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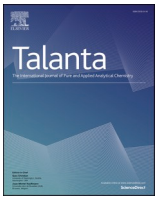

Rapid, reliable and easy-to-perform *chemometric-less* method for rice syrup adulterated honey detection using FTIR-ATR

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ABSTRACT

The adulteration of honey (*Apis mellifera*) is a global problem due to its economic, commercial and health implications. The world's leading beekeeping organisation, APIMONDIA, considers that the detection of adulteration in honey is a problem that has not yet been resolved. This evidence of the importance of the intensive development of analytical techniques that allow the unequivocal detection of adulterants in honey, especially those whose use as honey adulterants has recently emerged. This work aims to develop a fast, easy-to-perform, low-cost analytical method to qualitatively and quantitatively determine rice syrup using the Fourier transform infrared spectroscopy (FTIR) technique with attenuated total reflectance (ATR) mode without complex mathematical procedures and sophisticated sample preparation. This study involved the analysis of 256 intentionally rice-syrup-adulterated honey samples and 92 pure honey samples of bee multifloral honey from Spain. The method, based strictly on the determination of the absorbance directly from the samples, at 1013 cm^{-1} The methodology used no need for previous treatments or preparations and demonstrated the scope for the unequivocal detection of rice syrup in adulterated honey containing equal to or higher than 3% (m/m) or more of this adulterant. Using the Exponential Plus Linear model ($r = 0.998$) shows high accuracy and precision, in terms of relative error (0.32%, m/m) and coefficient of variation (1.4%). The results of this study have led to the establishment of a maximum absorbance threshold of 0.670 for honey without rice syrup.

1. Introduction

1.1. Honey adulteration problematic and consequences

Economically motivated adulteration (EMA) occurs when someone intentionally leaves out, removes, or substitutes a valuable ingredient or part of food [1]. EMA also happens when someone adds substances to food, making it appear better or of more excellent value. For example, when manufacturers add cheaper sweeteners to expensive pure honey but sell the product as 100% honey, they are cheating their customers. We refer to this type of EMA as food fraud. Food fraud is a common type of EMA that the United States Food and Drugs Administration (USFDA) deals with. Some kinds of EMA are also misbranding violations [1]. Adulterated honey is a fine example of EMA, even though its labels represent their food as a pure product.

In recent decades, honey has become the target of adulteration with cheaper sweeteners. Many countries have reported at least 21 different cheap sweeteners used for the direct adulteration of honey [2]. Honey adulteration has become so intensive that it is now the third most adulterated product, after milk and olive oil, in the European Union and worldwide [3]. The main reason for the adulteration of honey is the high demand for honey compared to the limited availability of honey, which is relatively expensive [4].

Honey adulteration is not just an ethical and commercial-economic problem. There are studies that have confirmed the variety of the adverse health impact of adulterated honey consumption: increased blood sugar, insulin hormone release and type II diabetes, increasing blood lipid levels and abdominal weight, obesity, and high blood pressure are notable examples [5]. In addition, adulterated honey consumption may lead to serious affections on internal organs, like fatty liver, acute and chronic kidney injury and elevated visceral fat pads and total body fat, which may cause death [5].

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The adulteration of honey by adding sweeteners is a very serious problem, not only because it is an unfair trade practice or for the implications of constituting commercial and economic fraud. In addition, the adulteration alters honey's chemical and biochemical properties, such as enzymatic activity, conductivity electricity and the content of specific compounds that give it its quality and characteristic properties. It cannot be ignored either the possibility that contaminants can be transferred to honey by the addition of rice syrup made from rice contaminated with toxic substances such as pesticides, and heavy metals, among other emerging contaminants such as phytotoxins, mycotoxins, antibiotics and their metabolites, and allergenic compounds.

1.2. Analytical methods for adulterated honey detection

Over the years, a wide variety of analytical methods have been developed for the determination of honey adulteration. These analytical methods are based on using multiple analytical techniques, both for the determination of specific adulterants and for the simultaneous detection of several adulterants.

Among the most commonly used analytical techniques for adulterated honey detection are [6]: Polymerase Chain Reaction PCR; Thin layer chromatography (TLC). Isotope Ratio Mass Spectrometry coupled with Elemental Analyser and Liquid Chromatography (EA-LC-IRMS) or coupled with High Performance Liquid Chromatography (HPLC-IRMS). Gas Chromatography with a Photoionization detector (GC-FID) or coupled with Mass Spectrometry (GC-MS). High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD), Refractive Index Detector (HPLC-RID) or Electrochemical Detector (HPLC-ECD). High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detector (HPAEC-PAD) or coupled with Solid Phase Extraction (SPE-HPAEC-PAD). Ultra-High Performance Liquid Chromatography-Quadrupole-Time of Flight Mass Spectrometry (UHPLC-Q-TOF-MS). Proton Nuclear Magnetic Resonance (^{1}H NMR), 2D/3D Nuclear Magnetic Resonance (2D/3D-NMR). Fourier Transformed Raman Spectroscopy (FT-Raman). Rapid Evaporative Ionization Mass Spectrometry (REIMS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Visible Near-Infrared Spectroscopy (VIS-NIR). Laser-Induced Breakdown Spectroscopy (LIBS). Fourier Transformed Infrared techniques (Near-Infrared (NIR). Attenuated Total Reflectance (FTIR-ATR). Horizontal Attenuated Total Reflectance (FTIR-HATR). Refractive index sensors, biosensors (E-tongue and E-nose). Stable Carbon Isotope Ratio Analysis (SCIRA) and SCIRA coupled with isolated honey protein levels, which is the official international method (AOAC 998.12) for detection of adulterants in honey [7].

