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Food and beverage

Determining the geographical and botanical origins of honey by harnessing the power of triple quadrupole ICP-MS technology

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Keywords

Honey, authenticity, elemental composition, food safety, profiling, Inductively Coupled Plasma Mass Spectrometry, transition metals

Goal

This application note will highlight how botanical and geographical origins of honey can be predicted using a workflow that includes automatized sample preparation, accurate analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), and statistical analysis.

Introduction

Different types of honey have distinct flavors, colors, and nutritional profiles, which are influenced by the plants from which the bees collect nectar and in turn by geographical origin. However, fraud is a growing concern worldwide, and fraudulent producers resort to chemical adulteration. For example, dyes, artificial flavors, or preservatives are added to enhance the appearance, texture, or preservation of honey. Studies have been conducted to understand the scale of the problem, revealing that a large percentage of samples analyzed are potentially adulterated with the addition of extraneous sugar.[1] The falsification of the geographical origin of honey, by mixing with honey from different locations despite making a local origin label claim, is also common.

Determining the botanical and geographical origin of honey is important for various reasons. It helps ensure the authenticity and quality of the honey, enabling consumers to make informed choices based on their preferences and dietary needs. Honey authenticity testing is also crucial for traceability, food safety, and compliance with local applicable quality standards and regulations. This information is particularly important for people with allergies, as they can avoid honey from regions where specific allergenic plants are prevalent. It also supports the preservation of biodiversity and local ecosystems, encouraging sustainable beekeeping practices and helps protect native plant species that are crucial for pollinators' survival.

Government authorities, international organizations, and the beekeeping industry are working together to combat honey fraud.[2] Quality control measures, laboratory tests, and stricter regulations are put in place to ensure the authenticity of honey and protect consumers. The use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is widespread because it allows reliable quantitative analyses of trace elements, as well as indispensable isotopic analyses for authenticity studies.

This study outlines a comprehensive method for honey authentication using the elemental composition, complimentary to traditionally used methods based on isotope ratio measurements.[3] A full workflow is presented for a large sample suite of >100 honey samples of different geographical and botanical origin, including sample preparation, elemental analyses, and statistical data management.

Experimental

A Thermo Scientific™ iCAP™ TQ ICP-MS and a SC-4DX Autosampler with FAST valve (Elemental Scientific, Inc., Omaha, NE, USA) were used for analysis of 37 elements in 122 honey samples. Analysis was performed in kinetic energy discrimination (KED) mode using pure helium and using oxygen as the reactive gas in triple quadrupole (TQ-O_2) mode. The instrument was operated using the configuration and parameters highlighted in Table 1. This analysis can also be carried out using a Thermo Scientific™ iCAP™ MTX ICP-MS with equivalent results and even better performance.

Table 1. iCAP TQ ICP-MS configuration and parameters

Parameter	Value		
Nebulizer	MicroMist™ nebulizer		
Interface cones	Nickel sample and skimmer		
Spray chamber	Quartz cyclonic spray chamber		
Spray chamber temp.	2.7 °C		
Injector	Quartz injector 2.5 mm		
Torch	Ceramic Torch Plus		
RF power (W)	1,550		
Sampling depth (mm)	8		
Peri pump speed (rpm)	15		
Auxiliary flow ($L \cdot min^{-1}$)	0.8		
Cool gas flow ($L \cdot min^{-1}$)	14		
Nebulizer flow ($L \cdot min^{-1}$)	1.16		
Sample tubing	PVC standard pump tubing, ID 0.762 mm		
Internal standard tubing	PVC standard pump tubing, ID 0.381 mm		
Insert	High sensitivity		
FAST loop	3 mL		
QCell setting	**SQ-N/A**	**SQ-KED**	**TQ-O_2**
QCell gas flow	No gas	100% He 4.60 $mL \cdot min^{-1}$	100% O_2 0.32 $mL \cdot min^{-1}$
Kinetic energy barrier	N/A	+ 3 V	N/A
CR/Q3 bias difference	-1 V	-	-6 V
Scan setting sweeps	0.03 to 0.1 s dwell time, 10 sweeps, 3 main runs		

Software

The Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software was used for the ICP-MS analysis, including method definition, data acquisition, and evaluation. To statistically evaluate all results, Minitab™ Statistical Software (Minitab, LLC, State College, Pennsylvania, United States) was chosen. The flexible and easy data modelling options along with the large number of samples analyzed help to build a solid and reliable database. The software easily combines resulting concentration data from the ICP-MS and automatically generates statistical models based on the data. Minitab Statistical Software determines the contributions of each element and builds tree diagrams based on the type of model selected. The software automatically selects the most relevant elements to establish a prediction on the geographical and floral origins of unknown samples.

Sample selection

A total of 122 honey samples were analyzed in this study, selected based on the certainty of their precise geographical origin as well as their monofloral origin. Precise knowledge of the samples and their authenticity is vital in creating a suitable statistical database. Most of the samples were provided by AB Labo (Gan, France), a French laboratory that characterizes the purity and authenticity of honeys to guarantee the best origins and the purest harvests. Other honey samples were selected in a local supermarket based on their certified geographical and botanical origin, as listed in Table 2.

