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# Physicochemical parameters and mineral components in Vietnam honey as a promising tool for classifying biological origin of honeys

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***Abstract:***

**Honey is known as a natural sweetener agent with high nutritional value and health benefits, especially premium honey because of its desirable flavour and medicinal properties. Because of this, honey has been a target of adulteration through the mixing of low-quality honey and mislabelling of the honey's origin. The aim of this research considers the potential of using mineral and physicochemical data to authenticate the origin of honey. To this end, 40 samples of 8 botanica collected from 18 different regions of Vietnam were analysed for its metal contents (Na, K, Mg, Ca, Al, Cu, Fe, Mn, Zn, Pb, Cd, Ni, Cr, Co, As, Hg) and physicochemical parameters (pH and electrical conductivity). The data were processed by multivariate analysis, which allowed the classification of honey according to its botanical origin.**

***Keywords:*** **authenticity, honey, mineral, multivariate analysis, physicochemical parameters.**

***Classification number:*** **2.2**

## Introduction

Vietnam was ranked the sixth largest in the world and the second largest in Asia in the amount of honey exported in 2018. However, honey export has recently suffered a dramatic decrease in terms of production, quality, and value. A statistic reported that Vietnam exported honey at a comparative price with India in 2013, but in 2017, prices became 10% cheaper than India. In 2018 the export value of the natural honey of Vietnam was approximately 67.7 million US dollars, which was down 49.2% from 2014 when it fell into the twelfth position on the world market [1]. The highest priced honey products come from New Zealand at 23.25 €/kg, while the honey price in Vietnam only reached 1.22 €/kg, which is the lowest price for honey products for export [2]. Vietnamese honey is also facing the risk of losing of both import and export markets. In the domestic market, Vietnamese honey either lacks or is underqualified for specific compositions or is mixed with illegal products to increase commercial profits. For those reasons, potential customers are now showing suspicion and hesitancy thus limiting their purchasing power. While the average honey consumption level in the world is about 700 g per person per year, the figure is just 30-40 g per person per year in Vietnam. This is due to the lack of a good policy to control the quality of honey, especially to detect fake or artificially produced honey. The sale of unidentified honey is also unregulated, which makes consumers hesitant to purchase honey products. Therefore, a simple and effective quality control method, as well as one that improves the traceability of honey, is essential. Methods assessing the authenticity of foodstuffs and honey in particular has become an indispensable issue in quality control and food safety. Honey's properties and compositions, both wild and farmed, depend not only on the nectar but also on other factors such as bee strains, geographic areas, seasons, storage methods, and even cultivation technology and harvesting conditions. Therefore, it is not trivial to correctly identify the source of honey. In previous works, different parameters such as moisture, electrical conductivity, carbohydrate, pH, and mineral content in honey were discriminated by multivariate data analysis, and the results exhibited high classification power [3-5]. Oroian, et al. (2017) [6] reported the coupled use of honey parameters and chemometric analysis is a powerful method to determine the origin of honey as well as other food products.

The main objective of this study is to measure some

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common physicochemical parameters and elements of honey samples cultivated in various known botanical origins. The results were to evaluate the quality of honey from several provinces in Vietnam and to perform multivariate statistical analysis to find the relation of these parameters with the botanical origins of the honeybee.

## Methodologies

### *Sampling and sample storage*

In this study, 40 samples of eight botanical origins of honey (acacia, coffee flower, jungle flower, longan flower, lychee flower, rambutan flower, rubber, and mutifloral) were taken from 18 different regions of Vietnam. The samples were provided by the individual beekeepers and natural honey hunters from 2016 to 2019. Their botanical and geographic origins are shown in Table 1. After delivery to the laboratory, the samples were kept at room temperature in plastic or glass bottles until analysis. To reduce the viscosity and ensure representability of the sample, the honey container was sonicated and later a mass of honey was digested or diluted prior to the measurement [7].