Another analytical approach for the detection of adulterated honey is based in the study of the honey physical chemistry parameters, determined with harmonised methods, a combined study of physical chemistry parameters of honey with microscopic pollen examination, the study of honey physical chemistry parameters in combination with other instrumental techniques, or a variety of diverse coupled analytical instrumental techniques. Recently, novel hyperspectral imaging [8] and spectrofluorometric [9] methods have been developed to detect adulteration in honey, however, neither contemplates detection with rice syrup.

Because these adulterants are produced from C4 plants, they are easily detectable with the AOAC 998.12–1998 official method [7]; since bees use C3 plants for honey production, this official method is capable of differentiating adulteration with this type of C4 plant adulterants. However, in the last decade, various studies have detected the presence of rice syrup in honey produced in multiple countries, which constitutes an important analytical problem since the available analysis techniques for the control of adulteration of the honey are based on the identification of the sugars that come from the C4 type of plant with which they are manufactured. However, the adulteration of honey with rice syrup is indeed a practice that has been carried out globally, making it difficult to attribute responsibility and address the problem specifically. Recently, Chinese scientists detected the presence of rice syrup in commercial honey [10,11], which significantly aggravates honey adulteration with rice syrup because China is the world's leading honey producer [10].

Due to the recent appearance of commercial rice syrup as an adulterant in honey in the Chinese [11,12] and other countries market its presence is undetectable with the relatively old Official Method 998.12–1998 due to the C3 plant-based sweeteners, like rice syrup, cannot be detected by this official method [2]. In addition, false positives have been reported with honey produced by bees that may have fed on C4 plants analysed by SCIRA [13], not to mention the questionable detection limit of this official method [2]. In addition to the aforementioned drawbacks, with the official method, USFDA has reported that their laboratories do not have the instrumental capability to analyse adulterants in honey according to AOAC Official Method 998.12 (SCIRA coupled with isolated honey protein levels). Due to the requirement of an isotope isotopic ratio mass spectrometer to perform the Official Method 991.41 on which SCIRA is based [14]. To date, this USFDA important alert has been updated to allow honey traders to perform SCIRA analysis in third-party laboratories in accordance with International Official Method 991.41.

Accordingly, determining rice syrup in honey adulterated with this sweetener remains an unsolved problem. Even with the wide range of analytical techniques available for determining adulteration in honey, researchers have had to work hard to identify specific markers that can aid in establishing rice syrup adulteration in honey [11]. Despite the diversity of analytical techniques and methodologies currently available, the last resolution of the European Commission (2019), on the perspectives and challenges for the beekeeping sector of the European Union, warns about the lack of analytical techniques for detecting adulteration in honey [15].

On the other hand, using the aforementioned techniques requires the application of advanced and complex data analysis methods, like chemometrics, learning machines, deep learning algorithms, like artificial neural networks or their combination with chemometrics, and sophisticated instrumental analytical techniques.

Although all the analytical techniques we have discussed are indeed modern and sophisticated, this constitutes a double-edged sword that causes another significant problem in the fight against honey adulteration due to various factors such as 1) The variation in analytical performance depending on the adulterant to be identified and quantified, especially in techniques that analyse several adulterants simultaneously. 2) The high cost of the instrumentation and its maintenance. 3) The availability and cost of the specialised personnel required. 4) The complicated and tedious treatment of samples and the expenses generated by this (solvents, reagents, etc.). 5) Its restricted detection limits for low levels of adulteration, especially in the case of rice syrup. 6) Its limited transferability to portable detection devices; and especially, 7) the intricate and complex chemometric algorithms that must be used in almost all available analytical techniques. In this sense, the following question arises: why is honey adulteration a problem that is becoming increasingly complicated and common if there is an important diversity of analytical techniques to determine adulteration in honey? The answer is simple: they are too complicated, expensive and time-consuming to be used in the real world by common people.

All this evidence supports the development of reliable, accurate, fast and easy-to-perform by people without advanced knowledge, analytical methods to detect adulterants in honey, especially rice syrup that has been detected as an important adulterant in honey coming from the world's largest honey producer. All this is to safeguard and guarantee not only the quality and the characteristically special composition of honey. In addition, to ensure that the population can continue to enjoy the valuable properties of this product and for the improvement of the constant and rapid monitoring of its quality by the authorities that regulate it, the circumstances constitute the motivation for our research.

On this line, the main objective of this work has focused on the

characterisation of honey with different botanical origins and the detection of small amounts of rice syrup in specifically adulterated samples using a rapid, reliable and easy-to-perform method based on FTIR-ATR without complex chemometric mathematical process.

