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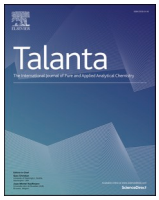

FTIR-ATR detection method for emerging C3-plants-derivated adulterants in honey: Beet, dates, and carob syrups

J. Cárdenas-Escudero [a,c], D. Galán-Madruga [b], J.O. Cáceres [a,*]

[a] Laser Chemistry Research Group, Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, Plaza de Ciencias 1, 28040 Madrid, Spain
[b] National Centre for Environmental Health. Carlos III Health Institute, Ctra. Majadahonda-Pozuelo km 2.2, 28220, Majadahonda, Madrid, Spain
[c] Analytical Chemistry Department, FCNET, University of Panama, Ciudad Universitaria, Estafeta Universitaria, 3366, Panama 4, Panama City, Panama

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ABSTRACT

The European Union Publications Office has recently presented a report on the European Union's coordinated action with the Joint Research Centre to determine certain fraudulent practices in the honey sector, in which it has been indicated that 74% of the samples analyzed, imported from China, and 93% of the samples analyzed, imported from Turkey, the two largest honey producers worldwide, presented at least one indicator of exogenous sugar or suspicion of being adulterated. This situation has revealed the critical state of the problem of honey adulteration worldwide and the need to develop analytical techniques for its detection. Even though the adulteration of honey is carried out in a general way with sweetened syrups derived from C4 plants, recent studies have indicated the emerging use of syrups derived from C3 plants for the adulteration of honey. This kind of adulteration makes it impossible to analyze its detection using official analysis techniques. In this work, we have developed a fast, simple, and economical method based on the Fourier transform infrared spectroscopy technique, with attenuated total reflectance, for the qualitative, quantitative, and simultaneous determination of beetroot, date, and carob syrups, derived from of C3 plants; whose available bibliography is very scarce and analytically not very conclusive for its use by the authorities. The proposed method has been based on the establishment of the spectral differences between honey and the mentioned syrups at eight different points in the spectral region between 1200 and 900 cm^{-1} of the mid-infrared, characteristic of the vibrational modes of carbohydrates in honey, which allows the pre-discrimination of the presence or absence of the syrups studied, and their subsequent quantification, with precision levels lower than 2.0% of the relative standard deviation and relative errors lower than 2.0% (m/m).

1. Introduction

1.1. Honey adulteration problematic

Honey is a delightful and viscous substance produced by *Apis mellifera* bees by collecting nectar from the flowers of plants, which is mixed with their saliva and stored in their honeycombs for maturation [1–3]. The production of honey is such a special process that it gives honey a characteristic composition, mainly of sugars and water. However, enzymes, minerals, vitamins, proteins, and amino acids can be found in honey as a minority [4]. This composition gives honey properties that have been of special benefit to the world's population since ancient times. This fact is supported by a great diversity of studies carried out by many researchers who have demonstrated its pharmaceutical [5–8], antibacterial [9,10], anti-ulcerative [9,11], gastroprotective [9,10,12], cardioprotective [10,13], anti-diabetic [9,10], anti-inflammatory [9,10,14], antioxidant [9,10,12,15,16], regenerative properties [17], and many others properties [9,10,18–22]; generally associated with the content of polyphenols present in honey [23].

In recent decades honey has become the target of adulteration with cheaper sweeteners, and a large number of countries have reported at least 21 different cheap sweeteners that have been used for the direct adulteration of honey [24]. Furthermore, honey adulteration has become an increasingly complicated problem due to the spread of fraudulent practices of honey adulteration with cheap sugar syrups, which have been detected in several countries, including China [24], the

* Corresponding author.
E-mail address: jcaceres@ucm.es (J.O. Cáceres).

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world's leading honey producer [25]. As a result, honey has become the third most adulterated food product worldwide, after milk and olive oil [26]. 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 [27,28]. Also, due to its increased demand as a sugar substitute, the limitations inherent to its production, its nutritional value, and the media proliferation of its properties thanks to the development of social networks.

Although the general population may understand the addition of sugar syrups to pure honey as a situation without major consequences, the reality is that honey adulteration is a major economic and health problem, given the severe consequences associated with the trade and consumption of adulterated honey. According to the United States Food and Drugs Administration (USFDA) [29], honey adulteration with sugar syrups constitutes an economically motivated fraud due to the fraudulent increase in both honey production and the economic profits of packers and distributors. Furthermore, adding sugar syrups causes honey to significantly change its characteristics, such as enzymatic activity, electric conductivity, and the content of specific compounds that give it its quality and characteristic properties [30,31], which results in harm its beneficial properties, nutritional value, and accurate cost.

As if that were not enough, scientific studies have presented overwhelming evidence of the impact on health and its complications directly associated with the consumption of adulterated honey, which can cause significant health problems such as increased blood sugar, insulin hormone release, and type II diabetes, increasing of blood lipid levels and abdominal weight, obesity, and high, blood pressure is notable examples [31–34]. In addition, adulterated honey consumption may cause death [32,35] related to severe affections on internal organs, like fatty liver, acute and chronic kidney injury [34], and elevated visceral fat pads and total body fat. On the other hand, since honey adulterants are generally cheap sugar syrups, there is a significant possibility that they can transfer pathogenic substances such as mycotoxins, phytotoxins, antibiotics and their metabolites, heavy metals, industrial contaminants, and allergens to honey, which further aggravates the health scenario of adulterated honey consumption.

