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Original Research Paper

Determination of Honey Origin Using Mineral Profiling and Multivariate Statistical Analysis

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Abstract

Honey authenticity and traceability have become increasingly important due to rising concerns over adulteration and mislabeling in the global market. This study aims to evaluate the potential of elemental composition for classifying honey from different origins using multivariate chemometric techniques. Six honey samples were analyzed for selected major and trace elements using spectrometric methods, followed by statistical evaluation through one-way ANOVA, Principal Component Analysis (PCA), and Hierarchical Cluster Analysis (HCA). The results showed significant differences in elemental concentrations, particularly calcium (Ca) and cesium (Cs) ($p < 0.001$), indicating strong discriminatory potential, while potassium (K) was dominant but highly variable. Univariate analysis exhibited limited classification capability due to overlapping distributions and small sample size. In contrast, chemometric approaches improved classification performance, where HCA showed partial clustering and PCA after standardization provided clearer separation among sample groups. This study concludes that multivariate analysis enhances the reliability of honey classification compared to univariate methods. The findings demonstrate that meaningful classification can be achieved using limited variables and small datasets, supporting the development of cost-effective and accessible approaches for honey authentication and traceability.

Keywords: Chemometric analysis, Honey authentication, Trace elements.

INTRODUCTION

Honey is a natural sweet substance produced by *Apis mellifera* from floral nectar, plant secretions, or excretions of plant-sucking insects (honeydew) (Kimindu et al., 2025), and it has significant nutritional, medicinal, and economic value (Pereira et al., 2020). Increasing global demand for honey is driven by consumer preference for natural and functional food products (Kristína Predanócyová & Peter Šedík, 2024). This trend has encouraged extensive research on honey characteristics Silva et al., (2025), particularly its physicochemical and mineral composition (Sapitri et al., 2025). Honey composition is strongly influenced by botanical origin, geographical conditions, climate, and environmental factors that determine nectar availability. (Inaudi et al., 2025) Mineral and trace element contents are important indicators reflecting environmental conditions and plant sources (Rosmiatinnafiz et al., 2026). These characteristics make honey composition a key parameter for assessing quality and authenticity.

Trace element analysis has emerged as an effective approach for honey authentication based on botanical and geographical origin (Biswas et al., 2026). Mineral composition, including macroelements, microelements (Galić et al., 2025), and rare earth elements, provides a geochemical fingerprint that represents both natural environmental conditions and anthropogenic influences (Putu Eka Gunadi et al., 2026). Advances in analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) enable sensitive multi-element detection (Putu Eka Gunadi et al., 2026). Integration of chemometric methods, including principal component analysis (PCA) and hierarchical cluster analysis (HCA) (Taha et al., 2025), improves the interpretation of complex multivariate data (Halimatussoleha et al., 2026). These techniques allow pattern recognition and classification of honey samples based on compositional similarities (Álvarez-Suárez et al., 2025). The combination of elemental analysis and chemometrics has become a standard approach in modern honey authentication studies.

Limitations remain in the application of these approaches, particularly related to high-cost instrumentation and analytical complexity (Alves et al., 2025). Most studies rely on large datasets with numerous variables (Kim et al., 2025), whereas research using limited variables and small sample sizes is still scarce (Hu et al., 2025). This condition indicates a gap between advanced analytical methods and the need for simpler, more practical applications. Novelty in this study lies in the use of a limited number of elemental variables combined with a small dataset to achieve reliable classification results. This approach offers a cost-effective and efficient alternative without compromising analytical performance. The proposed strategy addresses the need for accessible honey authentication methods, especially for region-specific applications.

This study aims to develop a simplified and cost-effective method for honey classification based on mineral composition using multivariate statistical techniques. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are applied to evaluate the discriminatory power of a limited set of elemental variables. Six honey samples are analyzed to identify compositional patterns and similarities (Santos-Buelga & González-Paramás, 2025). The main objective is to demonstrate that meaningful classification can be achieved using small datasets. The results are expected to contribute to the development of accessible and efficient honey authentication approaches. This contribution supports improved consumer trust, product authenticity, and sustainable honey production systems.