**Table 1. Botanical and geographical origin of the 40 honey samples analysed.**

| Sample No. | Geographic origin | Biographic origin | No. of sample |
|---|---|---|---|
| BG | Bac Giang | Lychee flower | 2 |
| BP | Binh Phuoc | Rubber | 1 |
| BT | Ben Tre | Mutifloral | 1 |
| BT2 | Ben Tre | Rubber | 1 |
| BT3 | Ben Tre | Longan flower | 1 |
| CG | Can Gio | Jungle | 1 |
| CM | Ca Mau | Jungle | 1 |
| CM2 | Ca Mau | Acacia | 1 |
| DB | Dien Bien | Jungle | 2 |
| DL | Dak Lak | Coffee flower | 1 |
| DN | Dong Nai | Rambutan flower | 1 |
| GL | Gia Lai | Jungle | 1 |
| GL2 | Gia Lai | Rubber | 1 |
| KT | Kon Tum | Jungle | 1 |
| KT2 | Kon Tum | Coffee flower | 1 |
| LD | Lam Dong | Coffee flower | 14 |
| NA | Nghe An | Jungle | 1 |
| PQ | Phu Quoc | Acacia | 1 |
| QN | Quang Ngai | Acacia | 1 |
| ST | Soc Trang | Longan flower | 2 |
| TG | Tien Giang | Longan flower | 1 |
| TN | Tay Ninh | Longan flower | 1 |
| T | Blended honey | Longan+Lychee flower | 2 |

### *Sample analysis*

*Electrical conductivity and pH determination:*

The electrical conductivity of the honey was measured using an InLab 731-ISM conductivity electrode connected to a Mettler Toledo SevenExcellence meter (USA). According to the method proposed by the International Honey Commission (IHC) [8], the electrical conductivity measurement was performed on a 20% (w/v) diluted honey solution at 20°C. To achieve this, first 20 g dry honey was dissolved in milli-Q water. This solution was transferred quantitatively to a 100-ml volumetric flask and milli-Q water was added. Afterward, 40 ml of the sample solution was transferred to a 100-ml beaker, which was temperature controlled at 20±0.5°C. The conductivity electrode was then immersed in the sample solution and the electrical conductivity of this solution was measured in units of $\mu S.cm^{-1}$ after equilibrium was reached. All samples were measured in triplicate.

The pH values of the honey samples were determined according to the method proposed by the IHC [8] using an Inlab Expert Pro-ISM pH electrode (Mettler Toledo, USA). First, 10 g of the honey sample was dissolved in 75 ml of milli-Q water in a 250-ml beaker to obtain a 10% (w/v) honey solution. The solution was vortexed for 2 min and the pH value was obtained. Each sample was measured twice and the results were given as mean values.

*Elemental analysis:*

Digestion procedures for determining minerals: because the majority of the organic matrix could interfere with the precision of analytical results, it is necessary to pre-treat the samples prior to analysis. There are a range of methods to remove the predominance of sugar in the honey such as solid-phase extraction, wet digestion, dry ash, and microwave digestion.

High temperature dry ashing was used to digest the organic substances and release the metals into the solution before determining the metal ion content using flame atomic absorption spectroscopy (F-AAS) and inductively coupled plasma mass spectrometry (ICP-MS). The sample was initially homogenized by vortexing. Five grams of the sample was placed in a porcelain crucible, then 1 ml $NH_4HPO_4$ (200 $mg.l^{-1}$) and 1.0 ml concentrated $H_2SO_4$ (Merck) were added as matrix modifiers. This mixture must be heated for 4 h to remove water by using the medium setting of a hot plate. Next, the dried sample was transferred to a furnace and the temperature of the furnace was slowly increased from 250 to 500°C at a heating rate of 2°C/min and kept at its

final temperature for 12 h to ensure the organic matter was fully destroyed. The residuals left were diluted by adding 5 ml $HNO_3$ (25% solution) and was made up to a volume of 10 ml by using $HNO_3$ (1% solution). This solution was measured for its metal ion contents via AAS and ICP-MS techniques.

