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Optimization of sample preparation of Brazilian honeys for TQ-ICP-MS analysis

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ABSTRACT

Mass spectrometry-based techniques have been used to study the chemical profile of honeys to authenticate entomological, botanical and geographical origins. Sample preparation is a crucial step of the analysis to obtaining reliable data and minimizing interference owing to matrix effects. The present work studied the best sample digestion procedure for elemental analysis of Brazilian honeys from *Tetragonisca angustula* (Jataí) and *Apis mellifera sp* (Apis) by triple quadrupole inductively coupled plasma mass spectrometry (TQ-ICP-MS). A central composite design with 2^2 factorial and 3 center points considering different volumes of HNO_3 and H_2O_2 was investigated. There was no statistically significant influence of the amounts of HNO_3 and H_2O_2 on the recoveries of Ag, Al, As, Ba, Be, Ca, Cd, Ce, Co, Cr, Cs, Cu, K, La, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Th, U, V and Zn mass fractions. Machine learning algorithms (Multilayer Perceptron, Random Forest and Support Vector Machine) allowed discriminating entomological origin of honeys based on chemical profile with a classification accuracy of 99%.

1. Introduction

Brazil, known as the country with the greatest biological diversity in the world, is at the top among the 17 megadiverse countries, holding 70% of the animal and plant species cataloged worldwide [1]. Comprising six distinct biomes, the country offers suitable environments with a rich variety of vegetation for the 3,000 different species of honeybees and stingless bees that produce honeys with specific chemical characteristics, depending on the seasons and regions of the country [2]. Produced by forage individuals, the composition of honey reflects the current state of conservation of the original environment. Each bee species has its particular behavior of exploiting all available environmental resources, not just the nectar or pollen of different flowers, but plant resins and cerumen, mud, water and carcass, and even flying for greater distances to achieve nutritional variability [3].

It is estimated that Brazil has 300 native species of stingless bees out of the 500 species catalogued worldwide. The honey produced by stingless bees can present a full variety of colors, aromas and viscosities, usually with high water content and an opaque shine. Its species *Tetragonisca angustula* (popularly called Jataí) is widespread in the country, being also found from southern Argentina to northern Mexico. The Jataí honey is highly appreciated by consumers in view of its special taste and recognition of nutraceutical and pharmacological properties. In fact, the COVID-19 pandemic has drawn attention to the regular honey consumption based on the favorable inducing responses in the human body, such as anti-inflammatory, antiviral, antihyperlipidemic, enzyme production and regulation of metabolic pathways [4]. For such evidences, honey has been considered an immunity-enhancing food [5], boosting the world market. Brazil, the 5th largest honey trader in the world, has seen demand for exports of its products increase by 50% in 2020 [6].

Honey is the third most adulterated product in the world [7]. Faced with the complexity of the honey matrix [8,9] and the expansion of sophisticated types of adulteration over time, a multitude of analytical methods have recently been deployed to authenticate honey [10]. Evaluating the authenticity of honey is still a challenge, however the elemental analysis-liquid chromatography-isotope ratio mass spectrometry (EA-LC-IRMS) has been widely accepted for its use by scientific communities and transparent international metrological assessment, validation and proficiency testing [11].

Currently, three European Union schemes, known as Protected

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Table 1
Instrumental parameters for the microwave oven digestion.

Step	Power (W)	Temperature (°C)	Ramp time (min)	Hold time (min)
1	1000	200	10	
2	1000	200		15
3	Cooling 30 min			

Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Specialties Guaranteed (TSG), attest to the specific region of origin of the product and the traditional production process [12]. The Brazilian seal "ARTE", released by the Ministry of Agriculture, Livestock and Food Supply in 2021 [13], attests to the honey from stingless bees and honeybees that might be originated exclusively in their geographical areas of natural occurrence, and the producing process must maintain the original characteristics of product, not allowing any kind of additive. All these certifications intend to guarantee the safety and authenticity of honey composition in order to face the technical barriers to world trade.

Many analytical approaches have been used to study the chemical profile of honeys, describing physicochemical characteristics [14], organic [15] and inorganic constituents [16–18]. Although mass spectrometry-based techniques are considered state-of-the-art, adequate sample preparation is extremely necessary to avoid major systematic errors, induced by changing the ionization efficiency of the target analytes, loss or increase of response, respectively ion suppression and ion enhancement [19]. Such alterations often occur due to changes in the viscosity and density of the matrix analyzed. In order to reduce this error, certified reference materials with matrices similar to the analyzed are used as analytical quality control.

Described as a food matrix, honey is a viscous substance, with a Newtonian fluid characteristics [20,21] considered a supersaturated solution of sugars [20]. An important source of concern is the absence of certified reference material of honey to validate the sample preparation procedure. Several authors use certified reference materials of analogue composition to avoid commutability problems, like tomato [22–24] since it has high amount of sugars and inorganic elements commonly present in honey, and skimmed milk [25] that is rich in carbohydrates. Therefore, the characteristics of the investigated matrix, honey in this case, is essential to develop the customized process to obtain the best sample digestion procedure.

