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Espectroscopia de impedância elétrica como base para uma metodologia alternativa para detecção de adulteração em mel

Governador Valadares

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Artigo elaborado na disciplina de Trabalho de Conclusão de Curso, requisito obrigatório à obtenção de menção da disciplina e título de bacharel em farmácia.

Orientador: Wesley William Gonçalves Nascimento

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Electrical impedance spectroscopy as the basis for an alternative methodology for detecting honey adulteration

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ABSTRACT

Quality control for the identification of honey adulterations is a very important topic in food safety, especially because honey are constantly target of adulterations. A common fraud in honey samples is the addition of syrup composed of water and sugar, with the illicit intention of increasing its volume. The traditional analytical methods for quality control, such as chromatography or spectrophotometric methods, are sometimes not effective on an industrial scale because demand a long time for experimentation and qualified professionals. Recently, our research group has proposed Electrical Impedance Spectroscopy as a fast and promising technique for quality control in honey. Thus, the objective of this work was to study changes in electrical impedance spectra in pure honey upon addition of sucrose syrup. The honey sample, certified by the Federal Inspection Service from MAPA were purposely adulterated with additional syrup (20%w/w sucrose solution) up to weight percentages of 0, 2.5, 5.0, 10.0, 15.0, 20.0 and 25.0%. Measurements were carried out using a HIOKI electrical impedance analyzer, in the frequency range of 10 Hz to 5 MHz. with an electrode of a parallel plate of platinum, which has been adapted to the instrument for measurements in liquids. Additionally, rheology measurements were carried out to support the discussions. The results demonstrated that there was a significant change in all spectra profiles due to the syrup additions, allowing a mathematical model to be adopted for practical application The effects indicated that there was a reduction in the electrical resistance of the samples and this is due to the reduction in viscosity and the consequent increase in ionic mobility in the medium. A modeling with equivalent circuits was realized to expand knowledge about the phenomena studied and all presented a correction factor above 0.96 and an error of less than 1,1%, indicating good adjustments. The results obtained demonstrate the potential of measuring electrical impedance as an alternative in the development of methodologies applied to the detection of adulteration in honey by syrup.

Keywords:

Technological innovation; Food adulterations; Food quality control; Electroanalytical techniques

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1 Introduction

Honey is a viscous, sweet-tasting liquid produced by *Apis mellifera L.* bees for their food. It is mainly composed of carbohydrates, such as fructose, glucose, and sucrose. Other components are enzymes, amino acids, lipids, vitamins, and minerals (Pentós & Łuczycka, 2017). However, this arrangement is quite diverse as there are many interfering factors in production, such as the bee species, soil, climate, the honey's state of ripeness, and, above all, the floral origin (Fernandes et al., 2022).

The honey importance is related to its nutritional, medical and economic value. Nutritionally, honey is a source of energy and the amount of micronutrients varies according to the variables already mentioned above, even though there are trace elements in honey, they are essential for the proper functioning of the body (Ranneh et al., 2021). Their beneficial effects on human health are due to some nutrients prebiotic, antioxidant, antibacterial and antimutagenic effects (Pentós & Łuczycka, 2017). Indirectly, honey is linked to 9.5% of Brazil's agricultural production since 70% of the plants consumed depend on pollination, mainly done by bees (Aguiar et al., 2023). The production of honey in Brazil was highlighted in 2020 when the rise in the dollar made Brazilian honey attractive to the international market, increasing Brazilian exports by 52% compared to the previous year, resulting in more than R$ 600 million for the national economy (Trevisol et al., 2022).

According to Everstine et al. (2024), honey is the third most adulterated food worldwide because, its limited availability and moderately high price make it attractive for unfair producers to add commercial sugars to honey, such as sucrose syrup, inverted beet syrup, brown sugar syrup and corn syrup which are lower-cost sweeteners (Silva et al.,2022).

Adulteration may be associated with a decrease in food safety, reducing not only the nutritional value but also, because it is an illegal practice, the lack of control over what has been added to the food may lead to some health problems (Machado et al., 2021).

Although national honey consumption is only 25% of the world average per year, (Trevisol et al., 2022), Brazil is the 9th largest producer, according to the Food and Agriculture Organization of the United Nations (FAO, 2022), the quality of the product is extremely important, mainly because most of it is destined for export.

The addition of sugar or any other component that alters the original composition of the product is illegal, as established nationally by the Ministry of Agriculture and Supply (MAPA, 2000) and internationally by the Association of Official Analytical Chemists (AOAC, 1998). These entities also describe the standardization of honey through normative instruction 11/2000

(MAPA, 2000) and AOAC (1998), which establish the minimum quality requirements including the sensory, physicochemical and packaging characteristics, as well as methods of analysis, such as chromatography, spectrophotometry and titrimetry.

