

Comparative Compositional Characterization of New Zealand Manuka Honey and Selected Chinese Honeys Using Multiple Chemical Indicators

Haiquan Chen

Graduate School of Bioagricultural Sciences, Nagoya University

Bo Chen

Food Inspection and Quarantine Center, Shenzhen Customs

Fengqi Wu

Food Inspection and Quarantine Center, Shenzhen Customs

Baohui Jin

Food Inspection and Quarantine Center, Shenzhen Customs

Zhi Yan

Food Inspection and Quarantine Center, Shenzhen Customs

Xiuwen Zhou

Department of Ocean Science and Engineering, Southern University of Science and Technology

Karyne M. Rogers

Department of Soil and Environmental Sciences, Lincoln University

Duoyong Zhao

Institute of Quality Standards & Testing Technology for Agro-Products, Xinjiang Academy of Agricultural Sciences

Hao Wu

haowu@xmu.edu.cn

Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, College of the Environment and Ecology, Xiamen University

Research Article

Keywords: stable isotopes, carbon and nitrogen contents, saccharide composition, mineral element composition, botanical origin, chemometrics, New Zealand manuka honey

Posted Date: September 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10979615/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

New Zealand manuka honey is a high-value honey that has attracted increasing attention in the Chinese market. Comparing its chemical characteristics with those of selected Chinese honeys provides valuable information for understanding compositional variability among honey samples. In this study, stable isotope ratios, carbon and nitrogen contents, saccharide composition, mineral element composition, and chemometric analyses were integrated to investigate compositional differences between New Zealand manuka honey and selected Chinese honeys of different botanical types. Clear compositional differences were observed among the predefined honey sample groups within the present dataset. Stable isotope ratios, carbon and nitrogen contents, saccharide composition, and mineral element composition each provided complementary information for characterizing honey samples, with mineral element composition making an important contribution to sample classification. Chemometric analyses, including principal component analysis (PCA), Fisher discriminant analysis (Fisher-DA), and artificial neural network (ANN) models, further showed that integrating multiple compositional indicators substantially improved classification performance, with the supervised models clearly distinguishing Chinese honey samples from New Zealand manuka honey. This study provides a comprehensive comparative compositional analysis of New Zealand manuka honey and selected Chinese honeys based on multiple complementary chemical indicators, providing comparative compositional data and an integrated analytical strategy for future studies on honey characterization, quality evaluation, and comparative analysis of commercial honey samples.

1. Introduction

Honey is a natural, sweet product produced by bees from the nectar of flowers and is mainly composed of glucose and fructose[1], together with minor components such as vitamins, minerals, amino acids, enzymes, and other bioactive compounds[2, 3]. Among commercial honeys, New Zealand manuka honey[4] has attracted particular attention because of its distinctive compositional characteristics, recognized health-promoting properties, and high market value. In recent years, the growing demand for manuka honey has been accompanied by increasing concerns regarding product quality, authenticity, and mislabeling[4, 5]. Consequently, reliable analytical approaches are increasingly required for the characterization and quality evaluation of manuka honey and for comparison with selected Chinese honeys.

To meet this demand, various analytical approaches have been developed for honey characterization and origin evaluation, including spectroscopic techniques, nuclear magnetic resonance (NMR), chromatographic analysis, stable isotope analysis, elemental profiling, and pollen analysis[6]. Among these approaches, pollen analysis remains a widely used method for determining the floral origin of honey[7] and is an important reference method for manuka honey authentication[4, 5]. However, pollen analysis requires specialized expertise, and its application to manuka honey may be complicated by the morphological similarity between manuka and kanuka pollen[4]. These limitations have encouraged the development of complementary chemical approaches for honey characterization.

Stable isotope analysis has become an important analytical approach for characterizing honey composition and evaluating its botanical and geographical characteristics[8–10]. Stable isotope ratios reflect differences in plant metabolic pathways and environmental conditions[8], providing valuable compositional information for honey characterization. Carbon isotope ratios are closely associated with plant photosynthetic pathways and can provide information on plant-derived carbon sources, making them valuable for evaluating honey composition and detecting the addition of C4 plant-derived sugars, such as cane or corn syrup[11, 12]. Nitrogen isotope ratios provide complementary environmental information, as they are influenced by soil nitrogen sources, fertilizer application, biological nitrogen fixation, land-use practices, and climatic conditions[8, 9]. Owing to the high analytical precision and good reproducibility demonstrated for isotope-ratio measurements of honey saccharides, stable isotope analysis provides reliable compositional information for honey characterization[13].

Mineral element composition provides another important source of chemical information for honey characterization[14, 15]. Elemental profiles are influenced by soil characteristics, geological background, environmental conditions, and regional ecosystems, enabling them to reflect the geographical characteristics of honey production areas[16, 17]. Environmental contamination may also influence trace- and heavy-metal concentrations in honey, allowing mineral profiles to reflect local environmental conditions[17, 18]. ICP-MS enables the simultaneous determination of multiple macro- and trace elements and has been widely applied to elemental fingerprinting and origin discrimination[19, 20].

In addition to stable isotope and mineral element analyses, a wide range of analytical techniques has been employed to characterize honey composition, including near-infrared spectroscopy[7, 21, 22], middle infrared spectroscopy[23], infrared spectroscopy[24, 25], and NMR[26, 27]; fingerprinting and barcoding of proteins in honey[28, 29]; chemical composition analysis[30, 31], analysis of specific components[32] and volatile chemical components in honey[33–35]; and physicochemical indices[36, 37, 35, 3, 38]. These analytical techniques provide complementary information on the chemical characteristics of honey from different analytical perspectives and have substantially improved the characterization of honey composition. Among the major compositional indicators, fructose and glucose, the two predominant saccharides in honey[39, 1], are important indicators of nectar-derived carbohydrate composition and compositional variation among honey samples. Similarly, measurements of the extracted protein fraction and bulk honey provide additional compositional information alongside the corresponding stable isotope ratios, allowing different aspects of honey composition to be evaluated jointly[40, 10].

Increasing attention has been directed toward integrating multiple complementary analytical approaches to achieve a more comprehensive characterization of honey[6, 4]. Representative examples include the combination of physicochemical indices with ATR-FTIR[25]; volatile compounds with physicochemical parameters[41]; isotope ratio mass spectrometry (IRMS) with site-specific natural isotope fractionation by NMR (SNIF-NMR)[42]; and the integration of stable isotope and elemental analyses[16, 8]. Collectively, these studies suggest that combining complementary analytical information provides a more robust characterization of honey than any individual analytical technique alone[19, 6]. Building on this concept,

stable isotope ratios together with carbon and nitrogen contents, saccharide composition, and mineral element composition can be integrated to capture complementary information on plant-derived carbon sources, carbohydrate characteristics, and environmental and geological influences[6, 19]. The resulting multidimensional datasets can be further evaluated using chemometric methods, which facilitate the identification of characteristic variables, visualization of sample relationships, and classification of predefined honey sample groups[6, 43, 44]. Accordingly, the combined use of complementary chemical indicators and chemometric analysis offers a comprehensive analytical framework for honey characterization and sample classification.

Based on the above considerations, this study integrated stable isotope ratios together with carbon and nitrogen contents, saccharide composition, and mineral element composition with chemometric methods to comprehensively compare the chemical characteristics of New Zealand manuka honey and selected Chinese honeys. This integrated analytical strategy is intended to provide a comprehensive characterization of honey composition by combining complementary chemical information from multiple analytical dimensions. Chemometric methods were employed to evaluate sample relationships and classification performance based on multidimensional compositional data. The overall aim of this study was to establish a comprehensive analytical framework for comparing the chemical characteristics of New Zealand manuka honey and selected Chinese honeys using multiple complementary chemical indicators.

2. Materials and Methods

2.1 Sample Collection and Preparation

A total of 85 honey samples were analyzed from selected regions in China (n = 69) and New Zealand (n = 16). The Chinese samples were sourced from Beijing (n = 12), Hebei (n = 14), Shandong (n = 21), and Shaanxi (n = 22). The sample details are shown in Table 1. The protein extraction method followed the PRC National Standard GB/T 18932.1–2002[45].

Table 1
Information on Honey Samples

Country	Quantity	Honey type	Origin
China	12	black locust honey	Shunyi, Beijing
China	14	black locust honey	Pingshan, Hebei
China	21	black locust honey	Tai'an, Shandong
China	22	rape honey	Yangxian, Shaanxi
New Zealand	16	manuka honey	New Zealand

Table 2

One-way analysis of variance (ANOVA) of honey from five sample groups and three botanical origins using F/G (Fructose/Glucose) and F + G (Fructose + Glucose). The values in the table are expressed as mean $\pm$ standard deviation.