The precision, sensitivity and lower limits of detection for a multitude of elements achieved by triple quadrupole inductively coupled plasma mass spectrometer (TQ-ICP-MS), together with neutron activation analysis (NAA), a method that direct measures the chemical elements in the solid sample [26], can result in a combination to validate the analytical procedure for determining chemical elements in honeys. This work aimed to evaluate the best sample digestion procedure for elemental analysis of Brazilian honeys by TQ-ICP-MS allowing to obtain discrimination models using chemical profiles for entomological classification purposes.

2. Material and methods

2.1. Sampling

Honey samples were collected directly in a beekeeper from Piracicaba, São Paulo state, Brazil, during the month of June 2019. Ten samples from *Tetragonisca angustula* ("Jataí") and 10 samples from *Apis mellifera* ("Apis") were collected from distinct honeycombs of each species.

Table 3
Central composite design for sample preparation.

Experiment	HNO_3 (70% w/w)	H_2O_2 (30% w/w)
1	5 mL	2 mL
2	5 mL	0 mL
3	1 mL	2 mL
4	1 mL	0 mL
5	3 mL	1 mL
6	3 mL	1 mL
7	3 mL	1 mL

Table 2
Instrumental parameters for determination of chemical elements by TQ-ICP-MS Agilent 8900.

Parameter Cell mode	Setting No gas (Ar) mode	He mode	He mode	HEHe mode	O_2 reaction mode
Scan type	Single Quad (SQ)	Single Quad (SQ)	Single Quad (SQ)	Single Quad (SQ)	MS/MS mode
Plasma condition	General Purpose	General Purpose	HMI mode	General Purpose	General Purpose
RF power (V)	1600	1550	1600	1550	
Sampling depth (mm)	10				
Nebulizer gas (L/min)	0.68	1.05	0.68	1.05	
Gas switch	Dilution gas 0.27 L/min Makeup gas	Dilution gas 0.27 L/min	Makeup gas		
Extract 1 (V)	-17				
Extract 2 (V)	-250				
Omega bias (V)	-130				
Omega lens (V)	8.2				
Cell gas flow	0.0 mL/min	5.5 mL/min	10 mL/min	30%	
Energy discrimination (V)	5.0	5.0		7	-7
Isotopes	^{9}Be, ^{23}Na, ^{27}Al, ^{238}U	^{23}Na, ^{24}Mg, ^{27}Al, ^{39}K, ^{44}Ca, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{78}Se, ^{85}Rb, ^{88}Sr, ^{107}Ag, ^{111}Cd, ^{133}Cs, ^{137}Ba, ^{138}Ba, ^{139}La, ^{140}Ce, ^{206}Pb, ^{207}Pb, ^{208}Pb ^{232}Th, ^{238}U	^{23}Na, ^{24}Mg, ^{39}K, ^{44}Ca, ^{56}Fe	^{56}Fe, ^{75}As, ^{78}Se	^{75}As, ^{78}Se
Internal standard	^{6}Li, ^{209}Bi	^{72}Ge, ^{74}Ge, ^{103}Rh, ^{115}In, ^{209}Bi	^{72}Ge, ^{74}Ge, ^{103}Rh	^{72}Ge, ^{74}Ge, ^{103}Rh	^{72}Ge, ^{74}Ge