Determination of total metal concentration: an Agilent 7700x inductively coupled plasma mass spectrometer was used to measure the concentrations of zinc (Zn), copper (Cu), aluminium (Al), iron (Fe), manganese (Mn), chromium (Cr), nickel (Ni), cobalt (Co), lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) with $^{103}Ru$ and $^{197}Au$ as internal standards, while a Shimadzu AA-6650 flame atomic absorption spectrophotometer was used to determine the concentrations of sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca).

FAAS was chosen to determine Na, K, Mg, and Ca contents because this method provides high accuracy and sensitivity to alkaline and earth-alkaline metals. Samples were diluted to ensure the measured results were the most accurate. In addition, FAAS utilizes an air/acetylene flame with a relatively low atomic temperature that could limit ionization interference for K and Na.

A certified reference material is not commercially available due to the practical problem representative of this kind of sample. In this study, a spike recovery was done to assess the accuracy of the digestion procedure. The results of the recovery study are presented in Table 2. The recovery percentages ranged between 81 and 102%, which fall within the normal acceptable range of a good recovery. Therefore, the methods used in this study achieved the required accuracy and reliability levels of all the metal contents in this study.

**Table 2. Recovery of the metal elements in this study.**

| Elements | Concentration | $C_{sample}$ | $C_{spike}$ | $C_{found}$ (mean±SD) | Recovery (%) |
|---|---|---|---|---|---|
| Na | mg.kg$^{-1}$ | 71.32 | 70 | 66.0±2.9 | 94.3±7.5 |
| K | mg.kg$^{-1}$ | 577.4 | 600 | 152±22 | 86±7.5 |
| Mg | mg.kg$^{-1}$ | 1.93 | 2.3 | 2.20±0.02 | 97.3±1.4 |
| Ca | mg.kg$^{-1}$ | 21.3 | 27.5 | 48.1±0.4 | 97.5±2.8 |
| Mn | mg.kg$^{-1}$ | 1.20 | 1.00 | 0.99±0.05 | 98.7±2.9 |
| Fe | mg.kg$^{-1}$ | 2.73 | 3.00 | 2.55±0.09 | 84.9±6.1 |
| Cu | mg.kg$^{-1}$ | 0.66 | 0.60 | 1.15±0.01 | 81.1±1.2 |
| Zn | mg.kg$^{-1}$ | 0.51 | 1.00 | 0.86±0.03 | 86.3±5.9 |
| Al | mg.kg$^{-1}$ | 2.84 | 5.00 | 5.10±0.12 | 102.1±4.0 |
| Cr | µg.kg$^{-1}$ | 23.47 | 25.04 | 23.4±1.1 | 93.6±8.5 |
| Cd | µg.kg$^{-1}$ | 0.68 | 2.05 | 1.6±5.9 | 82±13 |
| Pb | µg.kg$^{-1}$ | 12.74 | 15.03 | 12.2±0.6 | 81.7±8.5 |

### *Data processing*

The analysed data combined with multivariate statistical analysis including principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) were applied to the characterization of the botanical and geographical origins of the studied honeys. In this study, all PCA and PLS models and data pre-treatments were done by SIMCA-P software (Umetrics, Sweden).