2. Material and methods

2.1. Samples and sample preparation

The study was conducted using 92 samples of pure multifloral honey collected directly from beekeepers from diverse locations in Spain, by the Spanish Beekeeping Association and delivered to our laboratory. Thirty-two samples were randomly selected for the preparation of the working solutions. For each of the 32 selected samples, 256 working solutions spiked to 0, 3, 5, 10, 15, 20, 50 and 100% (m/m) of commercial rice syrup were prepared (32 × 8 levels = 256 working solutions) using a model 125 A Precisa Instruments A.G. (Dietikon Switzerland) analytical balance to weigh the components of the standards. Sterile plastic containers with lids were used to weigh the honey, the balance was tared, and the determined mass of rice syrup was added. Then, to ensure the standards' homogeneity, they were placed in a model AU-32 Argo Lab. (Capri, Italy) analogical ultrasonic cleaner bath, previously tuned to 38 °C, for 1 h. Subsequently, they have shaken in a model 7000384 J. P. Selecta (Barcelona, Spain) vibrator shaker, or vibromatic, with a speed setting of 950 u/min for 1 h. The homogenisation procedure was also carried out on the remaining 60 samples of pure multifloral honey.

2.2. FTIR-ATR method

Infrared spectra of working solutions (256) and pure multifloral honey samples (60) were obtained at room temperature using an Alpha II model Bruker Optics GmbH & Co. KG (Ettlingen, Germany). FTIR spectrometer equipped with a high-resolution deuterated triglycine sulfate (HR-DTGS) detector, coupled with a model Alpha II-P attenuated total reflectance (ATR). Platinum module with a 2 × 2 mm diamond crystal sampling surface. To drive the instrument, we used version 7.5 (build 7, 5, 18 (20140810)) OPUS software installed on the computer connected to the spectrometer. The spectrometer was set with a resolution: scan ratio of 4:40 and a spectral readout range of 4000 to 400 wavenumber (cm^{-1}). Before use, the spectrometer was kept on for at least 24 h to ensure the stability of the energy source and was kept on during the period in which the experimental work was carried out. A background spectrum against air was recorded before each sample spectrum to minimise the influence of temporal baseline shifts. All spectra were processed (baseline correction and smoothing) using version 1.2.15. Of the optical spectroscopy software Spectragryph (Oberdstdorf, Germany).

2.3. Statistical analysis method

From the spectral information of each pure honey sample (n = 60) and working solutions (n = 256), the average absorbance values were obtained at 1013 cm^{-1}. Their standard deviations (SD), variation coefficients (CV) and confidence interval (IC), with a 95% confidence level, were calculated for the study of the dispersion of the analytical method. Then, the average absorbance (n = 92) of all the pure honey samples was calculated, with their respective SD, CV and IC, with a 95% confidence level. With the information obtained from the working solutions (256) and pure honey samples (92), we studied the variation of the signal intensity of each set of working solutions and pure honey samples using a simple scatter plot. Finally, with the help of version 2.7.3 Curve Expert Professional software, the mathematical model that best-correlated absorbance was determined as a function of the degree of adulteration. The mathematical and statistical treatment of the obtained data and all the images of this work were processed with version 2021 OriginPro software designed by OriginLab Corporation (Massachusetts, USA).

3. Results

3.1. FTIR-ATR spectra of pure and rice syrup adulterated honey

Several authors have extensively studied the FTIR-ATR spectrum of honey. Fig. 1 shows the spectrum obtained from the 92 samples of multifloral honey studied in this research. Significant signals are observed at 3254, 2930, 1641 and 1020 $cm^{-1,}$ corresponding to O–H stretching, C–H stretching, O–H deformation and C–O and C–H stretching, respectively. In addition, signals are observed in the surrounding region 1343 cm^{-1} corresponding to C–C–H and H–C–O deformation. It is to be expected that, due to the high composition of sugars and water in honey, the IR spectrum of honey exhibits peaks related to the vibrational modes of sugars and water [16].

By assigning bands in the honey spectrum, we can infer and confirm the presence of its components. For example, the first broad peak observed at 3264 cm^{-1} is characteristic of water in honey [4,17–19]. The peak observed in the region between 3000 and 2800 cm^{-1} corresponds to the vibrational modes of carbohydrates (sugars) [19–21], amino acids [20,22] and carboxylic acids [4,23,24]. Moving towards the fingerprint region (1500-700 cm^{-1}), characteristic of various vibrational modes of carbohydrates and ketones [25], we observe signals at 1343 and 1020 cm^{-1} attributable to the stretching and bending vibrational modes of C–OH, C–H and C–O bonds of carbohydrates [4,18,19,26,27]. In this sense, we can confirm that the significant components of honey are water, carbohydrates and other sugars, as seen in previous studies [4,17–20,23,26,28–30]. It is important to highlight the spectral coincidence of these signals for each of the 92 samples of multifloral honey, which could be directly related to the characteristic composition of the flora of the Spain region.

Fig. 2 shows the average spectrum (n = 92) of pure honey (PH) and the spectrum of commercial rice syrup (RS). Despite the similarity of the spectra, a clear phase shift is observed in the peaks corresponding to the signals at 3260, 2925, 1358 and 1010 cm^{-1}, with respect to the average spectrum of pure honey.