1.2. C3-plant-derivated-syrups and honey adulteration analytical problematic

The most used honey adulterants, such as high fructose corn syrup and corn syrup [24], come from C4 plants, which has facilitated their identification since bees use the nectar of C3 plants to produce honey. This fact has allowed the development of multiple analytical methods for the detection of this kind of C4-plants-derivated adulterants, especially the official method of analysis 998.12 based on the determination of the Carbon Isotope Ratio ($^{13}C/^{12}C$) by isotope ratio mass spectroscopy (IRMS) [36]. However, this method cannot detect adulterations with syrups derived from C3 plants [24]. In addition to the official method, a wide variety of analytical methods are currently used for detecting honey adulteration, like nuclear magnetic resonance (NMR) techniques [37]. However, detecting adulterants derived from C3 plants is an analytical complication for IRMS and NMR-based methods [38]. This fact has resulted in unscrupulous traders using sugar syrups derived from C3 plants for honey adulteration due to the difficulty of analytical detection of this type of syrups.

Some studies have detected the presence of C3-plant-derivated sugar syrups in honey, such as rice syrup, using advanced techniques such as HPLC-DAD [39]. Other syrups derived from C3 plants have been detected as adulterants in honey. Such is the case of beet [40,41], date [42,43], and carob [44] syrups. However, the literature on the detection of these three syrups is extremely scarce and limited to qualitative determinations, generally applying statistical methods of analysis based on differences in physical properties [42], but which are not capable of inferring concrete results on the identity of the adulterant and its quantification in honey. These detection methods require the use of one [41] or two [43] advanced analytical techniques, in addition to complex chemometric treatment [40,42], not to mention the complicated sample preparation stages [40] using solvents [41] that constitute a potential danger to human and environmental health, in addition to the problems of waste treatment. So, these methods are costly alternatives from an instrumental point of view and require personnel with advanced knowledge, are complicated to carry out, are not fast, and are not an efficient option from the point of view of resource consumption, nor do they offer analytically convincing results to combat the problem of honey adulteration. And in this sense, the most important organization of beekeepers in the world, APIMONDIA, considers that the problem of detecting honey adulteration has not yet been effectively resolved [45]. For its part, the European Commission, 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 [46]. Therefore, the lines of research related to developing analytical techniques and methods for detecting honey adulteration are currently necessary and justified. Even more so with the recent report from the Joint Research Centre that has qualitatively detected the presence of at least one marker of sugars exogenous to honey in 74% of samples analyzed imported from China; and in 93% of samples analyzed from Turkey [47], the two largest honey producers worldwide [25].

In this sense, the purpose of this research has been oriented toward the development of a technique for the qualitative and quantitative detection of honey adulterants derived from C3 plants, specifically beet, date, and carob syrups, by using the Fourier Transform Infrared Spectroscopy technique with attenuated total reflectance (FTIR-ATR), quickly and easily, without the need for complex chemometric or mathematical treatments, with a relatively easy sample preparation step, without the use of expensive solvents; so that it can constitute a fast, easy and economical alternative for the detection of the mentioned syrups used as emerging adulterants in honey.

2. Material and methods

2.1. Samples and sample treatment

To carry out this study, 15 samples of multifloral honey collected in various places in Spain, directly from beekeepers, by the Spanish Beekeeping Association and delivered to our laboratory were used. In addition, the beet, date, and carob syrups used in the study were purchased commercially. Initially, the pure honey samples were incubated in a DIGITRONIC model J.P. Selecta (Barcelona, Spain) universal precision oven at 40 °C overnight to eliminate the crystallized phase in some samples. Next, the honey was shaken vigorously using a 7000384 model J. P. Selecta (Barcelona, Spain) vibrating shaker, or vibromatic, with a speed setting of 950 u/min for 1 h to guarantee homogeneity in pure honey samples. Subsequently, all the honey samples were adjusted to a standard solids content of 70 °Brix, adding Type 1 water (18.2 MΩ • cm) produced by IQ 7000 model Merck Milli-Q ultrapure water system (Darmstadt, Germany), and with the help of a 068621 ATC model Dominique Dutscher (Alsace, France) handheld refractometer. The latter is to avoid spectral variations due to natural differences in sugar concentration in the honey used in the study. After this simple preparation, we proceeded to prepare the working solutions or intentionally adulterated honey samples, with each of the selected syrups, in an content of 0, 3, 5, 10, 15, 20, 50, and 100% (m/m), with the aid of a 125A model Precisa Instruments A.G. (Dietikon Switzerland) analytical balance to accurately and precisely weigh components. Also, ten samples of commercially obtained honey were used for this study, four with a protected designation of origin (DOP) and six labeled as pure honey.

2.2. Instrumentation and analytical procedure

All samples of pure honey, syrups, working solutions and commercially obtained honey were analyzed at room temperature using an