## Results and discussion

### *Physicochemical properties*

According to international regulations (CODEX and EU standards), the determination of the conductivity of honey is carried out at a 20% (w/v) concentration of honey. Conductivity is closely related to the concentration of mineral and organic acids and shows high variability both internally and externally between honey groups. The electrical conductivity of honey is limited by the CODEX standard for honey, which states that the conductivity should be no more than 800 µS.cm$^{-1}$ [9]. The conductivity of the honey samples can range from 96.50 to 630.05 µS.cm$^{-1}$ and are described in Fig. 1. The data indicated the lowest conductivity was found in jungle flower honey from Dien Bien (96.50 µS.cm$^{-1}$), while the highest conductivity was found in samples from the forest regions, namely QN, NA, and BT3, which exceeded the maximum value of 800 µS.cm$^{-1}$ stipulated by CODEX. High electrical conductivity often indicates an increased content of mineral compounds. It is possible that the environment surrounding honeycombs or the beekeeping process could have changed the honey's composition through the nectar, which is the main food source of honeybees. Therefore, in order to more precisely explain this issue, it is necessary to find out more information from the beekeepers about the nest position as well as the bees' food composition. For the Quang Ngai (QN) and Nghe An (NA) samples, due to their limited number, no conclusion can be given until the number of analysed samples is increased.

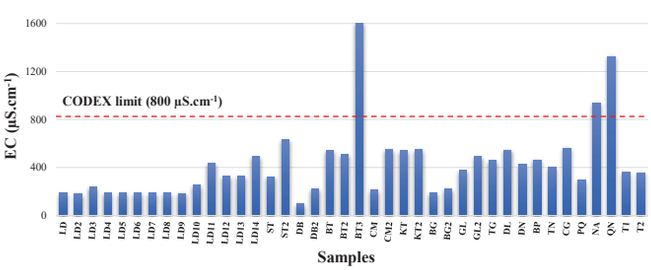


**Fig. 1. Electrical conductivity of 40 collected samples performed at 10% (w/v) dilution ratio of honey.**

Other studies have recently shown that the pH of honey depends on the free acid and total acid parameters, which could significantly contribute toward locating the biological origin of honey. Honey contains a number of acidic compounds including various amino acids (0.05-0.1% by weight) and organic acids (0.17 to 1.17%, averaged at 0.57% by weight). The average pH value of honey is 3.9 and usually varies between 3.4 to 4.35 [9]. As apparent from Fig. 2, the honeys have relatively low pH values, which limit the growth of microorganisms. All of 40 analysed honeys met permissible pH values set by the CODEX 2001 [10] and EU standards (pH 3.2-5.5). The pH values obtained in this study (3.44 to 4.35) were equivalent to those previously reported in Algeria, Poland, and Portugal honeys, which vary between pH 3.50 and 4.58 [11-13].

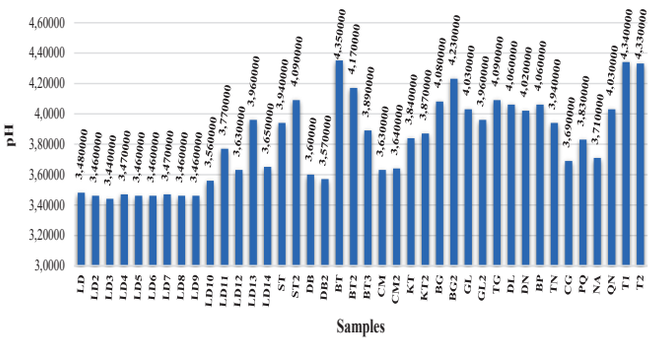


**Fig. 2. pH values of 40 collected samples performed at 20% (w/v) dilution ratio of honey.**

### *Mineral components in Vietnam honey*

The mineral components of the 40 Vietnamese honeys are shown in Table 3. As depicted in the table, potassium is the predominate element, which ranged between 200 and 2851 mg.kg$^{-1}$, which agrees with previous works [14]. The highest K content was found in the Ben Tre (BT) sample, followed by the Phu Quoc sample with 1544 mg.kg$^{-1}$. Along with K, some other minerals such as Na, Ca, and Mg could be considered as major metal components, which ranged from 3.18 to 535.5 mg.kg$^{-1}$, 3.63 to 115.0 mg.kg$^{-1}$, and 2.21 to 114.7 mg.kg$^{-1}$, in the honey samples, respectively.

Other essential elements including Al, Fe, Cu, Zn, and Mn were found at minor content levels. In particular, Al and Fe contents ranged from 1 to 30 mg.kg$^{-1}$ in all honey samples. It was also found that Cu, Zn, and Mn had mean values lower than 8 mg.kg$^{-1}$.