Table 2. Geographical origin of the honey samples

Geographic origin	Number of honey samples	Botanical origin	Number of honey samples
Argentina	6	Acacia	15
Bulgaria	3	Chestnut	17
China	13	Rapeseed	5
Spain	5	Lavender	10
France	74	Mountain	8
Hungary	2	Linden	8
Romania	1	Sunflower	7
Ukraine	10	All flowers	49
Vietnam	8	All flowers (Vitex)	3
Total	**122**	**Total**	**122**

Sample preparation

Honey samples have different physical properties, for example viscosity, that depend on botanical origin, sugar composition, or the presence of starch. However, constant and reproducible sample preparation is essential for sample-to-sample comparability and for creating an accurate database. Thus, microwave-assisted acid digestion, with a fully automated workflow offering high reproducibility and productivity, was chosen as the most suitable and universal sample preparation approach for all honey samples.

Due to the capacity of the ultraWAVE microwave digestion system (Milestone™ Srl., Sorisole, Italy) to digest high sample amounts, 1.0 g of honey was weighed in the quartz ultraWAVE tubes. The EasyFILL (Milestone Srl.) acid dispenser was used to automatically add 2.5 mL of HNO_3, 0.5 mL of HCl, and 0.5 mL of H_2O_2 to each tube, thus minimizing operator interaction and contamination (Figure 1). The samples were digested at 220 °C for 20 minutes.

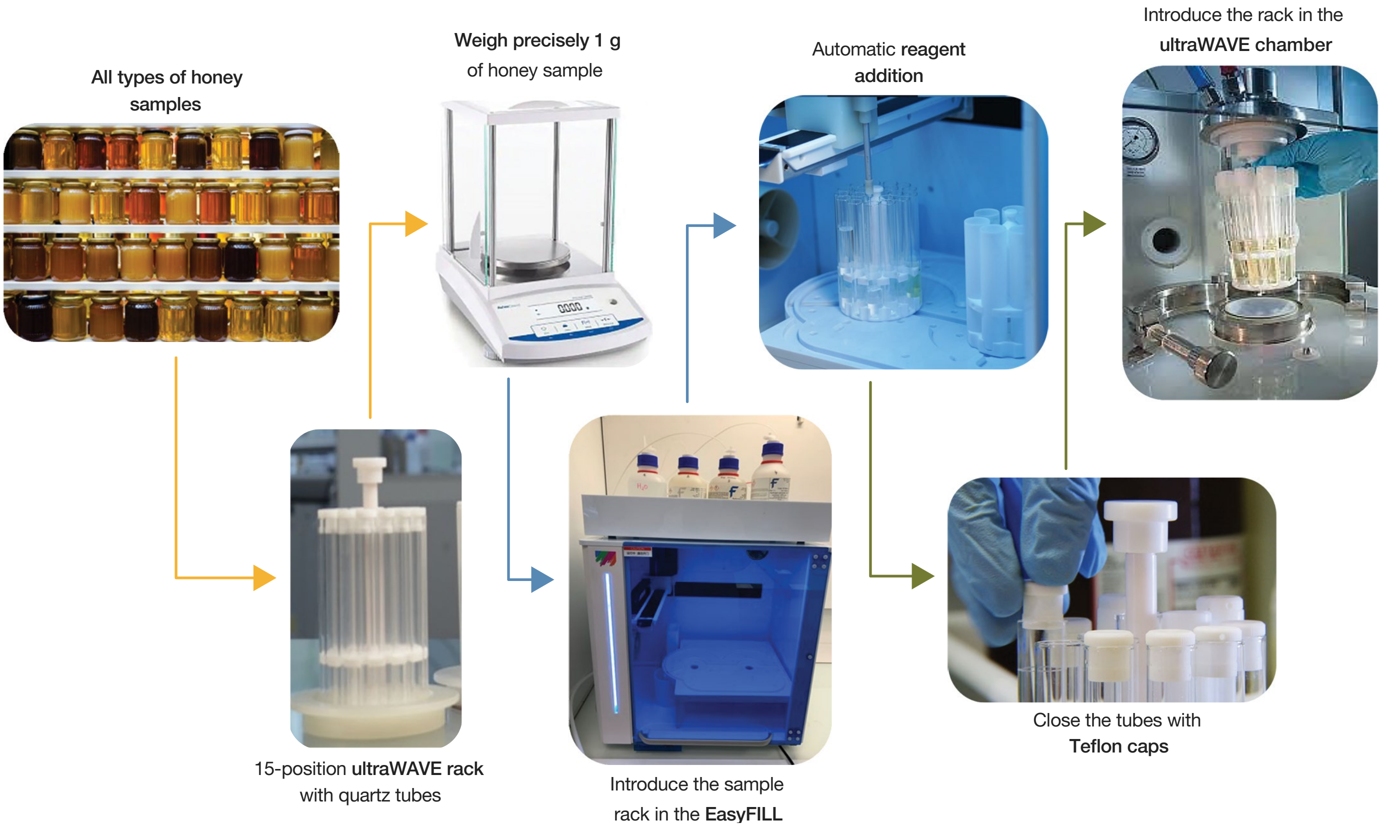

Figure 1. Honey sample preparation workflow

Each sample was prepared in duplicate to check the sample preparation reproducibility and to populate the database with more than 240 entries for more accurate modelling. The workflow automation ensures minimal contamination during sample preparation and thus allows the construction of a precise and reproducible database.