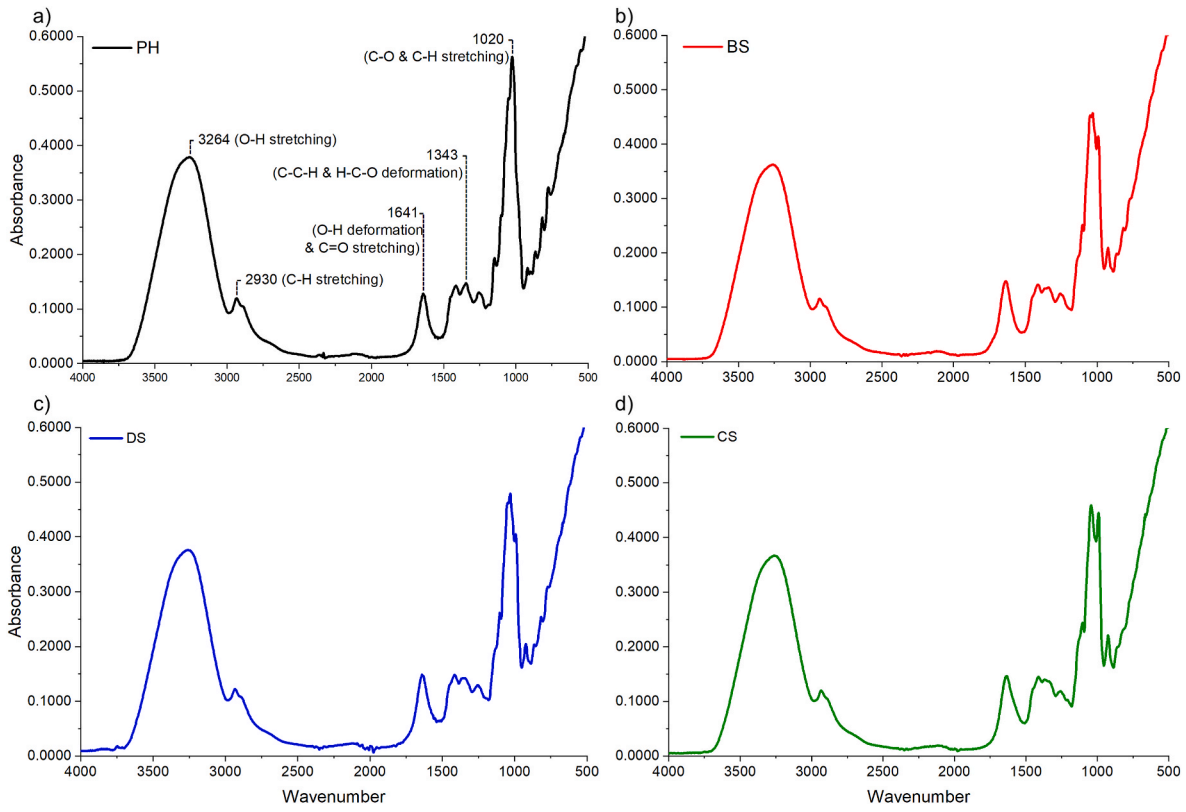


Fig. 1. FTIR-ATR average (n = 20) spectra obtained from a) multifloral honey (PH), b) beet (BS), c) dates (DS) and d) carob (CS) syrups; from 4000 to 500 cm^{-1}. The spectrum of multifloral honey is shown with its main FTIR-ATR spectral features.

Alpha II model Bruker Optics GmbH & Co. K.G. (Ettlingen, Germany) FTIR spectrometer equipped with a high-resolution deuterated triglycine sulfate detector (HR-DTGS), coupled with a platinum Alpha II-P model attenuated total reflectance (ATR) accessory from the same brand house, equipped with a 2 × 2 mm monolithic diamond crystal sampling surface. The spectrometer was turned on 24 h before making the records to ensure maximum stability of the measuring instrument's power source, and it was kept turned on throughout the period in which the experimental work was carried out. The diamond crystal's surface was cleaned with water and acetone between each record and immediately dried. To perform the spectral records, the native software of the spectrometer, version 7.5 (build 7.5.18 (20140810)) OPUS, was executed in its. The spectrometer was configured with a resolution of 4 cm^{-1} and 40 scans (4:40) and a measurement range between 400 and 4000 cm^{-1} (this configuration was obtained in a previous analysis by studying the time of spectral record as a function of the smallest coefficient of variation). Before each record, a background correction was done using the OPUS software's Background option, with the diamond crystal's surface clean and dry. Twenty spectra (replicates) were recorded for each sample used in this study (multifloral honey, syrups, working solutions and commercially obtained honey). All the obtained spectra were subsequently pre-processed for offset-correcting of straight baselines that deviate from zero (linear baseline correction) and using as smoothing algorithm a 3rd order Savitzky-Golay smoothing [48]; with the assistance of the SpectraGryph optical spectroscopy software [49] version 1.2.16.1 (Oberdstdorf, Germany). Finally, the average spectrum (n = 20) of each sample was calculated.

2.3. Data analysis

2.3.1. C3-plants-derivated syrups detection

From the spectral information of the pure honey and beet, dates, and carob syrups, it is possible to identify the points where spectral differences were observed in the region of interest, from 1200 to 900 cm^{-1}. Once these points were established, a principal component analysis was carried out using the absorbance information of pure honey and syrups at the points of the spectrum where significant spectral differences were observed. This was obtained using the 2021 version OriginPro software designed by OriginLab Corporation (Massachusetts, U. S.A.).

2.3.2. C3-plants-derivated syrups quantification

From the spectral information of the pure honey samples intentionally adulterated at 0, 3, 5, 10, 15, 20, 50, and 100% (m/m) for each of the three adulterants used (working solutions), the first derivative of the spectra in the range of 1200–900 cm^{-1} was determined to accurately establish the spectral points in which the maximums occur in the region of interest. The coefficient of determination (r^2) between the degree of adulteration as a function of absorbance was determined using the maximums peak signals previously delimited by the first derivative spectra, in the region of interest, for each spectrum for the three adulterants used in the study. Having calculated the determination coefficients, the highest one was selected to generate the calibration graphs for each of the three adulterants selected in this study, using the OriginPro software. Because honey can be adulterated with a syrup content that can vary widely, calibrations were used for an adulterant content range of 0–100% (m/m), consisting of two sub-ranges. The first range of adulterant content was distributed from 0 to 20% (m/m), specifically in 5% (m/m) increments, i.e. 0, 5, 10, 15 and 20% (m/m) of syrup content derived from C3 plants, also including a 3% (m/m) point in the calibration to verify the suitability of the method, in terms of accuracy and precision, in the quantification of low adulterant concentrations. The second sub-range was between 20 and 65%, in 15% (m/m) increments of adulterant content, specifically 20, 35, 50 and 65% (m/m). Finally, a last point of 100% (m/m) adulterants derived from C3 plants was used in the calibration to verify the linearity of the

