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Electrical impedance spectroscopy for potassium content analysis and botanical origin identification of honey

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ABSTRACT

Minerals are reported to dominate the electrical properties of honey and indicate its botanical and geographical origins. In this study, Electrochemical Impedance Spectroscopy (EIS) was used to assess the relation between mineral elements, electrical properties and botanical origin using three honey varieties - *Citrus* sp., *Eucalyptus* sp., and *Erica* sp. These varieties are identified through pollen analysis and market labelling. Flame atomic absorption and emission spectroscopies were used to quantify the concentrations of eight elements (potassium, sodium, calcium, magnesium, manganese, zinc, copper, and iron). Among all the mineral elements, potassium showed a consistent correlation with impedance. The potassium estimation in honey and standard solutions (calibration curve) had similar sensitivities of 153.43 nF/mM and 132.68 nF/mM, respectively. Additionally, the analysis revealed that potassium dominates the mineral composition, with the other species present in minimal quantities. The EIS technique showed high sensitivity to potassium and other ionisable species, making it possible to classify the botanical origin of these three honey types. The EIS technique proved to be both time and cost effective, yielding a classification rate higher than that achieved by analysing mineral composition.

1. Introduction

There are two main types of fraud in the honey market. The first one is mislabelling the origin, which can be either botanical or geographical. The second one is adulteration with sugar syrup. Mellissopalynology is still the gold standard method for determining botanical origin. This is because it determines the exact proportions of pollen species present in the honey (Guzelmeric, Ciftci, Yuksel, & Yesilada, 2020; Nicolson, Massimo, & Ettore, 2007; Tsagkaris et al., 2021). The application of mellissopalynology can be extended to the determination of geographical origin when endemic species have been identified (Araújo & de Novais, 2023). Additionally, the mineral composition, generally assumed to be conditioned by the composition of the soil, has been proposed as an indicator of the geographical origin of honey (Anklam, 1998). Minerals are absorbed by the roots of the plants and find their way into the nectar or honeydew and eventually into the honey (Vanhanen, Emmertz, & Savage, 2011). Water content is another factor that determines the concentration of minerals in nectar and is indirectly related to geography. In dry climates, the accelerated evaporation of water leads to higher concentrations of the various elements in nectar. This phenomenon is particularly pronounced when the flower morphology lacks structures that protect the nectar from evaporation (Nocentini, Pacini, Guarnieri, & Nepi, 2012).

However, the above assertion about the connection between the mineral content and the geographical origin of the honey should be treated with caution. This is because other factors, in particular the botanical origin of the honey, must also be taken into account (Bogdanov, Haldimann, Luginbühl, & Gallmann, 2007). The concentration of

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minerals in the plant is also influenced by plant physiology and homeostasis, not only by the direct transport from the soil to the nectar. For example, the nectar of the black locust (*Robinia pseudoacacia* L.) shows a consistent and well-defined enrichment of metals, regardless of soil composition (Jovetić, Trifković, Stanković, Manojlović, & Milojković-Opsenica, 2017). A more detailed analysis of the mineral profile of honey also indicates that the predominant element is always K, irrespective of geographical origin. In darker honey, the reported values of K content can reach up to 60% (Kek, Chin, Tan, Yusof, & Chua, 2017) or even 70% (González-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia, 2005) of the total mineral content. It is worth noting that heavy metals can still serve as a good indicator of geographical origin when a link with anthropic activity is established (Bogdanov et al., 2007).

Given the complexity of mellissopalynology and mineral analysis, the International Honey Commission (IHC) recommends using electrical conductivity as a reliable indicator of honey's mineral content and botanical origin. This recommendation is justified as some honey types have electrical conductivities in well-defined ranges (Bogdanov et al., 2007; Bogdanov, Martin, & Lullmann, 1997). The predominance of K in honey suggests that it is the element that determines electrical conductivity. Although K concentration has been reported to correlate with electrical conductivity (Aazza, Lyoussi, Antunes, & Miguel, 2013; Bogdanov et al., 2007; Imtara, Elamine, & Lyoussi, 2018), the contribution of other minerals has not been clearly indicated.

Mineral analysis and measurement of electrical conductivity in honey are performed using different methods. Electrical conductivity is measured with a standardised approach using a 20% honey solution (Bogdanov et al., 1997). In contrast, mineral analysis, including K, involves a comprehensive sample preparation process (Elamine et al., 2019). This process consists of ashing the samples to concentrate the minerals in smaller volumes of solvent, followed by applying techniques such as flame atomic absorption and emission spectroscopy compared to standards of the elements of interest. As mineral concentrations in honey are typically in the mg/kg range, their direct correlation with electrical conductivity is questionable.

Impedance spectroscopy is a promising technique that eliminates the need for complex sample preparation. Our previous work showed that an impedance-based sensor can accurately measure electrical conductivity at remarkably low thresholds (Elamine et al., 2019). We suggested that the sensor is sensitive to ionizable species, including mineral elements.

In this study, we suggest that the significance of individual mineral elements in determining the electrical characteristics of honey is frequently ignored. As a result, we carried out a complete investigation to examine the correlation between mineral content, electrical properties, and their usefulness in identifying the source of honey. The findings are presented in the following manner. Firstly, the contribution of each mineral element to the overall electrical conductivity of honey is presented and discussed, highlighting the crucial role of K. Secondly, we introduce an impedance-based sensor for potassium quantification. Lastly, we evaluate the predictive capability of mineral content in conjugation with classification algorithms for identifying the botanical origin of honey. We then compare the performance of this approach with the data obtained from the impedance-based sensor.