Other methodologies for detecting adulteration are also being studied, such as microscopy combined with real-time PCR, three-dimensional fluorescence spectroscopy coupled with multivariate calibration, nuclear magnetic resonance and electronic tongue (Naila et al., 2018).

Consequently, all of the above-mentioned techniques used to determine the physicochemical quality parameters of honey are complicated, time-consuming, destructive, expensive and require qualified personnel. For this reason, electrical impedance measurements have emerged as an alternative due to their speed of analysis, practicality, low cost and the possibility of being portable. There are already other studies carried out by our research group that apply electrical impedance spectroscopy to quality control, especially to detect fraud, both in honey, by Pereira et al., 2020 and Lima et al., 2022, and in milk, by Silva et al., 2022.

This work aims to study the electrical characteristics of honey, such as impedance, admittance, real and imaginary parts of impedance, compare them with equivalent electrical circuits to characterize the material and apply them to quality control. For this work, sucrose syrup was chosen as our adulterant due to its ease of access, it was prepared and added to pure honey to simulate adulteration, the electrical difference between the systems was evaluated and a mathematical model was developed to identify the fraud. Based on the results, equivalent electrical circuits were also assembled to complement our study.

2 Materials and methods

2.1 Materials

The experiments were conducted with pure eucalyptus honey purchased from local shops at Governador Valadares MG-Brazil. The product has the Federal Inspection Service (SIF), guaranteeing the specifications required by current legislation that is, with the following specifications: reducing sugar < 65%, humidity < 20%, apparent sucrose < 6%, water-insoluble matters < 0.1%, ash < 0.6%, presence of pollen grains, acidity < 50k equivalents /Kg, diastase activity at least 8 on the Gothe scale, hydroxymethylfurfural < 60mg/kg (MAPA, 2000; AOAC,1998).

2.2 Characterization of honey

For honey to be marketed for human consumption, in Brazil, it must follow the technical regulations for its identity and quality, including specifications stablished by AOAC and MAPA. According to the product label, the honey used as the basis for the work is multifloral with a predominance of eucalyptus flowers. As the MAPA has inspected it, it has the SIF seal, guaranteeing its sensory characteristics - color, taste, consistency - as well as its physicochemical characteristics, mentioned in section 2.1.

2.3 Preparation of sucrose syrup

The sucrose syrup was prepared at 20% w/v. 20g of cristal sugar was manually dissolved in 100 mL of distilled water until no sugar crystals were visible. This mixture was used to simulate frequent fraud as Pentós and Łuczycka (2017) described.

2.4 Addition of sucrose syrup to honey

The purposely adulterated honey samples were prepared by adding sucrose syrup to the pure honey in concentrations of 2.5, 5.0, 10.0, 15.0, 20.0, and 25.0% w/w, the solution was mixed manually until visually homogenized. The experiment was performed in triplicate. The choice of dilutions for evaluating small additions was based on articles on frequent adulteration such as Silva et al. (2022) and Sivakesava and Irudayaraj (2001).

2.5 Sample pre-treatment

The high viscosity of honey limits electrical characterization techniques due to presenting signals with high noise and low reproducibility, such as electrical conductivity and impedance. Therefore, we opted for a 20% pre-treatment for all samples in this work. To determine this concentration, the pure honey was previously diluted in different concentrations and the conductivity and electrical impedance were measured at room temperature (25°C).

2.6 Determination of the electrical impedance parameters

After pre-treatment, to investigate the interactions between sucrose syrup (adulterant) and honey, electrical impedance measurements were carried out on each prepared sample and the pure honey sample. The samples at concentrations of 2.5, 5.0, 10.0, 15.0, 20.0, and 25.0 w/w and their duplicate were subjected to oscillatory impedance tests, the electrical properties studied were real (Re[Z*]), imaginary (Im[Z*]) parts, admittance module ([Y*],) and impedance module ([Z*]). The room temperature was kept constant at 25°C; For this, a HIOKI model 3170 impedance analyzer was stimulated by an alternating sinusoidal (AC) external electric field, with 1 V peak to peak, in the frequency range of 10 Hz to 5 MHz. A platinum parallel plate electrode with a geometric constant equal to $1cm^{-1}$ was adapted to the equipment for measurements in liquids, as described by Silva et al., (2022). OriginPro 9.0 (64-bit) SR2 b87 software was used to analyze the spectra and ZView was used to draw the equivalent circuits.