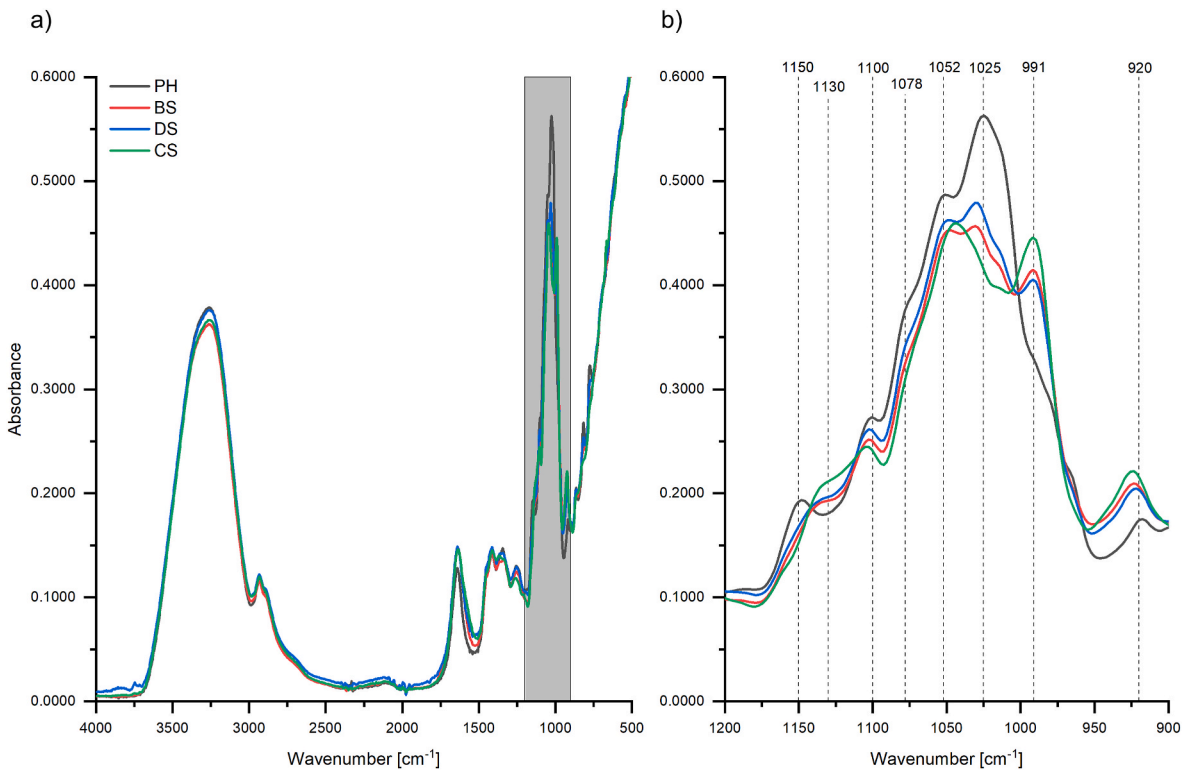


Fig. 2. FTIR-ATR spectral differences between pure honey (PH) and selected C3-plants-derivated syrups. a) Comparison of FTIR-ATR spectra (4000–500 cm^{-1}) of pure honey (PH) and beet (BS), dates (DS), and carob (CS) syrups. The grey zone indicated the region of interest. b) Zoomed region of interest spectra from 1200 to 900 cm^{-1} with labeled spectral features.

quantification method over the entire range 0–100% (m/m) adulterants derived from C3 plants. For the validation of the calibration model, due to the impossibility of obtaining certified standards of adulterated honey, working solutions were used, whose degrees of adulteration are known. Although the authors know the origin and degree of adulteration of all the samples, these variables are completely unknown to the instrument and the calibration model, and the results obtained do not depend on this knowledge, but on the spectral characteristics of the sample attributed to the presence or absence of the adulterants used in this study.

2.3.3. Commercial honey samples analysis

The samples were prepared before their analysis according to the protocol described in section 2.3.1. After their preparation, the FTIR-ATR spectra of commercially obtained honey samples were directly recorded in the FTIR spectrometer according to the procedure described in section 2.3.2.

3. Results and discussion

3.1. Pure honey and selected C3-plant-derivated adulterants spectra

Several authors have extensively studied the FTIR-ATR spectrum of honey [24,50–59]. Fig. 1a shows the average spectrum obtained from the 15 samples of multifloral honey studied in this investigation. At 3254, 2930, 1641, and 1020 cm^{-1}, significant signals correspond to O–H stretching, C–H stretching, O–H deformation and C═O stretching, and C–O and C–H stretching, respectively. Other significant signals around the 1343 cm^{-1} region correspond to the C–C–H and H–C–O deformation.

By performing a simple band assignment in the spectrum of honey, we can infer and confirm the presence of its components [60]. For example, the broadband observed at 3264 cm^{-1} is a characteristic sign of water and sugars present in honey [55], while the peak observed in the region between 3000 and 2800 cm^{-1} corresponds to vibrational modes of carbohydrates [57], amino acids [50] and carboxylic acids [61] in honey. When reviewing the spectrum of honey, the region of the fingerprint (1500 - 700 cm^{-1}), which is characterized by indicating various vibrational modes of carbohydrates and ketones [55], signals are observed at 1343 and 1020 cm^{-1}, which are attributed to vibrational modes of stretching and bending of the C–OH, C–H, and C–O bonds of carbohydrates [62,63]. Thus, it is confirmed that the main components of honey are water, carbohydrates, and other sugars, as has been inferred in similar previous studies [50,51,55,57,58,60–65].

On the other hand, Fig. 1b, c, and d show the average FTIR-ATR spectra (n = 20) of beet, dates, and carob syrups, respectively. We observed remarkable spectral similarity when carefully observing the spectra of syrups and honey. However, in the region between 1200 and 900 cm^{-1}, spectral differences can be observed between them. Since this region includes characteristic vibrational modes of carbohydrate bonds and given the difference in the sugar composition of honey and selected syrups, the study of this spectral region is of special interest.