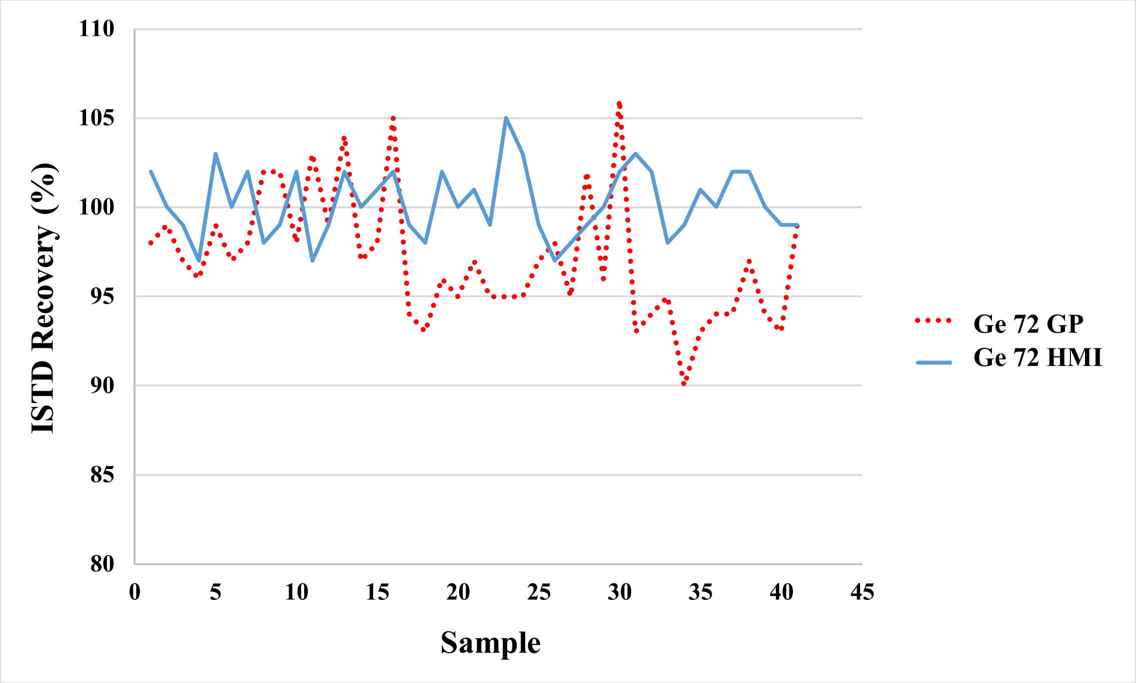

Fig. 1. Stability of the internal standard solution (ISTD) in General Purpose (GP) and High Matrix Introduction (HMI) modes.

2.2. *Triple quadrupole inductively coupled plasma mass spectrometry (TQ-ICP-MS)*

2.2.1. *Reagents and solutions*

All the reagents used were of ultrapure analytical grade. Hydrogen peroxide (30% w/w) and nitric acid (70% w/w) were purchased from Sigma-Aldrich. The nitric acid was purified using the Milestone subCLEAN PTFE sub-boiling distillation system. All standards and solutions were prepared with deionized water (18.2 MΩ and 4.0 ppb TOC at 24.8 °C) obtained from an ultrapure water system Milli Q IQ 7000. Tuning solution (p/n 5190-0465), internal standard solution – ISTD (p/n 5188-6525) and multi-element standard solution (p/n 8500-6940) were purchased from Agilent (Santa Clara, CA, USA).

2.2.2. *Instrumentation*

Digestion was done using a Milestone ETHOS UP high performance microwave digestion system equipped with MAXI-44 easy TEMP high-throughput rotor, infrared temperature sensor (200 °C maximum temperature) and programmable power control (1800 W maximum power). The instrumental parameters used are shown in Table 1.

Chemical elements were determined using an Agilent 8900 Series (Agilent Technologies, Japan) triple quadrupole inductively coupled plasma mass spectrometer TQ-ICP-MS. Tuning solution 1 μg L^{-1} containing Ce ($m/z = 140$), Co ($m/z = 59$), Li ($m/z = 7$), Tl ($m/z = 205$) and Y ($m/z = 89$) was used for corrections of the torch axis, detector, plasma and lens voltage for maximum sensitivity. Oxide formation and double charges were also evaluated with the autotune, allowing to obtain the best configuration of the equipment under the proposed analysis conditions. Internal standard solution containing Li ($m/z = 6$), Sc ($m/z = 45$), Ge ($m/z = 72$), Ge ($m/z = 74$), Rh ($m/z = 103$), In ($m/z = 115$), Tb ($m/z = 159$) and Bi ($m/z = 209$) was used to correct variations in the plasma stability and disturbance in the sample introduction system that may interfere with the result. All operating conditions are shown in Table 2. TQ-ICP-MS was equipped with a collision/reaction cell to reduce spectral interferences from polyatomic ions.

2.2.3. *Experimental*

The central composite design containing a 2^2 factorial design with 3 center points was carried out to obtain the best condition of sample preparation. The center points play a fundamental role in the experimental design, allowing estimating the error and precision of the model [27]. Seven experiments with 10 honey samples of each bee species (Jataí and Apis) were performed using distinct amounts of nitric acid and hydrogen peroxide (Table 3).

Nitric acid was added for 12 h predigestion of analytical portions of 1 g of fresh honey directly conditioned into closed microwave TFM vessels. The volume of the final solution in each vessel was made up to 8 mL with Milli Q quality deionized water.

Calibration curves for Ag, Al, As, Ba, Be, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, K, La, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Th Ti, U, V and Zn, at mass fractions of 0, 1, 2, 5, 10, 20, 40, 80, 150, 300, 500, 800 and 1000 μg kg^{-1} for General Purpose (GP) mode, and for Ca, Fe, K, Mg, and Na at mass fractions of 0, 0.5, 1, 1.5, 2, 2.5, 3, 5 and 10 mg kg^{-1} for High Matrix Introduction (HMI) mode were prepared from the multi-element standard solution (10 mg L^{-1}). HMI mode adds a flow of argon gas to dilute the sample aerosol, improving the plasma robustness compared to conventional liquid dilution [28]. Chemical mass fractions and respective uncertainties were calculated by triplicate sample analysis [29] using MassHunter 4.4 Workstation Software for 8900 ICP-QQQ Version C.01.04 Patch 5, provided by Agilent. The levels of the calibration curve used for each chemical element in each sample depended on its mass fraction, so that the average of the points on the curve is as close as possible to the determined mass fraction, thus reducing the uncertainty due to the calibration curve.