Standards

For the calibration standards, a multi-element solution containing 27 trace elements and single element solutions for mercury and the major elements were used to achieve the concentrations reported in Table 3. Mercury was added at a lower concentration to the calibration solutions due to its minimal presence in honey samples and to mitigate potential memory effects. The calibration standards were prepared in 5% HNO_3 / 1% HCl / 1% H_2O_2 to match the acid concentration of the samples after the microwave-assisted digestion.

Analytical method performance

The developed quantitative method can analyze both major elements present at high concentrations (mg/L), as well as trace elements present at very low concentrations (µg/L) in the same run. A combination of the three operating modes—SQ-N/A, SQ-KED, and TQ-O_2—is necessary to handle the isobaric and polyatomic interferences and to obtain the best quantification limits for trace elements and major elements. The acquisition mode by elements and the analytical performances of the quantitative analysis are given in Table 4.

Method robustness

Throughout the duration of the analyses (calibrations, control points, and samples), the internal standard mixture containing 500 µg·L^{-1} of germanium, and 20 µg·L^{-1} of indium, rhodium, and iridium was introduced online using a dedicated inlet of the 7-way valve of the FAST system. The introduction of internal standards ensures the correction of drift, often related to the matrix effects. Even with a high sample weight of 1.0 g, the data shows the excellent internal standard stability during 8 hours of analysis including more than 120 honey samples (Figure 2). Stability and robustness are key points to creating a reliable and updatable database.

Table 3. Calibration standards for all elements analyzed with the quantitative method

Elements	Unit	Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Ag, As, Ba, Be, Bi, Cd, Ce, Co, Cr, Cu, Fe, La, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sn, Sr, Ti, Tl, U, V, Zn	µg·L^{-1}	0.00	0.01	0.05	0.20	1.00	10.0	100.0
Hg	µg·L^{-1}	0.00	0.0001	0.0005	0.002	0.01	0.10	1.00
Al, B, Ca, Mg, Na, P, S	mg·L^{-1}	0.00	0.05	0.10	0.50	1.00	5.00	-
K, Si	mg·L^{-1}	0.00	0.50	1.00	5.00	10.0	50.0	-

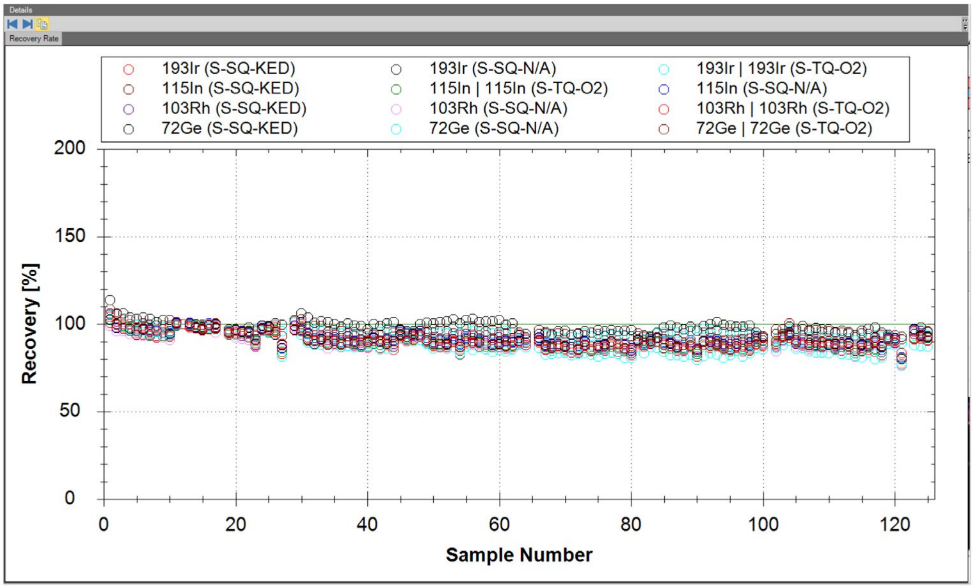

Figure 2. Stability of internal standards Ge, Rh, In, and Ir in all measurement modes (S-SQ-N/A, S-SQ-KED, S-TQ-O_2) during an 8 h analysis of honey samples

Table 4. Analytical figures of merit of the developed method. Blank equivalent concentration (BEC), limit of detection (LOD), and method quantification limit (MQL) are given in μg·kg^{-1} for trace elements, and in mg·kg^{-1} for major elements (shown in bold blue).