By overlapping the spectra of the honey and the syrups studied (Fig. 2a), a clear differentiation is observed in the surrounding signals at 1020 cm^{-1}. Specifically, by strictly studying the overlapping spectra's 1200 - 900 cm-1 region (Fig. 2b), we observed eight significant differences in the observed band within the region of interest. The difference in the sugar content between the honey and the syrups studied gives these spectral features. It is important to note that the spectra of the syrups show a very similar spectral performance between them, evidence on which the subsequent classification and identification mechanism of C3-plants-derivated syrups was based.

3.2. Identification of emerging C3-plants-derivated adulterants in honey

A simple principal component analysis was performed using a differentiation parameter of the absorbances given for the eight sites of

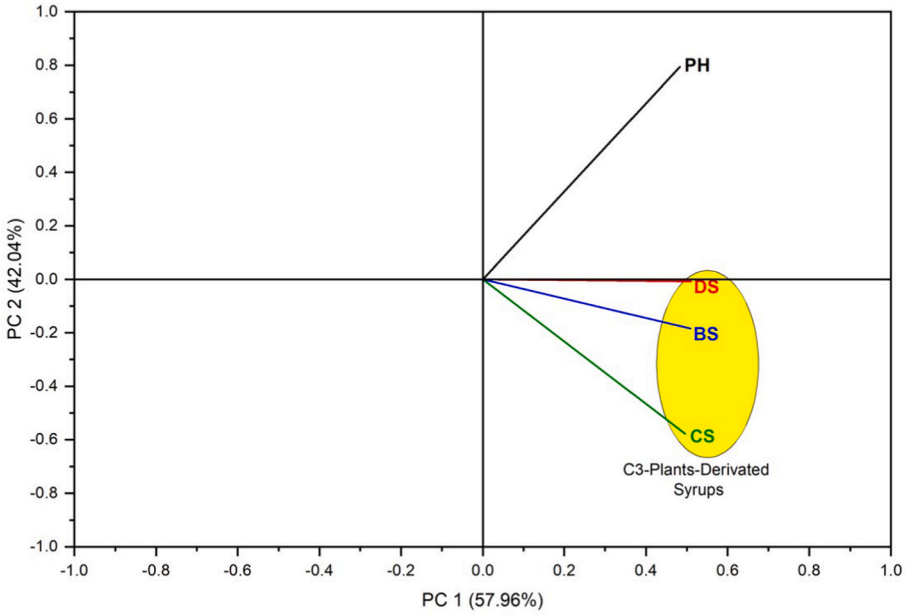


Fig. 3. results of the PCA analysis performed using the absorbances of pure honey and beet, date, and carob syrups for the eight different sites where spectral differences are observed in the region of interest: 1150, 1130, 1100, 1078, 1052, 1025, 991 and 920 cm^{-1}.