RESEARCH METHODS

Time and Study Area

This study was conducted in 2025 in an analytical chemistry laboratory equipped with spectrometric instruments for mineral analysis. Sample preparation and measurements were carried out under controlled laboratory conditions to ensure accuracy and reproducibility. All experimental procedures followed standard operational protocols for food chemical analysis.Sample Preparation

Research Design

This study employed a laboratory-based experimental design with a quantitative approach to analyze the mineral composition of honey and evaluate its classification potential using chemometric techniques (Prananda et al., 2025). A multivariate approach was applied to identify similarities and differences among samples based on elemental profiles (Abdelsalam et al., 2025). Comparative analysis was performed to assess the discriminatory capability of selected variables (Udayani et al., 2025).

Population and Samples

The population of this study consisted of natural honey originating from various botanical and geographical sources (Liaqat et al., 2025). The samples included six types of honey representing different floral and/or geographical origins. A purposive sampling technique was applied to ensure sample authenticity and representation of variability (Samuel & Merkebu, 2026), as commonly recommended in food authentication studies (Danezis et al., 2016). The research variables included concentrations of major and trace elements such as K, Na, Ca, Mg, Fe, Zn, and Cu. Data were obtained through laboratory analysis using spectrometric instruments, while materials included honey samples, certified multi-element standard solutions, and chemical reagents such as HNO_3 and H_2O_2.

Research Procedure

Honey samples were collected directly from local beekeepers and certified producers to ensure authenticity and minimize industrial processing effects. Samples were stored in sterile amber glass containers at room temperature and protected from light until analysis. Prior to analysis, samples were homogenized to ensure uniform distribution of components. Sample preparation was conducted using a wet acid digestion method with nitric acid (HNO_3 65%) and hydrogen peroxide (H_2O_2 30%), followed by gradual heating until a clear solution was obtained. The digested solution was cooled, filtered, and diluted with deionized water to a fixed volume. Mineral content was determined using Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Instrument calibration was performed using certified standard solutions, and each sample was analyzed in triplicate to obtain mean values and standard deviations.

Data Analysis

Elemental concentration data were analyzed using both univariate and multivariate statistical approaches. One-way ANOVA was applied to evaluate significant differences among samples at a 95% confidence level ($p < 0.05$) (Pytlakowska et al., 2012). Principal Component Analysis (PCA) was used to reduce data dimensionality and identify key contributing variables, with components selected based on eigenvalues greater than 1 (Kaiser criterion). Prior to PCA, data were normalized using z-score standardization to eliminate scale differences. Hierarchical Cluster Analysis (HCA) was conducted using Euclidean distance and Ward's linkage method to group samples based on compositional similarity. All statistical analyses were performed using SPSS (version 25) and R software (packages: factoextra, stats, and cluster). Data interpretation focused on evaluating the discriminatory power of selected variables and the effectiveness of small datasets for honey classification.

RESULTS AND DISCUSSION

The present study demonstrates that elemental profiling combined with multivariate chemometric techniques provides a robust framework for differentiating honey samples with varying botanical and geographical origins. The observed variability in elemental composition among honey types reflects the strong influence of environmental conditions, floral source, and geochemical background on honey mineral profiles, consistent with previous studies reporting honey as an environmental bioindicator (Ellis et al., 2008;Pohl, 2009).

Elemental Variability and Environmental Fingerprinting

Figure 1 illustrates the distribution of sodium (Na) concentrations across six honey types, showing considerable variability among samples that reflects differences in botanical and geographical origin. This variation indicates that environmental factors such as soil composition, nectar sources, and local ecosystem conditions influence the mineral content of honey. The boxplot reveals differences in median values among groups as well as the presence of outliers, suggesting internal heterogeneity within certain honey types (Tibebe et al., 2022). These outliers may be associated with localized environmental conditions or variations in floral sources. The scatterplot demonstrates overlapping distributions among honey groups, indicating that Na concentrations are not distinctly separated between sample types. This overlap suggests that Na values share similar ranges across different honeys, limiting their effectiveness as a single discriminating parameter. Such patterns are typical in datasets with high variability and highlight the limitations of univariate approaches in resolving complex compositional differences (Pacifico et al., 2025).