2.3. *Neutron activation analysis*

Ten samples of each of the two types of honey were transferred to inert bisphenol A free plastic vessel and then placed in an ultra-freezer (-85 °C) for 1.5 days and freeze-dried for 72 h. Due to the high hygroscopicity of the honey, they were put back into ultra-freezer and kept until encapsulation. Analytical portions of 200 mg of dry honey were weighed into ultrapure polyethylene capsules from Posthumus Plastics, Beverwijk, The Netherlands. Fragments of a Ni-Cr [30] alloy with known chemical composition were placed between capsules to measure the neutron flux. Irradiation was carried out for 4 h in the nuclear research

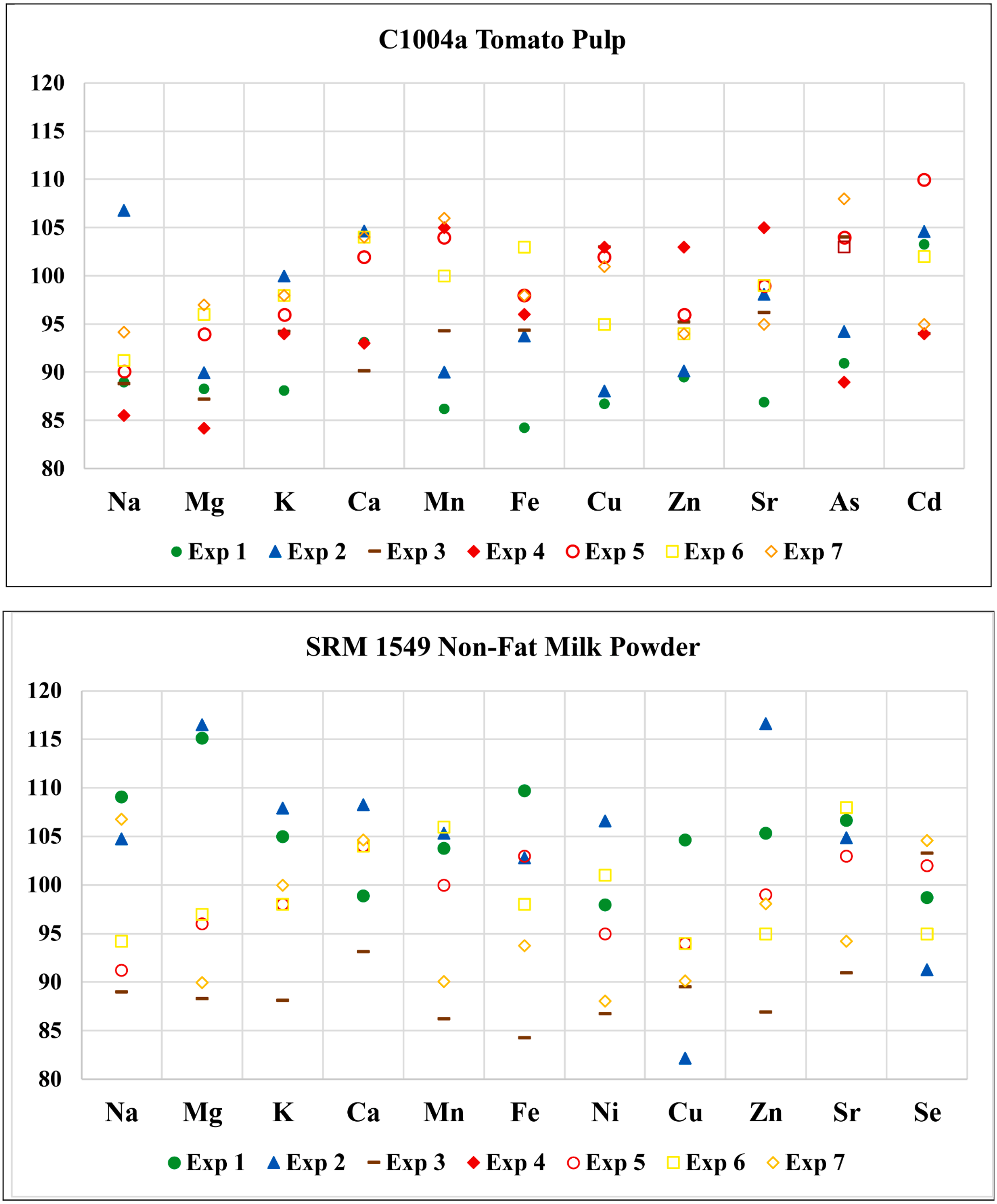

Fig. 2. Recoveries of certified values of chemical elements determined by TQ-ICP-MS for the 7 experiments.

reactor IEA-R1 of the Nuclear and Energy Research Institute, Brazilian Nuclear Energy Commission (IPEN/CNEN) located in São Paulo, SP. Measurements of induced activity, with decay times of 3, 7, 15 and 30 days, were performed to quantify the maximum number of radionuclides by high-resolution gamma ray spectrometry at the Radioisotopes Laboratory of CENA/USP, using Ortec HPGe coaxial detectors with relative efficiencies of 50% and 55% for ^{60}Co photopeak of 1332 keV. Mass fractions of elements and respective uncertainties were calculated using the software package Quantu based on the k_0 method [31].