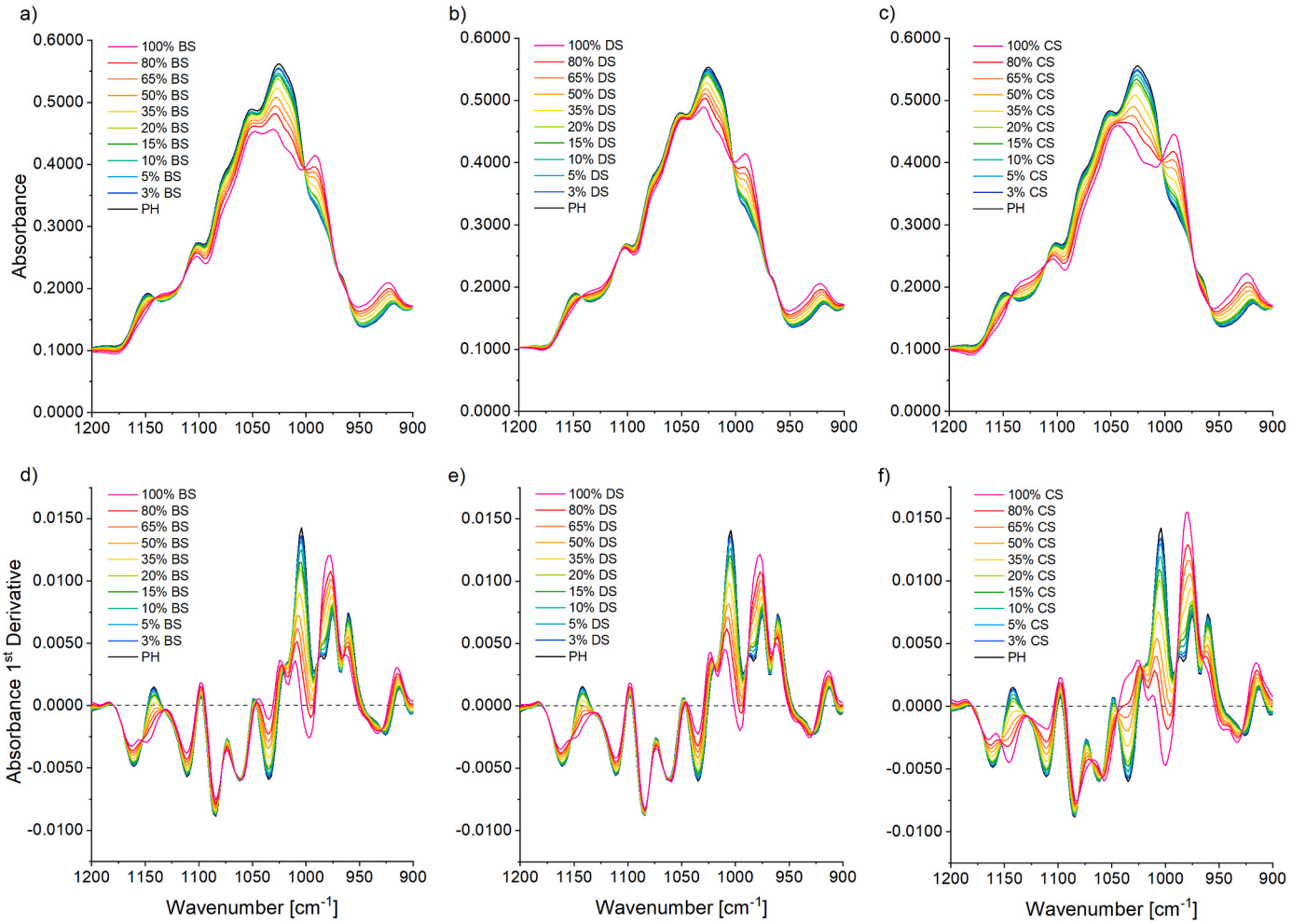
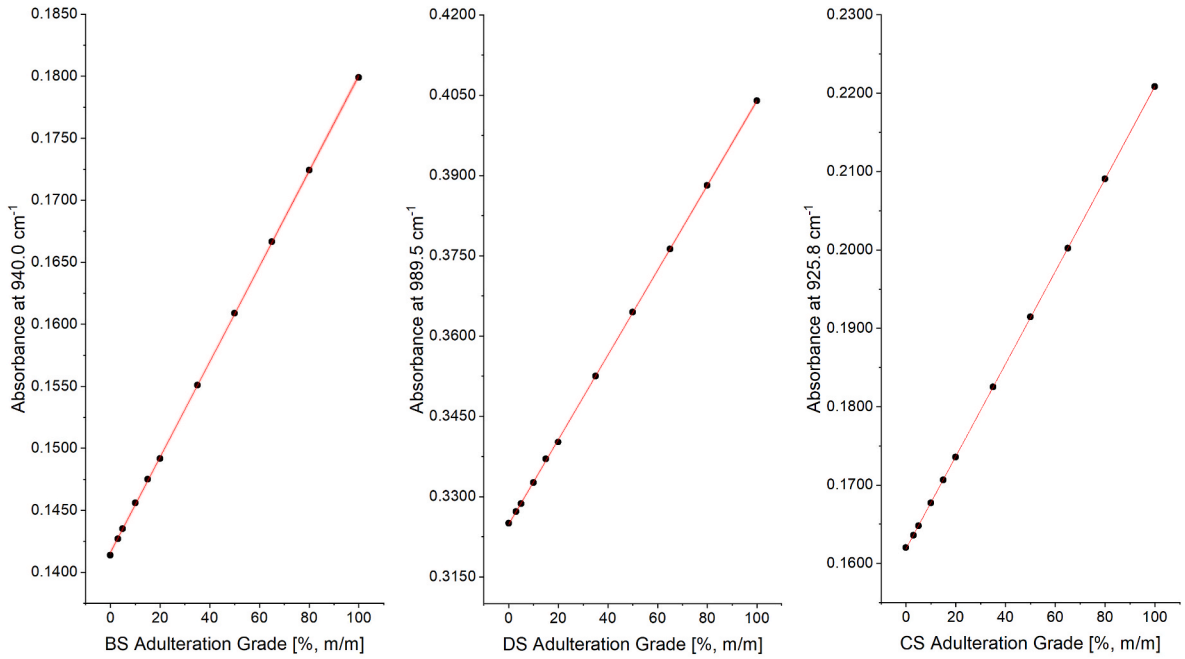


Fig. 4. Average spectra (n = 20) of pure honey adulterated at 0, 3, 5, 10, 15, 20, 50, and 100% (m/m) with: a) beet syrup, b) date syrup, c) carob syrup; with their respective first derivative: d) adulterations with beet syrup, e) adulterations with date syrup and f) adulterations with carob syrup.



Equation	y = a + b*x
Plot	Beet syrup
Weight	No Weighting
Intercept	0.14161 ± 1.23971E-4
Slope	3.85418E-4 ± 3.04515E-6
Residual Sum of Squares	4.51102E-7
Pearson's r	0.99981
R-Square (COD)	0.99963
Adj. R-Square	0.99956

Equation	y = a + b*x
Plot	Dates syrup
Weight	No Weighting
Intercept	0.32481 ± 1.14732E-4
Slope	7.91829E-4 ± 2.81822E-6
Residual Sum of Squares	3.86374E-7
Pearson's r	0.99996
R-Square (COD)	0.99992
Adj. R-Square	0.99991

Equation	y = a + b*x
Plot	Carob
Weight	No Weighting
Intercept	0.16185 ± 4.20348E-5
Slope	5.90135E-4 ± 1.03252E-6
Residual Sum of Squares	5.18628E-8
Pearson's r	0.99999
R-Square (COD)	0.99998
Adj. R-Square	0.99998

Fig. 5. Calibration curves for the quantification of a) beet syrup, b) date syrup, and c) carob syrup, each with its corresponding statistical information. Note that for each of the three calibrations, the standard deviation of the intercept, slope, and residual of the sum of squares are small enough to indicate the good statistical quality of the calibrations.

spectral difference in the region of interest (1150, 1130, 1100, 1078, 1052, 1025, 991, and 920 cm^{-1}). Fig. 3 shows the results.

The results of this simple PCA analysis, based on the spectral difference in the region of interest, show the visual classification capacity based on the absorbance of the eight points in which the spectral signals of honey differ concerning the syrups studied in the region of interest. Note that the C3-plant-derived syrup vectors are sufficiently separated from the honey vector in Fig. 3 and that the syrup vectors are relatively close together, unlike honey, indicative of their spectral similarity, which we can associate with using the same photosynthetic route to produce sugars in the C3-plants-derivated syrups.