ANOVA			
Origin		F/G	F + G(g/100 g)
Sample Groups	Beijing	1.52 $\pm$ 0.16d	66.75 $\pm$ 2.133a
	Hebei	1.49 $\pm$ 0.09d	69.54 $\pm$ 1.44b
	Shandong	1.38 $\pm$ 0.12c	66.66 $\pm$ 2.83a
	Shaanxi	0.94 $\pm$ 0.05a	68.85 $\pm$ 3.50ab
	New Zealand	1.28 $\pm$ 0.04b	73.04 $\pm$ 1.89c
	<i>p</i> value	< 0.001	< 0.001
Botanical Origin(g/100g)	black locust honey	1.45 $\pm$ 0.14C	67.54 $\pm$ 2.62A
	rape honey	0.94 $\pm$ 0.05A	68.85 $\pm$ 3.50A
	manuka honey	1.28 $\pm$ 0.04B	73.04 $\pm$ 1.89B
	<i>p</i> value	< 0.001	< 0.001
^{a-e} and ^{A-C} Different superscript letter values indicate a significant difference among groups (<i>p</i> <0.05)			

2.2 Stable Isotope Analysis

Stable isotope values of $\delta^{15}\text{N}_{\text{protein}}$ [10], $\delta^{13}\text{C}_{\text{protein}}$ [45], and $\delta^{13}\text{C}_{\text{honey}}$ [46] along with the elemental contents of $\text{N}_{\text{protein}}$, $\text{C}_{\text{protein}}$, and C_{honey} in the honey samples, were measured using an Isotope Ratio Mass Spectrometer (IRMS) (DELTA δ , ADVANTAGE, Thermo Scientific, Bremen, Germany) equipped with an Organic Elemental Analyzer (FLASH 2000). An appropriate amount of each dried protein or homogenized honey sample was weighed into a tin capsule to obtain a sample signal intensity comparable to that of the reference gas. The samples were then analyzed at a combustion temperature of 850°C using helium as the carrier gas. IAEA-600 caffeine ($\delta^{13}\text{C}_{\text{VPDB}} = -27.771\text{‰}$; $\delta^{15}\text{N}_{\text{Air-N}_2} = +1.0\text{‰}$) was used as the reference material for calibration of $\delta^{15}\text{N}_{\text{protein}}$, $\delta^{13}\text{C}_{\text{protein}}$, and $\delta^{13}\text{C}_{\text{honey}}$. Isotope values were calibrated using a single-point calibration approach rather than multi-point calibration. Sulfadiazine (C = 41.81%, N = 16.25%; Thermo Fisher, USA) was used to monitor the accuracy of C and N elemental content measurements for $\text{N}_{\text{protein}}$, $\text{C}_{\text{protein}}$, and C_{honey} . The $\delta^{13}\text{C}_{\text{Fru}}$ and $\delta^{13}\text{C}_{\text{Glu}}$ values were determined using an LC-IRMS system consisting of a Dionex UltiMate 3000 HPLC, an LC Isolink interface, and a DELTA V Advantage IRMS. Fructose and glucose were separated on a Rezex RCM-Monosaccharide Ca^{2+} column (300 $\times$ 7.8 mm, Phenomenex, USA) using ultrapure water as the mobile phase at a flow rate of 1.0 mL min⁻¹, a column temperature of 60°C, and an injection volume of 1–3 μL . The separated compounds were oxidized online to CO_2 before isotope measurement. IAEA-CH-6 sucrose ($\delta^{13}\text{C}_{\text{VPDB}} = -$

10.449 ± 0.043‰) was used as the reference material for calibration of $\delta^{13}\text{C}_{\text{Fru}}$ and $\delta^{13}\text{C}_{\text{Glu}}$, also using single-point calibration. The long-term analytical precision for $\delta^{15}\text{N}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{protein}}$ in a working standard protein derived from honey was 0.2‰ and 0.1‰, respectively, and that for $\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{Fru}}$, and $\delta^{13}\text{C}_{\text{Glu}}$ in honey was 0.1‰.

2.3 Saccharide Analysis

Fructose and glucose contents were measured using HPLC (1290 Infinity, Agilent, Waldbronn, Germany) with a RID detector (1290 RID, Agilent, Waldbronn, Germany) and a chromatographic column (Carbohydrate ES, 5µm 250×4.6mm, ECHWAY, China), following PRC National Standard GB 5009.8–2023[47]. Calibration standards ranged from 1 mg/L to 10 mg/L for fructose (D-(-)-Fructose, ≥ 99.5%, Sigma) and glucose (D-(+) Glucose powder, ≥ 99.5%, Sigma).

2.4 Mineral Element Analysis

Sixteen mineral elements (Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Cu, Zn, Rb, Sr, Ba, Pb)[48] were quantified using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Scientific, iCAP-Q).

Approximately 1.0 g of honey was weighed and digested using a microwave digestion system. Samples were digested in Teflon vessels with 10.0 mL of HNO_3 (AR, 65%, w/w, Merck, Darmstadt, Germany) for 2 hours. The digestion temperature program included an increase from room temperature to 100°C, then to 180°C over 15 minutes, followed by an increase to 240°C over a further 15 minutes, maintaining 240°C for 10 minutes before cooling. The mineralized extracts were redissolved to a total volume of 25 mL with Milli-Q water (Integral 5, Milli-Q, Millipore, USA) and analyzed using ICP-MS. Calibration standards were prepared using multi-elemental standard solutions (Multi-Element Calibration Standard 3, PerkinElmer, USA) in 0.5% nitric acid (AR, 65%, w/w, Merck, Darmstadt, Germany) with concentrations ranging from 1.0 µg/L to 1.0 mg/L. Ge and Rh (50 µg/L) were used as internal standards to correct for instrument drift.

2.5 Statistical Analysis

Data processing was conducted using SPSS Statistics 18 (IBM, USA), while figures were prepared using Origin 2018 (OriginLab, USA) and GraphPad Prism 10 (GraphPad Software, USA). One-way analysis of variance (ANOVA) was employed to compare the predefined honey sample groups, followed by the least significant difference (LSD) test for post-hoc comparisons. Differences were considered statistically significant at $P < 0.05$. Principal component analysis (PCA) was used to visualize the overall distribution patterns of the predefined honey sample groups. Fisher discriminant analysis (Fisher-DA) and an artificial neural network (ANN) were used as supervised classification models. Three-dimensional score plots were generated using PCA and Fisher-DA to visualize sample distributions.

3. Results and Discussion

3.1 Stable Isotope Ratios among Predefined Honey Sample Groups

Stable isotope ratios were determined for both the extracted protein fraction and bulk honey samples. After protein flocculation and isolation, $\delta^{15}\text{N}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{protein}}$ were measured for the protein fraction, whereas $\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{Fru}}$, and $\delta^{13}\text{C}_{\text{Glu}}$ were measured for bulk honey. The $\delta^{15}\text{N}_{\text{protein}}$ values differed significantly among the five predefined sample groups (Table S1; Fig. 1a). Hebei ($1.56 \pm 1.35\text{‰}$) and New Zealand ($1.36 \pm 1.36\text{‰}$) formed one statistical group, whereas Beijing ($3.19 \pm 0.91\text{‰}$), Shandong ($3.63 \pm 0.69\text{‰}$), and Shaanxi ($3.12 \pm 0.53\text{‰}$) formed another ($p < 0.05$). Similarly, the four carbon isotope variables ($\delta^{13}\text{C}_{\text{protein}}$, $\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{Fru}}$, and $\delta^{13}\text{C}_{\text{Glu}}$) differed significantly among the predefined sample groups (Table S1; Fig. 1b–e) and exhibited highly consistent variation patterns. Shaanxi had the most negative $\delta^{13}\text{C}_{\text{protein}}$ value ($-27.37 \pm 0.47\text{‰}$), whereas Hebei had the least negative value ($-23.19 \pm 0.52\text{‰}$). $\delta^{13}\text{C}_{\text{honey}}$ values were generally less negative than the corresponding $\delta^{13}\text{C}_{\text{protein}}$ values, except in Shaanxi. Similar trends were observed for $\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{Fru}}$, and $\delta^{13}\text{C}_{\text{Glu}}$ (Fig. 1c–e). Although $\delta^{13}\text{C}_{\text{honey}}$ was separated into four statistically distinct groups, $\delta^{13}\text{C}_{\text{Fru}}$ and $\delta^{13}\text{C}_{\text{Glu}}$ exhibited three similar statistical groupings, indicating highly consistent carbon isotope signatures for the two major monosaccharides. Shaanxi had the most negative mean $\delta^{13}\text{C}_{\text{Fru}}$ and $\delta^{13}\text{C}_{\text{Glu}}$ values among the five groups ($-27.50 \pm 1.02\text{‰}$ and $-27.65 \pm 1.00\text{‰}$, respectively), while Hebei had the least negative mean values ($-23.39 \pm 0.50\text{‰}$ and $-22.88 \pm 0.52\text{‰}$, respectively).

When grouped according to botanical origin, $\delta^{15}\text{N}_{\text{protein}}$ also separated the three honey types into two statistical groups ($p < 0.05$), with manuka honey showing the most negative mean value ($1.36 \pm 0.85\text{‰}$), whereas black locust and rape honeys exhibited more positive values of $2.90 \pm 1.32\text{‰}$ and $3.12 \pm 0.53\text{‰}$, respectively. The four carbon isotope variables showed a similar pattern, with rape honey exhibiting the most negative values, black locust honey the least negative values, and manuka honey intermediate values. Rape honey had the most negative mean $\delta^{13}\text{C}_{\text{protein}}$, $\delta^{13}\text{C}_{\text{honey}}$, $\delta^{13}\text{C}_{\text{Fru}}$, and $\delta^{13}\text{C}_{\text{Glu}}$ values ($-27.37 \pm 0.47\text{‰}$, $-27.43 \pm 0.90\text{‰}$, $-27.50 \pm 1.02\text{‰}$, and $-27.65 \pm 1.00\text{‰}$, respectively), black locust honey had the least negative values ($-24.14 \pm 1.12\text{‰}$, $-23.42 \pm 1.15\text{‰}$, $-23.60 \pm 1.04\text{‰}$, and $-23.21 \pm 0.99\text{‰}$, respectively), and manuka honey had intermediate values ($-26.70 \pm 0.55\text{‰}$, $-25.20 \pm 0.33\text{‰}$, $-25.43 \pm 0.47\text{‰}$, and $-25.18 \pm 0.50\text{‰}$, respectively). Previous studies have indicated that black locust honey tends to have more positive carbon stable isotope values than other honey types[11], and the present results are consistent with these observations, with black locust honey exhibiting less negative $\delta^{13}\text{C}$ values than rape and manuka honeys.