2.7 Determination of the rheological parameters

The rheological behavior of the honey solutions was investigated by stationary rheology in an automated rheometer DHR1 from TA Instruments (USA). Evaluation of the flow profile was obtained by evaluating flow and viscosity curves by scanning shear stress as a function of shear rate, isothermally at 25 °C . The flow experiments were performed using plate-to-plate geometry, with a 500 µm gap and shear rate ranging from 1 to 1000 s^{-1}. The control of temperature was supplied by a Standard Peltier Plate system coupled with the DHR1 rheometer.

The data were collected and processed using TRIOS® software from TA Instruments. The data were exported to the Microcal Origin® 9.0 software (OriginLab Corp., Northampton, MA, USA), and they were fitted using the linear relation of rheology applying Newton's law of viscosity so that the profile of all curves was practically linear. In Equation 1, τ is the shear stress (Pa), $\eta$is the viscosity (Pa.s) and φ is the shear rate (s^{-1}).

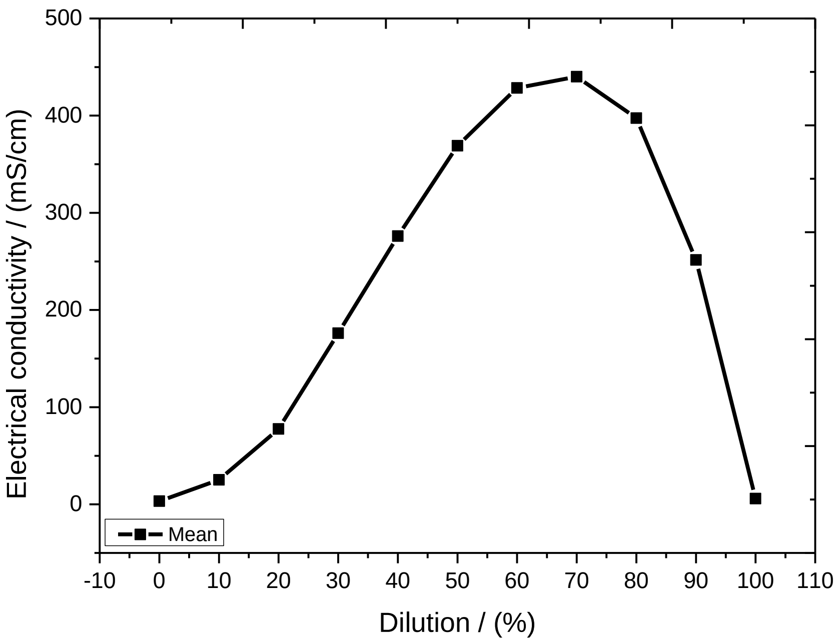
$$\tau = \eta\varphi \qquad \text{Eq. 1}$$

3 Results and discussion

3.1 Pre-treatment

The high viscosity of the honey makes the electrical characterization technique difficult to analyze, as it causes regions of noise in the reading, preventing repeatable results, which is why it was necessary to determine a pre-dilution, related to the level of fraud. The pre-treatment was determined based on the conductivity of the samples. Pure honey was diluted in distilled water at concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90%, then, the conductivity was measured as shown in Figure 1.

Figure 1: Relationship between conductivity and honey dilution.



From the graph, it is possible to separate it into two parts: the increase in conductivity, followed by its decay. Initially, this increase is associated with the predominance of ionic mobility. The addition of water reduces the viscosity, thus allowing for greater ionic mobility, and consequently conductivity, but from 70% the predominant effect comes from the concentration, so even if ionic mobility increases, the ionic concentration decreases to the point where it has no considerable effect, and so conductivity falls.

Considering that large additions can be detected visually, due to the considerable reduction in viscosity, we opted for the increasing range of electrical conductivity. To allow for the widest detection range, electrical measurements were taken of the samples on the left-hand side of the curve, and the first to show good reproducibility was the 20% sample, which was chosen for pre-treatment. Therefore, using electrical techniques, it is possible to detect up to 50% fraud in the samples analyzed.

3.2 Rheology

Viscosity is a measurement of the resistance to flow, directly dependent on concentration, moisture and the presence of macromolecules (Costa et al., 2013). Regarding honey samples, viscosity is one of the main physical-chemical parameters that define their sensorial acceptability (Oliveira et al., 2015). To characterize the rheological behavior of the samples, rheological experiments were conducted through the construction of flow curves (shear stress x shear rate) in the range of 1 to 1000 s^{-1} and the viscosity was determined through Equation 1. Typical flow curves are shown in Figure 2, where the linear relation between shear stress x shear rate indicates a Newtonian profile. Figure 3 shows the viscosity values plotted against the percentage of syrup, where an exponential profile can be observed.