2. Material and methods

2.1. Honey samples characterisation

Thirty-two honey samples of *Citrus* sp. ($n = 11$), *Eucalyptus* sp. ($n = 10$) and *Erica* sp. ($n = 11$) were purchased from local Portuguese beekeepers and associations. They were stored in tight glass flasks in the dark until analysis was required. The selection was based on previous work (Solayman et al., 2016), in which a clear distinction between the three types of honey could be made based on the total mineral content. To confirm the labelled botanical origin of the honey samples, pollen analysis was performed according to the International Commission on Bee Botany (ICBB) method (Louveaux, Maurizio, & Vorwohl, 1978). The light microscope Leitz Messtechnik GmbH (Wetzlar, Germany) with 400× and 1000× objectives was used to identify and count 1000 pollen grains for each of two duplicates of the analysed samples.

Mineral content was determined according to the procedures described in the reference (Elamine, Lyoussi, et al., 2019). Five grams of each honey sample were calcined by gradually increasing the temperatures up to 550 °C. After cooling, the obtained ash content (percentage of the remaining substance in the total calcined honey mass) was dissolved in 5 mL of 0.1 M nitric acid with hot stirring until completely dry. Another 10 mL of the same nitric acid solution was added. When solubilisation was assured, the volume was up to 25 mL with distilled water. Ca, Mg, Mn, Zn, Cu and Fe were measured by flame atomic absorption, while Na and K concentrations were quantified by flame emission spectrometry using an (air-acetylene) novAA 350 (Analytik Jena, Germany), and their values are expressed as mg/kg honey.

The water content was determined by Brix index (Bogdanov et al., 1997) and the results were used to determine the dry matter in each honey sample. The dry matter value was then used to prepare the samples for electrical conductivity, as a ratio of 20% (weight/volume) in water is required (Bogdanov et al., 1997).

2.2. Measurements of the electrochemical impedance

Electrochemical impedance measurements were performed in the frequency range (60 Hz − 1 MHz) using an RCL meter, a Fluke PM 6306 (Fluke Corporation, Everett, Washington, USA). The parallel small-signal capacitance (C_P) and the parallel resistance (R_P) were measured as a function of frequency. The electrode structure consists of a gold sensor electrode, which serves as the working electrode, and a platinum grid, used as the counter electrode. This impedance-based sensor integrates an e-tongue we have previously reported (Elamine, Inácio, et al., 2019). The impedance sensor was calibrated by measuring the impedance of different concentrations of potassium (ranging from 0 to 20 mM) in a 20% (*w/v*) solution of fructose. This was done to minimize any potential interference from sugars, present in honey at high concentrations.

2.3. Data analysis and statistical tools

Different statistical tools and data analyses were used for different purposes in this study, as explained below:

(a) One-way ANOVA followed by a Tukey test was used to compare the means of the assessed parameters. The aim was to find out how significant a parameter's contribution is in distinguishing between the honey groups.
(b) Correlation matrices were created to analyse the correlation pattern of the mineral elements in each honey group. The obtained correlation matrices were presented as heat maps, with significant values labelled asterisks (*) for clarity.
(c) A hierarchical clustering analysis was performed to understand the clustering patterns of honey samples based on their mineral content and impedance measurements. The first two principal components, derived from a principal component analysis (PCA) of each dataset, served as the basis for this clustering. The PCA was conducted to reduce the dimensionality of the data while retaining the variance critical for differentiating the samples. Dendrograms were used to visualize the resulting hierarchical structure and facilitate the visual differentiation of clusters. The dendrograms were colour-coded to distinguish between the botanical origins of the samples. The details of the PCA, including the explained variance for each principal component, were documented and are available in the supplementary material.

3. Results

3.1. Pollen analysis

A 45% or more percentage of pollen species indicates monofloral honey (Andrade et al., 1999); all samples analysed met this criterion, which is a crucial requirement for the present study. Of the identified pollen species, comprised a total of 43 identified pollen species, *Citrus* sp. pollen accounted for an average of 51.2 ± 5.5% in the *Citrus* sp. honey samples. *Eucalyptus* sp. pollen accounted for an average of 69.6 ± 4.5% of 43 identified species/families. In the case of *Erica* sp. honey, 47 different pollen grains were identified, with *Erica* sp. pollen averaging 50.3 ± 5.6%. Detailed data on pollen distribution in the analysed samples can be found in the supplementary information (SI1). The average values observed were of a similar order of magnitude to those reported in studies for *Citrus* sp. (Terrab, Díez, & Heredia, 2003), *Eucalyptus* sp. (Bobis et al., 2020) and *Erica* sp. (Pires, Estevinho, Feás, Cantalapiedra, & Iglesias, 2009).

3.2. The mineral content in each type of honey

The honey varieties in the present study were selected based on their different mineral profiles as reported by (Solayman et al., 2016). Therefore, the concentration of eight mineral elements was determined for each honey type, namely *Citrus* sp., *Eucalyptus* sp. and *Erica* sp. and presented in Fig. 1. The results show that the total mineral content maintained the same order as reported. *Citrus* sp. had the lowest mineral content, followed by the *Eucalyptus* sp., and then the *Erica* sp. samples with the highest values. However, the concentration of each element shows different profiles. Fig. 1a shows the variation in K concentration