Figure 2 illustrates the relationships among the stable isotope variables and the predefined honey groups. Figure 2a shows a clear separation among the three botanical honey types, indicating that the combination of $\delta^{15}\text{N}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{protein}}$ provides effective discrimination between honey varieties. Black locust honey from different geographical origins also showed partial separation, although a small overlap was observed between two groups. Figures 2b and 2c show strong positive relationships

between $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{honey}}$ ($R^2 = 0.78$) and between $\delta^{13}\text{C}_{\text{Glu}}$ and $\delta^{13}\text{C}_{\text{Fru}}$ ($R^2 = 0.97$), respectively. The stronger correlation between $\delta^{13}\text{C}_{\text{Glu}}$ and $\delta^{13}\text{C}_{\text{Fru}}$ is consistent with the similar isotopic characteristics of the two major monosaccharides, whereas the relatively weaker relationship between $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{honey}}$ reflects the different compositional fractions represented by protein and bulk honey. Despite these differences, both relationships demonstrate a high degree of consistency among the carbon isotope variables, indicating that each variable provides complementary compositional information for honey discrimination. Although partial overlap was observed among black locust honeys from different geographical origins, samples from the same botanical origin generally clustered within similar regions of the scatter plots.

The carbon isotope fractionation in honey was further examined by comparing the absolute differences in carbon stable isotope ratios between different honey components (Fig. 3). Box plots showed that the absolute differences between carbon isotope variables varied among the five predefined sample groups (Fig. 3a–e). The absolute differences between $\delta^{13}\text{C}_{\text{honey}}$ and $\delta^{13}\text{C}_{\text{protein}}$ were larger in the Beijing, Shaanxi, and New Zealand groups than in the Hebei and Shandong groups (Fig. 3a; $p < 0.05$), with the New Zealand manuka honey group showing the largest difference within the present dataset. Hebei and Shandong samples showed smaller and similar $\delta^{13}\text{C}$ differences. Differences in $\delta^{13}\text{C}$ values were also observed among black locust honey samples from Beijing, Hebei, and Shandong. In contrast, the absolute differences between $\delta^{13}\text{C}_{\text{Fru}}$ and $\delta^{13}\text{C}_{\text{Glu}}$ remained relatively small across all honey groups (Fig. 3D), indicating high isotopic consistency between the two major monosaccharides. Together, these results demonstrate distinct patterns in elemental composition among the investigated honey groups and provide complementary compositional information for honey characterization.

Previous studies have reported that the difference between $\delta^{13}\text{C}_{\text{honey}}$ and $\delta^{13}\text{C}_{\text{protein}}$ in authentic manuka honey is frequently greater than $\pm 1.00\text{‰}$ [44, 45]. A similar pattern was observed in the present study, with most manuka honey samples exhibiting $|\delta^{13}\text{C}_{\text{honey}} - \delta^{13}\text{C}_{\text{protein}}|$ values exceeding 1.00‰ , which is consistent with previous reports on the carbon isotope characteristics of manuka honey. Previous studies have also suggested that the difference between $\delta^{13}\text{C}_{\text{Fru}}$ and $\delta^{13}\text{C}_{\text{Glu}}$ in authentic honey is generally less than 1.00‰ [35]. Consistent with this observation, all samples except one from Shandong remained within this range, with only a single sample exhibiting a difference of 1.35‰ . Combined with the strong correlation between $\delta^{13}\text{C}_{\text{Fru}}$ and $\delta^{13}\text{C}_{\text{Glu}}$ observed in Fig. 2c, these findings indicate that the two major monosaccharides retain highly consistent carbon isotope signatures. Overall, the carbon isotope differences among honey components support the use of multiple stable isotope variables as complementary indicators for differentiating honey samples within the investigated dataset.

3.2 Carbon and nitrogen contents and saccharide composition of honey

The nitrogen and carbon contents of the extracted protein fraction and the carbon content of bulk honey differed significantly among the five predefined sample groups (Table S1; Fig. 4). New Zealand manuka honey exhibited the lowest $\%N_{\text{protein}}$ ($5.56 \pm 0.92\%$) but the highest $\%C_{\text{protein}}$ ($35.30 \pm 1.02\%$) and $\%C_{\text{honey}}$

($34.33 \pm 0.36\%$), with $\%C_{\text{honey}}$ being significantly higher than that of the other sample groups ($p < 0.05$). Three statistical groups were identified for $\%N_{\text{protein}}$, whereas $\%C_{\text{protein}}$ and $\%C_{\text{honey}}$ were separated into four groups. The $\%N_{\text{protein}}$ contents of Beijing, Hebei, and Shandong black locust honeys were similar ($7.23 \pm 0.14\%$, $7.38 \pm 0.11\%$, and $7.39 \pm 0.20\%$, respectively), indicating limited differentiation based on the nitrogen content of the extracted protein fraction alone. When grouped according to botanical origin, black locust honey exhibited the highest $\%N_{\text{protein}}$, whereas manuka honey exhibited the highest $\%C_{\text{protein}}$ and $\%C_{\text{honey}}$. Rape honey showed an intermediate $\%N_{\text{protein}}$ value but the lowest $\%C_{\text{honey}}$ value, while its $\%C_{\text{protein}}$ did not differ significantly from that of black locust honey. Together, these results demonstrate distinct patterns in carbon and nitrogen contents among the investigated honey groups and provide complementary compositional information for honey characterization.

Fructose and glucose contents also differed significantly among the investigated honey groups (Table S1; Fig. 4). Fructose content formed three statistical groups, with Shaanxi rape honey exhibiting the lowest value ($33.37 \pm 2.01\%$), whereas Beijing, Hebei, and New Zealand manuka honeys showed comparable fructose contents ($40.10 \pm 1.76\%$, $41.59 \pm 1.29\%$, and $40.94 \pm 1.10\%$, respectively). In contrast, glucose content was separated into four statistical groups, with Beijing black locust honey showing the lowest value ($26.64 \pm 2.40\%$) and Shaanxi rape honey the highest ($35.48 \pm 1.97\%$). Hebei and Shandong black locust honeys exhibited similar glucose contents ($27.94 \pm 1.17\%$ and $28.04 \pm 1.41\%$, respectively). When grouped according to botanical origin, fructose contents were comparable between black locust and manuka honeys but lower in rape honey, whereas glucose content was highest in rape honey and lowest in black locust honey. These differences in fructose and glucose contents further demonstrate distinct sugar composition patterns among the investigated honey groups and provide a basis for comparing F/G characteristics.

The fructose-to-glucose ratio (F/G) is an important compositional characteristic of honey that influences flavour and crystallization and varies with botanical origin, geographical location, and climatic conditions[3, 49, 35]. Therefore, both the F/G ratio and the total fructose and glucose content (F + G) provide useful compositional information for differentiating honey samples. Honey from Beijing and Hebei exhibited the highest F/G ratios (1.52 ± 0.16 and 1.49 ± 0.09 , respectively), whereas Shandong black locust honey showed a slightly lower ratio (1.38 ± 0.12). At the botanical level, black locust honey had a higher mean F/G ratio (1.45 ± 0.14) than manuka honey (1.28 ± 0.04) and rape honey (0.94 ± 0.05). The total F + G content did not differ significantly between black locust honey (67.54 ± 2.62 g/100 g) and rape honey (68.85 ± 3.50 g/100 g), whereas manuka honey exhibited the highest value (73.04 ± 1.89 g/100 g). Among black locust honeys, samples from Hebei had a higher total F + G content (69.54 ± 1.44 g/100 g) than those from Beijing (66.75 ± 2.13 g/100 g) and Shandong (66.66 ± 2.83 g/100 g). Overall, differences in carbon and nitrogen contents, saccharide composition, and F/G characteristics provide complementary compositional information that, together with the stable isotope variables, supports the multivariate discrimination of honey samples.

3.3 Mineral element composition of honey

Mineral element concentrations differed significantly among the investigated honey groups (Table S1; Fig. 5). Because mineral element composition in honey is strongly influenced by soil characteristics, water availability, and local environmental conditions[8, 17], the observed differences are more likely to reflect origin-related and geogenic characteristics than botanical-type-specific characteristics. Overall, New Zealand manuka honey exhibited the highest concentrations for most mineral elements; however, Fe and Zn differed from this general trend. The highest Fe concentration was observed in Beijing black locust honey ($3456.59 \pm 1072.16 \mu\text{g/kg}$), while the highest Zn concentration occurred in Hebei black locust honey ($1307.16 \pm 772.00 \mu\text{g/kg}$).

Among the investigated elements, Na, Mg, K, Ca, V, and Ba exhibited the greatest variability, being separated into three or four statistically distinct groups among the five sample groups, whereas Cr, Mn, Cu, Rb, Pb, Fe, and Zn exhibited relatively limited variability. Consequently, the former elements contributed more strongly to differentiating the investigated honey groups. This trend is also reflected in the standardized element profiles shown in Fig. 5, highlighting the stronger discriminatory potential of elements with higher ANOVA F-values.

When samples were grouped according to botanical origin, manuka honey generally exhibited significantly higher mineral element concentrations than both black locust and rape honeys. For most elements, black locust honey and rape honey belonged to the same statistical group, indicating relatively limited differentiation based solely on mineral composition. Overall, mineral element composition provided complementary compositional information that, together with the stable isotope and saccharide variables, supports subsequent multivariate analyses.

3.4 Statistical evaluation of compositional differences among honey sample groups

One-way ANOVA was performed using 26 compositional variables, including stable isotope ratios, carbon and nitrogen contents, saccharide composition, F/G, F + G, and mineral element concentrations, to evaluate compositional differences among the predefined honey sample groups and botanical origins (Tables S2 and 2). Significant differences were observed among the five predefined honey sample groups for all investigated variables, although Zn exhibited the weakest discrimination ($p = 0.003$). Similarly, all variables differed significantly among the three botanical groups except for Fe ($p = 0.010$) and Zn ($p = 0.274$). These results indicate that most investigated variables possess strong discriminatory potential within the present dataset.