**Table 3. The concentration of major metals (K, Na, Ca, Mg), minor metals (Zn, Cu, Al, Fe, Mn), and trace metals in honey from different countries.**

| Elements | | Vietnam[a] | Turkey | Spain | New Zealand |
|---|---|---|---|---|---|
| K (mg.kg$^{-1}$) | $C_{min}$ | 200.3 | 143 | 670 | 200 |
| | $C_{max}$ | 2851.9 | 6029 | | 3640 |
| Na (mg.kg$^{-1}$) | $C_{min}$ | 3.18 | 9.3 | 47.3 | 1.1 |
| | $C_{max}$ | 535.5 | 172 | | 110 |
| Ca (mg.kg$^{-1}$) | $C_{min}$ | 3.63 | 3.3 | 34.2 | 7.21 |
| | $C_{max}$ | 115.0 | 900 | | 94.3 |
| Mg (mg.kg$^{-1}$) | $C_{min}$ | 2.21 | 2 | 73.2 | 7.52 |
| | $C_{max}$ | 114.7 | 111 | | 86.3 |
| Zn (mg.kg$^{-1}$) | $C_{min}$ | 0.17 | <1 | - | 0.2 |
| | $C_{max}$ | 3.39 | 20.2 | - | 2.46 |
| Cu (mg.kg$^{-1}$) | $C_{min}$ | 0.11 | <1 | 0.393 | 0.09 |
| | $C_{max}$ | 0.86 | 3.5 | | 0.7 |
| Al (mg.kg$^{-1}$) | $C_{min}$ | 0.92 | <1 | - | 0.23 |
| | $C_{max}$ | 29.0 | 0.96 | - | 21.3 |
| Fe (mg.kg$^{-1}$) | $C_{min}$ | 0.70 | 0.04 | 3.62 | 0.67 |
| | $C_{max}$ | 18.9 | 19.7 | | 3.39 |
| Mn (mg.kg$^{-1}$) | $C_{min}$ | 0.17 | <1 | 2.514 | 0.18 |
| | $C_{max}$ | 7.76 | 74.2 | | 4.75 |
| Pb (µg.kg$^{-1}$) | $C_{min}$ | 5.86 | <1 | - | 3.01 |
| | $C_{max}$ | 145.47 | <1 | - | 40 |
| Cd (µg.kg$^{-1}$) | $C_{min}$ | 0.50 | <1 | - | <1 |
| | $C_{max}$ | 34.09 | <1 | - | 149 |
| Cr (µg.kg$^{-1}$) | $C_{min}$ | 1.46 | - | - | <1 |
| | $C_{max}$ | 170.02 | - | - | 370 |
| As (µg.kg$^{-1}$) | $C_{min}$ | 0.14 | - | - | <1 |
| | $C_{max}$ | 11.28 | - | - | 80 |
| Ni (µg.kg$^{-1}$) | $C_{min}$ | 18.41 | - | - | - |
| | $C_{max}$ | 618.1 | - | - | - |
| Co (µg.kg$^{-1}$) | $C_{min}$ | 1.24 | - | - | - |
| | $C_{max}$ | 29.76 | - | - | - |
| Hg (ng.kg$^{-1}$) | $C_{min}$ | 10.75 | - | - | - |
| | $C_{max}$ | 268.8 | - | - | - |

[a]: results of this study; -: either not detected or not provided by the studies.

The mean and range of the concentrations of each trace element in the honey samples are shown in Table 4. The order of the mean trace element content from low to high is Hg, As, Cd, Co, Cr, Pb, Ni. The amount of trace element concentrations varied widely in the honey samples but were all below the minimum limit allowed in honey set by the Standards of the Vietnam Health Ministry for food products (QCVN 8-2011-BYT) and CODEX Alimentarius Commission (As<1.0 mg.kg$^{-1}$, Pb<1.0 mg.kg$^{-1}$, Cd<2.0 mg.kg$^{-1}$, Hg<0.05 mg.kg$^{-1}$) [10,

15]. It is noticeable that some heavy trace metal elements, including lead, cadmium, and mercury, were found at a relatively high level, which is associated more with soil and environment contamination.