2.4. Data analysis

Statgraphics Centurion XV Version 15.2.14 was used to generate central composite design, order of the experiments and data processing. Mann-Whitney U test (RANOVA) was used to verify statistically significant differences between the chemical composition of honey samples originated from Jataí and Apis, at the 95% confidence level ($p < 0.05$). Principal Component Analysis (PCA) was implemented as multivariate exploratory data analysis. Chi-Square was used to determine the discriminative power of variables for classification between the two groups of honeys [32]. SAS OnDemand for Academics software was used

Table 4
Mean mass fraction (X), standard deviation (SD) and statistically significant difference (SSD) of chemical elements determined in fresh honey from Apis and Jataí by TQ-ICP-MS.

Analyte (Cell mode)	Apis (n=70)		Jataí (n=70)	
	X ± SD μg kg^{-1} *mg kg^{-1}	SSD	X ± SD μg kg^{-1} *mg kg^{-1}	SSD
Be (No gas)	0.37 ± 0.06	A	0.3 ± 0.1	B
*Na (He)	47 ± 8	A	35 ± 15	B
*Mg (He)	41 ± 9	B	65 ± 15	A
Al (He)	849 ± 284	B	3523 ± 985	A
*K (He)	1487 ± 243	A	1122 ± 242	A
*Ca (He)	110 ± 32	A	85 ± 13	B
V (He)	1.2 ± 0.4	B	8 ± 1	A
Cr (He)	6.9 ± 0.6	B	16.4 ± 0.8	A
*Mn (He)	4.4 ± 0.9	B	5.1 ± 0.2	A
*Fe (He)	5.1 ± 0.9	A	3.0 ± 0.4	B
Co (He)	9 ± 1	A	8.8 ± 0.3	B
Ni (He)	27 ± 6	B	62 ± 18	A
Cu (He)	132 ± 34	B	311 ± 93	A
*Zn (He)	6 ± 1	A	8 ± 1	A
*Rb (He)	7 ± 2	A	3 ± 1	B
Sr (He)	950 ± 225	A	711 ± 158	B
As (O$_2$)	0.75 ± 0.07	A	1.3 ± 0.3	B
Se (O$_2$)	5 ± 1	A	1.0 ± 0.3	B
Ag (He)	9 ± 1	A	1.4 ± 0.6	B
Cd (He)	79 ± 18	A	78 ± 17	A
Cs (He)	5 ± 1	B	12 ± 3	A
*Ba (He)	2.0 ± 0.5	A	0.7 ± 0.2	B
La (He)	0.8 ± 0.3	B	7 ± 2	A
Ce (He)	1.3 ± 0.8	B	14 ± 4	A
Pb (He)	139 ± 65	A	223 ± 78	A
Th (He)	0.4 ± 0.1	B	0.9 ± 0.3	A
U (He)	0.06 ± 0.03	B	0.24 ± 0.06	A

to carry out the RANOVA test. Subsets were constructed according to the discriminative power of the variable (chemical elements). Each subset was used to train the Multilayer Perceptron (MLP), Random Forest (RF) and Support Vector Machine (SVM) using 10-fold cross-validation. Weka (Waikato Environment for Knowledge Analysis) software version 3.8 was used to implement the classification algorithms.

2.5. Quality assurance

For analytical quality control of NAA and TQ-ICP-MS, the certified reference materials SRM 1549 - Non-Fat Milk Powder from the National Institute of Standards and Technology (NIST) and CRM-Agro C1004a Tomato Paste from the CRM-Agro Reference Materials for Agriculture, Husbandry and Toxicology (CENA/USP) were used. For TQ-ICP-MS, the SRM 1643d Trace Elements in Water from NIST was also analyzed. En Score was calculated for NAA analytical results according to ISO (2015) [33] while true value recovery was calculated for TQ-ICP-MS [29]. Standard solutions were used as Initial Calibration Verification (ICV) and Continuing Calibration Verification (CCV). Table S1 shows the reference materials and standard solutions used for analytical quality control.

The limit of detection (LOD) for NAA was calculated for each sample using the software package Quantu, as three times the square-root of baseline counts [34]. The limit of detection (LOD) and limit of quantification (LOQ) for TQ-ICP-MS were based on the measurement of 10 blank solutions [29]. The standard deviation of the blanks was multiplied by the background equivalent concentration (BEC) and divided by 100.

3. Results and discussion

The mass fractions of chemical elements determined in SRM 1643d and CCV presented recovery values between 80 and 120% (Table S2), demonstrating the performance of the Agilent 8900 TQ-ICP-MS during the analysis. The mass fractions of chemical elements determined in the SRM 1549 and CRM-Agro C1004a by NAA were in agreement with reported certified values, with En score between -1 and 1 (Fig. S1), thereby ensuring the analytical quality of results.

Fig. 1 shows the difference between ^{72}Ge internal standard recovery in General Purpose and High Matrix Introduction modes, for the measurement of Ca, Fe, K, Mg, and Na. Internal standard with ionic strength similar to that of the analyte of interest offers an adjustment that considers the stability of the plasma, which can be affected by changes in the analyzed matrix.