3.3. Emerging C3-plants-derivated adulterants quantification

The average spectra (n = 20) obtained from the adulteration of multifloral honey with the syrups selected in this investigation are shown in Fig. 4. Note that in the central zone of the region of interest, the absorbance of honey decreases with increasing adulterant content. However, in the extreme zone of the region of interest, below 1000 cm^{-1} it is observed that the absorbance increases with increasing adulterant content. This spectral behavior is evident in Fig. 4a, b, and c.

To verify this, in a similar way to how we have carried out a previous study with adulteration with rice syrup [60], the first derivatives of the spectra were established, in the region of interest, for each of the honey-adulterant groups, as shown for beet, dates and carob syrup in Fig. 4c, d and e, respectively. Regarding the linearity observed between absorbance and adulterant content, for each of the maximums established with the information of the inflection points of the first derivative of the spectra, the results were tabulated in Table S1. The coefficient of determination closest to 1 is shown in bold.

The results obtained show that the spectral points in which the absorbance of the sample shows greater linearity and a directly proportional relationship depending on the degree of adulteration was 940.0 cm^{-1} for beet syrup ($r^2 = 0.9996$); 989.5 cm^{-1} for date syrup ($r^2 = 0.9999$) and 925.8 cm^{-1} for carob syrup ($r^2 = 1.0000$). These results show the convenience of using the previously mentioned positions in the region of interest of the honey spectrum to determine the content of these three syrups derived from C3 plants.

Based on the above, calibrations were prepared for each syrup. The calibration curves and their corresponding linear model statistical aptitude information are shown in Fig. 5.

An analysis of the method's precision and accuracy for quantifying syrups derived from C3 plants was carried out. The results are consolidated in Table 1.

The results of the study of the accuracy and precision of the quantification method are significantly satisfactory since they indicate a very high precision, in terms of coefficient of variation, less than 5.0% for each one of the degrees of adulteration of each one of the three syrups used in this study; and a good accuracy gave the average relative error values below 1.0% for each of the adulterant syrups. These results show excellent analytical aptitude, in terms of accuracy and precision, for the quantification of beet, date, and carob syrups.

3.4. Analysis of commercially acquired honey samples

Using the mechanism employed in section 3.2, we proceeded to determine the potential presence of the studied adulterants in the commercially acquired honey samples, both for the 4 samples with protected denomination of origin (DOP1 – DOP4) and for the 6 samples of commercial honey labeled as pure honey (CH1 – CH6). The results (Fig. 6) show that samples DOP1, DOP4, and CH5 present significant spectral differences in the spectral region of interest (1200 - 900 cm-1)

Table 1
Results of the precision and accuracy analysis of the quantification method for beet, date, and carob syrups. All parameters for n = 20.

	Syrup Content (%, m/m)		Interval of Confidence (α = 0.05)	Standard Deviation (%, m/m)	RSD (%)	Relative Error (%, m/m)
	Nominal	Experimental				
Beet	0.00	−0.54	±0.09	0.10	2.8	0
Syrup at 940.0 cm^{-1}	3.00	2.99	±0.07	0.14	4.8	−0.40
	5.00	4.96	±0.05	0.10	2.1	−0.80
	10.00	10.17	±0.06	0.13	1.3	1.66
	15.00	14.89	±0.06	0.13	0.9	−0.74
	20.00	19.86	±0.07	0.15	0.8	−0.69
	35.00	34.95	±0.09	0.20	0.6	−0.13
	50.00	50.05	±0.08	0.17	0.3	0.11
	65.00	64.68	±0.08	0.18	0.3	−0.49
	80.00	81.09	±0.07	0.15	0.2	1.37
	100.00	100.68	±0.04	0.07	0.1	0.68
	Mean Results			**0.14**	**1.3**	0.71
Date	0.00	0.36	±0.08	0.16	4.1	4.76
Syrup at 989.5 cm^{-1}	3.00	3.14	±0.06	0.13	4.0	4.76
	5.00	5.21	±0.07	0.15	2.8	4.11
	10.00	9.84	±0.06	0.12	1.2	−1.60
	15.00	15.31	±0.09	0.19	1.3	2.06
	20.00	20.20	±0.06	0.12	0.6	0.98
	35.00	35.68	±0.09	0.19	0.5	1.95
	50.00	49.85	±0.05	0.10	0.2	−0.30
	65.00	66.04	±0.06	0,13	0.2	1,59
	80.00	80.20	±0.05	0,10	0.1	0,25
	100.00	100.13	±0.06	0.13	0.1	0.13
	Mean Results			**0.14**	**1.1**	**1.77**
Carob Syrup at 925.8 cm^{-1}	0.00	−0.60	±0.08	0.12	4.1	0
	3.00	3.06	±0.04	0.09	2.8	2.08
	5.00	4.95	±0.05	0.10	2.0	−1.02
	10.00	10.15	±0.05	0.11	1.1	1.51
	15.00	15.05	±0.04	0.09	0.6	0.36
	20.00	20.04	±0.05	0.10	0.5	0.18
	35.00	35.51	±0.08	0.17	0.5	1.45
	50.00	50.33	±0.04	0.08	0.2	0.67
	65.00	65.10	±0.05	0.10	0.2	0.16
	80.00	79.88	±0.08	0.18	0.2	−0.16
	100.00	99.95	±0.05	0.10	0.1	−0.05
	Mean Results			**0.11**	**0.8**	0.76