The two-sample Kolmogorov–Smirnov test further compared Chinese honey samples with New Zealand manuka honey (Table S2). Fe was the only variable that did not differ significantly between the two groups ($p = 0.931$), whereas all remaining variables showed significant differences ($p < 0.001$). Overall, the univariate statistical analyses confirmed substantial compositional differences among the predefined honey groups and demonstrated that most investigated variables provided useful discriminatory information for subsequent multivariate analyses. However, the relative contributions of geographical origin and botanical type could not be separated within the present experimental design.

3.5 Chemometric classification of honey samples

To further evaluate the discriminatory performance of the combined compositional dataset, principal component analysis (PCA), Fisher discriminant analysis (Fisher-DA), and artificial neural network (ANN) models were applied. PCA was used to visualize sample distribution patterns, whereas Fisher-DA and ANN were employed to assess classification performance.

PCA was first applied to visualize the overall distribution of honey samples based on the combined compositional dataset. The first three principal components explained 76.2% of the total variance, increasing to 87.44% for the first six principal components. As shown in the three-dimensional score plot (Fig. 6A), samples from the same predefined honey group generally clustered together, although partial overlap remained among the three black locust honey groups from Beijing, Hebei, and Shandong. By contrast, rape honey from Shaanxi and New Zealand manuka honey formed more distinct clusters with limited overlap, and Chinese honey samples were also clearly separated from New Zealand manuka honey. These results indicate that PCA captured the major compositional variation among the investigated honey groups, although discrimination among the closely related black locust honey groups remained limited. To further improve group discrimination, supervised classification models were subsequently applied.

Fisher discriminant analysis (Fisher-DA) was subsequently applied to evaluate the classification performance of different compositional datasets (Table S3). For discrimination among the five predefined honey sample groups, classification accuracy increased from 80.0% using stable isotope ratios alone to 90.6% using carbon and nitrogen contents combined with saccharide composition and 96.5% using mineral element composition, with the composite dataset achieving 100% original classification accuracy. Cross-validation produced slightly lower accuracies but showed the same overall trend, indicating good model robustness. Compared with PCA, Fisher-DA provided more compact clustering and clearer separation among the predefined honey groups in the discriminant space (Fig. 6B). In particular, Chinese honey samples and New Zealand manuka honey were clearly separated in the discriminant space, reflecting pronounced compositional differences between the two groups within the present dataset. Similarly, Fisher-DA also achieved excellent classification performance for the predefined botanical groups. Both the original and cross-validated classification accuracies reached 100% for the stable isotope, carbon and nitrogen contents combined with saccharide composition, and mineral element composition datasets. Using the composite dataset, only one manuka honey sample was misclassified as rape honey during cross-validation, resulting in a cross-validated accuracy of 98.8%. However, because botanical type and geographical origin were not independently controlled in the present dataset, these results should be interpreted as dataset-specific classification performance rather than as independent evidence of botanical effects.

Artificial neural network (ANN) models further demonstrated excellent classification performance, achieving 100% training and testing accuracies for discrimination among the five predefined honey sample groups, Chinese and New Zealand honey samples, and the predefined botanical groups (Table

S3). Normalized variable importance (Fig. 7) showed that the variables contributing most to classification differed among the three classification tasks. For discrimination among the five predefined honey sample groups, %C_{protein} together with several mineral elements (Rb, Mn, Cu, and K) contributed most strongly. In contrast, discrimination between Chinese honey samples and New Zealand manuka honey relied primarily on mineral element composition, particularly Mn, Cr, Rb, V, and Pb. For the predefined botanical groups, glucose was the dominant variable, with a normalized importance approximately twice that of the second-ranked variable (Zn), indicating a greater contribution of saccharide composition to botanical classification within the present dataset.

Overall, the supervised Fisher-DA and ANN models achieved higher classification performance than PCA, reflecting the advantage of supervised learning when multidimensional compositional information is available. In particular, both models achieved excellent classification performance for Chinese honey samples and New Zealand manuka honey, consistent with the pronounced differences in stable isotope ratios, carbon and nitrogen contents, saccharide composition, and mineral element composition observed in the preceding univariate analyses. However, because the present dataset was limited in sample size and did not independently separate geographical origin from botanical origin, these classification results should be interpreted as dataset-specific rather than as universally applicable classification models. Future studies should include larger and more diverse honey datasets, particularly manuka honey from different production years and geographical regions, together with additional complementary chemical indicators. Such efforts would further improve the comprehensiveness of honey characterization, strengthen the applicability of integrated chemometric models for honey quality evaluation, and enhance consumer confidence in commercial manuka honey.

4. Conclusions

This study integrated stable isotope ratios, carbon and nitrogen contents, saccharide composition, mineral element composition, and chemometric analyses to investigate compositional differences among predefined honey sample groups and to compare New Zealand manuka honey with selected Chinese honeys. The results demonstrated that these complementary compositional indicators revealed clear compositional differences among the investigated honey sample groups, with mineral element composition showing particularly strong discriminatory capability among the individual compositional datasets.

The different compositional indicators provided complementary insights into honey characteristics. Stable isotope ratios reflected differences associated with plant-derived carbon sources and environmental conditions, whereas mineral element composition provided important information related to regional and environmental characteristics. Carbon and nitrogen contents together with saccharide composition provided additional compositional information that complemented stable isotope and mineral element analyses, enabling a more comprehensive comparison of the chemical characteristics of New Zealand manuka honey and selected Chinese honeys.

Overall, this study provides comparative compositional data for New Zealand manuka honey and selected Chinese honeys based on multiple complementary chemical indicators. The resulting compositional dataset and integrated analytical strategy provide useful reference information for future studies on honey characterization, quality evaluation, and comparative analysis of commercial honey samples.

Declarations

Competing interests

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.

Funding

This study was supported by the Open Research Project of Laboratory of Quality & Safety Risk Assessment for Agro-Products (Urumqi), Ministry of Agriculture and Rural Affairs (Grant No. xjnkylwdzc-2025002-06-kt2)

Author Contribution

H.C. contributed to formal analysis, data curation, visualization, and writing the original draft. B.C. contributed resources. F.W., B.J., and Z.Y. supervised the study. X.Z. contributed to visualization. K.M.R. and D.Z. contributed to reviewing and editing the manuscript. H.W. contributed to conceptualization, supervision, project administration, and reviewing and editing the manuscript. All authors reviewed and approved the final manuscript.

Acknowledgement

This work was supported by the Open Research Project of Laboratory of Quality & Safety Risk Assessment for Agro-Products (Urumqi), Ministry of Agriculture and Rural Affairs (Grant No. xjnkylwdzc-2025002-06-kt2)

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. Doner LW (1977) The sugars of honey—a review. *J Sci Food Agric* 28(5):443–456.
<https://doi.org/10.1002/jsfa.2740280508>
2. Meo SA, Al-Asiri SA, Mahesar AL, Ansari MJ (2017) Role of honey in modern medicine. *Saudi J Biol Sci* 24(5):975–978. <https://doi.org/10.1016/j.sjbs.2016.12.010>
3. El Sohaimy SA, Masry SHD, Shehata MG (2015) Physicochemical characteristics of honey from different origins. *Annals Agricultural Sci* 60(2):279–287. <https://doi.org/10.1016/j.aosas.2015.10.015>
4. Hegazi NM, Elghani GEA, Farag MA (2022) The super-food Manuka honey, a comprehensive review of its analysis and authenticity approaches. *J Food Sci Technol* 59(7):2527–2534.
<https://doi.org/10.1007/s13197-021-05181-7>
5. Burns DT, Dillon A, Warren J, Walker MJ (2018) A Critical Review of the Factors Available for the Identification and Determination of Mānuka Honey. *Food Anal Methods* 11(6):1561–1567.
<https://doi.org/10.1007/s12161-018-1154-9>
6. Tsagkaris AS, Koulis GA, Danezis GP, Martakos I, Dasenaki M, Georgiou CA, Thomaidis NS (2021) Honey authenticity: analytical techniques, state of the art and challenges. *RSC Adv* 11(19):11273–11294. <https://doi.org/10.1039/d1ra00069a>
7. Bodor Z, Kovacs Z, Benedek C, Hitka G, Behling H (2021) Origin Identification of Hungarian Honey Using Melissopalynology, Physicochemical Analysis, and Near Infrared Spectroscopy. *Molecules* 26(23):7274. <https://doi.org/10.3390/molecules26237274>
8. Baroni MV, Podio NS, Badini RG, Inga M, Ostera HA, Cagnoni M, Gautier EA, Garcia PP, Hoogewerff J, Wunderlin DA (2015) Linking soil, water, and honey composition to assess the geographical origin of argentinean honey by multielemental and isotopic analyses. *J Agric Food Chem* 63(18):4638–4645.
<https://doi.org/10.1021/jf5060112>
9. Kropf U, Golob T, Necemer M, Kump P, Korosec M, Bertoneclj J, Ogrinc N (2010) Carbon and nitrogen natural stable isotopes in Slovene honey: adulteration and botanical and geographical aspects. *J Agric Food Chem* 58(24):12794–12803. <https://doi.org/10.1021/jf102940s>
10. Schellenberg A, Chmielus S, Schlicht C, Camin F, Perini M, Bontempo L, Heinrich K, Kelly SD, Rossmann A, Thomas F, Jamin E, Horacek M (2010) Multielement stable isotope ratios (H, C, N, S) of honey from different European regions. *Food Chem* 121(3):770–777.
<https://doi.org/10.1016/j.foodchem.2009.12.082>
11. Shuai M, Li H, She S, Wu M, Chen L (2023) C/H/O stable isotope of rape honey and its components combined with machine learning algorithms to identify geographical origin. *Rapid Commun Mass Spectrom* 37(15):e9539. <https://doi.org/10.1002/rcm.9539>
12. Lao MR, Bautista Vii AT, Mendoza NDS, Cervancia CR (2021) Stable carbon isotope ratio analysis of Philippine honeys for the determination of adulteration with C4 sugars. *Food Anal Methods* 14(7):1443–1455. <https://doi.org/10.1007/s12161-021-01968-8>
13. Aries E, De Rudder O, Kaklamanos G, Maquet A, Ulberth F (2021) Results of an Interlaboratory Comparison of a Liquid Chromatography-Isotope Ratio Mass Spectrometry Method for the