Using dilution with solvent in General Purpose mode, the effect of diluted solutions on the stability of the plasma was observed, since it has to deposit more energy to evaporate water. It can be observed that for points 18 to 29, corresponding to samples of honey from Apis bees, the ISTD recovery differs from the first 17 points, corresponding to the calibration curve and CCV. The same behavior is observed in the stability of ISTD recovery for points 32 to 42, corresponding to samples of honey from Jataí. The HMI, showing greater stability for the ISTD recovery, was used for determining Ca, Fe, K, Mg, and Na. Se and As were determined using the O$_2$ reaction cell mode. In this mode, the interferences of the dimer (KK) and the chloride (KCl) on Se mass fraction and the argide (ClAr), chloride (ArCl and CaCl) and oxide (CoO) on As mass fractions were minimized.

Fig. 2 shows the recovery values for the certified reference materials obtained by TQ-ICP-MS varying between 80% and 120% (85 – 110% for CRM C1004a Tomato Pulp and 82 – 116% for SRM 1549a Non-Fat Milk Powder).

Mass fractions of Ag, Al, As, Ba, Be, Ca, Cd, Ce, Co, Cr, Cs, Cu, Fe, K, La, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Th, U, V and Zn were measured by TQ-ICP-MS (Table 4). The mass fractions of Br, Ca, Co, Fe, K, Na, Rb, and Zn were measured by NAA (Table 5). The average moisture obtained for Apis and Jataí honeys were 28.5% and 30.7%, respectively. Since NAA allows direct measurement using solid samples [26], it can be used to

Table 6
Recovery of mass fractions of chemical elements determined by TQ-ICP-MS, *using the NAA results (in fresh honey basis) as reference values.*

	Recovery (%) Ca	Co	Fe	K	Na	Rb	Zn	
Apis	Experiment 1	103	90	101	97	91	107	98
	Experiment 2	104	91	102	96	92	105	100
	Experiment 3	102	90	99	96	97	105	97
	Experiment 4	105	94	101	98	93	101	98
	Experiment 5	104	95	101	95	93	99	99
	Experiment 6	102	92	100	99	96	99	102
	Experiment 7	100	93	101	94	99	103	99
Jataí	Experiment 1	98	97	99	95	94	108	101
	Experiment 2	89	95	97	99	90	107	95
	Experiment 3	93	90	98	96	93	112	96
	Experiment 4	94	95	98	95	98	108	105
	Experiment 5	92	90	100	94	97	107	99
	Experiment 6	91	96	100	96	93	109	96
	Experiment 7	95	98	101	96	91	108	94

Table 5
Mean mass fractions of chemical elements determined in fresh honey from Apis and Jataí by NAA.

	Br (mg kg^{-1})	Ca (mg kg^{-1})	Co (μg kg^{-1})	Fe (mg kg^{-1})	K (mg kg^{-1})	Na (mg kg^{-1})	Rb (mg kg^{-1})	Zn (mg kg^{-1})
Apis (n=10)	1.9 ± 0.1	109 ± 33	10 ± 4	5 ± 2	1577 ± 12	40 ± 2	6.6 ± 0.1	6.2 ± 0.4
Jataí (n=10)	1.85 ± 0.09	115 ± 36	12 ± 3	4 ± 2	1484 ± 15	48 ± 3	3.9 ± 0.2	8.7 ± 0.4

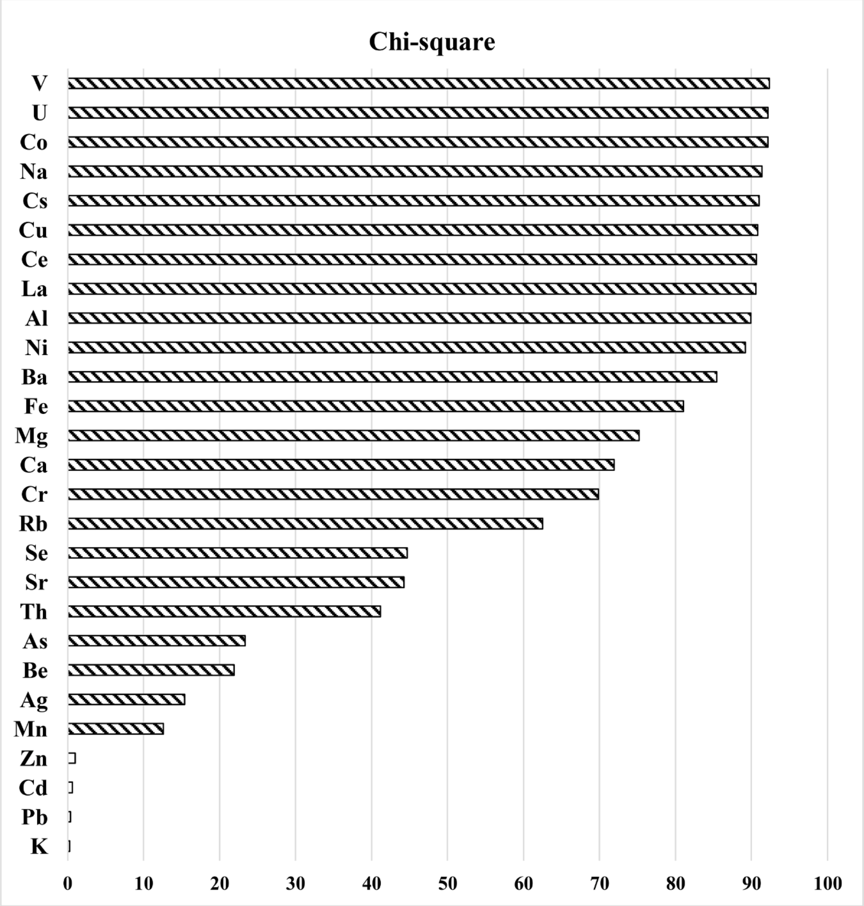

Fig. 3. Importance of variables for prediction of honeys according to the Chi-Square value.