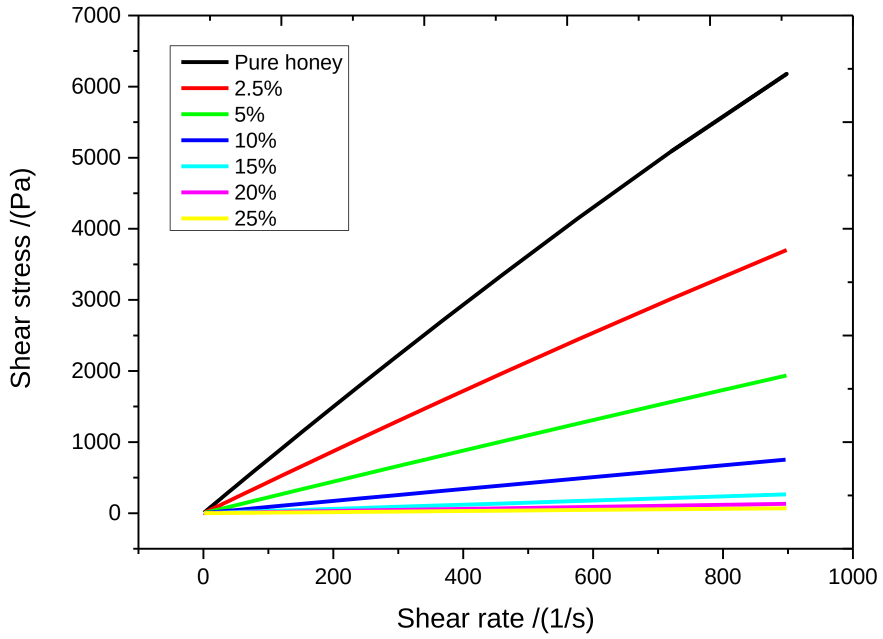


Figure 2: Relation between shear stress x shear rate.

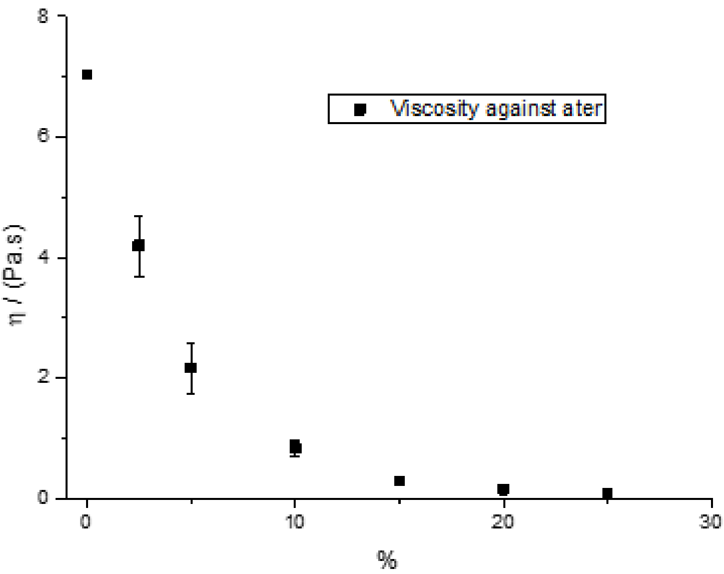


Figure 3: Relation between viscosity and percentage of syrup.

Initially, it is important to highlight that honey samples are especially Newtonian or quasi-Newtonian fluid (Boussaid et al., 2015; Pereira et al., 2020), showing a broad range of apparent viscosity (η_{ap}), depending on their origin, which in turn depends on their composition.

However, with the addition of a water/sucrose mixture, it was observed a sharp and non-linear reduction of viscosity, suggesting that a strong component of intermolecular interactions is also involved beyond the simple additive effect of dilution. It is believed that large aggregates are broken upon dilution during the addition of syrup, leading to a lower resistance to flow and consequent reduction of viscosity.

3.3 Electrical Impedance Spectroscopy (EIS)

Impedance spectroscopy is a very useful technique for electrically characterizing the behavior of a material, whether solid or liquid. An electrical stimulus is applied to the material to be studied, such as honey. The most common and used in the experiment was the sinusoidal type, where the real and imaginary parts of the impedance, impedance and admittance were measured as a function of frequency (Chinaglia et al., 2008).

Despite pure honey has low percentages of ionic compounds, the addition of syrup composed of water and sucrose to honey alters the electrical properties of the solution, because increases the polarity, since these two materials are polar, each dilution has a different electrical concentration and ionic mobility to the precursors (Campos et al., 2024; Silva et al., 2022).