In the spectrum of the pure honey samples (Fig. 1), it is observed that there is no total overlap in the peak at 3264 cm^{-1}. In comparison, a significant coincidence in the overlap of the spectra at 1020 cm^{-1} when superimposing the spectra of pure honey and rice syrup is displayed

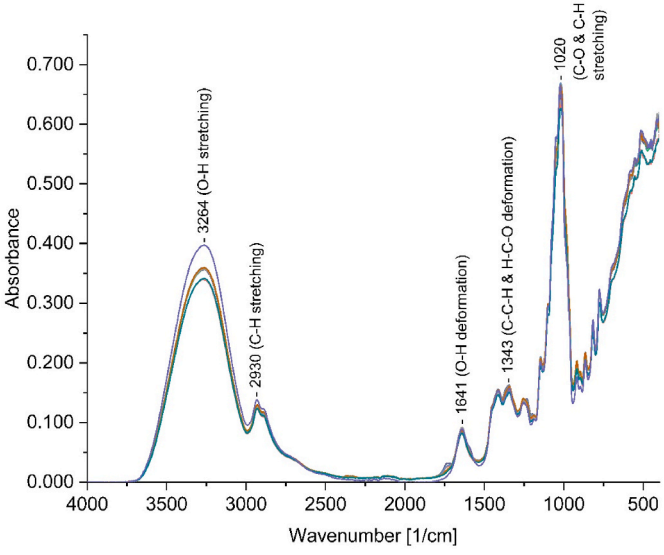

Fig. 1. FTIR-ATR of all tested samples (n = 92) of multifloral honey collected from different regions of Spain.

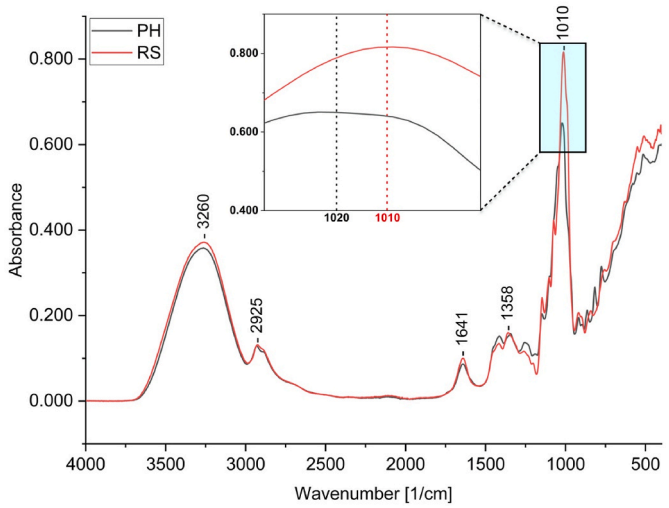

Fig. 2. Average (n = 92) FTIR-ATR spectra of pure honey (PH) and commercial rice syrup (RS) between 4000 and 400 cm^{-1}. The zoomed area shows more clearly the 10 cm^{-1} difference observed between the two interest maxima of pure honey (1020 cm^{-1}) and rice syrup (1010 cm^{-1}).

(Fig. 2). Also, can be observed a peak overlap, with a difference of 10 cm^{-1} in the 1020 cm^{-1} peak. This evidence sustains this spectral zone as a pertinent area to be studied to allow us to easily differentiate pure honey from honey adulterated with rice syrup in content $\geq$ 3% (m/m).

3.2. Classification of pure and rice syrup adulterated honey

The difference between the peaks of pure honey and commercial rice syrup is not only limited to a difference in position or wave number but a significant differentiation is observed concerning the intensity of the peaks. The peak at 1020 cm^{-1} of pure honey has an average absorbance (n = 92) of 0.652 ± 0.001, while the peak at 1010 cm^{-1} of the rice syrup spectrum has an average absorbance (n = 32) of 0.818 ± 0.001. These differences motivated us to prepare working solutions from 32 samples of pure honey containing 0, 3, 5, 10, 15, 20, and 100% commercial rice syrup. Spectra were obtained for each of the 256 stock solutions, and their respective average spectra (n = 32) by each adulteration grade are presented in Fig. 3.

Once established the significant differences between the ATR-FTIR spectra of pure honey and rice syrup (Fig. 2), we hypothesized that in pure honey spectra there would be a hyperchromism in the absorbance at 1013 cm^{-1} due to the presence of rice syrup and that this hyperchromism would increase proportionally with the intentionally added rice syrup content. The spectra of the working solutions (Fig. 3a and b) confirmed our hypothesis since it was observed that as the rice syrup content in the sample increases, the 1013 cm^{-1} peak starts to increase in absorbance. Fig. 3b in fact indicates that there are multiple overlapping peaks in this region, and what is observed as an apparent shift is in fact the result of the increasing prominence of the 1013 cm^{-1} peak as result of increasing rice syrup adulteration. To corroborate this fact, the second derivative of the absorbance of the working solutions was plotted (Fig. 3c), where the clear convergence of the signals around 1013 cm^{-1} is observed. This evidence substantiated our subsequent mechanism for the discrimination of honey adulterated with rice syrup. Table 1 compiles the resumed data about average absorbance obtained for the working solutions (n = 32) at the different concentration levels studied.