Element	Acquisition mode	Q1 isotope	Q3 isotope	R^2	BEC	LOD	MQL (dil50)
B	**SQ-KED**		**^{11}B**	**>0.9999**	**0.007**	**0.0007**	**0.115**
Li	SQ-NoGas		^{7}Li	0.9997	0.005	0.0019	0.317
Be	SQ-NoGas		^{9}Be	0.9999	0.0004	0.0013	0.209
Na	**SQ-KED**		**^{23}Na**	**>0.9999**	**0.002**	**0.0006**	**0.096**
Mg	**SQ-KED**		**^{24}Mg**	**>0.9999**	**0.0002**	**0.0001**	**0.012**
Al	SQ-NoGas		^{27}Al	>0.9999	0.978	0.01938	3.230
Si	**TQ-O$_2$**	**^{28}Si**	**^{28}Si.^{16}O**	**>0.9999**	**0.230**	**0.0445**	**7.413**
P	**TQ-O$_2$**	**^{31}P**	**^{31}P.^{16}O**	**>0.9999**	**0.001**	**0.00005**	**0.008**
S	**TQ-O$_2$**	**^{32}S**	**^{32}S.^{16}O**	**>0.9999**	**0.007**	**0.0005**	**0.080**
K	**SQ-KED**		**^{39}K**	**>0.9999**	**0.024**	**0.0019**	**0.316**
Ca	**SQ-KED**		**^{44}Ca**	**>0.9999**	**0.008**	**0.0034**	**0.562**
Ti	TQ-O$_2$	^{48}Ti	^{48}Ti.^{16}O	0.9998	0.021	0.0051	0.842
V	TQ-O$_2$	^{51}V	^{51}V.^{16}O	0.9999	0.081	0.0179	2.981
Cr	TQ-O$_2$	^{52}Cr	^{52}Cr.^{16}O	0.9999	0.047	0.0030	0.508
Mn	SQ-KED		^{55}Mn	>0.9999	0.030	0.0310	5.175
Fe	SQ-KED		^{56}Fe	>0.9999	0.348	0.0098	1.641
Co	SQ-KED		^{59}Co	>0.9999	0.001	0.0013	0.214
Ni	SQ-KED		^{60}Ni	>0.9999	0.009	0.0035	0.586
Cu	SQ-KED		^{63}Cu	>0.9999	0.064	0.0108	1.798
Zn	SQ-KED		^{66}Zn	0.9998	1.122	0.0807	13.443
As	TQ-O$_2$	^{75}As	^{75}As.^{16}O	>0.9999	0.095	0.0056	0.936
Se	TQ-O$_2$	^{80}Se	^{80}Se.^{16}O	0.9998	0.004	0.0028	0.467
Rb	SQ-KED		^{85}Rb	>0.9999	0.008	0.0049	0.824
Sr	TQ-O$_2$	^{88}Sr	^{88}Sr.^{16}O	>0.9999	0.010	0.0023	0.378
Mo	TQ-O$_2$	^{98}Mo	^{98}Mo.^{16}O	0.9995	0.009	0.0058	0.965
Ag	SQ-KED		^{107}Ag	0.9998	0.001	0.0005	0.080
Cd	SQ-KED		^{111}Cd	0.9998	0.0002	0.0009	0.152
Sn	SQ-KED		^{118}Sn	0.9999	0.013	0.0053	0.877
Sb	SQ-KED		^{121}Sb	0.9998	0.014	0.0014	0.238
Ba	TQ-O$_2$	^{138}Ba	^{138}Ba.^{16}O	0.9998	0.019	0.0038	0.629
La	SQ-KED		^{139}La	0.9999	0.0002	0.0004	0.070
Ce	TQ-O$_2$	^{140}Ce	^{140}Ce.^{16}O	0.9999	0.0002	0.0001	0.009
Hg	SQ-KED		^{202}Hg	0.9973	0.006	0.0013	0.212
Tl	SQ-KED		^{205}Tl	0.9999	0.002	0.0009	0.154
Pb	SQ-KED		^{208}Pb	0.9996	0.011	0.0014	0.236
Bi	SQ-KED		^{209}Bi	0.9997	0.021	0.0022	0.359
U	SQ-KED		^{238}U	0.9997	0.0001	0.0001	0.010

Results and discussion

Geographical origin

To determine the authenticity of European honey products, an initial model was established with all the analyzed honey samples, except 19 random samples. Unlike some commercial samples, most samples were of known and certified geographical origins, the accuracy of which affects the reliability of the model. The elements sodium, phosphorous, chromium, and potassium were automatically selected by the Minitab Statistical Software to establish five terminal nodes for the model and to determine the regional origin of the honey samples. This binary model is represented by a tree diagram (Figure 3) with a 2% error rate (5 samples misclassified out of 247 samples).

Given the quality and reliability of the model generated by Minitab Statistical Software, it is possible to perform a predictive analysis of geographical origins (EU or Non-EU) on unknown samples. For this, the remaining 19 randomly selected samples that were not used for generating the model were tested with this model and the prediction results are given in Table 5.

The model generates a prediction index under the category prediction event (PEvent) between 0 and 1, which is a measure of the probability of the honey sample coming from Europe. The closer the PEvent is to 1, the greater the probability that the sample is of European origin. For all the honey samples tested with this model, the predictive analyses are consistent for all samples with PEvent indices not less than 0.8 for EU samples, and not more than 0.1 for non-EU samples. These predictive results confirm the accuracy of the established model.