The discriminatory power of Na as an individual variable is therefore relatively low, as it does not provide consistent separation among honey types. This finding supports previous studies indicating that single-element analysis is often insufficient to capture the complexity of honey composition, particularly when sample size is limited and distributions overlap. Multivariate approaches offer a more effective solution by integrating multiple elemental variables simultaneously (Arteaga-Cabrera et al., 2025). Techniques such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) enable the identification of relationships among variables and improve classification performance (Pandhi & Kumar, 2025). The combination of multiple mineral elements enhances pattern recognition and provides clearer discrimination compared to single-variable analysis. This approach has been widely recognized as more robust for honey authentication and geographical differentiation.

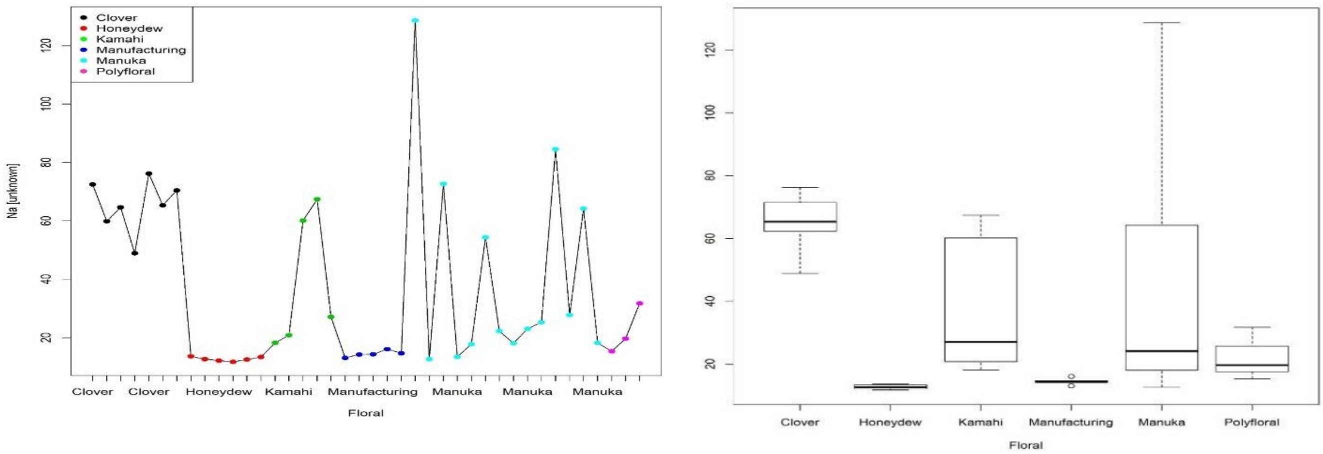

Figure. 1. Boxplot (right) and scatterplot (left) showing the distribution of sodium (Na) concentrations across different honey types (Clover, Honeydew, Kamahi, Manufacturing, Manuka, and Polyfloral). Units are reported as measured by the analytical method applied.

Figure 2 illustrates the distribution of magnesium (Mg) concentrations across six honey types, showing noticeable variability among samples that reflects differences in botanical and geographical origin. Variations in Mg content are likely influenced by soil mineral composition, plant uptake mechanisms, and environmental conditions associated with nectar sources (Doan, 2025). The boxplot indicates differences in median values among honey types, along with varying interquartile ranges that suggest differences in data dispersion. The presence of outliers in several groups indicates internal variability within specific honey types, potentially linked to localized environmental factors or heterogeneity in floral sources (Li et al., 2026). The scatterplot reveals partial overlap among sample groups, although some degree of separation is more apparent compared to sodium (Na). This pattern suggests that Mg may provide moderate discriminatory capability, but still lacks sufficient power as a single variable for clear classification.

The observed distribution indicates that magnesium contributes to distinguishing honey types to a certain extent, particularly where differences in median and spread are evident. However, overlapping values across groups limit its effectiveness in univariate classification. This finding aligns with previous studies showing that individual mineral elements often provide limited resolution when used independently in complex food matrices (Francis et al., 2025). Multivariate analysis remains essential to enhance classification performance by integrating Mg with other elemental variables (Çatal, 2025). Techniques such as Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) can capture combined variation patterns and improve discrimination among honey samples(El Hajj & Estephan, 2025). The inclusion of Mg as part of a multi-element profile strengthens the overall analytical framework and supports more reliable honey authentication.