When graphically comparing (Fig. 4a) the intensities of all unadulterated pure honey samples (n = 92), it was observed that all the samples presented an absorbance lower than 0.670. The same process with the intensities of the 256 working solutions (Fig. 4b) with different rice syrup adulteration grades showed that the adulterated honey maintained higher absorbance than 0.670 at 1013 cm^{-1}, and their intensities

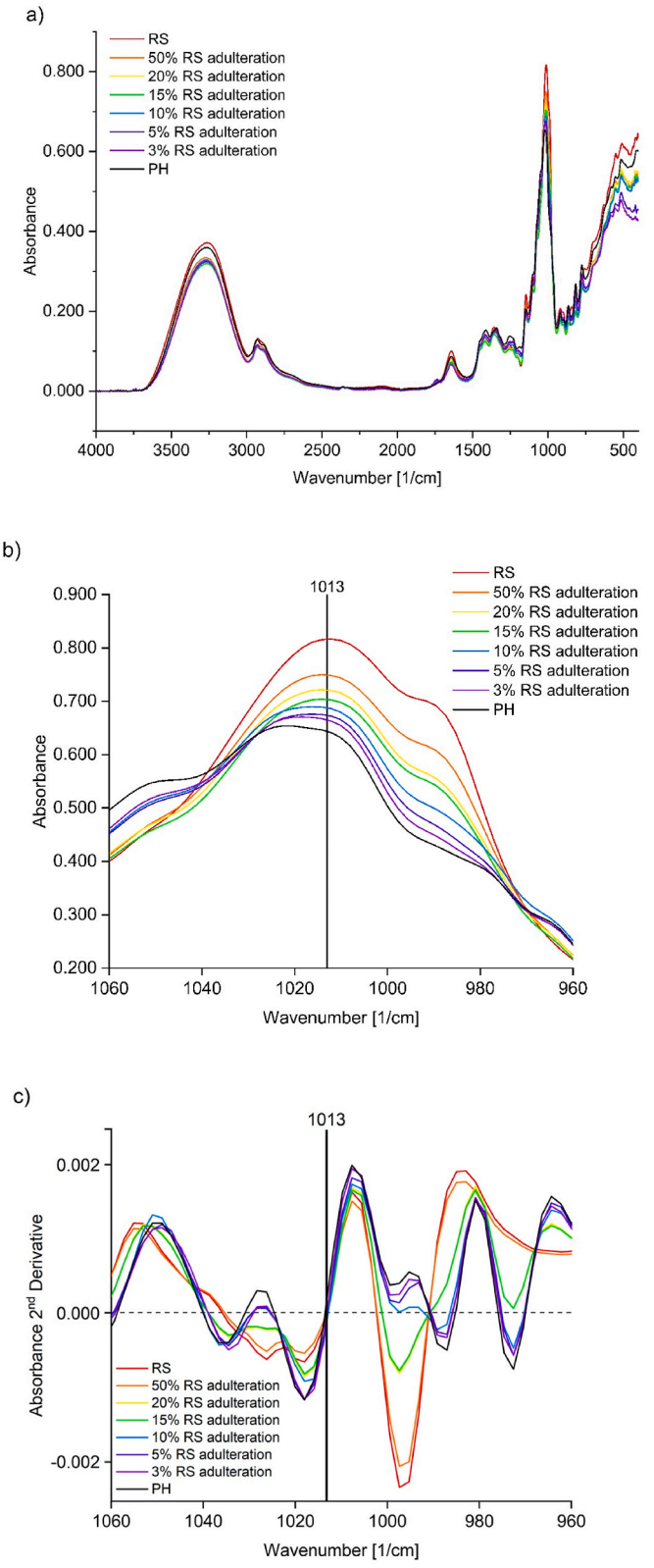

Fig. 3. FTIR-ATR average spectra (n = 32) by adulteration grade of selected honey samples. a) Whole spectra 4000–400 cm^{-1}). b) Zoomed zone between 1060 and 960 cm^{-1} shows the increase in the absorbance in the working solutions in order by adulteration grade. c) The Second derivative of the interest spectral zone, shows the convergence of the signals around 1013 cm^{-1}.

Table 1
Statistical parameters obtained from the 1013 cm^{-1} signals of the FTIR-ATR spectra of the working solutions (n = 32).

Parameter	Adulteration Grade (%. m/m)							
	0[a]	3.0	5.0	10.0	15.0	20.0	50.0	100.0[b]
Min Value	0.640	0.674	0.692	0.715	0.743	0.762	0.785	0.816
Max value	0.657	0.681	0.700	0.722	0.749	0.770	0.793	0.820
Average	0.650	0.678	0.697	0.720	0.747	0.767	0.790	0.818
SD	±0.003	±0.002	±0.002	±0.001	±0.002	±0.002	±0.002	±0.001
C.V. (%)	0.5	0.2	0.3	0.2	0.2	0.2	0.2	0.2
$IC_{95\%}$	±0.001	±0.001	±0.001	±0.001	±0.001	±0.001	±0.001	±0.001

[a] The absorbance of samples with 0% rice syrup content corresponds to the absorbance of the randomly selected pure honey samples. (n = 32).
[b] The absorbance obtained for the 100.0% degree of adulteration corresponds to the absorbance of commercial rice syrup obtained on 32 consecutive occasions.

oscillated in a defined range that can be easily seen. Table 1 also shows the ranges (min-max) in which the absorbance of the working solutions was observed. Several FTIR-ATR spectra obtained in previous studies of pure honey of different botanical origins (more than 1000 samples) and different countries (12, China included) have shown that the intensity of pure honey at 1013 cm^{-1} is lower than 0.670 [18,20,23,26,31–42]. Table 2 describes the detailed list of countries and botanical origin of the honey with the same spectral performance as the pure honey samples analysed in this work.