Country of origin

To identify the authenticity of unknown honey samples and determine their countries of origin, a second model with focus on the more precise origins of the honey samples was generated. This is a binary model in the form of a tree diagram generated by Minitab Statistical Software (Figure 4), built with data from French and Chinese samples only. The software automatically selects phosphorous and sodium as the main elements to identify the French and Chinese geographical origins of honey

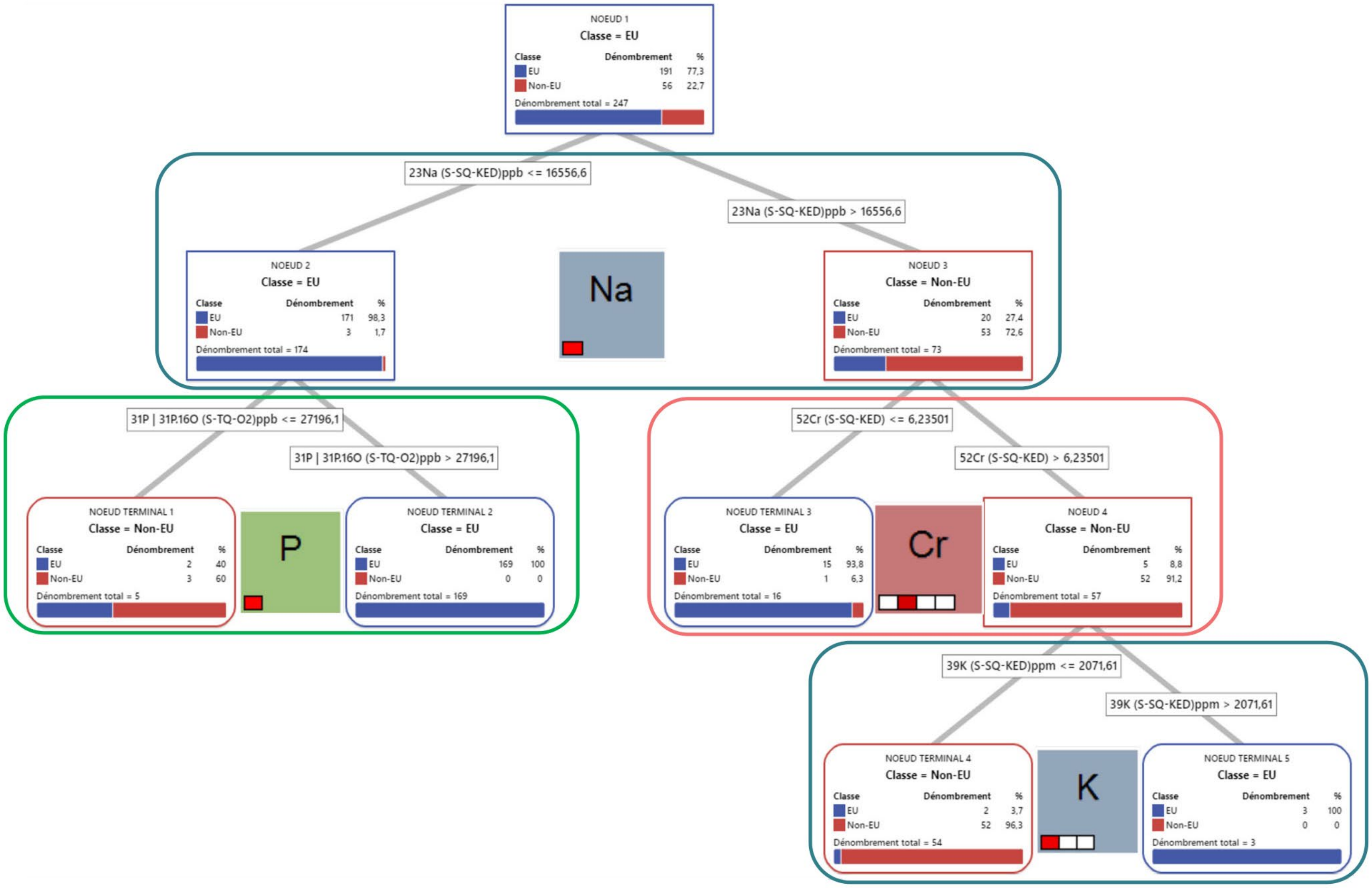

Figure 3. Tree diagram to determine European geographical origin for honey samples. This diagram is automatically generated by Minitab Statistical Software.

Table 5. Predictive analysis of the geographical origin of unknown honey samples

Honey ID	Certified geographic origin	Certified country of origin	Predictive event	Geographic origin
Partner lab 20	**Non-EU**	China	0	**Non-EU**
Partner lab 25	**Non-EU**	Vietnam	0	**Non-EU**
Partner lab 31	**EU**	France	1	**EU**
Partner lab 41	**EU**	Ukraine	1	**EU**
Partner lab 45	**EU**	Bulgaria	1	**EU**
Partner lab 64	**EU**	France	1	**EU**
Partner lab 71	**EU**	Hungary	1	**EU**
Partner lab 86	**EU**	France	1	**EU**
Partner lab 89	**EU**	France	1	**EU**
Partner lab 98	**EU**	France	0.8	**EU**
Partner lab 104	**EU**	France	1	**EU**
Partner lab 119	**Non-EU**	Argentina	0.1	**Non-EU**
Partner lab 121	**EU**	France	1	**EU**
Partner lab 123	**EU**	France	1	**EU**
Commercial honey C	**EU**	France	1	**EU**
Commercial honey D	**EU**	France	1	**EU**
Commercial honey E	**EU**	France	1	**EU**