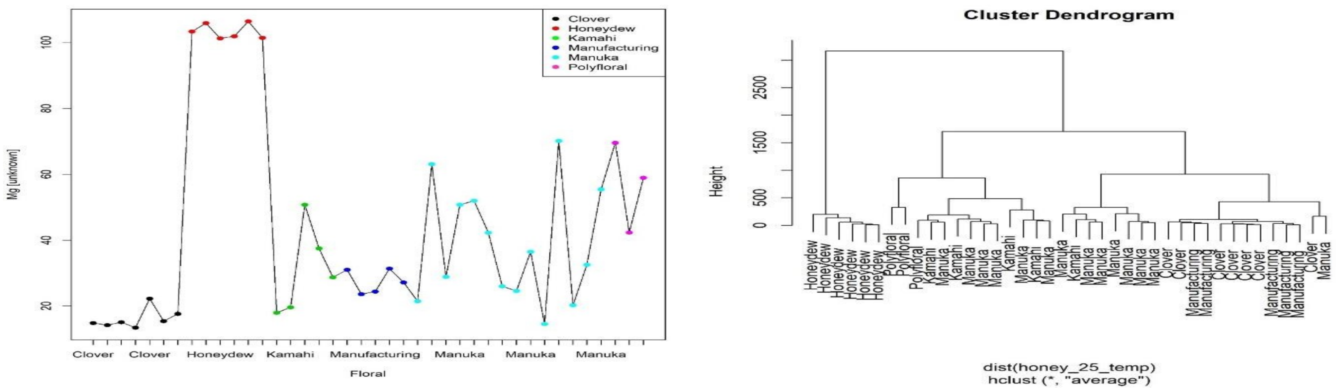

Figure. 2. Boxplot (right) and scatterplot (left) illustrating magnesium (Mg) concentration variability among honey samples from different floral and geographical origins, including Clover, Honeydew, Kamahi, Manufacturing, Manuka, and Polyfloral honeys.

Figure 3 presents the distribution of potassium (K) concentrations across six honey types, showing substantially higher variability compared to other analyzed elements. Potassium appears as the dominant mineral, with markedly elevated concentrations, particularly in Honeydew honey, which exhibits the highest values and widest range. This pattern reflects strong influence from botanical origin, as honeydew honey is typically associated with higher mineral content due to its derivation from plant exudates rather than floral nectar (Hajian-Tilaki et al., 2024). The boxplot reveals clear differences in median values among honey types, indicating that K has stronger discriminatory potential than elements such as Na and Mg. However, the large spread and presence of extreme values suggest high internal variability within certain groups (Conti et al., 2014). The scatterplot further highlights this variability, showing distinct clustering for some samples (e.g., Honeydew) while others remain partially overlapping.

Despite its strong contribution to differentiation, the dominance of potassium introduces scale-related bias, where high concentration values may overshadow the influence of other elements in the dataset. This effect can distort pattern recognition, particularly in multivariate analysis if data are not standardized (Blanusa et al., 2023). As a result, while K is highly informative, its standalone use may lead to overemphasis on certain groups. These findings indicate that potassium plays a critical role in distinguishing honey types, especially in identifying mineral-rich varieties such as Honeydew. However, reliable classification still requires multivariate approaches to balance the influence of dominant elements and capture the combined variability of multiple mineral components (Das, 2026). Integration of K with other trace elements enhances overall classification accuracy and supports more robust honey authentication.

