji Yun Nie:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2026.149224>.

Data availability

Data will be made available on request.

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