evaluate the efficiency of the sample digestion procedure by comparing the results from both techniques. Table 6 presents the recoveries of mass fractions of chemical elements determined by TQ-ICP-MS, taking the NAA results (in fresh honey basis) as reference values.

There was no statistically significant influence on the recoveries owing the variation in the amount of HNO_3 between 1 and 5 mL, when the amount of H_2O_2 varied between 0 and 2 mL and neither from the combined effect of the two variables. No significant interaction (p-value > 0.05) was observed between experiment and honey origin (produced by Apis or Jataí bees) for none of the quantified chemical elements. This allowed to analyze the experimental condition and the origin of honey factors independently.

Considering the honey samples from Apis and Jataí, there were no statistically significant differences (p-value > 0.05) for the mass fractions of Cd, K, Pb and Zn. The larger mass fractions of Ag, As, Ba, Be, Rb, Se and Sr were found in Apis honey and of Al, Ca, Ce, Co, Cr, Cs, Cu, Fe, La, Mg, Mn, Na, Ni, Th, U and V in Jataí honey. The variables (mass fractions of the elements) were ordered by importance to discriminate between the two groups of honeys, using the Chi-Square value (Fig. 3). The higher the Chi-Square value, greater is the discriminate power between classes of this variable [35].

Principal component analysis was generated (Fig. 4) considering 76% of the information and all chemical elements determined in the samples. Two groups corresponding to samples of Apis honey and Jataí honey were observed. Apis honey samples showed greater dispersion when compared to Jataí honey samples. Vectors corresponding to the mass fractions of the chemical elements that did not have statistically significant differences depicted direction perpendicular to the groups of samples of Apis honey and samples of Jataí honey, thus corroborating by a multivariate approach their low influence to differentiate between the groups of honey.

Twenty-seven subsets were established and used to generate the classification models (Fig. 5). The variables considered in each subset were chosen in order to have all variables in subset 1 and then being removed one by one according to their importance, as shown in Fig. 3. Therefore, in subset 27, only the variable V mass fraction was considered.

A highly statistically significant difference (p-value < 0.0001) between the mass fractions of the chemical elements Ag, Al, As, Ba, Be, Ca, Ce, Co, Cr, Cs, Cu, La, Mn, Na, Ni, Rb, Se, Sr, Th, U, and V in Apis and Jatai honeys was obtained. A great stability of classification accuracy was obtained, varying between 98 and 99%, even considering the mass fractions of the elements with less discrimination potential (Fig. 3). According to RF, MLP and SVM, using only the mass fraction of V, the three classification algorithms presented the highest honey classification accuracy (99%).

4. Conclusions

There was no statistically significant influence of the amounts of nitric acid and hydrogen peroxide on the recoveries of elemental mass fractions. The amounts of 3 mL HNO_3 and 1 mL H_2O_2 provided less residual acidity of the digestion solution, avoiding further dilutions and thereby reaching lower limits of detection and quantification. Recoveries between 80 and 120% when compared to NAA results indicate that the optimization of sample digestion of honeys was successfully achieved, regarding the measurement of Ca, Co, Fe, K, Na, Rb, and Zn. For the specific region studied, it was possible to differentiate the honeys by entomological origin as a function of elemental mass fractions with an accuracy of up to 99%, implementing classification algorithms MLP,