Electrical impedance analyses were carried out on samples of pure and purposely adulterated honey. Local honey, with the SIF, was used as a standard, as the aim was to investigate variations in parameter values due to the addition of syrup (adulterant). In this work, the modulus the impedance ([Z*]) and admittance ([Y*]), real part (Re[Z*]) and imaginary part (Im[Z*]) were chosen for discussion. Figures 4 and 5, show the spectra of [Z*] and [Y*] respectively.

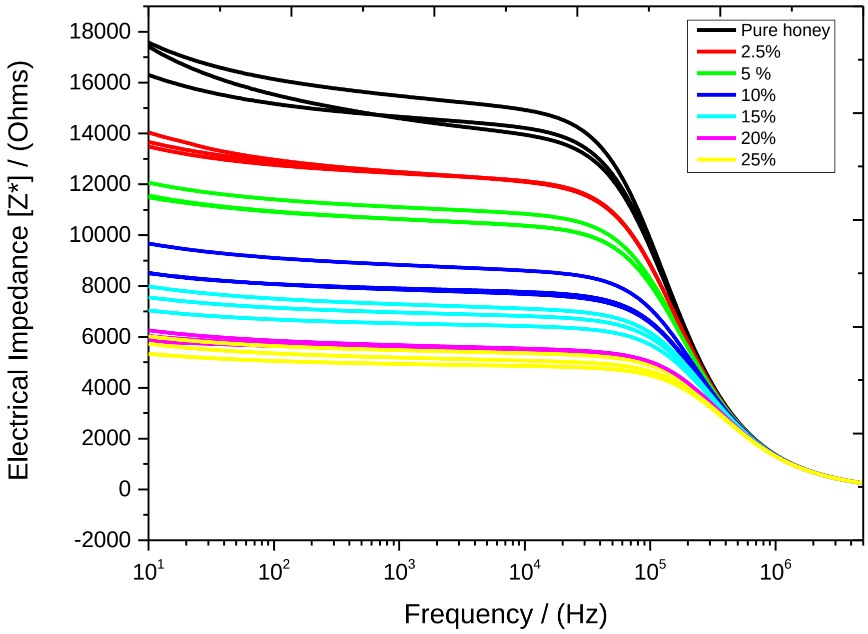


Figure 4: Impedance spectrum ([Z*]) of unadulterated and adulterated honey samples.

From the impedance results (Figure 4), it can be seen that all the curves are arranged towards zero at high frequencies (>10^6 Hz). This effect is consistent with theoretical impedance models for materials with resistive and capacitive behavior. According to Peko et al. (2013), this is due to the difficulty the charge carriers hold in keeping up with the external oscillation field at high frequencies. At 10^3 Hz< frequency < 10^5, the formation of plateaus is expected, which is in line with the theoretical model. At 10^5 Hz, there was a tendency for the electrical impedance of the samples to decrease as a function of the increasing dilutions. The addition of sucrose syrup caused differences in the composition of the honey, altering the viscosity, leading to lower [Z*] values of the adulterated honey rather than the pure honey.

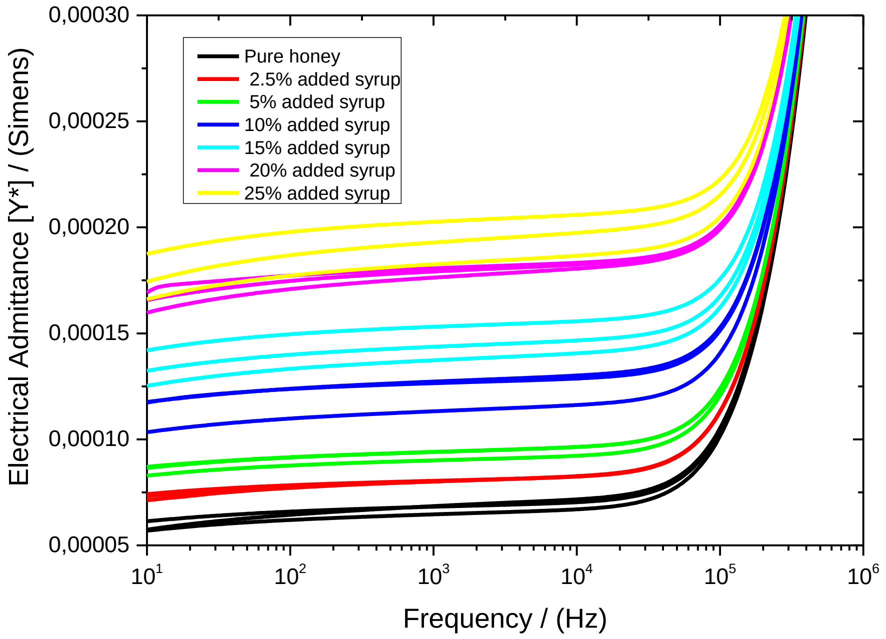


Figure 5: Admittance spectrometry ([Y*]) of unadulterated and adulterated honey samples.