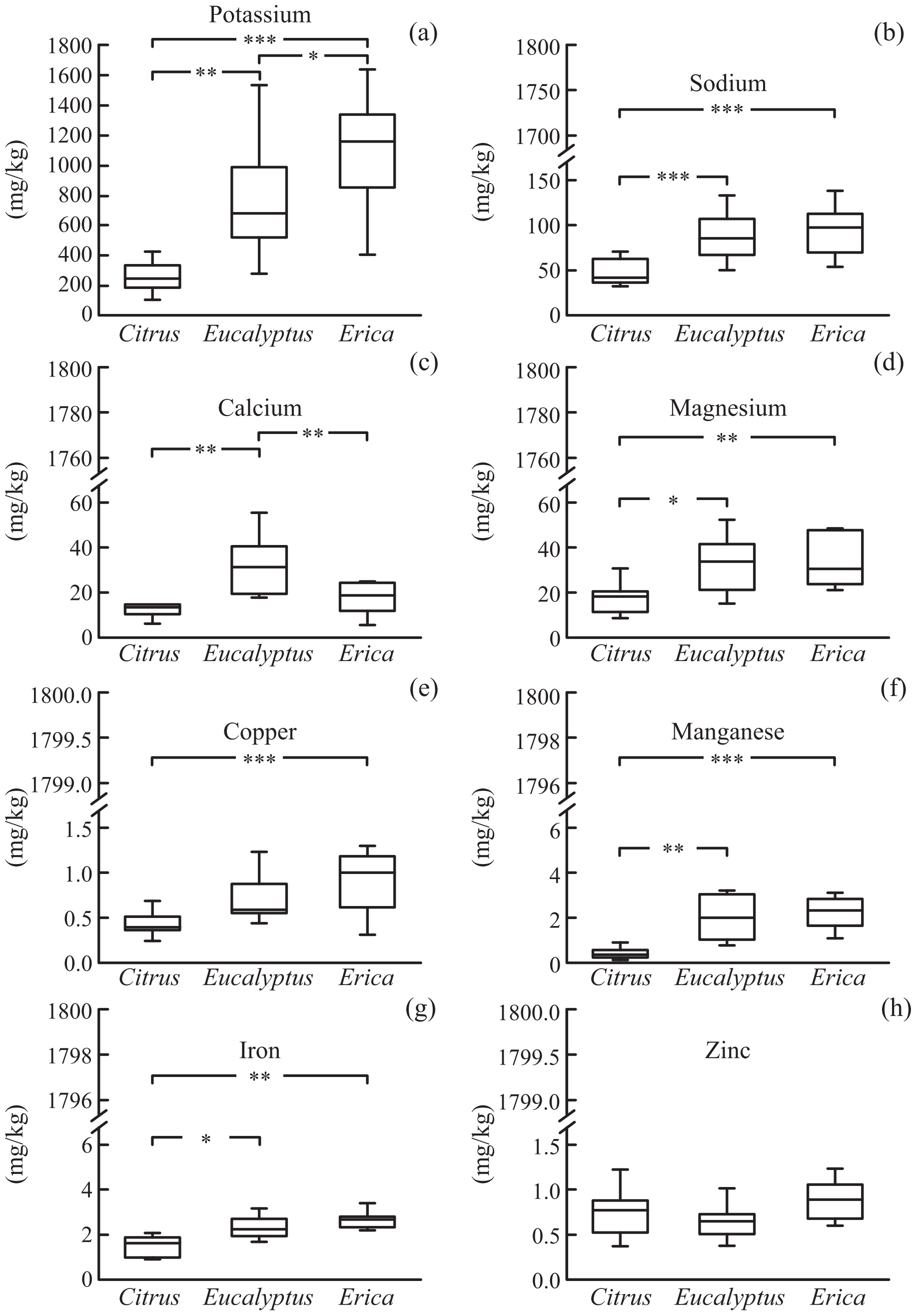

Fig. 1. The mineral content of the three types of honey. The mean values were compared using one-way ANOVA followed by the Tukey test. * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

between the different honey types. *Erica* sp. honey has the highest K concentration, while *Citrus* sp. honey has the lowest K concentration. K concentration is often two orders of magnitude higher than all other elements, and it is the only element that shows a significant difference between the different honey types.

The differences in concentration between the honey types are relatively small for all other elements. For example, the concentrations of Zn and Cu show only small differences between the different honey types (see Fig. 1g and h). The concentrations of Na, Cu and Mg (Fig. 1b, d and e) showed some differences between the different honey types, but not as marked as the K concentration. The honey of *Citrus* sp. and *Erica* sp. showed similar average values for Ca, while the concentration of mineral elements such as Fe, Mg, Mn, and Na showed no significant difference between *Eucalyptus* sp. and *Erica* sp. The presence of Zn (soil contaminants of anthropogenic origin (Bogdanov et al., 2007) does not allow important distinctions between the mean values of the three honey types.

3.3. Correlation profile among mineral elements is botanical origin dependent

Fig. 2 illustrates how the mineral elements intercorrelate and how they affect the total mineral content. In Fig. 2a, the correlation matrix of mineral concentrations includes all three honey varieties, while Fig. 2b, c and d focus specifically on the data of the *Citrus* sp., *Eucalyptus* sp. and *Erica* sp. varieties, respectively. A coloured map illustrates the correlation between the elements. A positive correlation is represented by a blue colour, while a negative correlation between the concentrations of two elements is shown in shades of red. The full-colour scale is shown above in Fig. 2.

The predominant positive correlation is obvious when analysing the combined data of all three honey types (Fig. 2a). Total mineral content (TMC) and ash content are plotted in the first and second position of the y-axis, respectively, while K is placed in the first position along the x-axis. These parameters show a strong positive correlation with all mineral elements (indicated by blue squares and R^2 close to 1). The exception is Ca, which shows no significant correlation with the other minerals.

When analysing the correlation data for the minerals, but this time for each individual honey type, the previously apparent strong positive correlations are no longer as pronounced as when considering all honey types together. However, there is a consistent positive correlation between TMC, ash and K. In the *Citrus* sp. samples (Fig. 2b), TMC, ash and K correlated positively with the concentration of Zn, Fe and Mg, but showed no correlation with Mn, Cu, Ca, and Na. In *Eucalyptus* sp. (Fig. 2c), TMC, ash and K correlated positively with all matrix elements, except Cu, which showed a negative correlation. In the honey variety *Erica* sp. (Fig. 2d), TMC and K show similar correlation profiles with a positive correlation with the concentrations of all elements. There were three exceptions for ash content, as no correlations were observed with Mg, Fe and Mn.