An easy interpolation of the absorbance at 1013 cm^{-1} of a honey sample in Fig. 4a and b can help establish whether it is a sample of pure honey or a sample of honey adulterated with commercial rice syrup. Fig. 4c shows that the data about the intensity at 1013 cm^{-1} is enough to discriminate correctly and precisely each level of adulteration. In this sense, the method has demonstrated its suitability for qualitatively determining multifloral honey or honey of various botanical origins.

3.3. Estimation of rice syrup content in honey with exponential plus linear (EPL) calibration model

Studying the intensities at 1013 cm^{-1} of the pure honey samples and those intentionally adulterated with rice syrup also allows us to estimate the rice syrup content. Calibrating the intensities at 1013 cm^{-1} as a function of the degree of adulteration with rice syrup, using the exponential plus linear (EPL) model whose fit has a coefficient of determination of 0.997953. This exponential mathematical model, selected by the Curve Expert software for its high correlation of intensity and degree of adulteration data, has the following form:

$$Abs\left(@1013\ cm^{-1}\right) = a + br^{\%} + c\% \quad (1)$$

Where the coefficients *a, b, c* and *r* are statistical parameters characteristic of the EPL model that describes the behaviour of the regression function. Fig. 5 shows the scatterplot of the intensities at 1013 cm^{-1} as a function of the degree of adulteration, adjusted with the EPL model fit (0.997953), and Table 3 shows and brief statistical overview of the fitting model results.

According to the interpolation of the threshold absorbance, we have established, that 0.670; corresponds to a rice syrup content of 3% (m/m). For rice syrup contents of 5, 10, 15, 20 and 50%, according to the EPL model, correspond to an absorbance of 0.690; 0.722; 0.742; 0.756; 0.788; respectively. According to the absorbance results of the 92 samples of Spanish honeys, we can infer that they are rice syrup-free honeys. Table 4 shows the precision and accuracy results obtained using the EPL model for the quantification of rice syrup content in intentionally adulterated samples.

According to the precision and accuracy results presented in Table 4, we observe that the quantitative method offers high accuracy, since the average relative error, for the determinations of rice syrup in honey, is 0.32%. The highest relative error observed was −3.3% for the nominal concentration of 3.0% (m/m) of rice syrup in intentionally adulterated honey. While the lowest relative error observed was −0.4% for the nominal concentration of 50% (m/m) rice syrup in intentionally adulterated honey. On the other hand, the average precision obtained in the quantitative method, in terms of coefficient of variation (C.V.), was 1.4%, relatively low if we compare it with the generally accepted C.V. values (<5%).

Although the literature reporting the content of rice syrup in honeys adulterated with this sweetener is scarce, it has been observed that the content of rice syrup as an adulterant in honey ranges between 20.0 and 65.0% [43], the method we have developed is able, with high accuracy and precision, to determine quantitatively the content of this adulterant in concentrations much lower than this range, with a high level of confidence (95%).

4. Conclusions

In this work, a high number of pure honey samples (92) and intentionally rice syrup adulterated honey samples (256) were analysed with a fast, reliable and easy-to-perform method for determining rice syrup adulteration in honey using FTIR-ATR without any complex mathematical or chemometric process. Therefore, the high and significant numbers of pure honey samples analysed, to the best of our knowledge and intensive bibliographical research, allows us to affirm that this work presents, for the first time, a simple and specific procedure to establish if a honey sample has been adulterated or not with rice syrup. The results have shown that detecting and estimating the adulteration in honey samples with values as low as 3% of rice syrup is possible in a simple way, with high precision and accuracy.

There are numerous studies aimed at detecting honey adulteration but in a qualitative manner. Although it is true that for the regulation of the authenticity of pure honey, a qualitative detection method is sufficient, it is important that the authorities and interested parties, such as repackages, have a method that also allows the quantification of adulteration with honey syrup. Rice in honey so that these levels of adulteration can be known exactly in a wide analytical range. This constitutes an important tool for honey regulatory authorities, for example, to be able to truthfully know to what extent honey is being adulterated and to develop regulations in order to fairly punish the crime of honey fraud and, in this way, combat it more efficient the problem of adulteration. In addition, the quantification of the degree of adulteration of honey with rice syrup offers support for the fair establishment of the purchase-sale price of honey and also as an instrument for litigation and other legal processes that may arise as a result of the identification of honey as adulterated with rice syrup. However, the difficulty of applying sophisticated and expensive instrumental analytical techniques has hindered its application for routine analysis and its possible adaptation for on-site analytical devices. This work paves the way for further development of this analytical method to be applied to a more significant number of samples from different countries of the European Union and other regions of the world that have interests in the honey production, export and import market. As mentioned above, the results of many papers in the literature confirm the observations made in this paper. Here we have extracted relevant information to detect adulteration in honey samples in a simple way and without the need for