According to Chinaglia et al. (2008), admittance is the inverse parameter of impedance, which explains the phenomenon observed in Figure 5, where admittance increases as sucrose syrup is added. Furthermore, in an alternating current circuit, admittance measures a material's ability to conduct (Lima et al., 2022). At low frequencies (< 10^4 Hz) it can be seen that pure honey has a lower admittance than honey with added syrup, and this value increases with the addition of syrup, while at high frequencies (> 10^5 Hz) all the samples tend towards infinity, according to the theoretical model for studying impedance in alternating current systems (Lima et al., 2022).

Figure 6 shows the behavior of the Im[Z*] and Re[Z*].

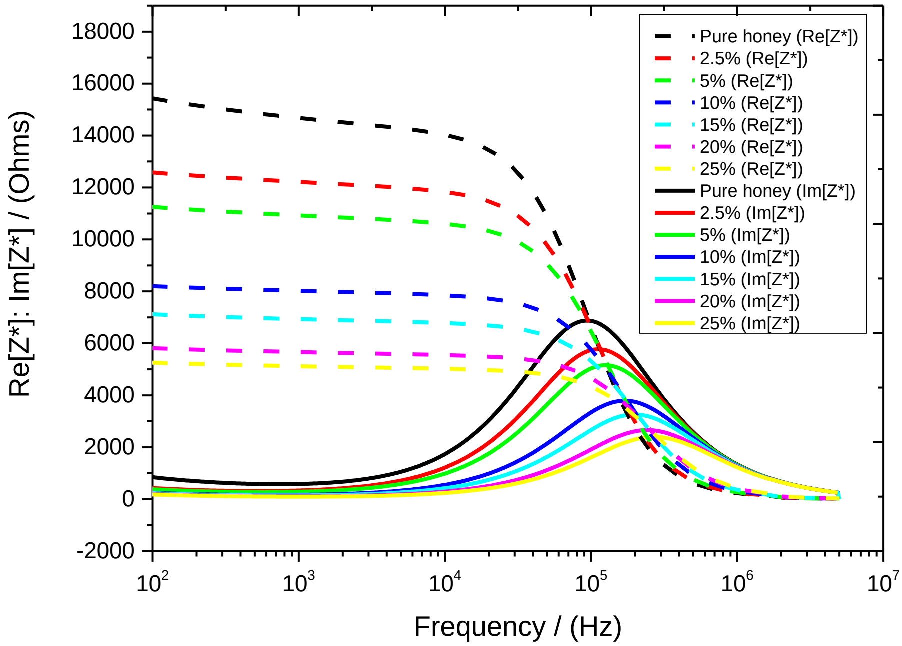


Figure 6: Real (Re[Z*]) and imaginary (Im[Z*]) impedance modulus spectrometry of unadulterated and adulterated honey samples.

Resistive and capacitive systems are characterized by an initial plateau in the real part, indicating the value of the material's electrical resistance, and by peaks in the Im[Z*] and a decline in the Re[Z*] as a function of frequency (Figure 6). It is observed that, in these systems, electrophoretic effects are due to the interaction between charge carriers and the medium. Moreover, viscosity and free ions are strongly related to these effects.

The spectrum profile observed in these samples is compatible with parallel resistor/capacitor (RC) circuits (Lima et al., 2022), this circuit was adjusted to the theoretical models (section 3.4).

The addition of syrup alters the capacitive and resistive effects, which depend on the composition of the material, as well as reducing viscosity as can be seen in Figure 3, which shows this reduction by increasing dilution, associated with this, it is also possible to state that it increases ionic mobility (μ), since, according to Peko et al. (2013) ionic mobility is inversely proportional to the radius of the diffuser charge (r) and the viscosity (η).

$$\mu = (6\pi\eta r)^{-1} \qquad \text{Eq. 2}$$

By plotting the imaginary part against the real part of the impedance (Figure 7), we can analyze the resistance of the system, indicated by the diameter of the semi-circle, and the imaginary part represented by the height of the semi-circle.