The mineral elements contribute to the total mineral content of the

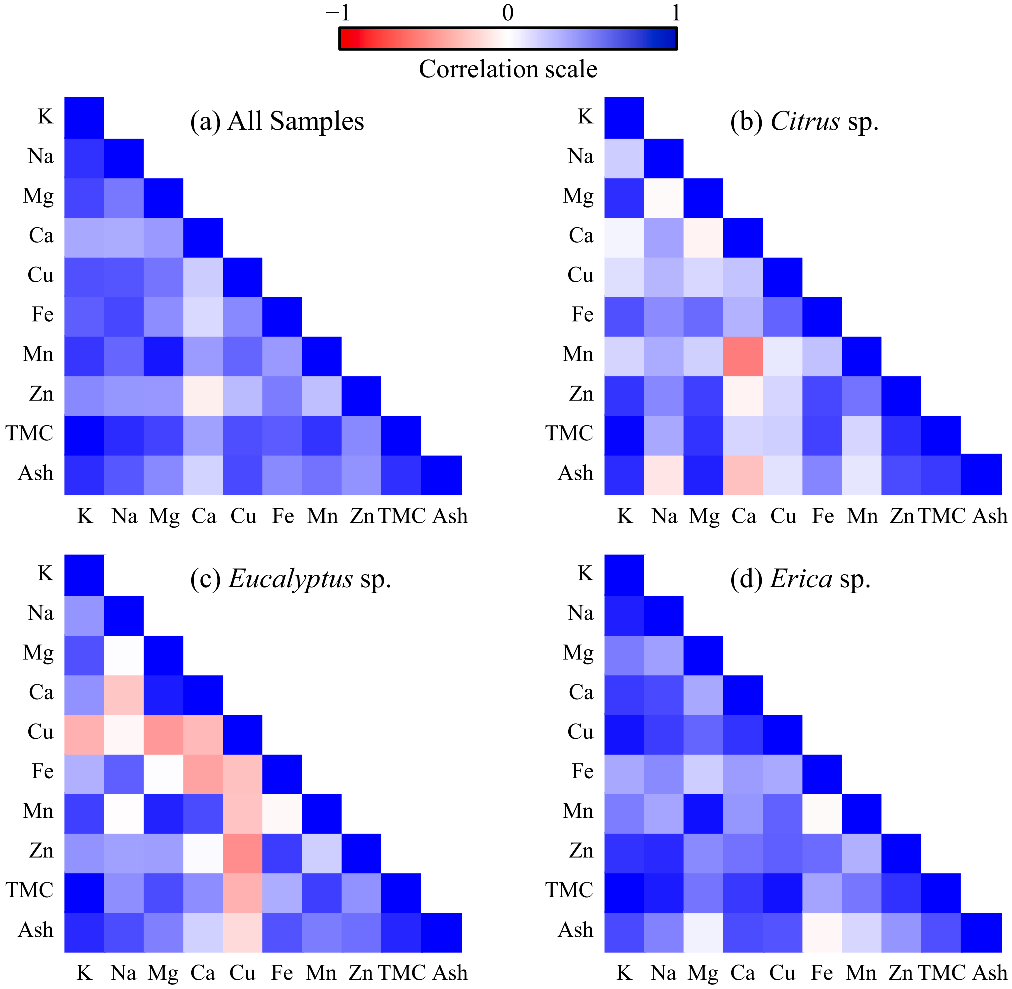

Fig. 2. Heat maps of the correlation matrices among the concentrations of the analysed mineral elements and the ash content: (a) All honey types together; (b) in *Citrus* sp. honey; (c) in *Eucalyptus* sp.; (d) in *Erica* sp. TMC: Total mineral content.

honey samples to varying degrees, calling into question their individual effects on mineral content-dependent parameters such as electrical conductivity. Theoretically, analysing other individual honey types or their combinations for correlation analysis should yield unique profiles. This observation is supported by correlation matrices documented in previous studies (Aazza et al., 2013; Imtara et al., 2018).

It should be emphasised that the claim provided here about the distinct correlation profiles is to question the actual contribution of mineral element to the TMC and ash content, and consequently to the electrical properties. The observed differences in the correlation profiles suggest that using minerals as input of multivariate methods such as principal component analysis will allow discrimination between honey types. Details on the extent to which mineral composition differentiates the three honey types will be presented later.

3.4. *Probing the electrical properties of honey samples*

3.4.1. *Electrical conductivity*

The relationship between the electrical conductivity and the mineral concentration within the three honey samples is shown in Fig. 3. *Citrus* sp. honey exhibited the lowest electrical conductivity, while the honey from *Erica* sp. showed the highest conductivity. The difference between *Citrus* sp. and the other honey types is significant ($p < 0.001$), while there is no significant difference in the electrical conductivity between *Eucalyptus* sp. and *Erica* sp. Looking at the correlation between individual elements and electrical conductivity (Fig. 3b), the relationship varies depending on the botanical origin. For example, in the case of *Citrus* sp. honey, Na, Ca and Mn show a negative correlation with electrical conductivity. For *Eucalyptus* sp., three elements, Mg, Ca and Zn, showed no relationship or negative correlation with electrical conductivity. The correlation profile for *Erica* sp. honey shows a positive correlation between the electrical conductivity and the concentrations of each element. The correlation profile between electrical conductivity and each individual mineral was identical to the correlation profile between the elements and ash, TMC and K.