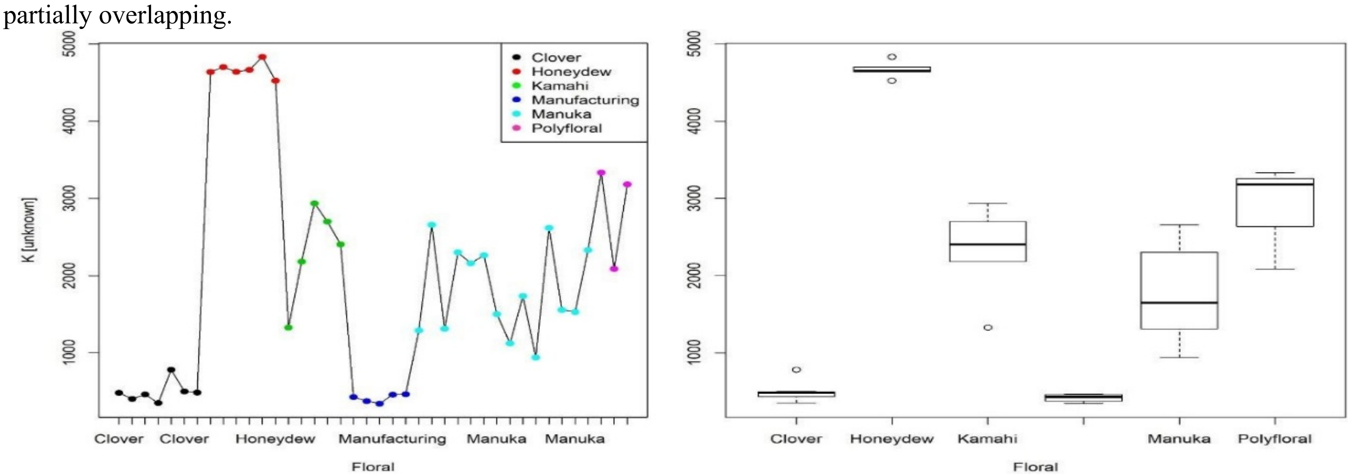

Figure. 3. Boxplot (right) and scatterplot (left) representing potassium (K) concentration distribution across honey types (Clover, Honeydew, Kamahi, Manufacturing, Manuka, and Polyfloral). Potassium shows relatively higher variability compared to other analyzed elements.

The results revealed substantial variability in major and trace elements, particularly potassium (K), magnesium (Mg), sodium (Na), calcium (Ca), and trace elements such as Cs. Potassium was the dominant element across all samples, consistent with its well-documented prevalence in honey matrices due to its high mobility in plant nectar and soil–plant transfer mechanisms (Smith et al., 2019). The elevated variability of K observed in this study further supports its sensitivity to floral origin and soil composition.

Conversely, sodium exhibited relatively inconsistent patterns across samples, suggesting limited specificity as a discriminating marker. This aligns with earlier findings indicating that Na levels in honey are more strongly influenced by environmental contamination or proximity to coastal regions rather than botanical origin (David et al., 2025). The significant variation observed for Ca and Cs ($p < 0.001$) highlights their potential as discriminative tracers. Calcium is closely associated with soil mineral composition and plant uptake efficiency, while Cs, although present in trace amounts, is known to reflect geogenic and anthropogenic inputs, making it highly sensitive for origin differentiation (Négrel et al., 2018). However, substantial within-group variability was also observed across all elemental distributions, indicating heterogeneity among samples of the same declared honey type. This variability may be associated with differences in local environmental conditions, soil composition, and bee foraging behavior.

Statistical Comparison Using ANOVA

One-way ANOVA was applied to assess statistically significant differences in elemental concentrations among honey types. The analysis revealed that calcium (Ca) and cesium (Cs) exhibited highly significant differences between groups ($p < 0.001$), indicating strong discriminatory potential for these elements in honey classification. Post hoc analysis further showed that Manuka honey differed significantly from Clover, Honeydew, Kamahi, Manufacturing, and Polyfloral samples. In contrast, sodium (Na) showed no significant differences, suggesting limited effectiveness as a single discriminating variable. Similar findings have been reported in recent studies, where Ca and trace elements demonstrated stronger classification capability compared to Na (Deng et al., 2020;Bao et al., 2022). Interpretation of these results requires caution due to limitations in dataset size and sample distribution. The number of observations per group was relatively small ($n < 30$), reducing statistical power. Visual inspection of boxplots indicated deviations from normality and potential heteroscedasticity, which may violate ANOVA assumptions and affect the validity of statistical inference (Arredondo Montero, 2026). These conditions may increase the risk of Type I or Type II errors, particularly in small datasets.