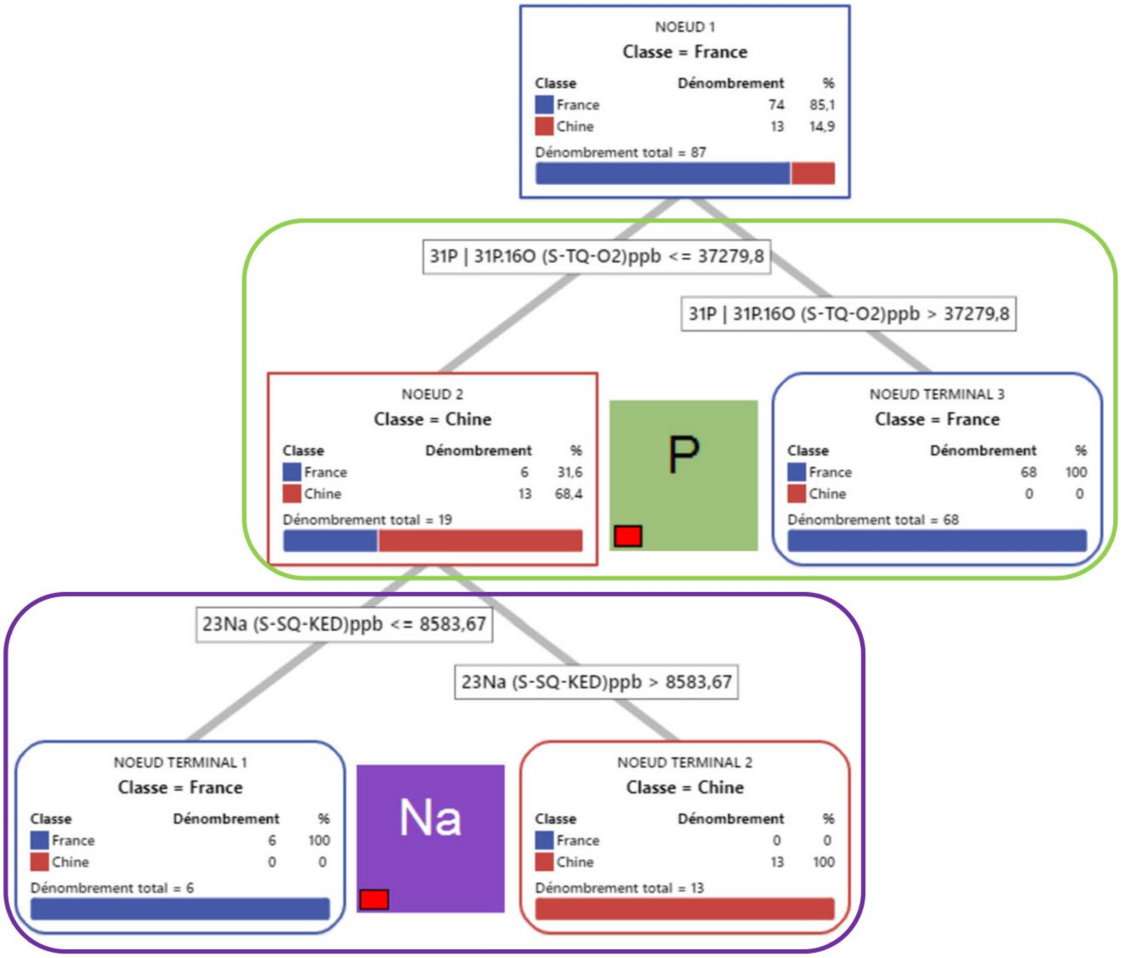

Figure 4. Tree diagram to determine French (blue) or China (red) honey geographical origin. This diagram is automatically generated by Minitab Statistical Software.

The TQ-O_2 mode of the iCAP TQ ICP-MS allows precise quantitation of the phosphorous concentration (based on $^{31}P|^{31}P.^{16}O$) while the SQ-KED mode ensures the quantification of the sodium concentration (^{23}Na). According to the model, the repartition is that out of a total of 88 unknown honey samples, 85% are from France and 15% are from China. The phosphorous concentration value of 37.3 mg·kg^{-1} is used as an indication by Minitab Statistical Software to identify 68 samples as honey from France (Terminal node ID 3). Out of the remaining 19 samples, the sodium limit value of 8.6 mg·kg^{-1} allows identification of the country of origin as France for 6 further samples (Terminal node ID 1) and as China for 13 honey samples (Terminal node ID 2).

Based on these results, the score diagram with both concentrations is an effective statistical tool to check if the model can highlight separated clusters for French and Chinese honey samples. The statistical software determines the first and second components based on the concentration found for phosphorous and sodium with the model and is able to build the score diagram shown in Figure 5. This score diagram confirms that considering the concentrations of P and Na ensures two distinct clusters for French and Chinese honey origins, which highlights the effectiveness of the model.

By using this model, it is possible to establish the predictive analysis of the geographical origin for unknown honey samples. To verify the model, 17 honey samples with certified geographical origin, as well as 3 commercial samples not considered when creating the model, were tested. The predictive results given in Table 6 describe the node ID from the tree diagram, the prediction event (PEvent=1 for France; PEvent=0 for China), and the country of origin prediction.