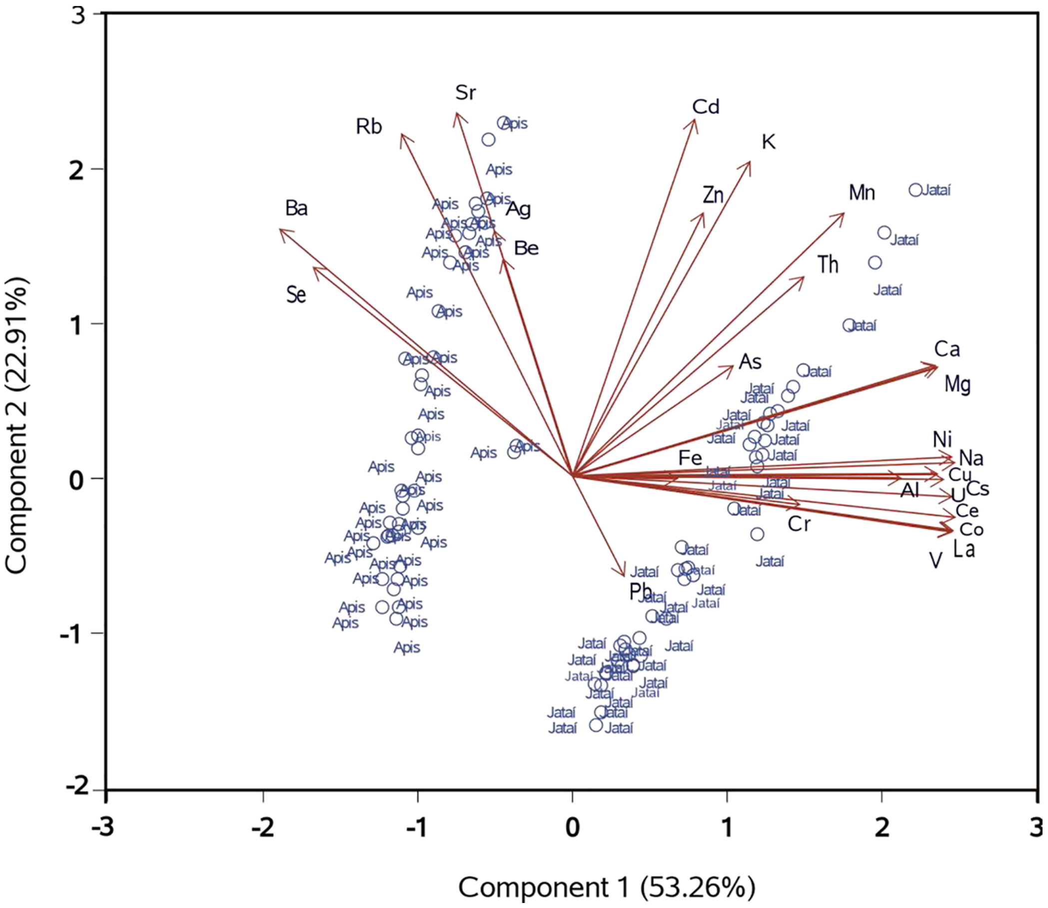

Fig. 4. Principal Component Analysis (PCA) to discriminate honeys of Jataí and Apis considering all element mass fractions.

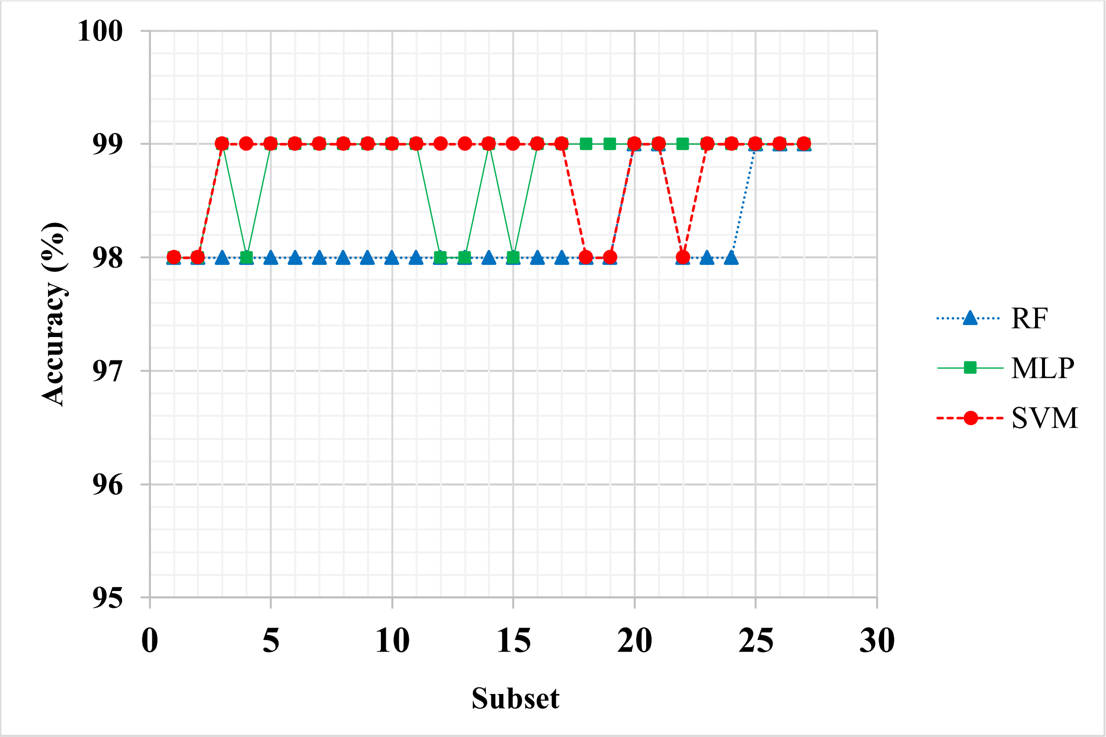

Fig. 5. Accuracy values achieved by Random Forest (RF), Multilayer Perceptron (MLP) and Support Vector Machine (SVM) using different subsets of variables.

RF and SVM. The isotopic techniques and machine learning approach can provide useful tools for authentication and certification of Brazilian honeys.

Declaration of Competing Interests

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

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