**Table 4. Concentration of trace metals in honey of different regions of Vietnam.** All results in µg.kg $^{-1}$ except Hg in ng.kg $^{-1}$.

| | Cr (µg.kg$^{-1}$) | Ni (µg.kg$^{-1}$) | Co (µg.kg$^{-1}$) | Pb (µg.kg$^{-1}$) | Cd (µg.kg$^{-1}$) | As (µg.kg$^{-1}$) | Hg (ng.kg$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Mean | 24.4 | 120 | 10.6 | 66 | 5.04 | 2.39 | 81.9 |
| Range | 1.46-170 | 18.4-618 | 1.24-29.8 | 5.86-91.8 | 0.5-34.1 | 0.14-11.3 | 10.8-269 |

Overall, the concentrations of the 16 metal elements in this study varied significantly with the geographical and botanical origins of the honey samples. Also noticeable are variations within samples with the same origin. Therefore, multivariate data analysis is required to evaluate the relationship between the metal contents and other parameters of the honey, as well as to develop a classification model based on the physicochemical and mineral contents according to the origin of the honey samples.

### *Characterisation of the honeybee*

In an attempt to establish the classification between 6 botanical origins and 18 graphical origins of honey, the PCA model was applied to the analysis data. PCA was able to evaluate the element composition of honey types. Fig. 3 shows the score plot of PCA corresponding to the data matrix. The two principal components (PCs) explained 85% of the variables of data set. The two PCs, PC1 and PC2, explained 65% and 20% of the variability, respectively. As seen in Fig. 3, the honey samples belonging to the following provinces tend to be separate from the rest: Lam Dong, Bac Giang, Gia Lai, and Kon Tum, although there was some overlapping of honey groups.

The model was slightly improved ($R^2$=0.861) after excluding 13 out of 18 analysis criteria, however, low classification ability was still observed ($Q^2$<0.5). This could be explained by the totally unsupervised nature of the PCA method, which might fail to achieve good separation in models that contain too many individual variables or classes. Therefore, the more supervised PLS-DA model should be considered to improve classification and explanation ability, in addition to supplying a prediction ability to the model.

To assess the PLS-DA model, a VIP (variable importance plot) was carried out to evaluate the level of contribution from each variable (VIP>0.7). Variables that did not distribute to the model were rejected to try for a better model. In the PLS model t[1] vs t[2], the $R^2$ and $Q^2$ were 0.858 and 0.812, respectively, after application of the stepwise PLS-DA to the data. The selected variables include pH, EC, K, Mg, Fe, Ni, Pb, and Cd.