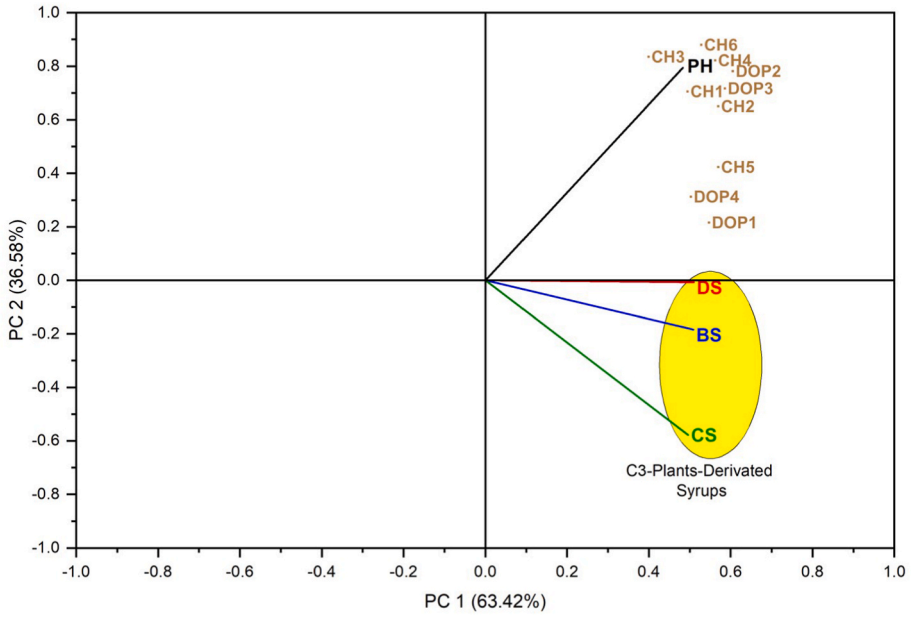


Fig. 6. Analysis of principal components (PCA) of commercially obtained honey, specifically, 4 with protected designation of origin (DOP1-DOP4) and 6 with the labeling of pure honey (CH1–CH6).

since their vectors in the PCA analysis, despite having similar magnitude, are distant from the vector of pure honey.

Moreover, the inference that these three samples may be adulterated with syrups derived from C3 plants is also given by the proximity of these samples to the area of the plane in which the group of syrups derived from C3 plants are found. Therefore, this result can be

Table 2
Qualitative and quantitative analysis results of commercially obtained honey samples for detecting C3-plants-derivated honey adulterants beet, dates, and carob syrups.

No.	Sample	Absorbance			C3-Plants-Derivated Syrup Detection			
					Quantitative (%, m/m)			Qualitative (+ or -)
		at 940.0 for BS	at 989.5 for DS	at 925.8 for CS	Beet Syrup	Date Syrup	Carob Syrup	
1	DOP1	0.1413	0.3270	0.1643	ND	2.76	4.16	+
2	DOP2	0.1368	0.3111	0.1581	ND	ND	ND	–
3	DOP3	0.1365	0.2939	0.1584	ND	ND	ND	–
4	DOP4	0.1412	0.3196	0.1636	ND	ND	2.97	+
5	CH1	0.1394	0.3097	0.1615	ND	ND	ND	–
6	CH2	0.1349	0.3095	0.1562	ND	ND	ND	–
7	CH3	0.1360	0.3062	0.1574	ND	ND	ND	–
8	CH4	0.1333	0.3048	0.1550	ND	ND	ND	–
9	CH5	0.1374	0.3264	0.1596	ND	2.01	ND	+
10	CH6	0.1360	0.3214	0.1579	ND	ND	ND	–

*ND = No detected.

considered presumptive for the presence of the syrups studied in this investigation. It is possible to infer that the rest of the commercially obtained samples present a spectral similarity concerning the average spectrum of pure honey due to the closeness of the plane of the PCA analysis.

In addition, the content of beet, date, and carob syrup in the commercially obtained honey was determined by interpolating the absorbance to the wavenumbers previously established for each syrup in section 3.3. The results are shown in Table 2.

4. Conclusions

The problem of honey adulteration has been complicated by the emerging use of syrups derived from C3 plants, which are impossible to detect by official methods of analysis and require many resources and significant analytical efforts for their detection. However, currently, the available literature regarding the detection of syrups derived from C3 plants, such as beetroot, date, and carob syrups, is already very limited.

In this work, a new method using Fourier transform infrared spectroscopy, by total attenuated reflectance, was developed for the qualitative and quantitative detection of syrups derived from C3 plants, specifically beet, date, and carob syrups. The method is based on the eight specific spectral differences that occur due to honey adulteration with C3-plant-derivated adulterants in the region between 1200 and 900 cm^{-1} of the mid-infrared spectrum, characteristic of vibrational modes of carbohydrates in honey.

The proposed method has shown an excellent analytical aptitude for detecting beetroot, dates, and carob syrups, simultaneously, quickly, efficiently, and economically without the need for cumbersome sample treatments or complicated chemometric procedures. In addition, the accuracy and precision evaluation of the proposed method has been satisfactory, given the low coefficient of variation and relative error results evidenced in the precision and accuracy analyses, which guarantees the reliability of its results. The development of this new and simple methodology has allowed the detection, for the first time, of the presence of date and carob syrups in Spanish commercial honeys, including in two samples of honey with protected designation of origin.

Finally, the presented method constitutes an analytically viable, simple, fast, and economical alternative that facilitates the determination of beet, dates, and carob syrups, simultaneously and with highly accurate and precise results. The FTIR-ATR technique which the method has great potential to be adapted to an in-situ analysis, which can be easily performed by the authorities that regulate the honey market and other stakeholders, such as producers, distributors, and packagers, that fight against the problem of adulteration at the global level.

CRediT authorship contribution statement

J. Cárdenas-Escudero: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **D. Galán-Madruga:** Conceptualization, Formal analysis, Writing – review & editing. **J. Cáceres:** Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no competing financial interest or conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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