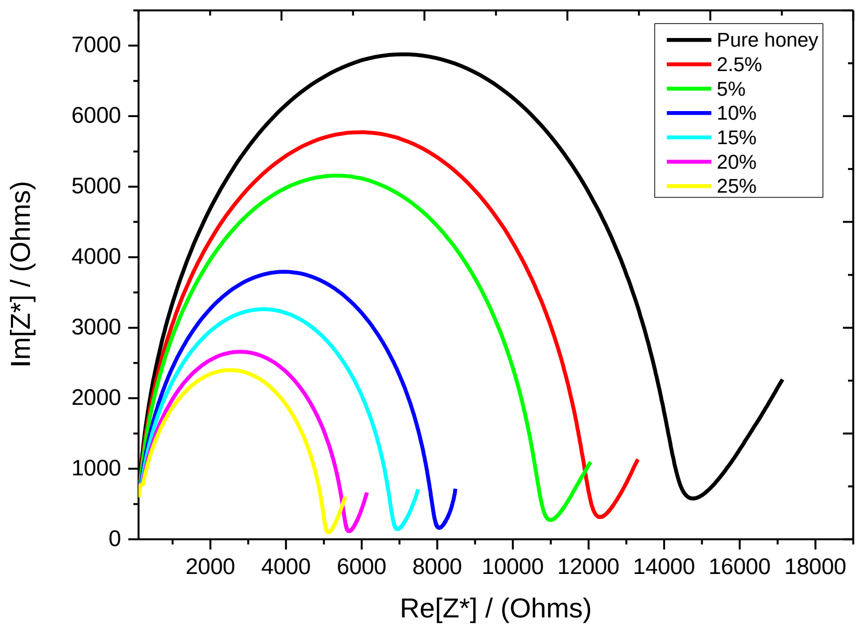


Figure 7: Modulus relationship between the imaginary (Im[Z*]) and real (Re[Z*]) parts of the impedance.

In the system studied, it is possible see that there is a reduction in resistance with the increase in the addition of syrup, due to the reduction in viscosity which allows for an increase in ionic mobility. On the y-axis it is possible to observe the reduction of the imaginary part with increasing syrup concentration, also due to the increase in ionic mobility, and therefore the number of free charges (q), causing the capacitance (C) of the system to grow, since according to Bauer et al. (2010):

$$C = |q/\Delta V| \qquad \text{Eq. 3}$$

However, since this is a parallel RC circuit, as discussed by Lima et al. (2022), we have that:

$$Z^* = \frac{R(1-j\omega RC)}{1+(\omega RC)^2} = \frac{R}{1+(\omega RC)^2} - \frac{\omega R^2 C}{1+(\omega RC)^2} j \qquad \text{Eq.4}$$

Analyzing the imaginary part, equation 4 shows, in these cases, for high frequencies the $\lim_{\omega\to\infty}$., the increase in capacitance causes a reduction in the Im[Z*], explaining the y-axis.

Other parameters that can be assessed are the relaxation time (τ) linked to the relaxation frequency (f_0). The τ indicates the time taken by the system to enter equilibrium when the electrical stimulus is removed and corresponds to the frequency of the top of the semi-circle, or the point where Re[Z*] coincides with Im[Z*] in Figure 6, according to Peko et al. (2013):

$$f_0 = 1/2\pi\tau \qquad \text{Eq. 5}$$

In other words, the reduction in viscosity causes f_0 increase (Figure 6), which implies a τ decrease, as shown the Equation 5. This occurs because the increase in ionic mobility causes the ions to adapt more quickly, reducing the time to equilibrium.

From these analyses, it can be seen that fraud alters the electrical signal of the material, making it possible to use it for quantitative detection of fraud in honey. To do this, a parameter and a frequency with greater sensitivity were selected ([Z*], frequency ~10^4 Hz), from which the mathematical model was fitted to an exponential function, as shown in Figure 8.