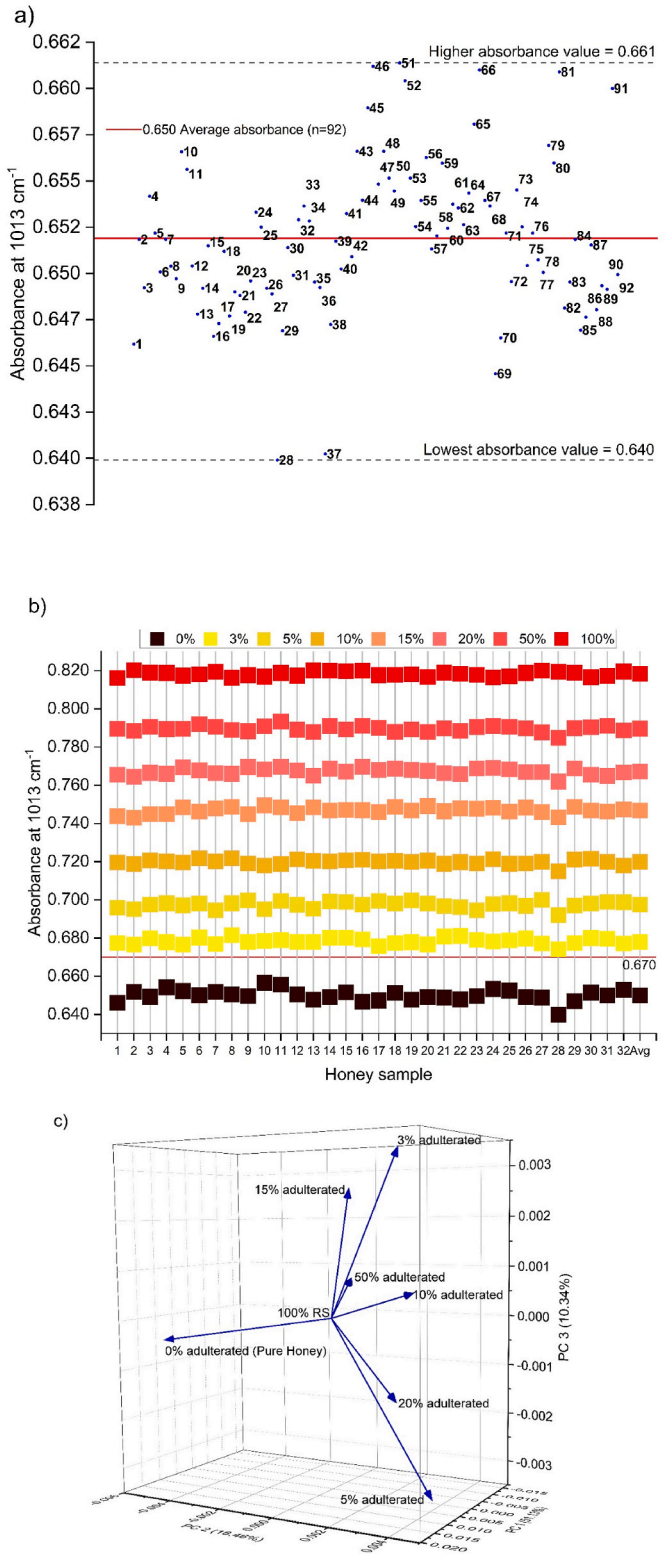

Fig. 4. a) Comparison of absorbance at 1013 cm^{-1} of 92 pure kinds of honey from the region of Madrid, Spain. b) Comparison of the absorbance at 1013 cm^{-1} of the 32 selected pure honey and their corresponding adulterations and commercial rice syrup. C) 3D-score plot of the PCA analysis of the working solutions.

Table 2
Previous studies show a detailed list of pure honey whose FTIR-ATR spectra have shown absorbance below 0.670 at 1013 cm^{-1}.