Mineral elements in a solution are known to increase electrical conductivity (Parasuraman et al., 2022). In honey negative correlations between conductivity and specific elements were observed in our data and in previous reports (Imtara et al., 2018). Analysis of Figs. 2 and 3 shows that elements that show a positive correlation with K also tend to correlate positively with ash content, TMC and electrical conductivity. This positive correlation supports the view that mineral content determines the electrical properties of honey (Bogdanov et al., 2007). The opposite holds true for elements not positively correlated with K. We suggest that elements sourced from the same botanical origin as K will exhibit a positive correlation with ash content, TMC, and electrical conductivity. In contrast, elements from different floral sources than K lead to a decrease in the concentration of K, displaying a negative correlation. This can create the impression that such elements reduce the ash content, TMC, and electrical conductivity of the honey.

3.4.2. *Relation of honey electrochemical impedance and electrical conductivity*

The electrochemical impedance of a honey solution is a parameter that is strongly dependent on the electrical conductivity of the honey. We have previously reported on an impedance-based electronic tongue that utilises an array of sensor electrodes immersed in dilute honey solutions (Elamine, Inácio, et al., 2019). By measuring the electrochemical impedance of all the sensing electrodes over a broad frequency range [60 Hz – 1 MHz], the electronic tongue could successfully distinguish different types of honeys. We proposed that the electrochemical impedance is highly sensitive to the presence of ionisable species due to the presence of soluble minerals. Herein we assess the contribution of individual elements to the two main impedance parameters, capacitance and resistance. Initially, we provided an impedance profile for each type of honey, and then utilized specific frequencies to indicate the distribution of the samples. In particular, we selected a frequency of 200 Hz to exhibit the capacitance data and 10 kHz for the resistance data.

Fig. 4a and b show the frequency (f) dependence of the parallel capacitance (C_P) and parallel resistance (R_P) for the three types of honey. C_P and R_P are the impedance parameters measured by the impedance analyser under the assumption that the electrode/electrolyte solution system behaves as a parallel RC circuit. The circuit in the inset of Fig. 4a relates the measured C_P and R_P to the internal structure of the system. The system consists of the electrodes immersed into the honey electrolyte solution. The electrical double layer (EDL) established at the interface between electrode and electrolyte is a high impedance region described by the parallel R_D and C_D network. The bulk electrolyte solution is described by the resistance R_B in series with the EDL. Both C_P and R_P decrease sharply with frequency. At low frequency the EDLs have an associated high capacitance and resistance However, as the frequency increases, the capacitance associated with the EDL becomes effectively

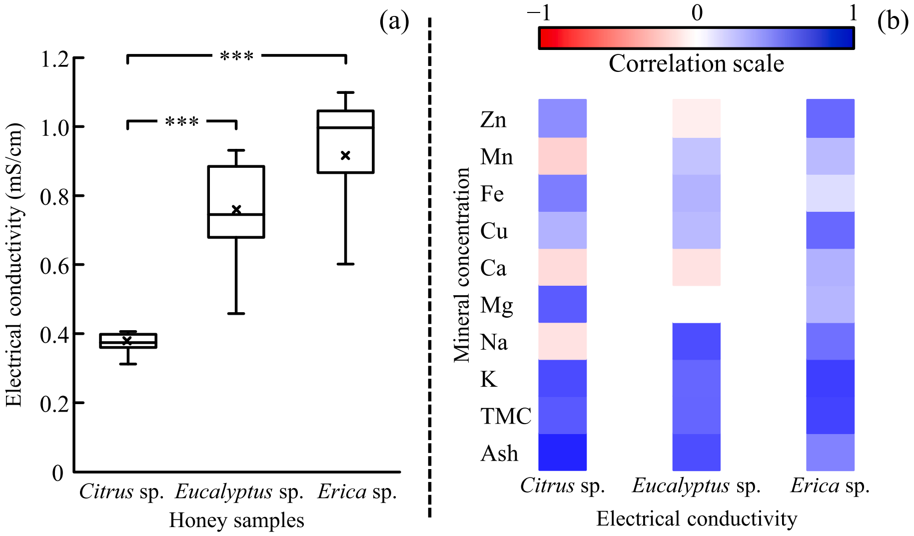

Fig. 3. Comparison of the electrical conductivity of the analysed honey samples and their correlation with the concentration of the analysed mineral elements. (a) Comparison of the mean electrical conductivity between the three types of honey using ANOVA ($p < 0.05$). (b) Correlation between the electrical conductivity and the concentration of mineral elements in the three types of honey. The colour scale represents the R^2 values, where blue corresponds to $R^2 = 1$, white to $R^2 = 0$ and red to $R^2 = -1$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