Interpretability of ANOVA results is inherently limited in complex multielement systems such as honey. Elemental composition reflects interdependent variables that cannot be fully captured through univariate analysis. Overlapping distributions among honey types further reduce the effectiveness of single-element comparisons. Previous studies have emphasized that mineral profiles in honey should be analyzed using multivariate approaches to better represent compositional complexity and improve classification accuracy (Gürbüz & Kıvrak, 2025). These findings indicate that ANOVA provides useful preliminary insights into elemental variability but is insufficient for reliable classification. Multivariate approaches such as PCA and HCA are required to enhance discrimination power and capture relationships among variables. Integration of these techniques has been widely recommended for food authentication studies due to their robustness in handling complex datasets and improving classification performance (Haider et al., 2024).

Hierarchical Cluster Analysis and Heatmap Patterns

Hierarchical cluster analysis (HCA), supported by heatmap visualization (Fig. 4), was used to evaluate similarity patterns among honey samples based on elemental composition. The heatmap revealed that honey samples could not be fully separated into clearly distinct clusters according to their declared floral types.

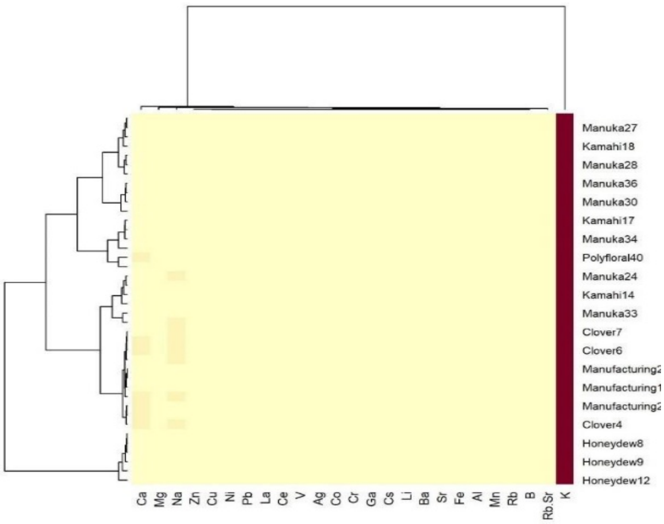

Figure. 4. The heatmap representing standardized elemental profiles, while the lower panels present dendrograms generated using complete-linkage (left) and average-linkage (right) methods. Clustering was performed based on Euclidean distance to evaluate similarity among honey types (Clover, Honeydew, Kamahi, Manufacturing, Manuka, and Polyfloral).

Instead, partial overlaps were observed among Clover, Kamahi, Manufacturing, Manuka, and Polyfloral samples, indicating shared elemental signatures. Interestingly, Honeydew samples appeared to be distributed across two distinct clusters, suggesting internal variability within this category, potentially due to differing environmental sources or production conditions. Both linkage methods ("complete" and "average") produced consistent clustering patterns (Fig. 4, lower panels), indicating stability of the clustering solution. However, the heatmap also revealed that potassium (K) exhibited substantially higher concentrations compared to other elements, which likely influenced distance-based clustering results. This dominance may introduce bias in HCA due to scale effects, where high-magnitude variables disproportionately affect similarity measurements. To address this limitation, data standardization (scaling and centering) is necessary prior to clustering to ensure equal contribution of all variables to the distance matrix (Jaeger & Banks, 2023).

Hierarchical cluster analysis (HCA) revealed partial grouping patterns; however, clustering was strongly influenced by high-magnitude variables, particularly potassium. This phenomenon reflects a known limitation of distance-based clustering methods, where variables with larger absolute values disproportionately affect Euclidean distance calculations (Liu et al., 2024). The observed clustering bias in the heatmap indicates that unscaled data may obscure true compositional relationships. This is particularly critical in food chemistry datasets, where element concentrations differ by several orders of magnitude. Consequently, without proper standardization, clustering results may reflect mathematical dominance rather than true chemical similarity. The consistent clustering patterns obtained using both complete and average linkage methods indicate methodological stability; however, the persistence of overlap among multiple honey types suggests limited discriminative resolution when using raw data

Principal Component Analysis (PCA)

To overcome the limitations of distance-based clustering and reduce the influence of scale imbalance, the elemental dataset was standardized prior to Principal Component Analysis (PCA). The PCA results (Fig. 5) revealed clearer grouping patterns among honey samples compared to HCA. The score plot of PC1 versus PC2 demonstrated the presence of distinct sample groupings. Clover and Honeydew samples formed relatively separated clusters, while Kamahi, Manufacturing, and Polyfloral samples showed substantial overlap, indicating similar elemental profiles. This overlap suggests that these honey types may share comparable botanical or environmental origins, or that their mineral compositions are not sufficiently distinct for separation using the selected elemental variables.