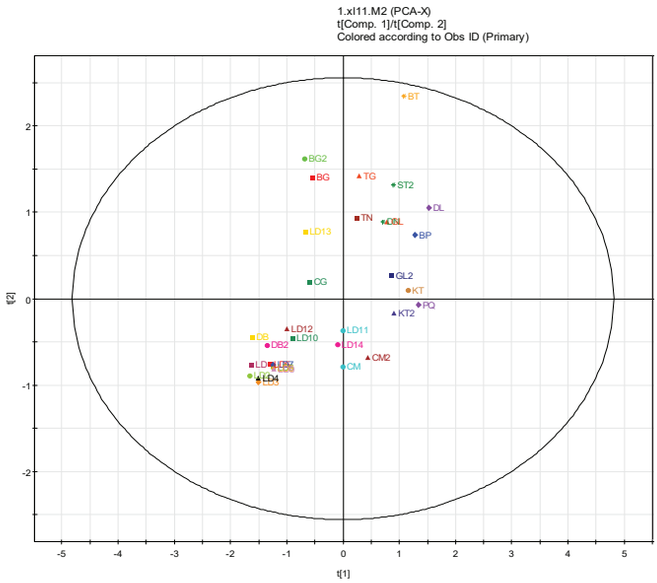


**Fig. 3. Principal component analysis of the multielement scores of graphical origin of honeys.** BG: Bac Giang, LD: Lam Dong, DB: Dien Bien, CM: Ca Mau, KT: Kon Tum, PQ: Phu Quoc, CG: Can Gio, TG: Tien Giang, ST: Soc Trang, DN: Dong Nai, GL: Gia Lai, BP: Binh Phuoc, DL: Dak Lak, TN: Tay Ninh.

Figure 4 presents the classification of honey in its corresponding botanical source with five separated groups (coffee, jungle, lychee, acacia, longan). There were some coffee honey samples that overlapped with the sample belonging to the jungle honey group. This can be explained by the fact that jungle honey is normally multifloral.

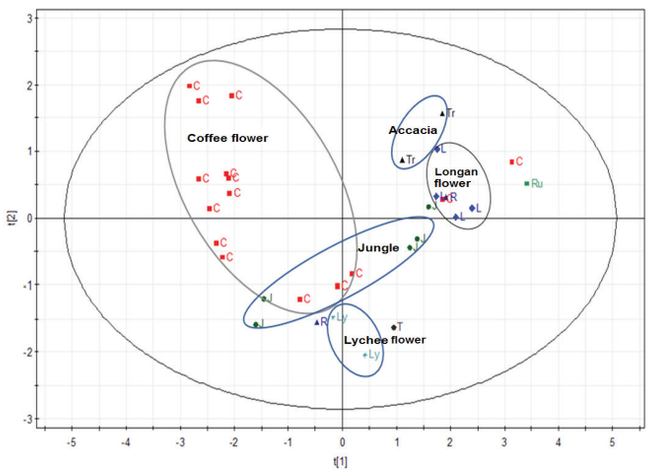


**Fig. 4. Score plot of PLS-DA analysis used to distinguish all honey samples (n=40).** C - coffee flower; J - jungle; Ly - lychee flower; L - longan flower; Ru - rubber; Tr - acacia; R - rambutan flower; T - blended honey.

The trueness of the model was checked by using two commercial blend samples from a beekeeper. Based on market demand, customers like to use honey products that are light in colour, dense, and scented. For example, unifloral honey, such as longan flower honey, has a fragrant scent but is less dense. Lychee honey is scented and is easily crystallized, while rubber honey is dark, not fragrant, and has a high density. Therefore, suppliers tend to mix these types of honey together but still want to ensure the quality of the honey. The two samples of honey used to evaluate the interpretation and prediction of the analytical model were mixes between the longan flower honey and the lychee flower honey. The score plot of the PLS-DA indicated that this blended honey (T) was located between the lychee group and the longan group and had characterizations of both groups. Therefore, it can be said that PLS-DA is a suitable tool for the differentiation and classification of studied honeys.

## Conclusions

In this work, all the studied Vietnamese honey samples met the regulations of the Codex Alimentarius Commission and the Ministry of Public Health of Vietnam in terms of pH, electrical conductivity, and concentrations of toxic heavy metals. The use of elemental and common physicochemical profiles of 8 plants from 18 different areas in Vietnam was coupled with multivariate analysis for classification purposes. Although the present results do not display a clear separation of honey types from a variety of geographic origins, the final PLS model had values of $R^2$=0.858 and $Q^2$=0.812 ($R^2$>0.7 and $Q^2$>0.5), which exhibited a potential approach for discriminating Vietnamese honeys from different botanical origins. From a market manager perspective, these results establish a fast tool for classifying of Vietnamese honey, which contribute to the formulation of food safety policies, ensure the success of national brands, create customer trust, and boost the sustainable development of beekeeping activities.

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## COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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