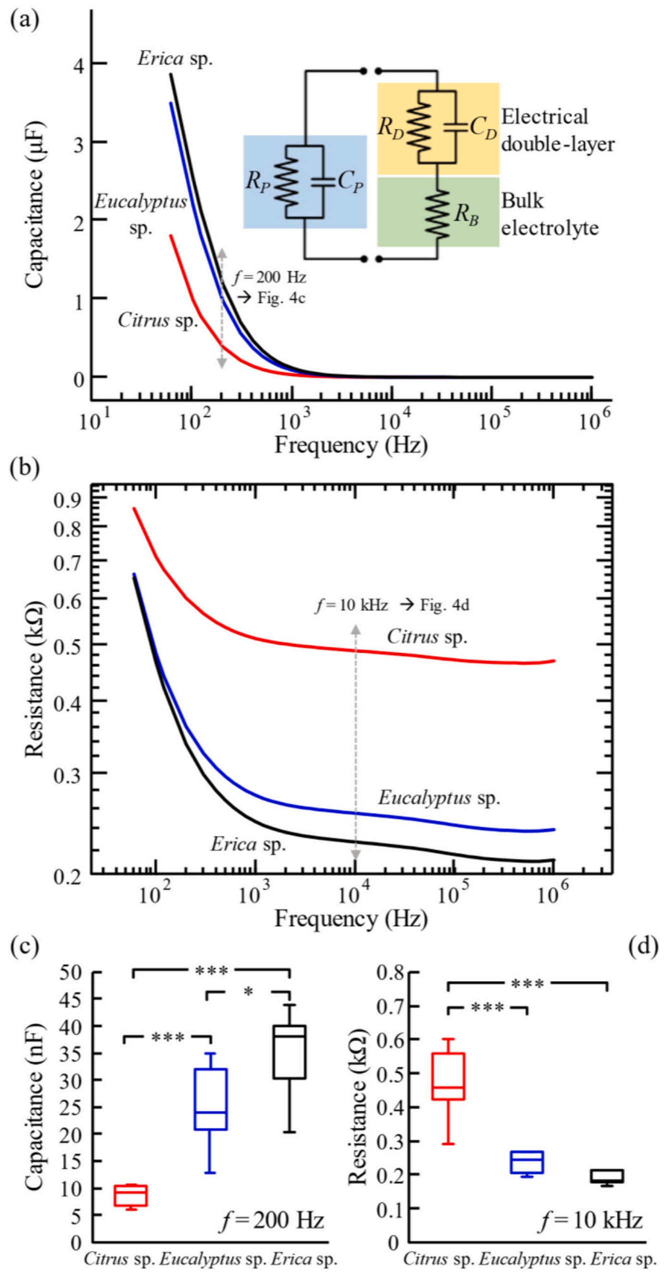

Fig. 4. Frequency dependence of the impedance parameters. (a) Frequency dependence of the parallel capacitance, C_P. (b) Frequency dependence of the parallel resistance, R_P. The inset is a simple equivalent circuit that explains the frequency dependence of the impedance. (c) Mean comparison of the capacitance at 200 Hz for the three honey types. (d) Mean comparison of the resistance at 10 kHz for the three honey types. The mean comparisons were performed using a one-way ANOVA, and the significance of the difference is accepted at $p < 0.05$. The significance levels are represented by * ($p < 0.05$) and *** ($p < 0.001$).

short circuited and the resistance decreases along with the associated capacitance. At reasonably high frequencies ($f > 10$ kHz), the impedance probes the bulk electrical conductivity of the honey solution and the small geometric capacitance between the sensing and the counter electrodes. This behaviour is simply described by the equivalent circuit on the inset of Fig. 4a. R_B is expected to be strongly dependent on electrolyte conductivity. The EDL also depends on the number of ionisable species that can contribute to the dipole layer at the electrode/electrolyte interface. Often in the literature, more intricate circuits employing constant phase elements (Taylor & Macdonald, 1987) are employed to model impedance data. Herein, the purpose of the simple equivalent circuit shown in the inset of Fig. 4a is solely to elucidate the frequency-dependence of the impedance. It serves to emphasize that, at high frequencies ($f > 10$ kHz), the ac signal primarily probes the bulk electrolyte conductivity, whereas, at low frequencies ($f < 1$ kHz), the signal probes the EDL.

On the grounds of the above description of the equivalent circuit, it is possible to appreciate that ionisable minerals influence both the dipole layer at the electrode/electrolyte interface (EDL) as well as the bulk electrical conductivity of the honey diluted solution. Fig. 4c shows both C_P and R_P for the three types of honey. Measured at low frequencies ($f = 200$ Hz), C_P reflects the properties of the EDL. On the other hand, R_P, measured at high frequencies ($f = 10$ kHz), reflects the bulk electrolyte conductivity (Fig. 4d). The plot of C_P at low frequencies for all the honey varieties is shown in Fig. 4c). The increase in capacitance for the different types of honey varieties is in line with the increase in the K concentration and electrical conductivity (previously shown in Fig. 1a and Fig. 3a). A similar analysis can be performed for the honey dependence of the high-frequency resistance (plot in Fig. 4d). Being K the dominant soluble element, it is reasonable to assume that it is the element of the honey that essentially determines the electrochemical impedance.

The correlation between the impedance and the mineral composition indicates that ionisable minerals (ash and TMC) are involved in the formation of the EDL. However, the contribution of each element, except K, varied according to botanical origin. This is consistent with what was observed in the case of electrical conductivity. The detailed correlation map between the mineral elements and the impedance data is provided in the heat map of the supplementary information Fig. SI2.

4. Discussion

4.1. Impedance-based sensor for potassium quantification and honey matrix effect

The proposal of K as the primary influencer of the electrical properties of honey finds support in prior research (Aazza et al., 2013; Guler, Bakan, Nisbet, & Yavuz, 2007). Previous multivariate analyses have shown that alongside electrical conductivity, K concentrations play a pivotal role in distinguishing between honey samples of various botanical origins (Terrab, González, Díez, & Heredia, 2003). However, as explained before, relaying on correlations to assess the contribution of the mineral element to the electrical conductivity can be misleading. The same holds true for the case of potassium. Therefore, we leveraged the sensitive measurement provided by the EIS to compare the impedance profile of honey samples and a series of K solutions. K dilutions were prepared in a fructose solution and in the same concentration range as measured in the honey samples. The sensitive capability of EIS at a frequency of 200 Hz to study the EDL at the interface between the electrode and the solutions was employed. Selecting 200 Hz as the optimum frequency was based on the significant differences in capacitance between the three types of honey, as shown in Fig. 3c. Additionally, plotting the slope of estimating K concentration as a function of frequency shows that shifting the measurements to higher frequencies reduces the sensitivity of the K estimate. This graph is provided as supplementary information in SI3.

Fig. 5 shows the change in capacitance at 200 Hz as a function of K concentration in honey samples and standard K solutions. The experimental data were fitted by a linear relationship. Eq. (1) represents the fit to the honey solutions, and Eq. (2) is the fitting to a well-defined series of K concentrations [K] in a fructose solution.

$$C_P(\text{nF}) = 153.43 \times [\text{K}]\ (\text{mM}) + 284.35 \tag{1}$$

$$C_P(\text{nF}) = 132.68 \times [\text{K}]\ (\text{mM}) - 111.05 \tag{2}$$

Where [K] is the concentration of K in mM.