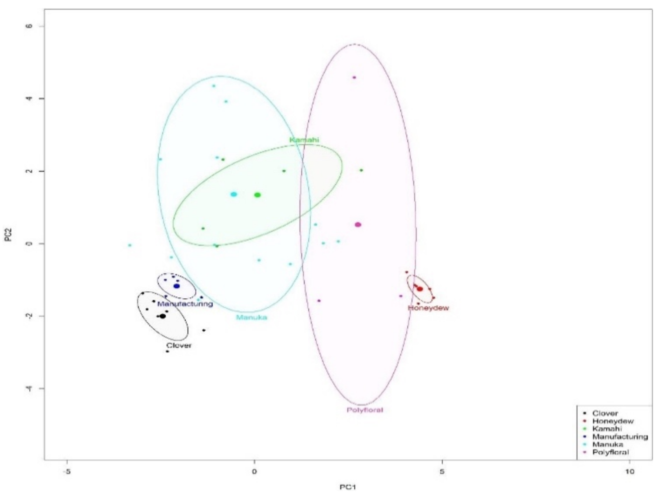

Figure. 5. Principal Component Analysis (PCA) score plot showing the distribution of honey samples based on the first two principal components (PC1 vs. PC2). Ellipses represent confidence regions for each honey type (Clover, Honeydew, Kamahi, Manufacturing, Manuka, and Polyfloral), highlighting group separation and overlap in multivariate space.

The PCA results further confirmed that multivariate projection methods are more effective than univariate or purely distance-based clustering approaches in resolving complex compositional datasets. This is because PCA reduces dimensionality while preserving maximum variance, allowing better visualization of underlying structure in the dataset (Chhikara et al., 2022). In contrast to HCA, Principal Component Analysis (PCA) provided clearer separation among honey samples after data standardization. PCA effectively reduced dimensionality while preserving the majority of variance, allowing underlying compositional structures to emerge more clearly.

The observed overlap among Kamahi, Manufacturing, and Polyfloral samples suggests similar elemental fingerprints, potentially indicating shared floral sources, overlapping geographical regions, or similar environmental conditions influencing nectar composition. Such overlaps are commonly reported in honey authentication studies, where botanical categories are not always chemically distinct due to environmental convergence (Schoder, 2026). Importantly, PCA mitigated the scaling bias observed in HCA by transforming correlated variables into orthogonal components. This allowed for more balanced representation of low- and high-abundance elements, improving classification interpretability. These findings reinforce previous reports that PCA is one of the most effective exploratory tools for food authentication and origin traceability (Frigerio et al., 2024).

Comparative Performance of PCA and HCA

A comparative evaluation of PCA and HCA indicated that PCA provided superior discrimination among honey samples compared to HCA. While HCA was sensitive to variable scale and dominated by high-concentration elements such as potassium, PCA mitigated this effect through standardization and variance-based projection.