- Determination of $^{13}\text{C}/^{12}\text{C}$ Ratios of Saccharides in Honey. *J AOAC Int* 104(6):1698–1702.
<https://doi.org/10.1093/jaoacint/qsab091>
14. Drivelos SA, Georgiou CA (2012) Multi-element and multi-isotope-ratio analysis to determine the geographical origin of foods in the European Union. *TRAC Trends Anal Chem* 40:38–51.
<https://doi.org/10.1016/j.trac.2012.08.003>
 15. Kelly S, Heaton K, Hoogewerff J (2005) Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. *Trends Food Sci Technol* 16(12):555–567.
<https://doi.org/10.1016/j.tifs.2005.08.008>
 16. Bontempo L, Camin F, Ziller L, Perini M, Nicolini G, Larcher R (2017) Isotopic and elemental composition of selected types of Italian honey. *Measurement* 98:283–289.
<https://doi.org/10.1016/j.measurement.2015.11.022>
 17. Schmidlova S, Javurkova Z, Tremlova B, Hernik J, Prus B, Marcincak S, Marcincakova D, Starha P, Cizkova H, Kruzik V, Bodor Z, Benedek C, Titera D, Borzikova J, Pospiech M (2024) Exploring the Influence of Soil Types on the Mineral Profile of Honey: Implications for Geographical Origin Prediction. *Foods* 13(13):2006. <https://doi.org/10.3390/foods13132006>
 18. Šerevičienė V, Zigmontienė A, Paliulis D (2022) Heavy Metals in Honey Collected from Contaminated Locations: A Case of Lithuania. *Sustainability* 14(15):9196. <https://doi.org/10.3390/su14159196>
 19. Soares S, Amaral JS, Oliveira M, Mafra I (2017) A Comprehensive Review on the Main Honey Authentication Issues: Production and Origin. *Compr Rev Food Sci Food Saf* 16(5):1072–1100.
<https://doi.org/10.1111/1541-4337.12278>
 20. Drivelos SA, Danezis GP, Halagarda M, Popek S, Georgiou CA (2021) Geographical origin and botanical type honey authentication through elemental metabolomics via chemometrics. *Food Chem* 338:127936. <https://doi.org/10.1016/j.foodchem.2020.127936>
 21. Woodcock T, Downey G, Kelly JD, O'Donnell C (2007) Geographical Classification of Honey Samples by Near-Infrared Spectroscopy: A Feasibility Study. *J Agric Food Chem* 55(22):9128–9134
 22. Chen L, Wang J, Ye Z, Zhao J, Xue X, Vander Heyden Y, Sun Q (2012) Classification of Chinese honeys according to their floral origin by near infrared spectroscopy. *Food Chem* 135(2):338–342.
<https://doi.org/10.1016/j.foodchem.2012.02.156>
 23. Formosa JP, Lia F, Mifsud D, Farrugia C (2020) Application of ATR-FT-MIR for Tracing the Geographical Origin of Honey Produced in the Maltese Islands. *Foods* 9(6):710.
<https://doi.org/10.3390/foods9060710>
 24. Gok S, Severcan M, Goormaghtigh E, Kandemir I, Severcan F (2015) Differentiation of Anatolian honey samples from different botanical origins by ATR-FTIR spectroscopy using multivariate analysis. *Food Chem* 170:234–240. <https://doi.org/10.1016/j.foodchem.2014.08.040>
 25. Ismail NF, Maulidiani M, Omar S, Zulkifli MF, Mohd Radzi MNF, Ismail N, Jusoh AZ, Roowi S, Yew WM, Rudiyanto R, Ismail WIW (2021) Classification of stingless bee honey based on species, dehumidification process and geographical origins using physicochemical and ATR-FTIR

- chemometric approach. *J Food Compos Anal* 104:104126.
<https://doi.org/10.1016/j.jfca.2021.104126>
26. Zhang J, Chen H, Fan C, Gao S, Zhang Z, Bo L (2020) Classification of the botanical and geographical origins of Chinese honey based on (1)H NMR profile with chemometrics. *Food Res Int* 137:109714. <https://doi.org/10.1016/j.foodres.2020.109714>
 27. Labsvards KD, Rudovica V, Kluga R, Rusko J, Busa L, Bertins M, Eglite I, Naumenko J, Salajeva M, Viksna A (2022) Determination of Floral Origin Markers of Latvian Honey by Using IRMS, UHPLC-HRMS, and (1)H-NMR. *Foods* 11(1):42. <https://doi.org/10.3390/foods11010042>
 28. Wang J, Kliks MM, Qu W, Jun S, Shi G, Li QX (2009) Rapid determination of the geographical origin of honey based on protein fingerprinting and barcoding using MALDI TOF MS. *J Agric Food Chem* 57(21):10081–10088. <https://doi.org/10.1021/jf902286p>
 29. Mohammed SEA, Azim MK (2011) Characterisation of natural honey proteins: implications for the floral and geographical origin of honey. *Int J Food Sci Technol* 47(2):362–368.
<https://doi.org/10.1111/j.1365-2621.2011.02847.x>
 30. Jandrić Z, Frew RD, Fernandez-Cedi LN, Cannavan A (2017) An investigative study on discrimination of honey of various floral and geographical origins using UPLC-QToF MS and multivariate data analysis. *Food Control* 72:189–197. <https://doi.org/10.1016/j.foodcont.2015.10.010>
 31. Castro-Vázquez L, Díaz-Maroto MC, de Torres C, Pérez-Coello MS (2010) Effect of geographical origin on the chemical and sensory characteristics of chestnut honeys. *Food Res Int* 43(10):2335–2340. <https://doi.org/10.1016/j.foodres.2010.07.007>
 32. Karabagias IK, Maia M, Karabagias VK, Gatzias I, Badeka AV (2020) Quality and origin characterisation of Portuguese, Greek, Oceanian, and Asian honey, based on poly-parametric analysis hand in hand with dimension reduction and classification techniques. *Eur Food Res Technol* 246(5):987–1006. <https://doi.org/10.1007/s00217-020-03461-8>
 33. Tedesco R, Scalabrin E, Malagnini V, Strojnik L, Ogrinc N, Capodaglio G (2022) Characterization of Botanical Origin of Italian Honey by Carbohydrate Composition and Volatile Organic Compounds (VOCs). *Foods* 11(16):2441. <https://doi.org/10.3390/foods11162441>
 34. Stanimirova I, Üstün B, Cajka T, Ridelova K, Hajslova J, Buydens LMC, Walczak B (2010) Tracing the geographical origin of honeys based on volatile compounds profiles assessment using pattern recognition techniques. *Food Chem* 118(1):171–176.
<https://doi.org/10.1016/j.foodchem.2009.04.079>
 35. Madas NM, Marghitas LA, Dezmirean DS, Bonta V, Bobis O, Fauconnier ML, Francis F, Haubruge E, Nguyen KB (2019) Volatile Profile and Physico-Chemical Analysis of Acacia Honey for Geographical Origin and Nutritional Value Determination. *Foods* 8(10):445. <https://doi.org/10.3390/foods8100445>
 36. Smetanska I, Alharthi SS, Selim KA (2021) Physicochemical, antioxidant capacity and color analysis of six honeys from different origin. *J King Saud Univ - Sci* 33(5):101447.
<https://doi.org/10.1016/j.jksus.2021.101447>

37. Scholz M, Quinhone Junior A, Delamuta BH, Nakamura JM, Baudraz MC, Reis MO, Kato T, Pedrao MR, Dias LF, Dos Santos DTR, Kitzberger CSG, Bianchini FP (2020) Indication of the geographical origin of honey using its physicochemical characteristics and multivariate analysis. *J Food Sci Technol* 57(5):1896–1903. <https://doi.org/10.1007/s13197-019-04225-3>
38. Acquarone C, Buera P, Elizalde B (2007) Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. *Food Chem* 101(2):695–703. <https://doi.org/10.1016/j.foodchem.2006.01.058>
39. Escuredo O, Dobre I, Fernandez-Gonzalez M, Seijo MC (2014) Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chem* 149:84–90. <https://doi.org/10.1016/j.foodchem.2013.10.097>
40. Bilic Rajs B, Flanjak I, Milojkovic-Opsenica D, Tesic Z, Cacic-Kenjeric F, Primorac L (2024) Analysis of delta(13)C and delta(15)N values in Croatian honey by EA-IRMS and possibility of their application in botanical origin verification. *J Food Sci Technol* 61(5):939–949. <https://doi.org/10.1007/s13197-023-05888-9>
41. Karabagias IK, Badeka A, Kontakos S, Karabournioti S, Kontominas MG (2014) Characterization and classification of *Thymus capitatus* (L.) honey according to geographical origin based on volatile compounds, physicochemical parameters and chemometrics. *Food Res Int* 55:363–372. <https://doi.org/10.1016/j.foodres.2013.11.032>
42. Dinca O-R, Ionete RE, Popescu R, Costinel D, Radu G-L (2014) Geographical and Botanical Origin Discrimination of Romanian Honey Using Complex Stable Isotope Data and Chemometrics. *Food Anal Methods* 8(2):401–412. <https://doi.org/10.1007/s12161-014-9903-x>
43. Brereton RG (2015) Pattern recognition in chemometrics. *Chemometr Intell Lab Syst* 149:90–96. <https://doi.org/10.1016/j.chemolab.2015.06.012>
44. Jolliffe IT, Cadima J (2016) Principal component analysis: a review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 374 (2065):20150202. <https://doi.org/10.1098/rsta.2015.0202>
45. Standardization Administration of the People's Republic of China (2002) Method for the determination of C-4 plant sugars in honey-Stable carbon isotope ratio method. GB/T 18932.1–2002. General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Beijing
46. Wu H, Zhou X, Chen H, Jin B, Yan Z, Xie L, Zhou H, Rogers KM, Lin G (2022) A comparative authentication study of fresh fruit and vegetable juices using whole juice and sugar-specific stable isotopes. *Food Chem* 373 (Pt B) 131535. <https://doi.org/10.1016/j.foodchem.2021.131535>
47. Standardization Administration of the People's Republic of China (2023) National Food Safety Standard Determination of fructose, glucose, sucrose, maltose, lactose in food. GB 5009.8–2023. State Administration for Market Regulation. National Health Commission of the People's Republic of China, Beijing