Honey Botanical Origin	Sample Quantity	Country	Reference
Acacia-Blossom[b]	1	Germany	[37]
Arbutus	1	Portugal	[18]
Astragalus (Radix astragali)	4	China	[20]
Balloon-flower (Platycodon grandiflorum)	2	China	[20]
Buckwheat	3	Poland	[23,31]
Carob	3	Portugal	[18]
Chinese knotweed (Polygonum chinense)	4	China	[20]
Citrus	16	Greece	[36]
Dandelion	2	Poland	[31]
Eucalyptus	5	Portugal	[18]
False indigo	36	Croatia	[26]
Fir	17	Greece	[36]
Goldenrod	1	Poland	[31]
Heath	14	Croatia	[26]
Honeydew	65	Poland, Croatia	[23,26,31,32]
Immortelle	6	Croatia	[26]
Lavander (Lavandula spp.)	90	Portugal	[34]
Lime	44	Croatia	[26]
Linden	3	China	[20,23]
Litchi	8	China	[20]
Locust	207	China, Croatia, India	[20,26,32,35]
Longan	2	China	[20]
Loquat	2	China	[20]
Magnolia vine (Schisandra chinensis)	2	China	[20]
Mandarin	16	Croatia	[26]
Manuka[b] (MGO 550+)	1	New Zealand	[40]
Maple-leaved Bayur (Pterospermum heterophyllum)	4	China	[20]
Matrimony vine	2	China	[20]
Motherwort	4	China	[20]
Multifloral	153	Portugal, Poland, Croatia, India, Indonesia	[18,23,26,31,35,41]
Multifloral[a]	26	Portugal	[18]
Multifloral	92	Spain	This work
Orange	2	Portugal	[18]
Pine	16	Greece	[36]
Pure[b]	9	USA	[39]
Rape	9	Poland	[31]
Rosemary	6	Portugal	[18]
Rosemary-heather	1	Portugal	[18]
Safflower	2	China	[20]
Sage	76	Croatia	[26]
Sahara (Euphorbia spp.)	1	Algeria	[42]
Sautsama (Citrus unshiu)	10	Croatia	[33]
Selfheal	4	China	[20]
Siberian apricot (Armeniaca sibirica)	2	China	[20]
Silver bar	2	China	[20]
Spur-flower (Plectranthus rugosus)	11	India	[35]
Sunflower	12	Portugal, Poland, Croatia	[18,23,32]
Sweet chestnut	110	Croatia	[26]
Sweet Osmanthus	2	China	[20]
Thyme	40	Portugal, Greece	[18,36]
Twigs of the chaste tree	2	China	[20]
Wax tree (Sapium sebiferum)	34	China	[20]
Winter	26	China	[20]
Winter savory	6	Croatia	[26]

[a] With an important percentage of Rosemary pollen but not enough to be considered monofloral honey.
[b] Commercially obtained.

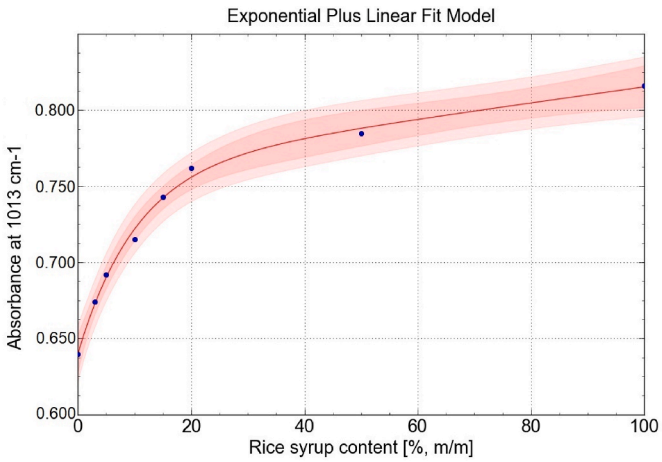

Fig. 5. Scatterplot of the average intensities of working solutions at 1013 cm^{-1} as a function of the degree of adulteration, adjusted with the EPL model fit (0.997953). The dark red shadow shows the confidence band, and the soft red shadow shows the prediction band. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Brief statistical overview of the fitting EPL model obtained with Curve Expert software.

Overview			
Name		Kind	
Exponential Plus Linear		Regression	
Family		Equation	
Yield-Density Models		y = a + b*r^x + c*x	
Standard Error		Weighting	
0.005005894784795632		Default	
Coeff. Of Determination (r^2)		Correlation Coeff. (r)	
0.995910483299878		0.997953	
DOF		AICC	
4		−78.299403	
Model Parameters			
Parameter	Value	Standard Error	Range (95%) confidence)
a	0,762575	0.010777	0.732655 to 0.792496
b	−0.122367	0.010516	−0.151564 to −0.093170
c	0.000527	0.000136	0.000149 to 0.000905
r	0.906664	0.014849	0.865436 to 0.947892

Table 4
Precision and accuracy results obtained using the EPL model for the quantification of rice syrup content in the intentionally adulterated samples (n = 32).

Rice Syrup Nominal Content (%, m/m)	Mean Content of Rice Syrup obtained with EPL model (%, m/m)	Interval of Confidence (α = 0.05)	Standard Deviation (%, m/m)	Variation Coefficient (%)	Relative Error with EPL Model (%)
0.0	0.02	±0.0004	±0.001	5.0	0
3.0	2.9	±0.02	±0.05	1.7	−3.3
5.0	5.1	±0.02	±0.06	1.2	2.0
10.0	9.9	±0.01	±0.04	0.4	−1.0
15.0	15.1	±0.02	±0.06	0.4	0.7
20.0	20.5	±0.02	±0.07	0.3	2.5
50.0	49.8	±0.1	±0.4	0.8	−0.4
100.0	102.1	±0.4	±1	1.2	2.1
Mean Results			**±0.2**	**1.4**	**0.32**

mathematical methods, which allows us to apply this methodology in a simple way that can be within reach of most packers and other stakeholders. In short, this study enables the identification of pure honey and the discrimination of honey adulterated with rice syrup, which provides a new direction for analytical alternatives that seek to solve the far-reaching problem of detecting adulterated honey.

Credit author statement

Jafet Cárdenas-Escudero: Conceptualization, Methodology, Validation, Formal analysis, investigation, writing the original draft, Writing e review & editing. David Galán-Madruga: Conceptualization, formal analysis, review & editing. Jorge O. Caceres: Writing e original draft, writing e review & Editing, Supervision, Project administration, and Funding acquisition.

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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