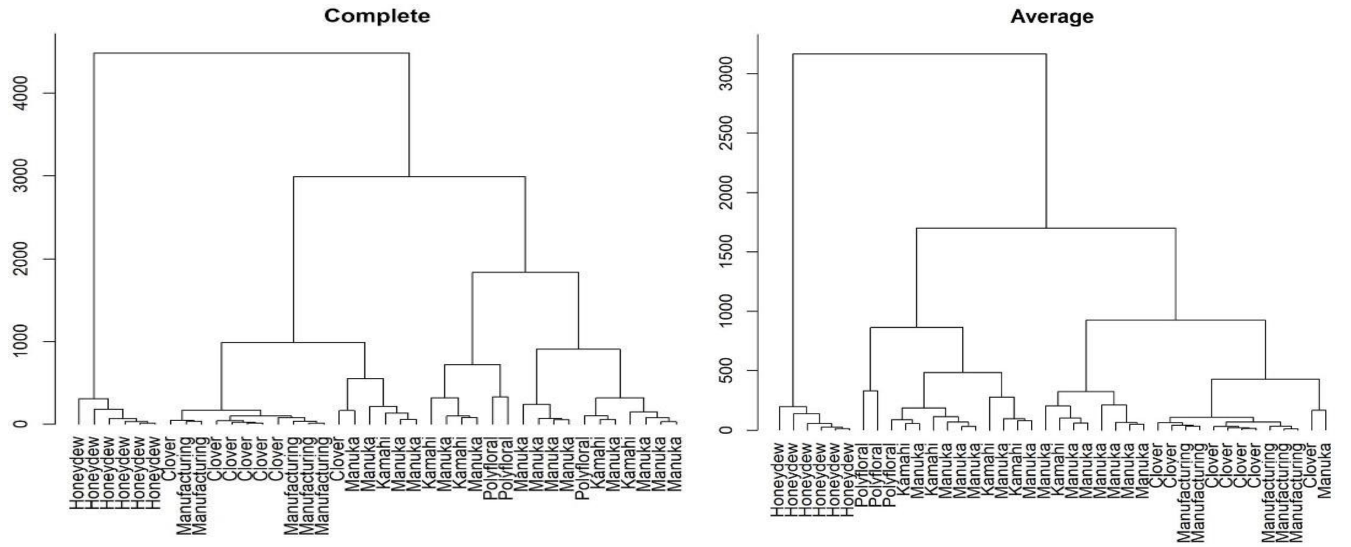

Figure 6. Hierarchical clustering heatmap and dendrogram of elemental concentrations in honey samples.

The comparative analysis between PCA and HCA highlights the complementary nature of these approaches. While HCA is useful for visualizing hierarchical similarity structures, it is highly sensitive to variable scaling and magnitude effects. PCA, on the other hand, provides a variance-driven projection that enhances class separability in multivariate space. The combined interpretation suggests that robust honey classification cannot rely on a single chemometric technique. Instead, integration of both methods improves interpretability and confidence in pattern recognition. This dual-approach strategy is widely recommended in food authentication studies to ensure both global structure detection (PCA) and local similarity grouping (Zaman et al., 2025) (HCA).

However, both methods consistently indicated partial similarity among Kamahi, Manufacturing, and Polyfloral samples, reinforcing the likelihood of shared compositional characteristics. These findings highlight that combining PCA and HCA provides complementary insights: HCA is useful for initial grouping, while PCA offers improved visualization and discrimination of multivariate structure. The findings of this study have important implications for developing simplified and cost-effective honey authentication frameworks. The results demonstrate that even a reduced set of elemental variables, when analyzed using appropriate multivariate tools, can provide meaningful discrimination among honey types.

This is particularly relevant for regions with limited access to high-resolution instrumentation such as ICP-MS. The combination of AAS/ICP-OES with chemometric analysis offers a viable alternative for routine quality control and traceability assessment. Furthermore, the successful application of PCA and HCA on a small dataset highlights the potential for rapid screening approaches in preliminary honey authentication studies. Despite the promising results, several limitations must be acknowledged. The relatively small sample size restricts statistical generalization and may limit model robustness. In addition, environmental variability within honey categories introduces internal heterogeneity that may obscure classification boundaries. Future studies should incorporate larger datasets across multiple seasons and geographical regions to improve model stability. Integration with additional chemical markers (e.g., isotopic ratios, phenolic compounds) and advanced machine learning algorithms could further enhance classification accuracy and predictive power.

CONCLUSION

This study confirms that elemental profiling combined with multivariate chemometric analysis is an effective approach for differentiating honey based on botanical and geographical origin. Among the analyzed elements, potassium, calcium, and selected trace elements showed the strongest discriminatory potential, while univariate analysis alone had limited capability due to sample variability and statistical constraints. In contrast, standardized multivariate techniques, particularly Principal Component Analysis (PCA), provided clearer classification patterns than Hierarchical Cluster Analysis (HCA), demonstrating their suitability for complex multielement datasets. Overall, the findings highlight that a limited set of elemental variables can still provide meaningful honey classification, offering a practical and cost-effective strategy for honey authentication and traceability, especially in resource-limited settings.

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