48. Standardization Administration of the People's Republic of China (2016) National Food Safety Standard Determination of multielement in food. GB 5009.268–2016. China Food and Drug Administration, National Health and Family Planning Commission of the People's Republic of China, Beijing
49. Kek SP, Chin NL, Tan SW, Yusof YA, Chua LS (2016) Classification of Honey from Its Bee Origin via Chemical Profiles and Mineral Content. Food Anal Methods 10(1):19–30.
<https://doi.org/10.1007/s12161-016-0544-0>

Figures

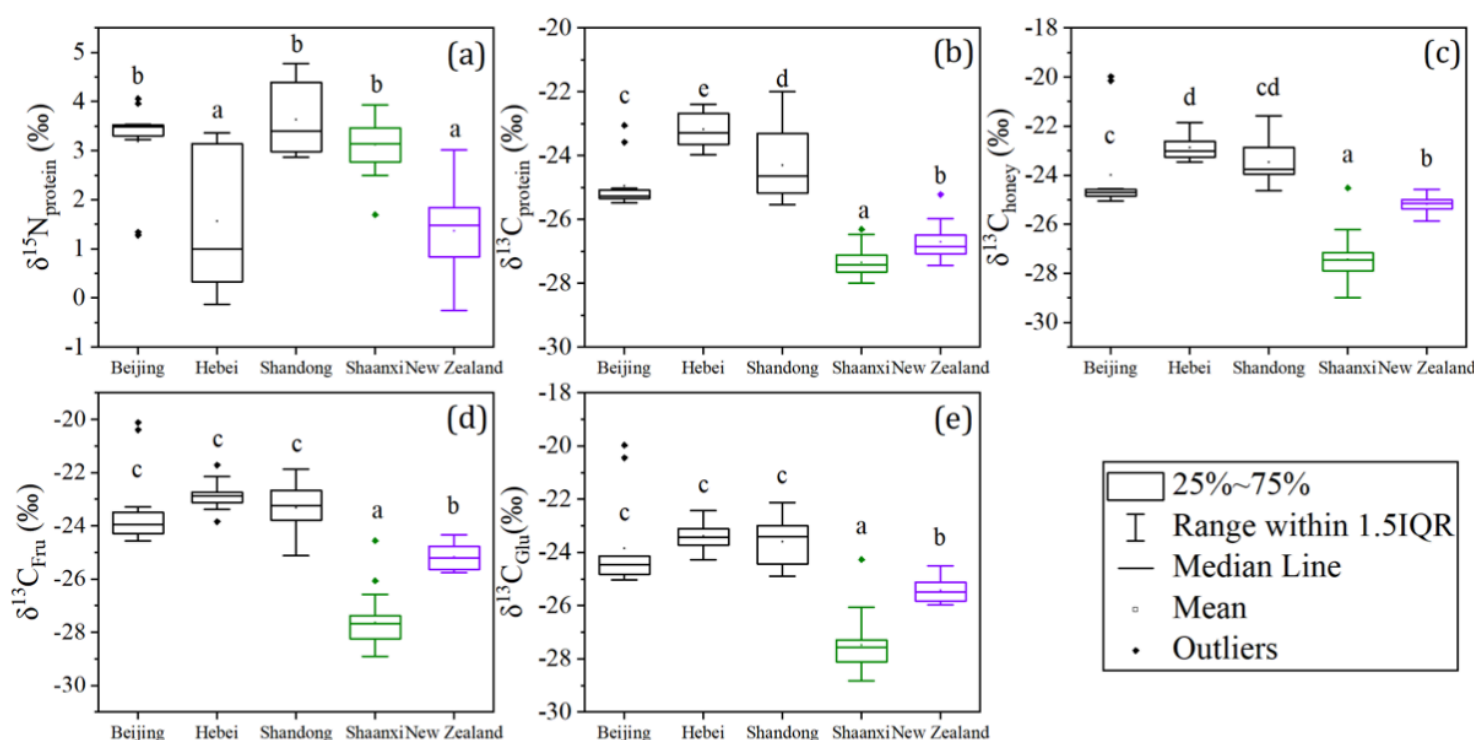


Figure 1

Box plots comparing five sample origins for (a) $\delta^{15}\text{N}_{\text{protein}}$; (b) $\delta^{13}\text{C}_{\text{protein}}$; (c) $\delta^{13}\text{C}_{\text{honey}}$; (d) $\delta^{13}\text{C}_{\text{Glu}}$; (e) $\delta^{13}\text{C}_{\text{Fru}}$ composition. The x-axis represents sample groups, and box colors indicate honey types: black, black locust honey; green, rape honey; and purple, manuka honey. The box in the plots represents data lying between the first quartile (Q1) and the third quartile (Q3). The range between Q1 and Q3 represents the Interquartile Range (IQR), which covers the middle 50% of the data. The box plots also display the median line, mean, and outliers. a-d Different lowercase letters indicate that significant differences were recorded in the honey from the five origins ($p < 0.05$).

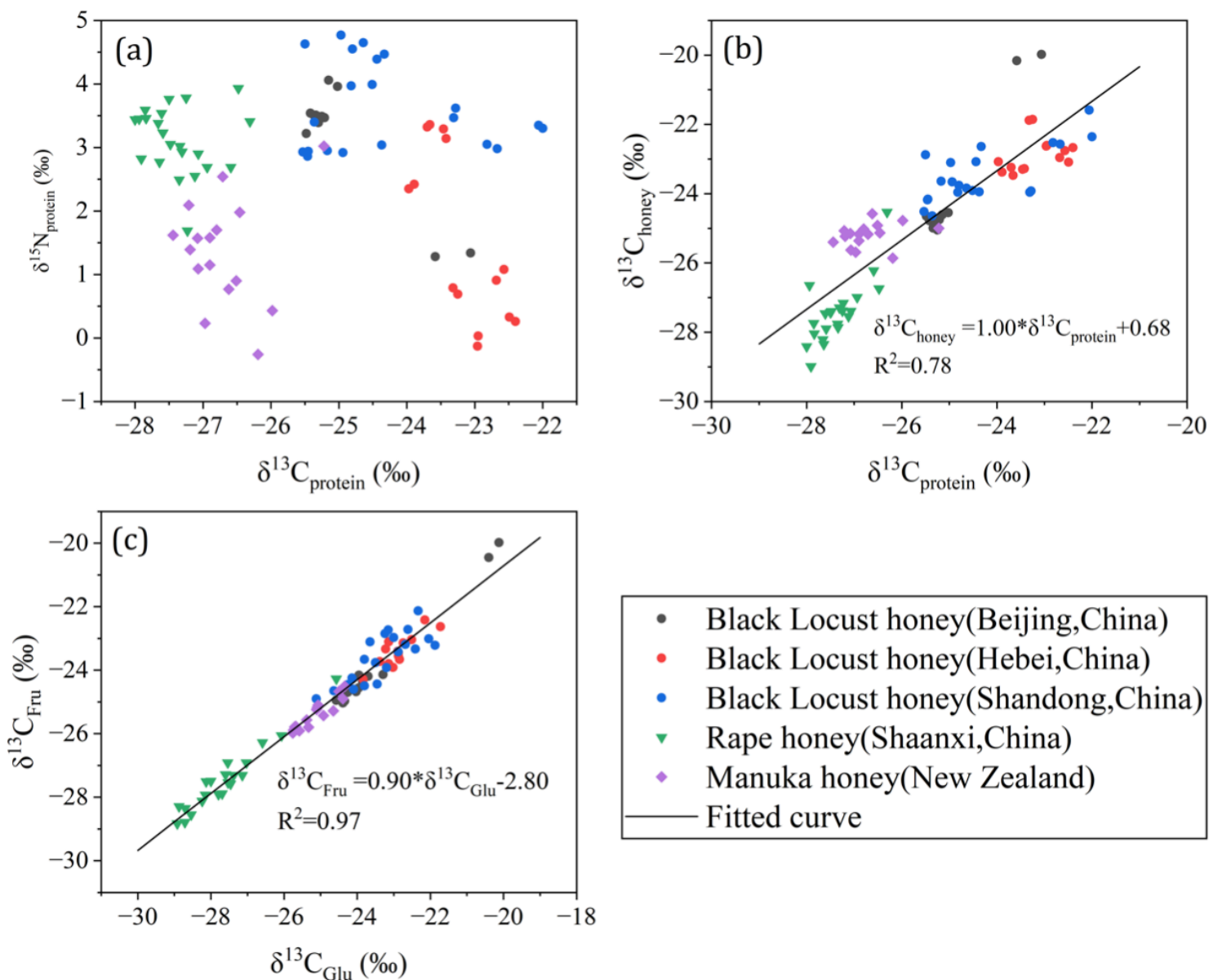


Figure 2

2D scatter plots of stable isotopes in honey samples from five sample origins and three botanical types (a) 2D scatter plot between $\delta^{15}\text{N}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{protein}}$; (b) 2D scatter plot between $\delta^{13}\text{C}_{\text{protein}}$ and $\delta^{13}\text{C}_{\text{honey}}$; (c) 2D scatter plot between $\delta^{13}\text{C}_{\text{Glu}}$ and $\delta^{13}\text{C}_{\text{Fru}}$.