For two honey samples, node 1 based on the concentration of phosphorus is sufficient to predict French origin. This prediction is confirmed by the high phosphorus concentration for these two honey samples, significantly higher than that of honeys from Chinese origin. For the other unknown honey samples, the sodium concentration is also taken into consideration to make the prediction. As a result, 100% of the samples (15 samples of French origin honey and 5 samples of Chinese honey) were correctly predicted compared to the certified country of origin of the samples.

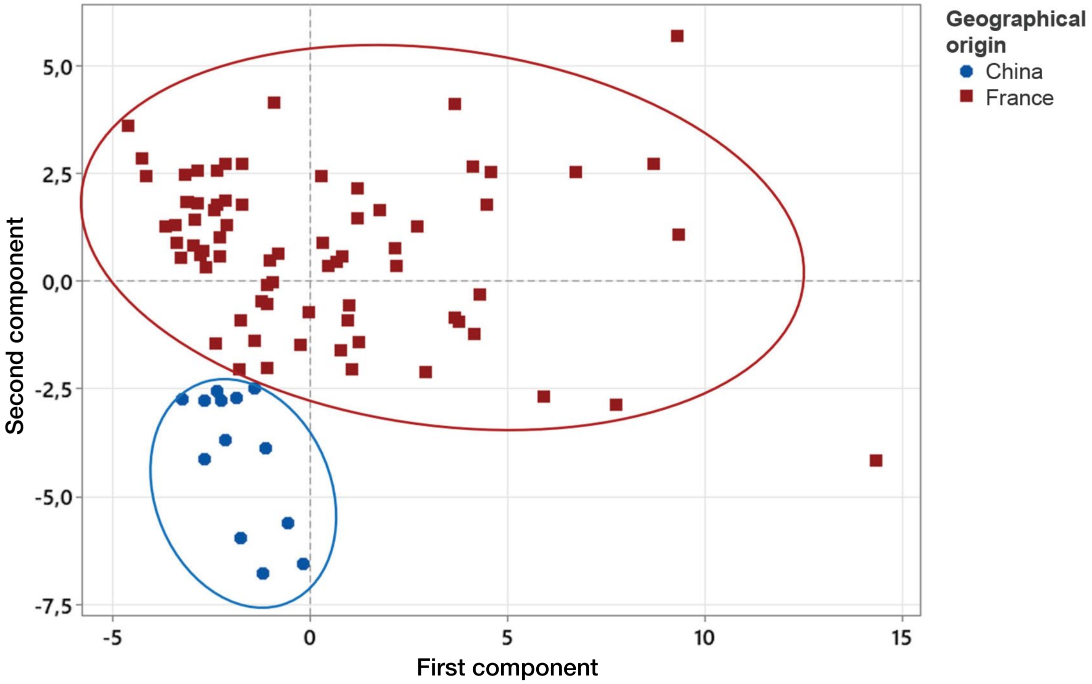

Figure 5. Score diagram for honey samples based on French or Chinese geographical origin

Table 6. Predictive analysis of the country of origin of unknown honey samples

Honey ID	Certified country of origin	Node ID	PEvent	Country of origin prediction
Partner lab honey 2	**France**	3	1	**France**
Partner lab honey 6	**China**	2	0	**China**
Partner lab honey 10	**France**	3	1	**France**
Partner lab honey 13	**France**	3	1	**France**
Partner lab honey 19	**China**	2	0	**China**
Partner lab honey 46	**China**	2	0	**China**
Partner lab honey 51	**China**	2	0	**China**
Partner lab honey 54	**France**	3	1	**France**
Partner lab honey 57	**China**	2	0	**China**
Partner lab honey 62	**France**	1	1	**France**
Partner lab honey 65	**France**	3	1	**France**
Partner lab honey 75	**France**	3	1	**France**
Partner lab honey 93	**France**	3	1	**France**
Partner lab honey 95	**France**	3	1	**France**
Partner lab honey 98	**France**	1	1	**France**
Partner lab honey 104	**France**	3	1	**France**
Partner lab honey 121	**France**	3	1	**France**
Commercial honey C	**France***	3	1	**France**
Commercial honey D	**France***	3	1	**France**
Commercial honey E	**France***	3	1	**France**

*Commercial honey samples with indicated origin on the label

Botanical origin

The botanical origin of honey can also be predicted based on the results. Indeed, the honey samples used to create the database have a certified origin in terms of their geographical origin but also from a floral point of view. It is therefore sufficient to create a model with these different criteria to obtain a dispersion, as well as a predictive analysis.

The best model is automatically built with seven elements (boron, potassium, magnesium, calcium, barium, cobalt, and aluminum) and eight terminal nodes. According to the tree diagram model, 114 out of 123 samples were correctly identified according to their botanical origin. Most of the samples identified as mis-classified (7.3% error) are mountain honey, mainly because mountain samples can come from different floral origins and not from a specific botanical origin.

Based on this model, dispersion diagrams of choice can be displayed (Figure 6). Given the large number of acacia honey samples, a study was conducted to determine the geographical origins of these samples. Figure 6A shows that the main components are different depending on acacia honey origins, thus generating a score diagram showing two distinct clusters for honeys from European origin and honeys originating from other regions. This allows the identification of the geographic origin and authenticity of the honey within the same floral family. Similarly, for a honey population of French origin, it is possible to display the dispersion of samples based on their botanical origin. Figure 6B displays three distinct clusters that are clearly visible to identify lavender, linden, and sunflower honeys.