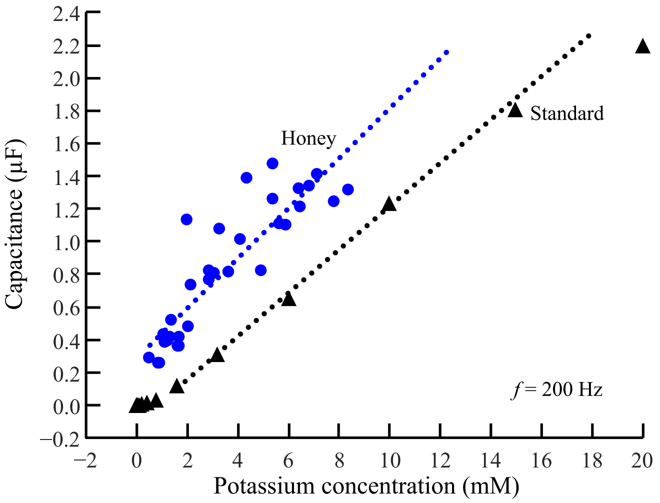

Fig. 5. Capacitance as a function of the K concentration ([K]) in honey samples and standard solutions.

The slope of the linear relations signifies the capacitance sensitivity to K concentration. In honey, the sensitivity is 153.43 nF/mM, whereas for fructose solutions, the sensitivity is 132.68 nF/mM. In honey samples, the intersect at the origin is 284.35 nF, while in the case of the fitting line to the fructose solutions, the intersect was −111.05 nF. This negative intercept value can be attributed to an artefact arising from capacitance saturation as it approaches the origin. The capacitance of the blank solution (20% fructose; 0% K) was 0.950 ± 0.005 nF ($n = 7$).

By definition, the limit of detection (LOD) and the limit of quantification (LOQ) are determined as follows: $LOD = 3 \times \mathrm{SD}/a$ and $LOQ = 10 \times \mathrm{SD}/a$, respectively; where SD is the standard deviation of the blank capacitance, and a is the slope of the fitting line. Accordingly, in a fructose solution, the sensor should detect K concentration as low as 0.13 mM and quantify a minimum value of 0.42 mM. Nonetheless, the capacitance saturation near the origin (Fig. 5) hinders reliable measurements below a K concentration of 1 mM. For clarification, the obtained LOD and LOQ corresponds to honey samples with K values of 25.41 mg/kg and LOQ = 82.11 mg/kg, respectively. The LOD is almost 5 times lower than the lowest K concentration in the analysed honey samples (102.68 mg/kg), measured in the *Citrus* sp. samples. It is worth mentioning that *Citrus* sp. honey exhibits lower mineral and K content compared to other varieties (Karabagias et al., 2017).

The K dilution performed with a 20% fructose solution aimed to mitigate any trend line shifts induced by the sugar matrix in the honey. Therefore, the observed shift of the fitting line towards a higher capacitance in honey samples is not due to the sugar content. The presence of conductive species other than minerals, such as organic acids (Živkov Baloš et al., 2018), probably contributes to the capacitive behaviour of the electrical double layer formed at the sensor/honey interface. In addition, the collective influence of mineral elements, apart from K, should not be neglected.

The utility of the impedance-based sensor for analysing K in honey is best understood from two different perspectives: (i) Efficiency and convenience: this sensor offers a fast, preparation-free analysis process that provides results within minutes. This contrasts with the traditional, more laborious methods of K determination, which take several days and rely heavily on specialised equipment, as explained in the Methods section. (ii) Comprehensive evaluation: The sensor not only analyses K but evaluates all charged substances over a broad frequency spectrum. This capability greatly expands its application and makes it a suitable tool for thorough authentication processes, including identification of botanical origin. The adulteration primarily affects the compositional profiles of honey samples, including K and other charged species. The ability of the sensor to use specific K concentrations and electrical conductivity ranges enables it to detect adulteration in instances where botanical origin is established. Nonetheless, the focus of this study is the determination of botanical origin which is presented and discussed in the following section.

4.2. Impedance sensor for honey botanical origin classification

The classification potential of the mineral content in honey was compared to that of the capacitive sensor. Fig. 6 illustrates a visual comparison of honey sample clustering based on mineral content (Fig. 6a) and impedance data (Fig. 6b). In Fig. 6a, the mineral content analysis generates three main clusters. The *Citrus* sp. samples predominantly constitute cluster C1, along with three *Erica* sp. and one *Eucalyptus* sp. sample. Cluster C2 comprises mainly *Eucalyptus* sp. samples with three *Erica* sp., while the third cluster is exclusively composed of three *Erica* sp. samples. Fig. 6b, derived from impedance data, also delineates three distinct clusters. Cluster C1 is primarily made up of *Erica* sp. samples with inclusion of four *Eucalyptus* sp. samples, cluster C2 is composed predominantly of *Eucalyptus* sp. samples with one *Citrus* sp. sample, and cluster C3 consists mainly of *Citrus* sp. samples with one sample each from *Eucalyptus* and *Erica* sp.

The clustering patterns from the mineral content analysis suggest a significant variation among the *Eucalyptus* sp. and *Erica* sp. honey, while *Citrus* sp. honey displays a unique mineral composition. The presence of different honey types within the same clusters may indicate similarities in their mineral profiles or point to potential cross-pollination or mixing during honey production. The clear segregation of *Citrus* sp. in both analyses underscores its unique chemical fingerprint, which could be exploited for authentication purposes. Conversely, the mixed clusters for *Eucalyptus* and *Erica* sp. highlight the complexity of using a single analytical approach for botanical classification and the need for a multimodal strategy that combines several data types for a more accurate determination.