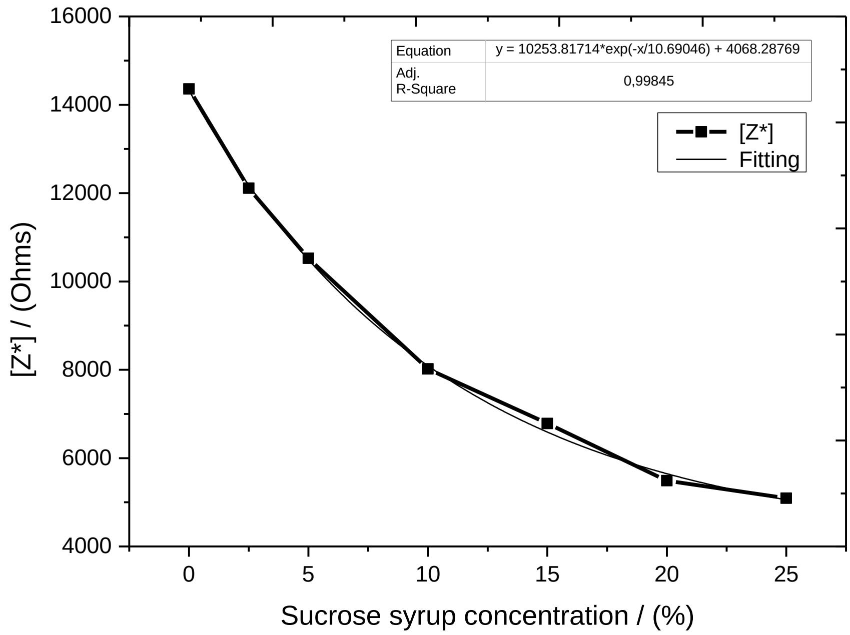


Figure 8: Impedance value at 1085Hz x concentration of added syrup.

The equation was inverted (equation 6), where X indicates the % of adulteration, while Y the value of [Z*].

$$X = -10.69 \ln y + (\ln 10253.8) + \ln (4068.3) \qquad \text{Eq. 6}$$

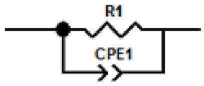
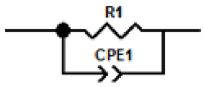
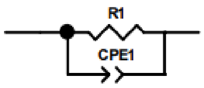
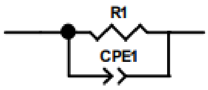
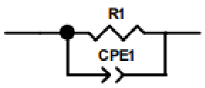
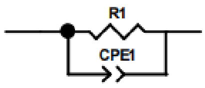
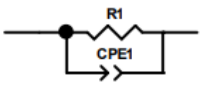
Therefore, by measuring the impedance of an unknown honey, it is possible to find out whether an adulterant has been added and in what percentage.

3.4 Equivalent circuits

Equivalent electrical circuit models were adjusted to increase understanding of the phenomena discussed. Table 1 shows the nominal values obtained from this modeling for each fraudulent sample. These values indicate the system resistance (R1), capacitance (CPE1-T), correction factor (CPE1-P), and their respective associated errors.

Table 1

Equivalent electrical circuit models for each sample studied.

	R1		**CPE1-T**		**CPE1-P**		
	Value	**Error (%)**	**Value**	**Error (%)**	**Value**	**Error (%)**	**Circuit model**
Blank	14326	0.0885	17.66E-9	0.7177	0.9716	0.0507	
2.5%	12013	0.0887	17.88E-9	0.7453	0.9710	0.0522	
5.0%	10472	0.1138	18.51E-9	0.9264	0.9687	0.0640	
10.0%	7915	0.1019	19.04E-9	0.9439	0.9670	0.0650	
15.0%	6820	0.0993	19.39E-9	0.9732	0.9658	0.0668	
20.0%	5569	0.1057	19.89E-9	1.0855	0.9642	0.0741	
25.0%	5033	0.0912	19.95E-9	1.0377	0.9639	0.0709	

As discussed, the resistance values decrease with the addition of syrup, while the capacitance values increase. In addition, the values showed an associated error of less than 1.1% with a correction factor close to 1, reaffirming that the system is associated with a resistor/capacitor system in parallel, which is in line with the graphs presented in this work.

4. Conclusion

The results show that electrical impedance spectroscopy can be used to assess honey's detect syrup fraud. Among the parameters analyzed (Re[Z*], Im[Z*], [Y*], [Z*]), all showed differences in their spectral profiles according to the amount of sucrose syrup added, allowing the creation of a mathematical model for investigating fraud in unknown samples. The equivalent electrical circuits with a correction factor greater than 0.96 show that the circuit is well adjusted, reinforcing that the electrical spectroscopy technique can identify the variation in the electrical signal due to the addition of adulterants, corroborating its use in the development of a methodology for assessing the quality of honey concerning detecting fraud.

CRediT authorship contribution statement

Gabriela Bicalho Magalhães: Writing – original draft, Visualization, Methodology, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Ângelo Marcio Leite Denadai:** Writing – original draft, Visualization, Methodology, Software, Investigation, Validation, Software, Data curation. **Jeferson Gomes Da Silva:** Visualization, Methodology, Investigation. **Juliano Pereira:** Visualization, Methodology, Investigation**. Wesley Willian Gonçalves Nascimento:** Writing – review and editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

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