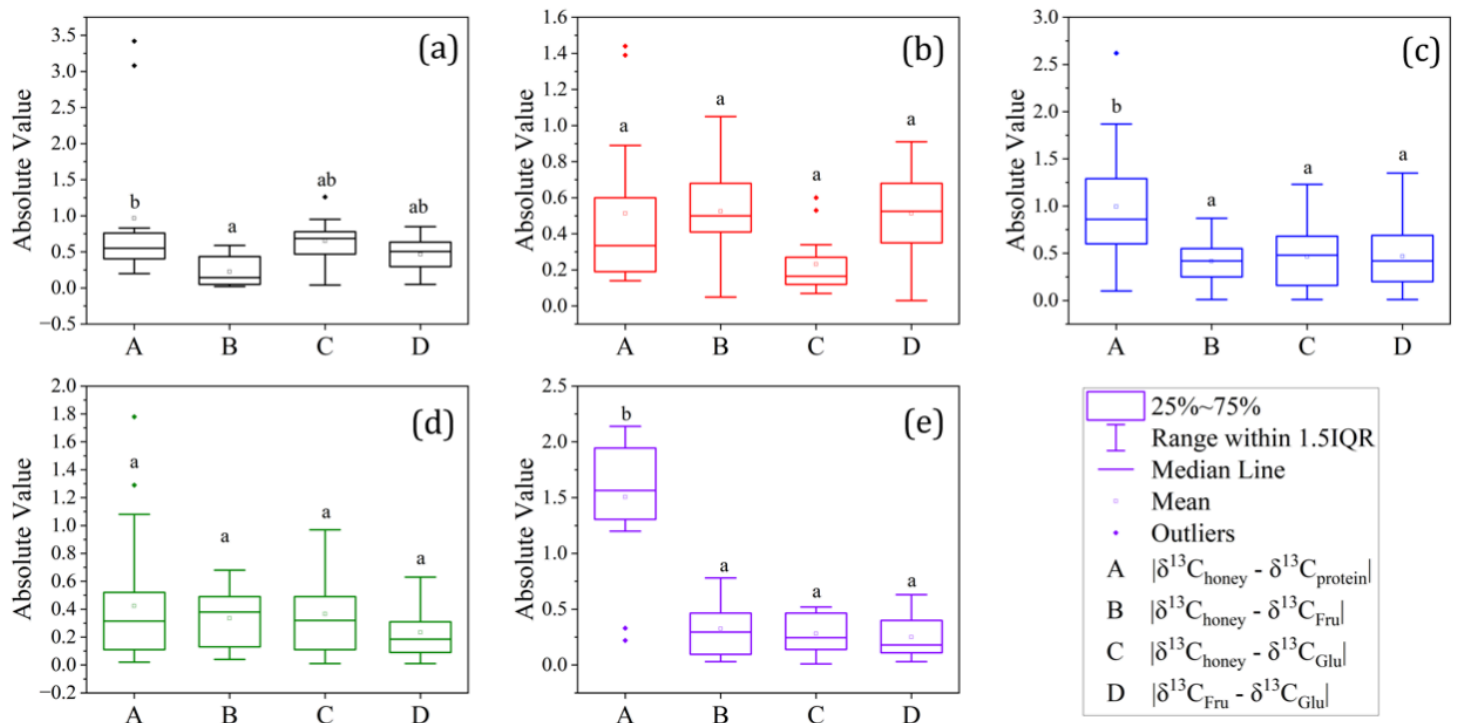


Figure 3

Box plots showing the absolute differences between different $\delta^{13}\text{C}$ variables in (a) Beijing black locust honey (n = 12), (b) Hebei black locust honey (n = 14), (c) Shandong black locust honey (n = 21), (d) Shaanxi rape honey (n = 22), and (e) New Zealand manuka honey (n = 16). A represents $|\delta^{13}\text{C}_{\text{honey}} - \delta^{13}\text{C}_{\text{protein}}|$; B represents $|\delta^{13}\text{C}_{\text{honey}} - \delta^{13}\text{C}_{\text{Fru}}|$; C represents $|\delta^{13}\text{C}_{\text{honey}} - \delta^{13}\text{C}_{\text{Glu}}|$; and D represents $|\delta^{13}\text{C}_{\text{Fru}} - \delta^{13}\text{C}_{\text{Glu}}|$. In the box plots, the main box shows data lying between the first quartile (25% or Q1) and the third quartile (75% or Q3). The range between Q1 and Q3 represents the Interquartile Range (IQR), which represents the middle 50% of the data. The box plots also display the median line, mean, and outliers. Different lowercase letters indicate significant differences among the four isotope differences within each honey group ($p < 0.05$).

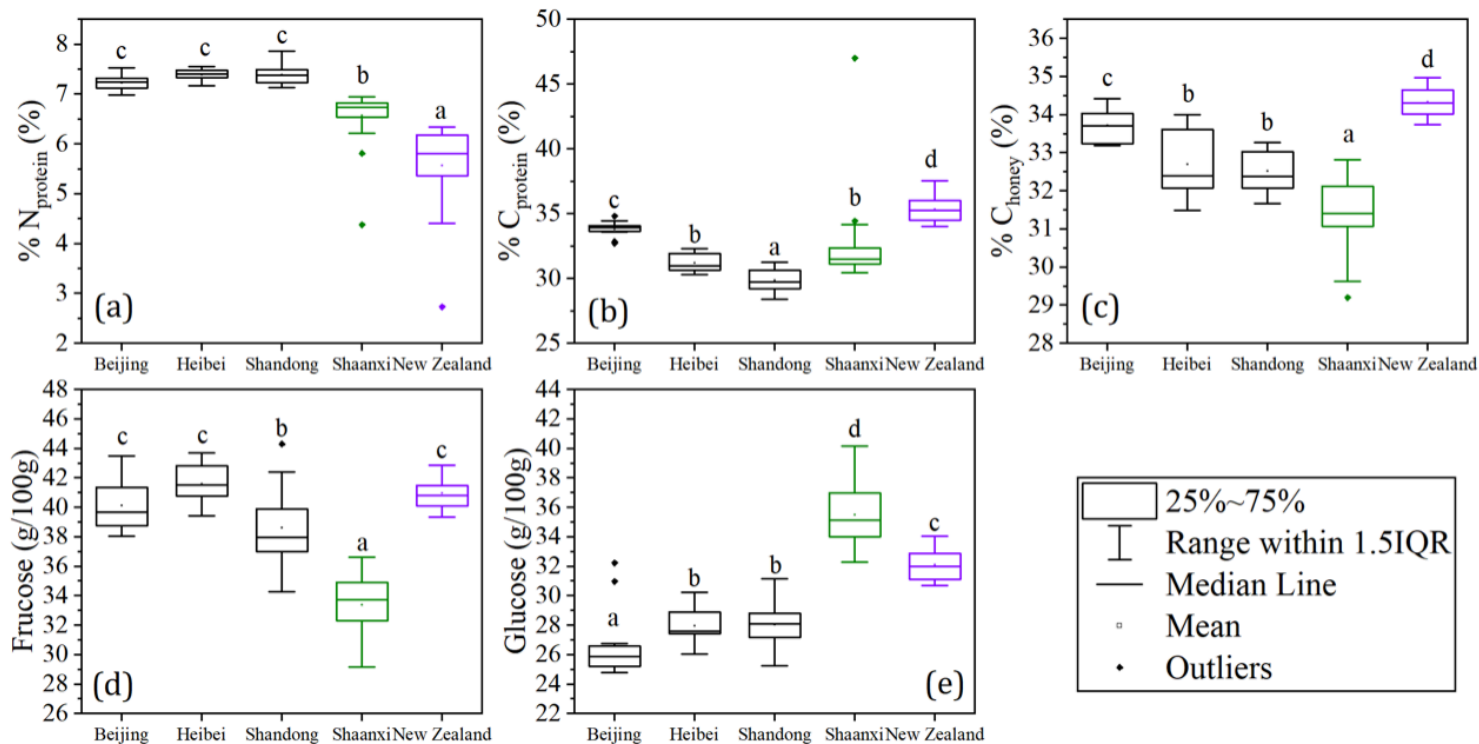


Figure 4

Box plots comparing (a) %N_{protein}; (b) %C_{protein}; (c) %C_{honey}; (d) Fructose; (e) Glucose compositions in honey from five sample groups. In the box plots, the main box shows data lying between the first quartile (25% or Q1) and the third quartile (75% or Q3). The range between Q1 and Q3 represents the Interquartile Range (IQR), which represents the middle 50% of the data. The box plots also display the median line, mean, and outliers. Lowercase letters (a-d) indicate significant differences recorded in the honey from the five sample groups ($p < 0.05$).