When evaluating the classification of honey samples based on whether they group with the majority of their type, the analysis reveals differing levels of accuracy between the two methods. The mineral content analysis correctly classified 57.58% of the samples, indicating a moderate effectiveness likely due to the variable mineral profiles among honey types. In contrast, impedance data analysis showed a higher accuracy, correctly classifying 69.70% of the samples. This suggests that impedance measurements, which capture the unique electrical properties of honey, provide a more reliable basis for distinguishing botanical origins. The results highlight the importance of choosing the right analytical method to achieve more accurate classification in honey authenticity studies. It is worth noting that these results are in accordance with previous reports stating that K hold most of the discriminatory power when using the mineral composition to classify the botanical origin of honey (Terrab, González, et al., 2003).

Impedance measurements and their incorporation as input data for classification evidently yields a significantly higher success rate. This superior performance of the impedance data set can be attributed to the fact that impedance measurements are carried out over a large frequency range (60 Hz − 1 MHz). Each frequency spectrum takes about 120 data points and explores the variations taking place both at the electrical double-layer (<1 kHz) as well as within the bulk honey electrolyte. We propose that this simultaneous probing of a polarized region and the bulk region contributes to enhancing the classifier performance. In addition, the impedance is sensitive to all the ionizable species present in the honey solution. Other charged molecules not only minerals, may contribute to the ionic conductivity.

When benchmarking the performance of the classification against others documented in the literature, we have found reported success rates solely for classifiers utilizing the total mineral content as a dataset. The reported success rate in these cases aligns closely with our findings in Fig. 6, standing at 76% (Bogdanov et al., 2007).

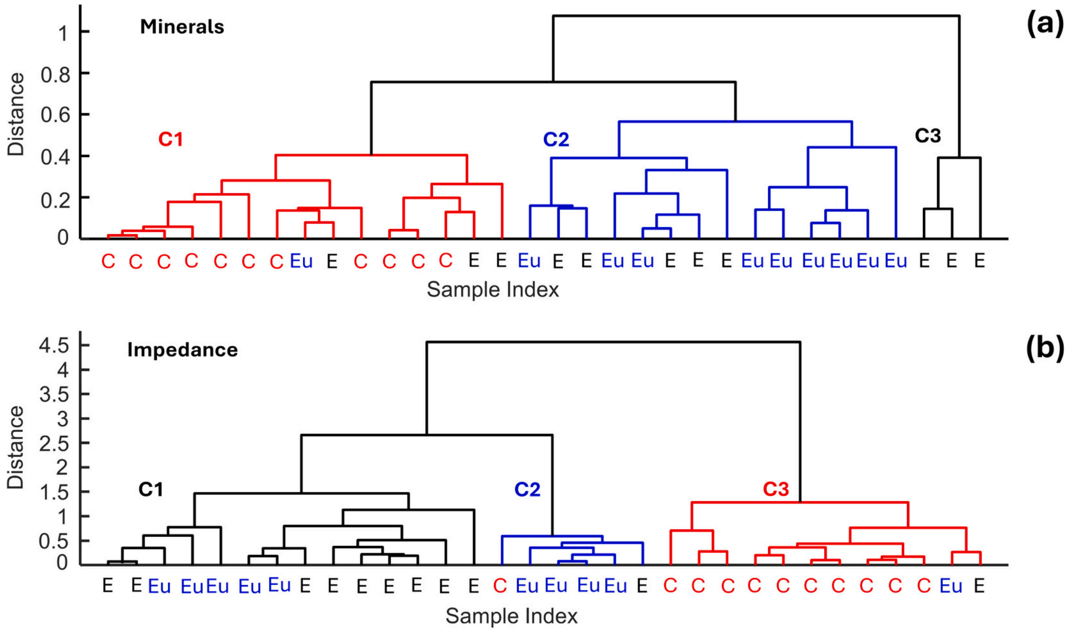

Fig. 6. Botanical origin discrimination using (a) mineral data and (b) impedance data. In both cases, the presented data were generated from PCA-reduced datasets focusing on the first two principal components. The sample indices were C (*Citrus* sp.), Eu (*Eucalyptus* sp.), and E (*Erica* sp.). The main clusters were indicated as C1, C2, and C3 and were attributed to the colour of the main honey type.

5. Conclusion

Herein we presented an EIS-based sensor as a sensitive tool for the determination of potassium in honey. The low-frequency capacitance exhibits a linear increase with K concentration. In a 20% fructose solution as matrix, the sensitivity of the capacitance to K concentration is 132 nF/mM. Extrapolating to honey, the estimated sensitivity reaches 152 nF/mM. With an impedance analyser, which has a resolution of 0.01 nF, a resolution of 0.6 μM in the K concentration can be achieved. However, the detection limit is highly dependent on the electrode design. The current electrode design has a detection limit of 0.42 mM.

By using a small signal impedance with appropriate electrode configurations, the electrical double layer and bulk electrolyte conductivity can be probed. This selectivity is achieved through appropriate frequency selection: low frequencies ($f < 1$ kHz), the impedance is used to measure changes in the electrical double layer, while at higher frequencies ($f > 10$ kHz), probe alterations in bulk conductivity. As such, classifying the botanical origin utilizing impedance datasets consistently demonstrates higher success rates in comparison to that obtained by mineral composition.

CRediT authorship contribution statement

Youssef Elamine: Writing – original draft, Validation, Methodology, Formal analysis, Conceptualization. **Pedro M.C. Inácio:** Visualization, Formal analysis, Data curation. **Maria da Graça Miguel:** Writing – review & editing, Supervision, Conceptualization. **Jorge D. Carlier:** Methodology, Formal analysis. **Maria Clara Costa:** Writing – review & editing, Supervision, Resources. **Leticia M. Estevinho:** Writing – review & editing, Resources, Methodology, Formal analysis. **Henrique L. Gomes:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139605.

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