With the objective of verifying the authenticity of the botanical information on honey packaging, a multinomial model check was carried out on 15 randomly selected honey samples. Table 7 describes the certified floral origins of the honey samples, as well as the terminal node of the model used to obtain the results of the predictive analyses. Except for two samples blended from different flower honeys for which there is no single botanical origin (commercial honeys A and D), the results of the model are in perfect agreement with the certified floral origins, thus demonstrating the accuracy of this multinomial model.

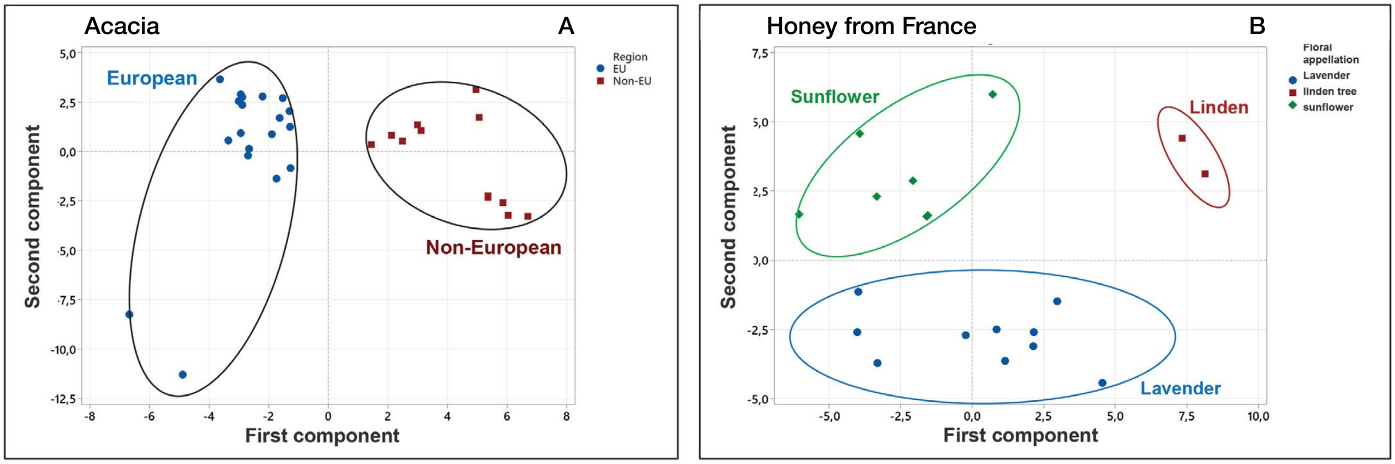

Figure 6. Score diagram for honey samples based on geographical origin for acacia honey (A) and botanical origin for French honey (B)

Table 7. Predictive analysis of the botanical origin of unknown honey samples

Honey ID	Certified floral origin	Country of origin	Node ID	Floral origin prediction
Partner lab honey 4	**Acacia**	China	1	**Acacia**
Partner lab honey 5	**Acacia**	China	1	**Acacia**
Partner lab honey 9	**Chestnut**	France	6	**Chestnut**
Partner lab honey 60	**Lavender**	France	3	**Lavender**
Partner lab honey 62	**Acacia**	France	1	**Acacia**
Partner lab honey 72	**Sunflower**	France	8	**Sunflower**
Partner lab honey 74	**Acacia**	France	1	**Acacia**
Partner lab honey 76	**Rapeseed**	France	7	**Rapeseed**
Partner lab honey 87	**Linden**	France	4	**Linden**
Partner lab honey 91	**Chestnut**	France	6	**Chestnut**
Commercial honey A	**Mixed flowers**	Arg. – Mex. – Bulg.	8	**Sunflower**
Commercial honey B	**Acacia**	EU & non-EU	1	**Acacia**
Commercial honey C	**Forest**	France	5	**Mountain**
Commercial honey D	**Mixed flowers**	France	8	**Sunflower**
Commercial honey E	**Lavender**	France	3	**Lavender**

Conclusion

This study describes a workflow and the results obtained for the authenticity analyses of honey samples using ICP-MS. The analysis of more than 120 honey samples with certified origins allows the construction of a substantial database for a reliable statistical model. An accurate prediction of the geographical and botanical origin of the unknown samples can be realized, giving consumers more confidence about the origin of the products consumed. Furthermore, this workflow can be easily implemented in testing laboratories to control the raw materials used during production, as well as control the authenticity of the finished products and discover potential fraud.

This study highlights the need to obtain an accurate and reliable database and the key points to constitute it.

- The automation of acid digestion ensured by the EasyFILL acid dispenser and the ultraWAVE microwave digestion system offers excellent precision and reproducibility of the sample preparation, necessary to ensure validity of results. This fully automated sample preparation can be adapted to other food sample matrices.

- The iCAP TQ ICP-MS is dedicated to run quantitative and isotopic analyses to cover all the needed parameters for the creation of the honey database.

- The robustness and the linearity range of the iCAP TQ ICP-MS allow simultaneous analyses of major and trace elements in a single run.

- The combination of helium and oxygen in a single method ensures the effective suppression of polyatomic and isobaric interferences to obtain an excellent accuracy for quantitative analyses.

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