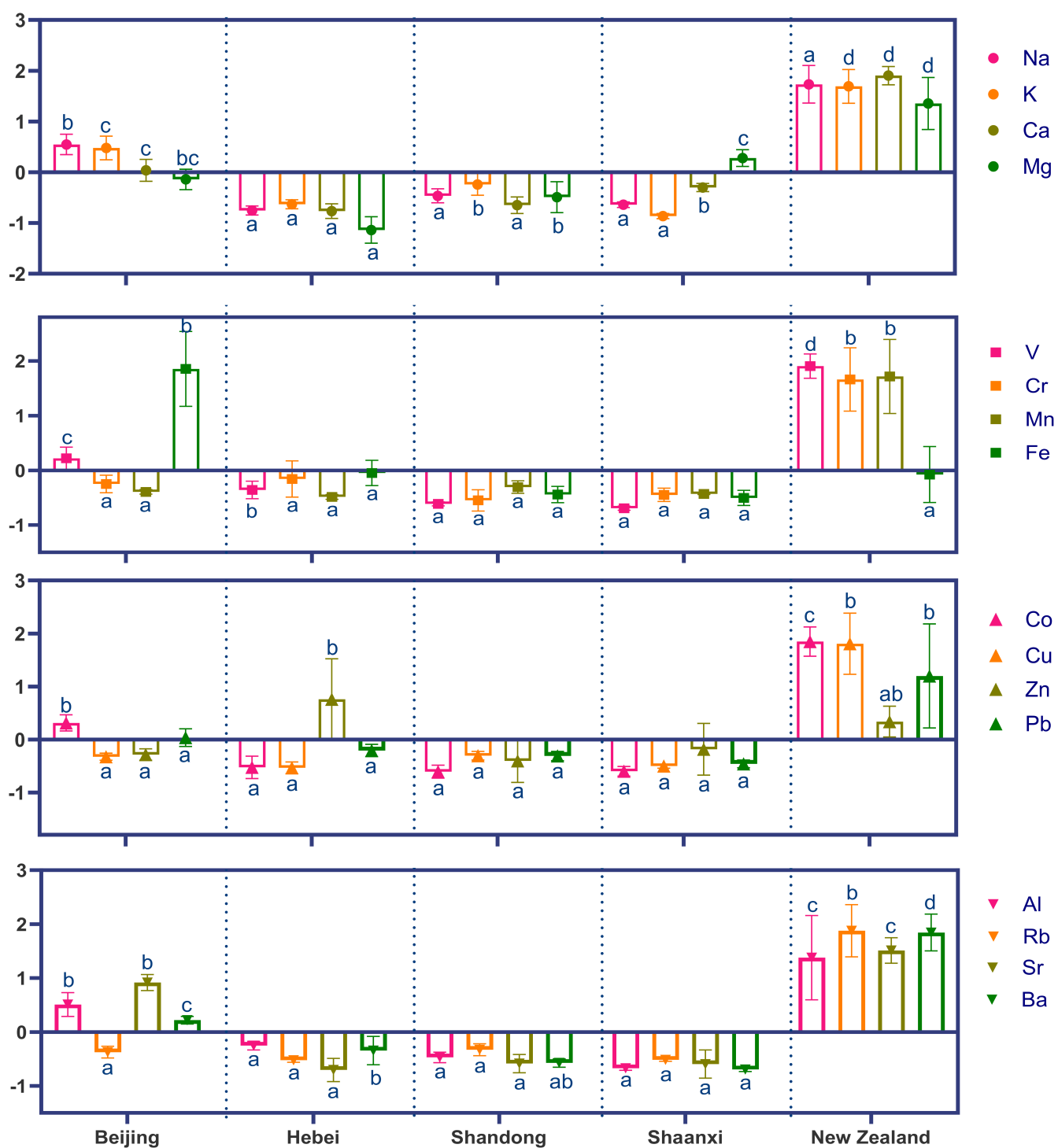


Figure 5

Histogram plots showing differences in 16 elemental compositions across five sample groups. The concentration of 16 mineral elements has been standardized in order to facilitate plotting, and the original results are shown in Table S1. a-d Different lowercase letters indicate significant differences were recorded in the honey from the five sample groups ($p < 0.05$). The order of the elements is sorted according to the F-values of significance analysis, from highest to lowest.

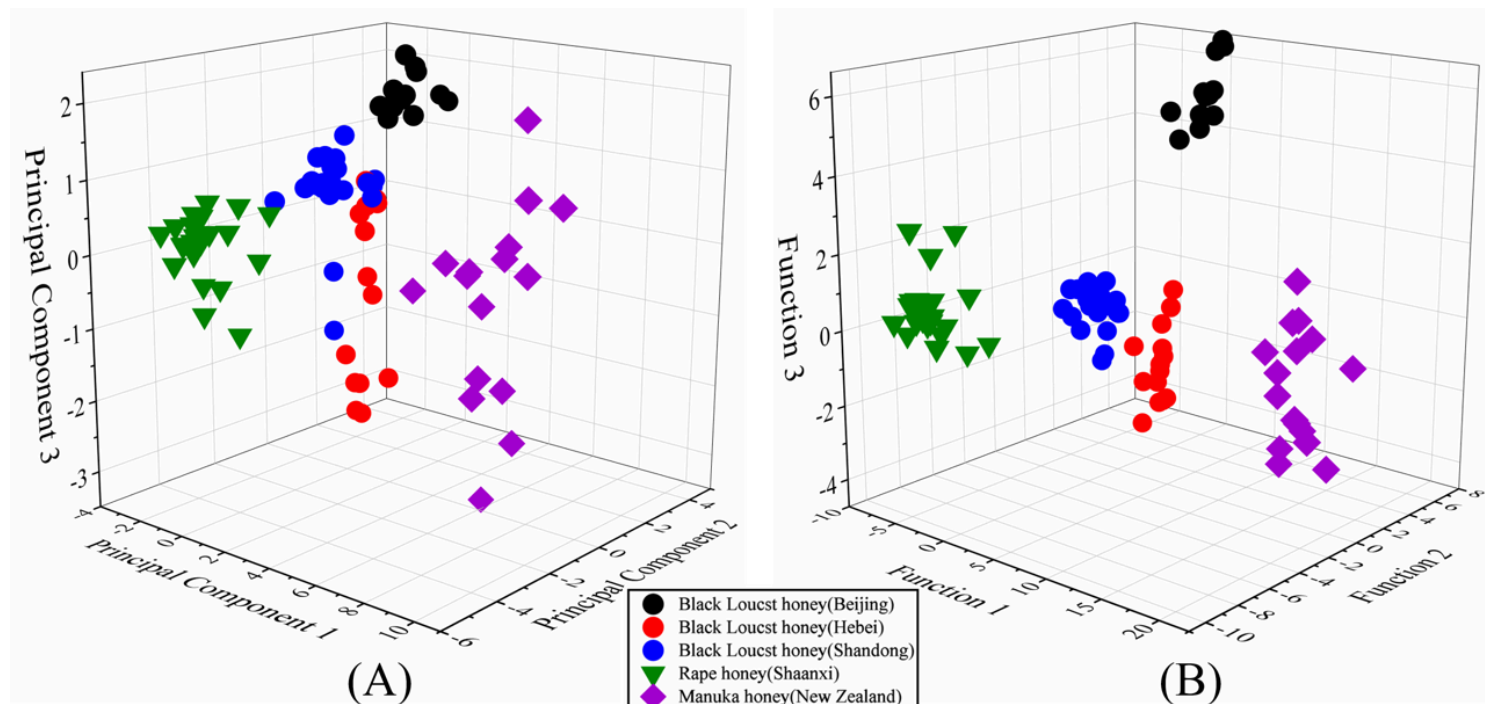


Figure 6

Three-dimensional scatter plots showing the distribution of honey samples in multivariate space based on the combined compositional dataset. (A) Principal component analysis (PCA). (B) Fisher discriminant analysis (Fisher-DA).

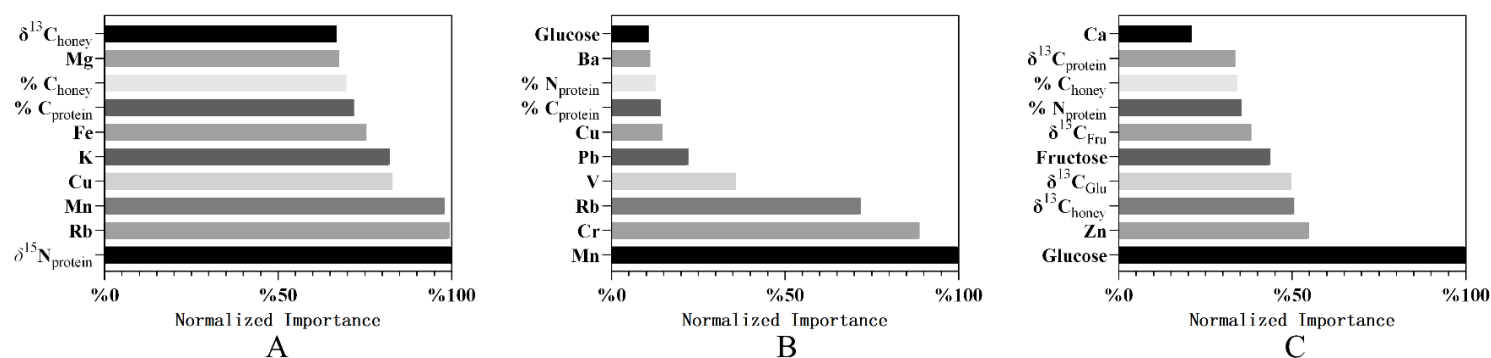


Figure 7

Stable isotopes and contents of saccharides and elements variable importance for the contribution towards origin verification using an ANN classification method. Plot A was top 10 of normalized importance of honey samples grouped by combined sample source and honey type, B was top 10 between honey samples from China and manuka honey from New Zealand, and C was top 10 of botanical origin.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [HaiquanChenManuscriptSupplementaryMaterial.docx](#)